Eric Lichtfouse
Editor

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Climate Change, Intercropping, Pest Control and Beneficial Microorganisms

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Climate Change, Society Issues and Sustainable Agriculture

Eric Lichtfouse

If you do not change direction, you may end up where you are heading.

Lao Tsu

Abstract Despite its prediction 100 years ago by scientists studying CO_2, man-made climate change has been officially recognised only in 2007 by the Nobel Prize Committee. Climate changes since the industrial revolution have already deeply impacted ecosystems. I report major impacts of climate change on waters, terrestrial ecosystems, agriculture and the economy in Europe. The lesson from the climate change story is that humans do not learn from scientists until it really hurts. Furthermore, all society issues cannot be solved anymore using the old, painkiller approach because all issues are now huge, linked, global and fast-developing. In that respect, actual society structures are probably outdated. Here, agronomists are the most advanced scientists to solve society issues because they master the study of complex systems, from the molecule to the global scale. Now, more than ever, agriculture is a central point to which all society issues are bound; indeed, humans eat food.

Keywords Climate change · CO_2 · Agriculture, Europe climate · Greenhouse gas · Temperature · Soil C · Plant · Birds · Flood · Growing season · Drought · Food price

1 A 100 Year-Old Prediction

More than 100 years ago, the Nobel Prize winner Svante Arrhenius (1859–1927) estimated that a doubling of atmospheric CO_2 concentration would cause a temperature rise of about +5−6°C (Arrhenius, 1896, Wikipedia). Remarkably, his crude

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estimate is higher but not largely different from the +2.0−4.5°C rise now estimated by the Intergovernmental Panel on Climate Change (IPCC, 2007). Combining his calculations with existing work suggesting that the burning of fossil fuels could significantly alter the concentration of carbon dioxide in the atmosphere (Högbom, 1894), Arrhenius later became the first person to predict the possibility of man-made global warming (Arrhenius, 1908; Weart, 2008).

Now, the recent record of atmospheric CO_2 levels at the Mauna Loa Observatory in Hawaii, known as the "Keeling curve", clearly shows a steady increase from 1958 (Fig. 1, Keeling et al., 2001, 2005; Scripps, 2008). Moreover, the comparison of modern CO_2 levels with ancient ones clearly shows that atmospheric CO_2 has now reached an unprecedented high level during the last 400,000 years, reaching 383 ppm in 2007 (Fig. 2). Despite accumulating evidence from various fields of science, the human origin of climate change has been challenged during the last 30 years. Finally, in 2007, the IPCC and Albert Arnold (Al) Gore Jr. were awarded the Nobel Peace Prize "for their efforts to build up and disseminate greater knowledge about man-made climate change, and to lay the foundations for the measures that are needed to counteract such change". In the next section I report some effects of climate change in Europe (see also Feehan et al., 2009; Jones et al., 2009; Lavalle et al., 2009).

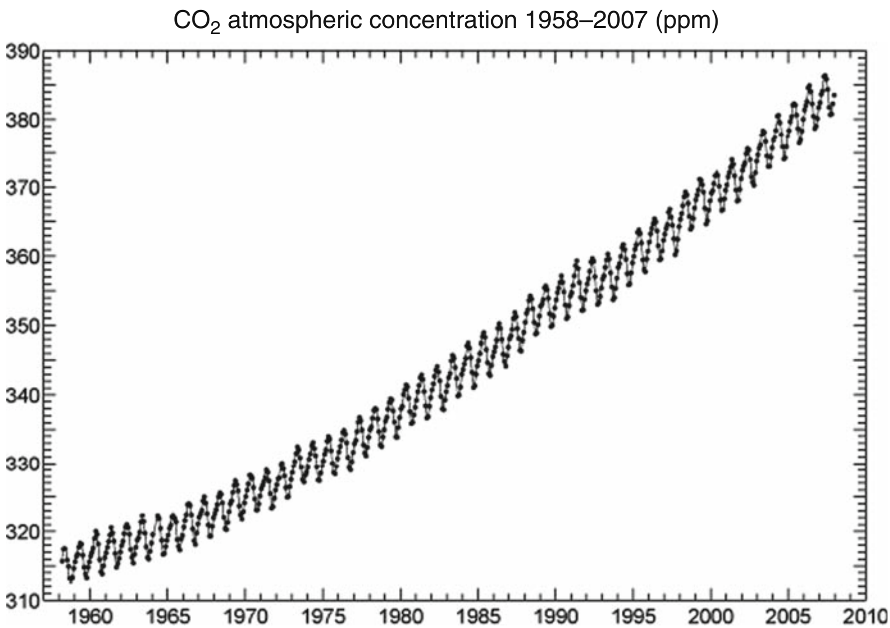

Fig. 1 The Keeling curve. Concentration of atmospheric CO_2 recorded at the Mauna Loa Observatory, Hawaii. The increase from 1958 to 2007 is driven by the burning of fossil fuels. The short annual variations reflect the seasonal growth and decay of land plants in the northern hemisphere. The data are from the Scripps CO_2 program. Reprinted with permission of Dr. Ralph Keeling

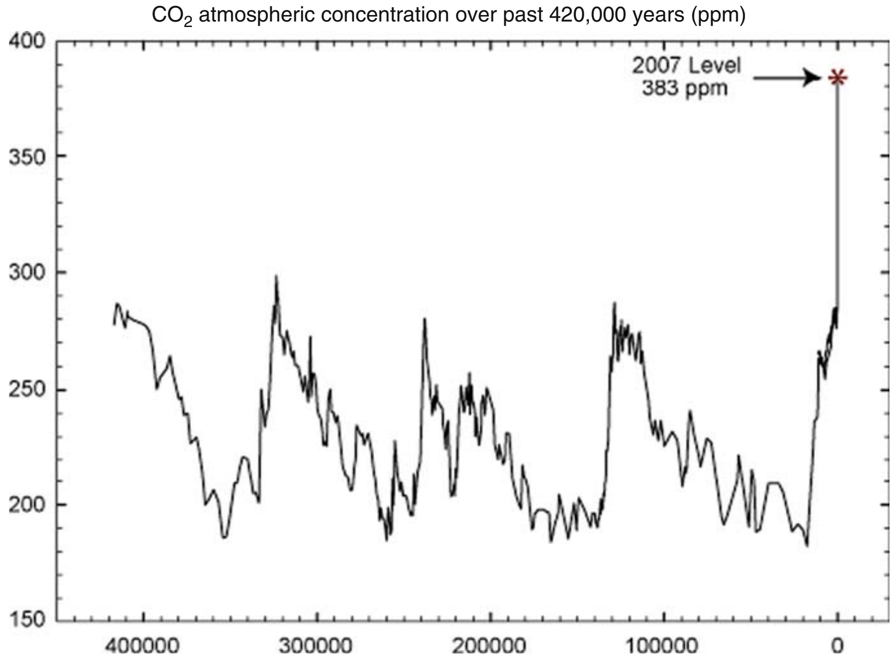

Fig. 2 CO_2 concentrations over the past 420,000 years. The figure shows that in 2007 the CO_2 level has reached an unprecedented high level of 383 ppm. During ice ages, the CO_2 levels were around 200 ppm, and during the warmer interglacial periods, the levels were around 280 ppm. Ancient data are based on reconstructions from polar ice cores. Modern data are from the Mauna Loa record of the Scripps CO_2 program. Reprinted with permission of Dr. Ralph Keeling

2 Climate Change in Europe

2.1 Impact on Climate and Water

The European Environment Agency has recently reviewed major observed and projected climate changes in Europe (EEA-JRC-WHO, 2008). Here I report the major changes that are relevant for agriculture. In Europe, the mean temperature for land has increased by +1.2°C compared with pre-industrial times. There have been more frequent hot extremes and less frequent cold extremes in the last 50 years. During the twentieth century, precipitation has increased by +10−40% in northern Europe and decreased by up to −20% in some southern parts. The intensity of precipitation events has increased in the past 50 years. Dry periods are projected to increase in southern Europe. Annual river flows have increased towards north and decreased towards south. Strong seasonal changes, such as lower flows in summer and higher flows in winter, are expected. Droughts will increase, notably in summer and in the south. Climate change has increased ozone concentrations in central and south-western Europe. European glaciers have lost 33% of their volume since 1850, with loss accelerating since 1980. Snow cover has decreased by 13% during the last 40 years. Mountain permafrost is reducing due to temperature increase. Temperatures of lakes and rivers have increased by +1−3°C during the twentieth century.

2.2 Impact on Terrestrial Ecosystems

Warmer temperatures, notably in winter, have shifted plant species northward and uphill. The rate of change will exceed the ability of many species to adapt. Seasonal events in plants are occurring earlier as shown by spring occurring 7 days earlier in 2000 than in 1971, on average. Birds, insects and animals are moving northward and uphill. The life cycles of many species such as birds, frogs and butterflies are advancing. Information on the effect of climate change on soil is scarce. Both rising temperatures and changing precipitation may lead to a decrease in soil organic carbon, and, in turn, an increase in CO_2 emissions. Rainfall changes should also increase soil erosion. In Mediterranean parts and central-eastern Europe intense soil degradation may lead to irreversible desertification.

2.3 Impact on Agriculture

Climate change is affecting the growing season and yields. In the north, the length of the growing season has increased, favouring the introduction of novel crops, whereas in the south, the length of the growing season has decreased locally. Flowering and maturity of plant species occur 2–3 weeks earlier, thus inducing a higher risk of frost damage in spring. Extreme climate events such as the 2003 heat wave and the 2007 spring drought have increased the variability in crop yields. In Mediterranean areas the water demand for agriculture has increased by +50−70%. In continental Europe, most forests are growing faster due to better management, higher N deposition, less acidification, increasing temperature, and increasing atmospheric CO_2 concentrations. Drought and warm winter increase pest populations, and temperature increase raises the risk of forest fires.

2.4 Impact on Economy

Since 1980, about 90% of natural disasters are due to weather and climate. They represent 95% of economic losses caused by catastrophic events. Losses due to climate events have increased during the past 25 years. The economic losses as a consequence of extreme flood in central Europe in 2002 were estimated at 17.4 billion Euro. The economic losses as a consequence of the hot 2003 summer were estimated at 10 billion Euro.

3 A Novel Approach to Solve Society Issues

The lesson from the history of climate change is that *humans do not learn from scientists' predictions until it really hurts*. This principle has always been true throughout history. It can easily be applied to most current society issues. What has changed, however, is the nature of today's negative impacts. While impacts were mainly small, local and slow to develop before the industrial revolution, they are now huge, global and fast. As a result, while it was previously possible to solve issues by treating only impacts, because impacts were rather isolated from the whole system, this "painkiller" or "fireman" approach does not work anymore because all issues are now closely interconnected in space and time. For instance, hunger and poverty of African nations is now closely linked to global warming, which, in turn, is mainly due to excess fossil fuel consumption of rich nations. Hunger and poverty in poor nations is also caused now by growing energy crops in rich nations. For instance, switching the use of maize from food to biofuel has dramatically increased maize prices in the world market. Therefore, providing food to poor countries, the painkiller approach, will not succeed in the end if the energy issue is not dealt with at the same time. In that respect ecological activists were right in the 1970's to claim that treating issue sources is better than treating impacts.

However, treating an individual issue source is not sufficient anymore today because all issue sources are closely linked. For instance, decreasing fossil fuel consumption in rich countries implies using cars less and less cars. This will be difficult on two grounds, at least. First, modern nations such as the United States of America have built their towns to fit with car use, with rather long distances from home to workplace and shopping malls. It will thus be difficult to turn back to using bicycles. Europe is actually adapting better in that respect because towns were built in a much more concentrated way in the Middle Ages when citizens were walking. Nonetheless, all citizens of rich nations should rethink their whole actual way of life, instead of applying the "I shop therefore I am" principle (Kruger, 1987). Second, the obesity issue in rich nations has increased so much that about half of the people will not even be able to turn back to walking and cycling on health grounds (Wall-E, 2008). Unexpectedly, poor nations may adapt better in that respect, because they are less "artificialised". To conclude, these examples demonstrate the dependence of seemingly independent, far-reaching issues: hunger in poor nations, global warming, energy crops, obesity in rich nations, spatial structure of modern towns, etc. Other recent, striking examples that show that society issues have no frontiers are World Wars and Chernobyl. Advices to solve major society issues are given in my previous article (Lichtfouse, 2009). It is suggested in particular to study artificialisation and to change sharply the society structures to adapt to current issues.

4 Sustainable Agriculture for Solving Society Issues

All society issues such as health, hunger, poverty, climate change, energy, economy, employment, politics and war are related to agriculture. Hunger and poverty in poor nations is related to crop production. Diseases in poor nations are linked to poverty and, in turn, to competitiveness of agriculture in a global market. Fast-emerging health problems such as cancer are partly due to food and water contamination by pesticides and other agrochemicals. Atmospheric pollution is caused in part by trucks and planes carrying food over long distances. Industrial agriculture is transferring soil carbon in the atmosphere as CO_2 and, in turn, increasing global warming. War is historically linked with agriculture because early agriculture was at the origin of territories and borders. War is economically related to agriculture, because wars are usually fought to control or develop markets. And so on. Agriculture is thus a central point to address most society issues. Here agronomists are thus the most advanced scientists because they are used to decipher complex scientific, social, political and economic issues at various levels in space and time (Lal, 2009a,b; Lichtfouse et al., 2009). Indeed, agronomists usually address a problem using the "system" approach which considers that a specific problem is never isolated and is part of a whole system of factors that can impact positively or negatively the problem. Now, by developing principles of sustainable agriculture, agronomists have thus the potential to solve not only common agricultural issues such as "low crop yield", but also world issues such as wars, poverty and climate change.

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Tragedy of the Global Commons: Soil, Water and Air

Rattan Lal

What is common to the greatest number gets the least amounts of care. Men pay most attention to what is their own: they care less of what is common.

Aristotle

Abstract Soil and water resources, essential to the wellbeing of humanity, are severely degraded and polluted. Degradation of natural resources, caused by misuse and mismanagement, is intertwined with poverty and desperateness. Poor people do not care about stewardship, and pass on their sufferings to the land. Extractive farming is the principal cause of mining soil fertility and depleting carbon resources. There is a need for developing strong ethics for sustainable management of soil, water, and natural resources.

Keywords Sustainable Agriculture · Soil Degradation · Food Security · Environmental Quality

The Prasna Upanishad, an ancient Sanskrit scripture, states "Kshiti (soil), jal (water), pawak (heat), gagan (sky), smeera (air); Panch (five) tatva (elements) yah (from) adham (made) sharira", or the human body is comprised of soil, water, heat, ether and air. The importance of the quality of soil, water and air for welfare of the biosphere in general and of the human in particular has been recognized for millennia. Yet, these three vital components of the environment have been taken for granted; misused, abused and exploited for short-term material gains; and often ignored and left to fend for themselves. The degradation and abuse of these common resources raises several questions:

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- Why are these basic truisms, e.g., Prasna Upanishad, so fundamental and vital to human welfare and recognized for millennia by most ancient cultures and civilizations, forgotten and ignored?
- Why was the desertification (dust-bowl)-related poverty and economic collapse in the USA serious in the 1930s but not so since the World War II and the latter half of the twentieth century?
- Why were poverty and environmental degradation serious issues in Iceland until 1950s but followed by an era of prosperity and land stewardship?
- Why are agriculture and economy thriving in Israel despite the harsh arid environments normally prone to desertification and poverty trap?
- Why did the Aral Sea and Lake Chad suffer a catastrophic demise since 1960s?
- Why did the once highly productive and profitable agriculture of Rhodesia/Zimbabwe collapse since 1980s?
- Why has it been difficult to break the vicious cycle of soil degradation-poverty-more degradation in Sub-Saharan Africa?
- Why nutrient mining at 20–40 kg/ha/yr of NPK has perpetuated at continental scale since 1950s in Sub-Saharan Africa with the attendant decline in soil productivity and increase in hunger and malnutrition?

Perhaps the answer to these and similar questions for other ecoregions lies in: (a) governance, political will and visionary leadership; (b) public awareness about land stewardship and land ethics; (c) use of modern technology; and (d) attitude towards management of the global commons. Garret Hardin (1968), in his essay “The Tragedy of the Commons”, reinforced what the Greek philosopher Aristotle (384 BC–322 BC) wrote “What is common to the greatest number gets the least amounts of care. Men pay most attention to what is their own: they care less of what is common”.

The tragedy of the environmental commons has exacerbated the problems of: (a) pollution of the oceans and rivers, (b) pollution of the atmosphere, (c) mining of soil carbon for plant nutrients (NPK) leading to emission of CO_2, (d) over-exploitation and poisoning of the ground water, and (e) deforestation of the world’s pristine forests including those in North America and Europe.

The good news is that we do now recognize that mismanaging the commons causes serious problems: deforestation causes flooding and landslides, fossil fuel combustion causes global warming, acid rain kills trees, and mining soil fertility causes hunger, malnutrition and social/political instability. The bad news is that we do not yet recognize the need for using the collective wisdom and for cutting-edge science to address the problems of the global commons. It is logical that we invest in common resources, e.g., soil, water and air, to restore and improve them, and to enhance their ecosystem services for present and the future generations. Institutional arrangements, nationally and internationally, are needed to identify policies based on “mutual coercion mutually agreed upon”. Institutional interventions can address the problem of soil degradation, global warming, hypoxia of coastal ecosystems, decline in biodiversity and extinction of endangered species including some soils, e.g., peat soils.

As a famous Latin proverb goes "Ex nihilo nihil fit" – from nothing comes nothing. Indeed, there is a price attached to every solution. Any strategy to address these commons reflects the value that our carbon civilization places on the benefits yielded by a given technological advance and the harm that we associate with the hazards foreseen. Such benefits and hazards must be objectively assessed, not just in monetary value but, more importantly, in terms of their ecological footprints. We must pay for all ecosystem services provided by world soils, e.g., water harvesting, water purification, biodiversity, mitigation of climate change, denaturing of pollutants, disposal of waste – nuclear, urban and industrial. The abuser/polluter must also be required to pay even more.

It is about time that we move away from the empty rhetoric's and capture reality, and develop global action plans. We must develop and respect the "Land Ethic". As Aldo Leopold (1981) stated "There is as yet no ethic dealing with man's relationships to land and the animals and plants which grow upon it. Land, like Odysseus' slave girls, is still property. The land-relation is still strictly economic, entailing privileges but not obligations."

The Sanskrit scriptures sum up human relation with nature: "Everything within the world is possessed by God. He pervades both the animate and the inanimate. Therefore one should only take one's fare share, and leave the rest to the Supreme" (Isa Upanishad, Mantra 1). The concept of stewardship is ingrained in all ancient cultures. Srimad Bhagavatam (10.22.23-35) states, "The whole life of these trees is to serve. With their leaves, flowers, fruits, branches, roots, shade, fragrance, sap, bark, wood and finally even their ashes and coal, they exist for the sake of others".

The importance of using modern and innovative technologies cannot be overemphasized. In the context of improving agriculture in Sub-Saharan Africa, it is important to refer to the Law of Marginality. It states that *marginal soils cultivated with marginal inputs produce marginal yields, support marginal living, and create marginal environment prone to physical, social and economic instability.* With the world population expected to increase from 6.6 billion in 2007 to 8 billion by 2020, there is no choice but to use cutting-edge science including nanotechnology, biotechnology, information technology and knowledge management to address issues of soil quality restoration, water purification, soil fertility enhancement and climate change. It is about time that we make an objective assessment of the over-reliance on traditional knowledge, which was decisively beneficial when the population was small and the societal expectations were low. At present, we have to build upon the traditional knowledge, and use the modern technology to address the modern issues of the twenty-first century. As Francis Becon once stated "He who will not apply modern remedies must expect new evils."

The Rediscovery of Intercropping in China: A Traditional Cropping System for Future Chinese Agriculture – A Review

Heike Knörzer, Simone Graeff-Hönninger, Buqing Guo, Pu Wang, and Wilhelm Claupein

Abstract Intercropping has a 1000-year old history in Chinese agriculture and is still widespread in modern Chinese agriculture. Nowadays, agricultural systems in China are stigmatized to exhaust high levels of input factors like N fertilizer or irrigation water and to contribute severely to environmental problems like desertification, river eutrophication, soil degradation and greenhouse effect. In this context, monocropping systems have to be revised and may not be the best performing systems any more, considering sustainability, income security and nutritional diversity in rural areas. Therefore, intercropping systems offer alternatives for a more sustainable agriculture with reduced input and stabilized yield. Especially in the last decade this cropping system has been rediscovered by scientific research. Studies showed increased yield of maize and wheat intercropped with legumes: chickpea facilitates P uptake by associated wheat, maize intercropped with peanut improves iron nutrition and faba bean enhances N uptake when intercropped with maize. China's intercropping area is the largest in the world. Nevertheless, there are only few international studies dealing with intercropping distribution, patterns and crops. Most studies deal with nutrient-use efficiency and availability. This study is a first approach to gain an overview of intercropping history, basic factors about interspecific facilitation and competition and distribution of Chinese intercropping systems. Finally, four intercropping regions can be distinguished and are explicitly described with their intercropping intensity, potential and conditions.

Keywords Arable crops · China · Intercropping · Sustainable agriculture

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1 Introduction

It may be referred to as an ancient and traditional cropping system, but has a serious potential to contribute to a modern and sustainable agriculture in China. Intercropping, defined as a kind of multiple cropping system with two or more crops grown simultaneously in alternate rows in the same area (Federer, 1993) while minimizing competition, is practiced in China for thousands of years. In general, intercropping can be done with field, vegetables and even tree crops. Available growth resources, such as light, water and nutrients are more completely absorbed and converted to crop biomass by the intercrop as a result of differences in competitive ability for growth factors between intercrop components. The more efficient utilization of growth resources may lead to yield advantages and increased stability compared to sole cropping and, hence, offers an option for a sustainable low-input cropping system and economic benefits. Though this practice may have some drawbacks, some may be overcome by proper intercrop selection and management. Table 1 lists possible benefits and uncertainties of intercropping compared to classical monocropping systems.

In China, estimations between more than 28 million ha (Li et al., 2007) and 3.4×10^7 ha (Li, 2001) of annually sown area are under intercropping, with a big share in agroforestry (Table 2).

Table 1 Benefits and uncertainties of intercropping systems

Benefits	Uncertainties
Maximized land use	Limited possibilities for production mechanization
Allows more than one harvest per year (e.g. with relay intercropping)	Harvesting produce more difficult
Diversification of crops for market supply	Higher management demand
Risks of crop failure may be reduced	No extensive production of stable or cash crops
Farmers may be better able to cope with price variability	A poorly chosen intercrop competes with main crop
Higher yield and improved resource efficiency	Intercropping may not significantly improve the soil nitrogen levels
Boost the soil nitrogen content in the medium-to-long term especially when legumes are involved	Herbicide use may be constrained
Soil structure may improve if plants with various root structures are grown	
Rotation effect and improving soil erosion control	
Reducing pests and weeds	
Reducing reliance on energy-intensive farming inputs and therefore less eutrophication and emission	

Table 2 Average farm size, intercropping practice and arable land in Africa, China and India*

Country	Farm size (ha)	Intercropping area	Intercropped species	Arable land (million ha) (of total land)	Population (million)
Africa	2	83% of all cropped land in Northern Nigeria; 94% in Malawi	Cowpea, cassava, plantain, yam, rice, sorghum, millet, maize, sweet potato, okra, cocoa, soybean, chickpea, pigeon pea, peanut, beans	182.3 (6%)	812.6
China	0.1	20–25% of arable land	Maize, soybean, peanut, potato, wheat, millet, faba bean tobacco, cotton, sorghum, sesame, garlic, vegetables, cassava	137.1 (16%)	1,320.9
India	1.2–2.7	17% of arable land	Peanut, pigeon pea, maize, soybean, sugarcane, jatropha, rubber, cabbage, coconut, banana, cassava, sorghum, rice, mustard, amaranth, potato, wheat	160.6 (57%)	1,081.3

*Source: American Society of Agronomy (1976); Beets (1982); Cohen (1988); FAO (2004); Li (2001); Li et al. (2007); Vandermeer (1989); Wubs et al. (2005).

Not only tradition and extent of intercropping practice in China are liable for the appearance of a great number of studies dealing with intercropping in China, but the alternatives and options of this cropping system for ecological and sustainable agriculture, especially over the last decade. Foremost in the last decade, Chinese researchers showed an increasing interest in this cropping system. However, research on intercropping has mostly been carried out in Africa, India and Australia, leading to a better understanding of these systems in these countries.

Internationally published studies dealing with intercropping convey the impression that the main interest of these systems lies upon plant nutrition as most of them consider the increased nutrient availability and uptake, the soil nitrate content and N leaching under intercropping in comparison with monocropping (Table 3). However, there is still a research gap considering crop production and cropping designs, especially as the studies about nutrient supply and availability in intercropping systems are mostly conducted under controlled conditions. The performance and behavior of intercropping systems in comparison to monocropping systems under field conditions are still fairly unknown. In addition, other aspects like N-efficiency, water-use efficiency, influence of tillage, pests and diseases or even the calculation of land equivalent ratios (LERs) have not been considered in the international literature so far. Further, in contrast to other countries like India or Africa, in China there is no special breeding of varieties suitable for intercropping comparable to a few approaches in some African regions. In rural areas, intercropping is practiced as a so-called unconscious cropping system, which forms a big part of the whole intercropping area in China: monocropped fields that are enclosed with one row of a different crop to separate them from neighbouring fields, limited field size turning the borderlines of one field to another to an intercropping pattern (Fig. 5). Of course, intercropping is common and widespread, but the main reason for a farmer to carry out intercropping is to use all available land for production, as arable land is scarce. Due to restricted land-use rights Chinese farmers are not able to increase the size of their farms and expand cropping areas. Hence, maximizing yields is only possible by optimizing crop management strategies leading to a better utilization of natural resources over space and time. Intercropping may be a suitable strategy to do so as multiple crops can be grown simultaneously over space and time offering the chance to better utilize solar radiation, nutrients and water over the growing period. Intercropping bears more advantages and is more than maximized field exploitation.

In China, two main systems of intercropping are common: strip intercropping and relay intercropping (Fig. 1, 3). Strip intercropping is defined as cropping two or more crops simultaneously in different strips, wide enough to permit independent cultivation but narrow enough for the crops to interact agronomically (Vandermeer, 1989). Relay intercropping means the maturing annual crop is interplanted with seedlings or seeds of the following crop (Federer, 1993). Intercropping can be practiced further as an additive or a replacement system (Fig. 2). Additive means that one crop is planted in a similar arrangement to its sole-crop equivalent, and a second crop is added, so that the total plant density increases (Keating and Carberry, 1993). In contrast, a number of a few rows of one crop can be replaced by a second crop with total plant density not necessarily changing.

Table 3 Overview over experiments and main researches dealing with intercropping cereals in China

System	Main research	References
wheat/maize	Spatial compatibility and temporal differentiation of root distribution	Li and Zhang (2006); Li et al. (2006);
	Competition-recovery production principle	Li et al. (2001b); Zhang and Li (2003).
	NO_3^- content in the soil profile or NO_3^- concentration in the rhizosphere or N uptake	Li et al. (2005); Song et al. (2007); Ye et al. (2005); Zhang and Li (2003).
	Inner- and border-row effects	Li et al. (2001).
wheat/soybean	N_2 fixation rate and/or N uptake	Li and Zhang (2006); Li et al. (2001b); Zhang and Li (2003).
	Competition-recovery production principle	Li et al. (2001b).
	Inner- and border-row effects	Li et al. (2001a).
wheat/faba bean or maize/faba bean or wheat/chickpea or maize/chickpea	Utilization, availability and uptake of P	Li and Zhang (2006); Li et al. (2003); Li et al. (2004); Li et al. (2004); Li et al. (2007); Zhang and Li (2003); Zhang et al. (2004).
	Utilization, availability and uptake of N	Li et al. (2003); Song et al. (2007); Xiao et al. (2004); Yu and Li (2007); Zhang et al. (2004).
	NO_3^- content in the soil profile	Li et al. (2005).
	Spatial compatibility and temporal differentiation of root distribution	Li et al. (2006).
maize/peanut	Reduction of Fe chlorosis	Inal et al. (2007); Zhang and Li (2003); Zhang et al. (2004); Zheng et al. (2003); Zuo et al. (2004).
	N_2 fixation rate and/or N uptake	Zuo et al. (2004).
	Nutrient (Fe, P, N, K, Ca, Zn, Mn) supply	Inal et al. (2007).

Two practical examples, first for strip or row intercropping, second for relay intercropping, should be mentioned which show the positive implications of intercropping. First, in China's northeast 0.3×10^6 ha of maize fields have been converted to intercropping with sweetclover. Even by planting one-third of the field with

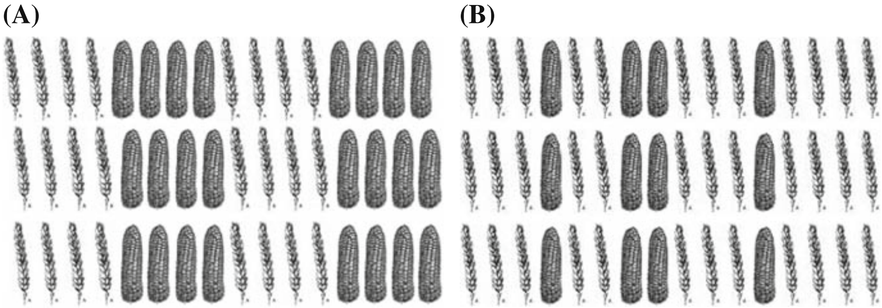

Fig. 1 Scheme of the two main intercropping systems in China: Strip intercropping (**A**), where two or more crops are grown simultaneously on the same field in different strips, e.g. four rows of crop A and four crops of crop B. Relay intercropping (**B**), where the maturing annual plant is interplanted with seeds of the following crop, e.g. 75% of wheat is sown in autumn and a few days or weeks before wheat harvest, maize is interplanted

sweetclover, the results in maize yields are about the same as those from monoculture. But there are additional 15 t/ha of sweetclover to feed three cows a year (Wen et al., 1992). Thus,without maize yield reduction, the amount and quality of pasture can be improved leading to an extension or an increase in livestock, as livestock farming is often based upon substrate fodder. Especially around the big cities Beijing, Tianjin and Shanghai and in the provinces Jiangsu, Zhejing and Fujian, cattle are mostly used for unimproved production and draught (Guohua and Peel, 1991). Improved production for milking is necessary to increase in nearly all provinces, and therefore, good and enough pasture is a big point. Second, relay intercropping showed that it is possible to increase grain yields of summer maize without a

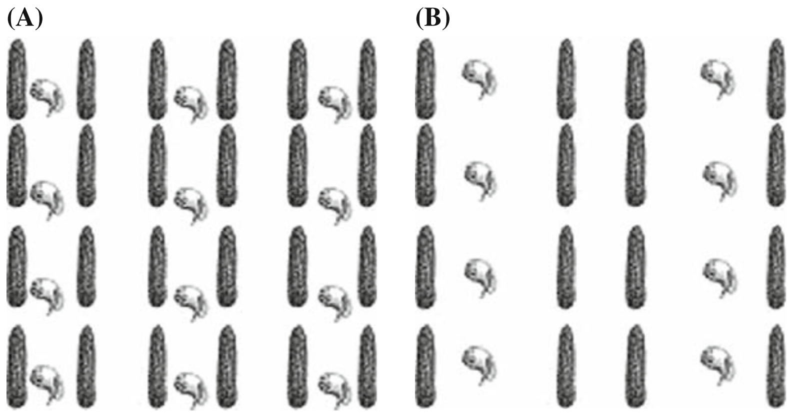

Fig. 2 Considering plant density, two main systems of intercropping exist. (**A**) In an additive intercropping system (l.), crop A is planted in a similar arrangement and amount to its sole-cropped equivalent, and a crop B is added. Total plant density increases. (**B**) In a replacement system (r.), a few rows of crop A are replaced by crop B. Total plant density does not necessarily change

(A) (B)

Fig. 3 Two examples of typical intercropping systems in the North China Plain: (**A**) Strip intercropping of maize and soybean (l.), where two or more crops are grown simultaneously on the same field in different strips. Three rows of soybean alternate with maize. These small strips often mark the borderline between two fields owned by different farmers and are the so-called unconscious intercropping. (**B**) Relay intercropping of wheat and maize, where the maturing annual plant is interplanted with seeds of the following crop (r.). Wheat and maize have a few days or weeks of overlapping growing season. When the wheat is harvested, the maize still grows for more than 3 months. In such a system plant density of maize is less than in monocropped systems (pictures: Zhang, F. and Feike, T.)

decrease in winter wheat productivity. This is remarkable, since only 75% of winter wheat sowing acreage is cultivated (Böning-Zilkens, 2004). But there is additional summer maize yield because of an elongated growing season.

2 General Questions About Competition and Facilitation

A brief basic introduction on intercropping, the influencing factors like competition for radiation, CO_2, water and nutrient availability, the ratio of competition and facilitation, resource capture and conversion efficiency is given by Vandermeer (1989) and Fukai and Trenbath (1993). Previous studies mainly dealt with competition (Keating and Carberry, 1993; Tsubo et al., 2001) and less with facilitation. However, interspecific competition and facilitation are two aspects of the same interaction, turning the system of intercropping to a successful one under some circumstances. Jolliffe (1997) pointed out that mixtures are significantly more productive than pure stands on an average of 12%. Current studies pronounce the interspecific facilitation (Li et al., 2001a,b, 2007; Zhang and Li, 2003) and hence, cultures or cereals suiting to each other for a better cropping management. Factors like root distribution (Li et al., 2006), root interactions (Inal et al., 2007; Li et al., 2003b; Zhang et al., 2004; Zheng et al., 2003), above- and below-ground interactions meaning row effects (Li et al., 2001a; Zhang and Li, 2003), N, P, K (Li and Zhang, 2006; Li et al., 2003b, 2004, 2007) and Fe (Zheng et al., 2003; Zuo et al., 2004) supply or modeling competition (Bauer, 2002; Kiniry et al., 1992; Piepho, 1995; Rossiter and Riha, 1999; Yokozawa and Hara, 1992) have been studied recently.

Competition or facilitation extents are difficult to class with their overall extent because of their intermingling. The success of intercropping is attempted to be measured by calculating the relative performance of the species. The most common parameter for judging the effect of intercropping is the land equivalent ratio (LER). A long, detailed and comprehensive list of international studies using the LER as an indicator of success is shown by Innis (1997). There, in almost all studies, LER was greater than 1 indicating that the intercropped species overyielded its monocropped counterparts. The LER is defined as

$$\mathrm{LER} = \sum_{\mathrm{j=1}}^{\mathrm{n}} Y_{\mathrm{ji}}/Y_{\mathrm{js}} \qquad \text{(Wubs et al., 2005).}$$

Y_{ji} = yield of component crop *j* in intercropping
Y_{js} = yield of component crop *j* in sole cropping

Jolliffe (1997) promoted the relative yield ratio (RYT) instead of the LER, but the RYT may be equivalent to LER as the same formula is used:

$$\mathrm{RYT} = \left[(Y_{\mathrm{i}})_{\mathrm{m}} / (Y_{\mathrm{i}})_{\mathrm{m}}\right] + \left[\left(Y_{\mathrm{j}}\right)_{\mathrm{m}} / \left(Y_{\mathrm{j}}\right)_{\mathrm{m}}\right] \qquad \text{(Jolliffe,1997).}$$

Y = yield
i, j = species 1 and 2
m = species mixture
p = pure stand

RYT and LER do not express the simple ratio of mixture to pure stands, nor do they involve equal populations and areas allocated to mixture and pure stands (Jolliffe, 1997). Instead, Jolliffe calculated the relative land output (RLO):

$$\mathrm{RLO} = \left(Y_{\mathrm{i}} + Y_{\mathrm{j}} + \ldots\right)_{\mathrm{m}} / \left(Y_{\mathrm{i}} + Y_{\mathrm{j}} + \ldots\right)_{\mathrm{p}} \qquad \text{(Jolliffe,1997).}$$

In order to get some information about the competitive ability of one species to another, the aggressivity (*A*) can be calculated:

$$A_{\mathrm{ab}} = \left[Y_{\mathrm{ia}}/\left(Y_{\mathrm{sa}} * F_{\mathrm{a}}\right)\right] - \left[Y_{\mathrm{ib}}/\left(Y_{\mathrm{sb}} * F_{\mathrm{b}}\right)\right] \qquad \text{(Li et al., 2001a).}$$

Y = yield
s = sole cropping
i = intercropping
F = proportion of the area occupied by the crops in intercropping
a, b = crop 1 and 2

When $A_{\mathrm{ab}} > 0$, the competitive ability of crop A exceeds that of crop B. In addition, the nutrient competitive ratio (CR) is given in the following equation:

$$CR_{ab} = \left[NU_{ia} / (NU_{sa} * F_a)\right] / [NU_{ib} (NU_{sa} * F_b)] \qquad \text{(Li et al., 2001a).}$$

NU = nutrient uptakes by species

When $CR_{ab} > 1$, the competitive ability in taking up nutrients of crop A is more efficient than that of crop B. At least, the cumulative relative efficiency index (REI_c) is a measure that compares the proportional change in total dry matter within a given time interval of one species relative to another:

$$REI_c = K_{\text{corp a}} / K_{\text{crop b}} \qquad \text{(Hauggaard} - \text{Nielsen et al., 2006).}$$

$$K_{\text{crop ab}} = \text{dry matter}_{ab} \text{ at time } t_2 / \text{dry matter}_{ab} \text{ at time } t_1$$

3 A Traditional Cropping System as a Contribution to Sustainable Agriculture in China

Historically, intercropping has already been proven for the Dong Zhou and Qin dynasties (770–206 BC) as a special form of crop rotation. Initiated from cropping of forests together with grains or cereals, the intercropping practice went further on with hemp, soybean, mung bean, rice and cotton as well as a system of intercropping of grains with green manure plants.

One of the early and important written documents about crop rotation and intercropping as a sub-item was the “Important Means of Subsistence for Common People”, dated back to the period of Wei and Jin dynasties (200–580 AD). It pointed out the possibility to improve soils by multiple cropping of red bean, mung bean and flax and described the theoretical and technical basis for proper rotations of leguminous plants and cereals. During the Ming Dynasty (1368–1644), the “Complete Works of Agronomy” was written by the agronomist Xu Guangqi, who summarized his experience on intercropping of wheat and broad bean (Gong et al., 2000). Besides, multi-component systems with some pertaining to intercropping are often reported in ancient literature, e.g. “Essential Farming Skills of the People of Qi” (∼600 AD), “Agricultural Treatise of Chen Fu” (1149) and “Complete Treatise on Agriculture” (∼1600) (Li, 2001).

In ancient Chinese agronomy literature it is postulated that farming activities should be in accordance with seasons, climate, soil conditions and nutrient input. Ellis and Wang (1997) showed in their regional field study on traditionally cultivated and used agroecosystems in the Tai Lake Region that these systems were capable of sustaining high productivity for more than nine centuries. Further, Chinese philosophers always pronounced the harmonious relationship between humans, nature and environment, e.g. Zhou Yi’s famous treatise “The Book of Change” or the Yin and Yang theory (Li, 1990), which had profound influence on the practice and formulation of integrated farming systems in modern Chinese agriculture policies, e.g. China’s Agenda 21. This Agenda, approved on 23 March 1994 in the form of

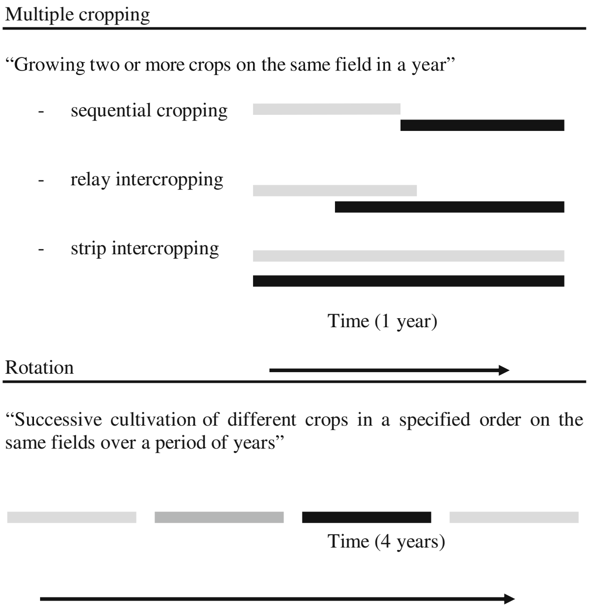

Fig. 4 Differences between and definitions of rotation, multiple cropping and intercropping (Graphic taken from Wageningen University, presentation 2002, definition of rotation added by the author)

the White Paper on China's Population, Environment and Development, lays down some basic principles for the comprehensive management of a sustainable agricultural development. As a consequence, Chinese Ecological Agriculture, not to confuse with European Organic Farming, puts a great deal of emphasis not only on the protection of the environment and the saving of resources but on the all-around development of the rural economy and specifically on rural income-generating activities (Sanders, 2000). Intercropping might be one suitable strategy.

In a public western view, modern agricultural development, except some European organic farming labels, has less to do with ancient philosophy, but more with intensive monocultures (cash crop), extensive use of energy, fertilizer and pesticides and machinery replacing manpower and human labour force. Considering the actual agricultural situation in China (Lin, 1998; Lüth and Preusse, 2007) – production as well as markets – intercropping as a well-adapted cropping system in this country is an option to solve the massive environmental problems caused by high use of fossil-energy-based inputs and a non-resource-preserving agriculture. Hence, a traditional cropping system could turn out to be a modern one (Lu et al., 2003; Zhen et al., 2005).

Intercropping is known as a system being more efficient in poorer soil and environmental conditions – because of a higher uptake and utilizing efficiency of resources like nutrients – and low-input cultivation, but losing this advantage in high-input cultivation. A theory about intercropping and input level says that the productivity of intercropping systems is higher than for pure crop situations when the input is low, but that this advantage decreases as the inputs increase (Wubs et al., 2005). Besides, seriously managed intercropping grants an option for a sustainable low-input cropping system with some kind of rotation effect, reducing pests and weeds, reducing reliance on energy-intensive farming inputs and therefore less eutrophication and emission, improving soil erosion control and, after all, giving economic benefits.

The enormous increase in grain yield and production per capita in the last 50 years in China appeared mostly due to the increased industrial energy inputs, especially in the form of chemical fertilizer (Tong et al., 2003) and irrigation water (Binder et al., 2007). It is not only to increase yield, but to compensate for the loss and degradation of the best lands through industrialization, erosion and soil misuse because of excessive irrigation, unadjusted cropping systems and chemical fertilizer.

For example, in the middle reaches of Heihe River in the Hexi Corridor region, the change of cultivation modes – crop–grass intercropping instead of monocropping – intimated a reduction in soil wind erosion and a halt in sand entrainment (Su et al., 2004). In this region it occurs that dust transport from farmlands is about 4.8–6.0 million tons per year and consequently higher than that of sandy desert dust transport in the same region.

In entire China, the use of mineral fertilizers grew more than 50 times from 1962 (0.63 million tons) to 1994 (33.18 million tons) with 80% being N fertilizer (Inal et al., 2007). The average fertilizer consumption in 2002 was 277.7 kg ha^{-1} arable land (FAO, 2006), rising in the irrigated areas to 450 kg ha^{-1}. Considering N-fertilization amounts, China ranks first in the world. Simultaneously, the yield per unit chemical fertilizer use decreased from 164 (rice), 44 (wheat) and 93 (maize) kg kg^{-1} in 1961 to 10 (rice), 6 (wheat) and 9 (maize) kg kg^{-1} in 1998 (Tong et al., 2003). This decline appears as a result of fertilizer saturation, soil degradation, strong soil, atmosphere and water pollution and poor and low-quality land use. Further, more than 100,000 people were poisoned by pesticides and fertilizers during 1992–1993, and more than 14,000 of them died (Sanders, 2000). Thus, low-input cropping systems sometimes yielding (economically) as high as high-input cropping systems are to be favored, further offering the change to save natural and ecological resources.

China is such a large country that any land use (Xiaofang, 1990) change would contribute greatly to changes in the global world (Tong et al., 2003). The country is known as an important source of methane from rice paddies as well as atmospheric nitrous oxide generated by the increasing use of large amounts of low grade and highly volatile ammonium bicarbonate fertilizers. In contrast, China has an urgent demand for food for a rapidly growing population (Gale, 2002; Lu and Kersten, 2005; Ministry of Agriculture of the People's Republic of China 2004). Only 25.7% of Chinese agricultural area is suitable for arable land usage, with 2.2% being permanent crops (FAO, 2006). With 1.32 million people, China has 20% of the world's population but only 9% of the world's arable cropland. There is only 0.1 ha per capita, which is one-third of the world's average (Wen et al., 1992). Following Tong et al. (2003), more than 60% of all cultivated land is poor in nutrients, and only 5–10% is free from drought, water logging or salinity. Two percent of China's total land area can be considered desertified by human-induced resource degradation (Sanders, 2000). This shows that China has a great demand for resource- and environment-saving agriculture systems being able to feed the country. Intercropping as a widely practiced and accepted cropping system could contribute to a more sustainable land use in this context.

In respect of the enormous environmental problems, like water shortage and pollution, over-fertilization, high nitrous emissions, leaching, soil degradation, erosion, etc., caused by agriculture in China, sustainability is an increasing factor to consider in future preventing natural catastrophes and preserving production levels (Lei, 2005). Climate change and resulting implications for sustainable development was a topic in the Regional Implementation Meeting of the United Nations Economic and Social Commission for Asia and the Pacific, held in November 2007. Around 15.5% of the GDP in 2006/2007 in China came from agriculture, animal husbandry and forestry, though more than 50% of the population depends on agriculture for their livelihoods and hence, a large proportion of the population depends on the climate and the climate change (Prabhakar, 2007). For 2050, the UN figures on scarcity of freshwater availability affecting more than a billion people in Central, South, East and South-East Asia. Additionally, crop yields are predicted to decline in parts of Asia between 2020 and 2050 about 2.5 to 30%. Freshwater availability, droughts and floods due to greenhouse gas emissions, water pollution and soil degradation will be the main problems. Therefore, natural resource and integrated ecosystem management are identified to be major priority actions. The Commission accentuated clearly (Prabhakar, 2007): "Revisiting the existing cropping patterns and systems is needed". As monocropping means higher risk, in terms of income security, nutritional diversity in rural areas and the possibility of severe impacts to large areas due to pest and disease outbreak in a changing climate, mixed and intercropping practices are the only alternative that may have multiple benefits.

4 The Nature and Extent of Chinese Intercropping

One-third of China's cultivated land area is used for multiple cropping (Fig. 4), and a half of the total grain yield is produced with multiple cropping (Zhang and Li, 2003). At present, about more than 70% of farm products are attributed to the improved multiple cropping systems like rotations or intercropping (Zhang et al., 2004). Between 1949 and 1995, the multiple cropping index, meaning the sown area:arable area ratio, increased from 128% to 158%, according to an increase of 2.7 $\times 10^7$ ha of farmland (Li, 2001). Although, intercropping is only one example of the various aspects of multiple cropping, it is substantial as China's intercropping area is the largest in the world. As an example, in 1995 the area under wheat intercropped with maize was about 75,100 ha in Ningxia, producing 43% of total grain yield for the area (Li et al., 2001a). While the most common agricultural land use in the Heihe River Basin before 1980 was to crop wheat, the intercropping of wheat together with maize increased after 1980. Today, 20% of the agricultural land in this region is sown with wheat, 40% with maize and 40% with wheat intercropped with maize (Yamazaki et al., 2005). Furthermore, intercropping has become the most common cropping system for peanut production in northern China (Zuo et al., 2004). Of 31 provinces 27 have land under peanut production. In 1991, the UNEP granted the Zhang Zhuang region the Global 500 award, given for sustainable farming systems in the sense of Chinese Ecological Agriculture (Sanders, 2000). In this region there were altogether ten different modes of intercropping, including cereals and oil crops and cereals and vegetables.

The most common intercropping types considering cereals or grains are those of wheat and maize, wheat and cotton, wheat and faba bean, wheat and soybean, maize and soybean, maize and faba bean and maize and peanut. Sorghum and maize are often used to enclose fields. In addition, there is a vast range of possible combinations of grains together with vegetables. Rice is mostly cultivated in the south where most of the high-quality land is found. In contrast, wheat is cultivated in central China and in the north, and maize is cultivated in central, the northeast and the north of China. The center of cereal production moved slightly towards northern China (Tong et al., 2003), with the North China Plain being China's granary. Various inter- or relay-cropping patterns are practiced mainly in the north, the northeast and northwest and the southwest, especially in Xinjiang, the corridor in the Gansu, Yinchuan plain, Hetao in Inner Mongolia, the Northeast Plain, the North China Plain, along the Yangtze and Yellow rivers, and also in lower dry lands and in hilly areas of South China (Ren, 2005). Wheat intercropped with maize has become increasingly popular in the irrigated area of the Hexi Corridor in the Gansu province,

Fig. 5 Average field size in China is very small, so the collectivity of field borders can be considered as intercropping in a larger scale (pictures: Feike, T.). Typical sequential borderlines in the North China Plain are between soybean, cotton, maize or sorghum, various vegetables, especially cabbage, and interjacent poplar trees

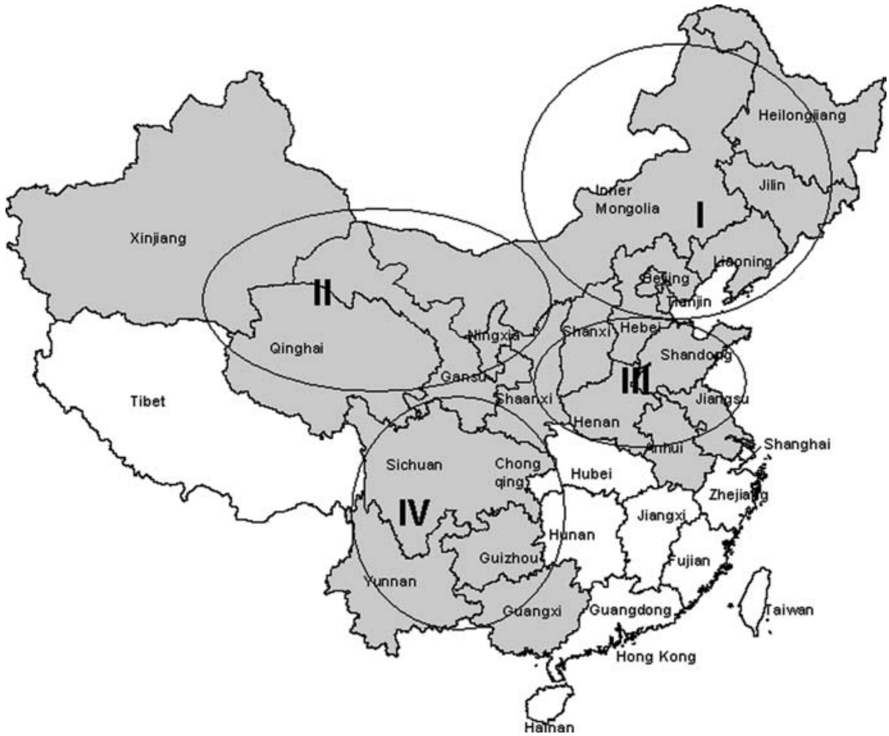

Fig. 6 Provinces where intercropping with cereals is popular (*grey*); regions where intercropping with cereals is less common (*white*) (Graphic taken from USDA, 2007, marking of intercropping regions added by the author). In a rough and simplified visualization, China can be classified into four intercropping regions I to IV: Going from Northeast and North (I) to Northwest (II) and Yellow-Huai River Valley (III) and finally to Southwest (IV) the cropping systems change from one crop a year (I + II) with a great potential for intercropping to relay intercropping (III) of especially maize and wheat and double cropping systems and at least three cropping seasons per year (IV) with different kinds of rotations, and rotations replacing intercropping

along the Huanghe River in Ningxia and in Inner Mongolia regions (Zhang and Li, 2003). In China's northwest, wheat/soybean and maize/faba bean intercropping systems are well established, and peanut/maize intercropping is widespread in the northern parts of the country (Zhang and Li, 2003). In the southwest, wheat–maize intercropping predominates within irrigated spring maize cropping systems. Also within rainfed spring maize cropping systems, intercropping is common. Especially in Sichuan province, wheat–maize intercropping is the most common agroecosystem model (Meng et al., 2006) (Fig. 6).

5 Intercropping Types and Regions

There are different approaches to divide China's agricultural land into specific agricultural regions. The most common is to partition the country into nine, respectively ten, agricultural regions (Guohua and Peel, 1991) situated in middle and eastern parts of China, depending on percentage of cultivation, climatic features and production systems. The agricultural region boundaries are not necessarily authoritative. The agriculture production system zone code of the PR China actually splits the country into 12 zones (FAO, 2007). As the title already tells, the production system – whether it is single or multiple cropping, cropping for uplands or paddy field – is decisive. Meng et al. (2006) analyzed the various cropping systems and potential production with regard to maize production, and divided China's cultivated land into six agricultural regions depending on significant differences in maize cropping patterns and practices.

Concerning intercropping, China's cultivated land can be classified into four main types and regions – illustrated in Fig. 6 – which is just a first and general approach to classify intercropping regions at all because there has been no detailed statistic or documentation dealing with intercropping in particular so far. This basic classification takes the underlying potential for intercropping and the intercropping practice into account (Atlas of the PR of China, 1989; Meng et al., 2006). In general, the agricultural regions in the north, northeast and northwest have more potential for intercropping than the regions in the south, even if the southeast has the highest average precipitation per year, a subtropical, humid and monsoonal climate and with around 135–242%, the highest multiple cropping index in China. The southwest is a very important agricultural region for China with a great diversity of crops, fruits and vegetables, but the climate conditions allow more flexible rotations, thus replacing intercropping systems. Going from north to south the cropping systems change from one crop a year with a great potential for intercropping to relay intercropping of especially maize and wheat and double cropping systems and at least three cropping seasons per year with different kinds of rotations and increasing number of paddy fields (Table 4).

5.1 Type I: Single Cropping with Great Intercropping Potential

The Northeast is characterized by a cold-temperate/semi-humid and temperate/humid climate zone in the north of the agricultural zone and temperate continental monsoonal climate zone. But there are also temperate, humid, monsoonal to subtropical, semi-humid, monsoonal climate conditions to be found.

In the North, there is a warm-temperate, semi-humid, monsoonal and also temperate continental climate. Dark brown soils, phaeozems, chernozem-castanozem-dark loessial soils, brown soils and cinnamon soils are predominant with high accumulation of organic matter. Especially in the Northeast, the soils tend to be slightly acidic or calcareous, with the latter being a problem for, e.g., peanut production. In calcareous soils, the Fe availability is weak because of immobilization of Fe in alkaline soils. Peanut is an important crop in this region, and the disadvantage of Fe availability can be remedied through intercropping as shown later.

In both, Northeast and North of China, with the provinces and municipalities Liaoning, Jinlin, Heilongjiang, parts of Inner Mongolia, Beijing, Tianjin, parts of

Table 4 Intercropping types and regions and their characteristics and potentials* in China

Agricultural region	Cropping conditions and characteristics	Intercropped species	Type of production
Northeast (NE) and North (N): • Liaoning • Jinlin • Heilongjiang • Parts of Inner Mongolia • Beijing • Tianjin • Parts of Hebei • Parts of Shanxi	*Climatic features: middle to warm temperate zone* *> 10°C accumulated temperature (°C)*: NE: 1,300–3,700 N: 200–3,600 *Average temperature (°C)*: –12 to –14 *Sunshine (hours)*: 2,300–3,200 *Frost-free period (days)*: 100–200 *Rainfall (mm/year)*: NE: 500–800 N: 200–600 *Altitude (m)*: 50–100 *Soils: siallitic and calcareous soils predominant* Dark brown soil and phaeozem zone; chernozem-castanozem-dark loessial soil zone; brown soil and cinnamon soil zone *Multiple cropping index (%)*: 0–135	Maize/**soybean Maize/peanut Maize/potato Wheat/broomcorn millet	*TYPE I: single cropping with great intercropping potential* • crops: maize, soybean, spring wheat, rice, sorghum, millet, sesame, potato, sugar beet, flax, peanut, ambary hemp, cotton • yield and production level of cereals: 5,155 kg ha^{-1} • irrigated area (1,000 ha): 1765.2 • consumption of chemical fertilizer (10,000 tons): 118.2

Table 4 (continued)

Agricultural region	Cropping conditions and characteristics	Intercropped species	Type of production
Northwest: • Gansu • Qinghai • Nigxia • Xinjiang • Parts of Inner Mongolia • Parts of Shaanxi	*Climatic features: cold temperate to subtropical zone* *> 10°C accumulated temperature (°C)*: 2,000–4,500 *Average temperature (°C)*: 0–12 *Sunshine (hours)*: 2,600–3,400 *Frost-free period (days)*: 140–170 *Rainfall (mm/year)*: 10–250 *Altitude (m)*: 300–3,000 *Soils: gypsum-bearing and calcareous soils predominant* subalpine meadow soil zone; brown desert soil zone; grey dessert soil zone; sierozem-brown calcic soil zone *Multiple cropping index*: 0–135	Maize/potato Maize/bean Wheat/maize Wheat/buckwheat Wheat/millet Wheat/tobacco Wheat/soybean	*TYPE II: single cropping for cold climate and semi-arid crops to double cropping for irrigation farming* • crops: maize, wheat, millet, broomcorn millet, oats, buckwheat, potato, highland barley, sorghum, rice, rape, soybeans, sugar beet, cotton, flax, hemp, peanut, pea, broad bean • yield and production level of cereals: 4,228 kg ha^{-1} • irrigated area (1,000 ha): 1438.2 • consumption of chemical fertilizer (10 000 tons): 75.6

Table 4 (continued)

Agricultural region	Cropping conditions and characteristics	Intercropped species	Type of production
Yellow-Huai River Valley: • Parts of Hebei • Parts of Shanxi • Shandong • Henan • Parts of Shaanxi • Parts of Anhui • Parts of Jiangsu	*Climatic features: warm temperate to subtropical zone* *> 10°C accumulated temperature (°C)*: 3,400–4,700 *Average temperature (°C)*: 10–14 *Sunshine (hours)*: 2,200–2,800 *Frost-free period (days)*: 170–220 *Rainfall (mm/year)*: 500–1,100 *Altitude (m)*: 50–100 *Soils: calcareous soils predominant* Brown soil and cinnamon soil zone; yellow brown soil and yellow cinnamon soil zone *Multiple cropping index*: 1–190	Wheat/maize Wheat/cotton Maize/soybean in rotation with wheat Wheat/garlic in rotation with maize	*TYPE III: double cropping with potential for relay intercropping* • crops: maize, wheat, soybean, peanut, cotton, vegetable, millet, potato, sorghum, sweet potato, rice, sesame, ambary hemp, tobacco, pea, sugarcane • yield and production level of cereals: 4,876 kg ha^{-1} • irrigated area (1,000 ha): 3369.2 • consumption of chemical fertilizer (10,000 tons): 297.9

Table 4 (continued)

Agricultural region	Cropping conditions and characteristics	Intercropped species	Type of production
Southwest: • Parts of Guangxi • Sichuan • Chongqing • Guizhou • Yunnan • Parts of Shaanxi	*Climatic features: subtropical to tropical zone* *> 10°C accumulated temperature (°C)*: 3,500–6,500 *Average temperature (°C)*: 15–18 *Sunshine (hours)*: 1,200–2,600 *Frost-free period (days)*: 240–360 *Rainfall (mm/year)*: 800–1,600 *Altitude (m)*: 200–3,000 *Soils: ferrallitic and ferro-siallitic soils predominant* Alpine meadow soil zone; subalpine meadow soil zone; read earth and yellow soil zone; lateritic red soil zone *Multiple cropping index*: 135–242	Maize/beans (sorghum) in rotation with wheat Maize/potato/wheat Wheat in rotation with maize/soybean (green bean) Maize/beans in rotation with wheat Maize/wheat Wheat in rotation with maize/sesame Wheat/vegetable in rotation with sweet potato/maize/soybean Maize/sorghum in rotation with wheat Maize in rotation with maize/sweet potato Maize/potato in rotation with wheat Rice/wheat Rice/rape Potato/maize Maize/cassava in rotation with soybean Maize/soybean in rotation with sunflower Wheat in rotation with maize/sesame-maize/potato Vegetable in rotation with maize/sweet potato Maize/sweet potato in rotation with wheat/vegetable Rape/maize Wheat/vegetable in rotation with maize/vegetable	*TYPE IV: three cropping seasons per year with rotations replacing intercropping* • crops: rice, maize, wheat, sweet potato, sorghum, rapeseed, sugarcane, peanut, tea, cotton, ambary hemp, tobacco, millet, cassava, soybean, pea • yield and production level of cereals: 4,605 kg ha^{-1} • irrigated area (1,000 ha): 1349.2 • consumption of chemical fertilizer (10,000 tons): 140.3

* Source: Atlas of the PR of China (1989); Meng et al. (2006); National Bureau of Statistics of the Peoples Republic of China, 2006; The National Physical Atlas of China = Chinese Academy of Sciences (1999).
** meaning “intercropping”.

Hebei and Shanxi, intercropping is widespread. The climatic features allow only one crop per year. The average precipitation is between 200 and 800 mm/year with rain falling mainly in summer, whereas spring droughts are frequent. The winters are long and cold, and there is a large daytime–nighttime temperature gap during the whole growing season. In addition, the average temperature is very low. The varieties grown in this region have to be fast-maturing varieties. Only crops which prefer semi-moist and warm conditions can be grown, thus reducing the cultivation range to especially maize, soybean, peanut, potato, spring wheat and millet. Generally, the production system is based upon rainfed conditions. In Hebei, Shanxi and around Beijing, irrigation is also practiced. In the northeast as well as in the north, intercropping can provide an optimal site utilization, a higher yield compared to monocropping and an improved diet diversification.

5.1.1 Intercropping Maize with Peanut

Peanut is the major oilseed crop in China constituting 30% of the land's total oilseed production and 30% of the cropped area (Zhang and Li, 2003). But especially in north China with its calcareous soils, iron deficiency chlorosis is often observed and Fe deficiency is one of the most common yield-limiting nutrients that causes serious economic problems in peanut monocropping systems (Zuo et al., 2004). Maize has a great potential to improve the Fe nutrition of peanut within an intercropping system by rhizosphere interactions (Fig. 7). In an experiment of Zhang and Li (2003), young leaves of peanut plants in rows 1–3 from the maize grew without visible symptoms of Fe deficiency, while those in rows 5–10 showed a variable degree of chlorosis. They were all chlorotic when roots were separated. Peanut is a strategy I, and maize a strategy II species. Strategy I plants respond to Fe deficiency with increased ferric reductase activity of roots and acidification of the rhizosphere by releasing protons from the roots. Strategy II plants excrete phytosiderophores into the rhizosphere thus being more efficient in Fe deficiency surroundings. They mobilize Fe (III) and benefit the iron nutrition of maize as well as peanut (Marschner, 1986).

The results indicate the importance of intercropping systems as a promising management practice to alleviate Fe deficiency stress (Inal et al., 2007), because soil amendments and foliar application of Fe fertilizers are usually ineffective or uneconomical for correcting Fe chlorosis. But in addition and especially in calcareous soils, the effects of soil moisture on soil iron availability under intercropping could be more complicated compared to monocropping. Zheng et al. (2003) showed that the Fe nutrition of peanut intercropped with maize could be affected by soil moisture condition. Root growth of peanut was significantly inhibited at 25% soil water content compared to those at 15% soil water content. Also, chlorophyll content in the new leaves of intercropped peanut decreased and leaves became chlorotic at 25% soil water content.

The improved Fe availability is the underlying reason for an increased N uptake. Competitive interactions between maize and peanut for N and improvement of Fe uptake by peanut were likely to be important factors affecting N_2 fixation of peanut (Zuo et al., 2004). Also the nitrate concentration in the soil rhizosphere of

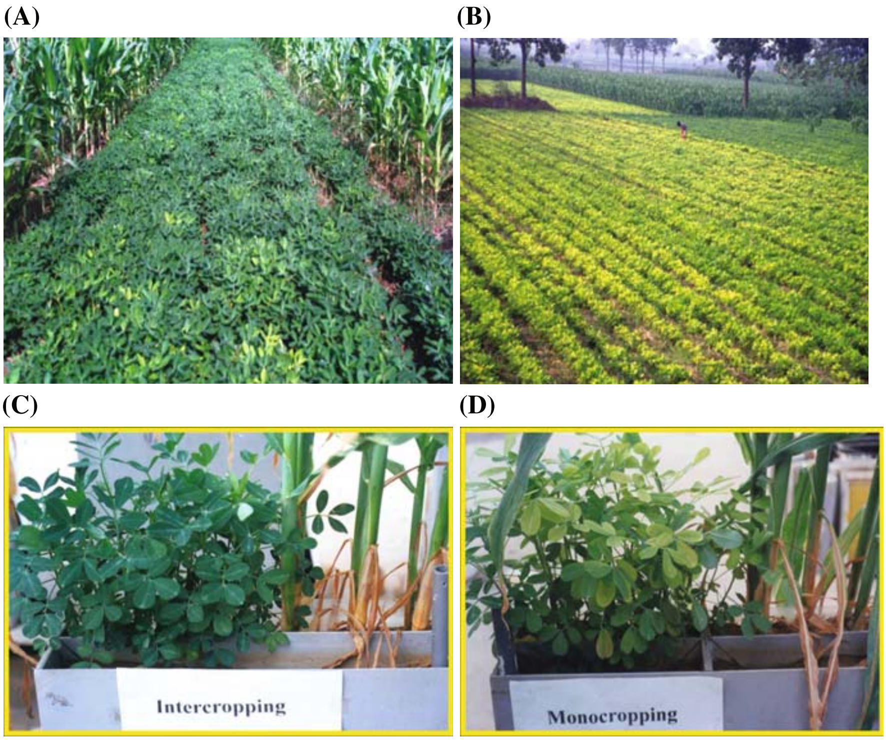

Fig. 7 Intercropping of maize and peanut reduces iron chlorosis in peanuts on calcareous soils (pictures: Zhang, F.). (**A/B**): Differences between (strip) intercropped (l.) and monocropped (r.) peanut in the field. (**C/D**): Differences between intercropped (l.) and monocropped peanut (r.) in a pot experiment. In the pot experiment as well as in the fields, peanut shows less Fe chlorosis when intercropped with maize

intercropped peanut did not increase, nor did the N uptake by peanut compared to sole stand. The authors concluded that the improvement in Fe nutrition was an important factor promoting N_2 fixation by peanut. The competition for N between maize and peanut was not the stimulating factor for N_2 fixation, but the increased Fe availability and uptake by peanut. Both peanut and the root nodule bacteria require Fe for many metabolic functions at several key stages in the symbiotic N_2 fixation. Furthermore, high levels of soil nitrate can be a potent inhibitor of N_2 fixation because then the legumes thrive without fixing atmospheric N (Zuo et al., 2004). Competition for N in a cereal/legume mixture acts as a stimulator for N_2 fixation.

5.2 Type II: Single Cropping for Cold Climate and Semi-Arid Crops to Double Cropping for Irrigation Farming

The north-western intercropping region includes the provinces Gansu, Qinghai, Nigxia, Xinjiang, parts of Inner Mongolia and parts of Shaanxi. In comparison to the provinces in the North and the Northeast, the Northwest of China has higher average temperature and more frost-free days, but the average precipitation with 10–250 mm per year is very low. The climate is temperate continental and subtropical, humid in the east and west of the agricultural region changing to dry, continental temperate in the middle. There are long, cold winters and short, hot summers with temperature shifting greatly from day to night as well as from season to season. The crops and varieties grown are fast-maturing ones. Usually, the region was a one harvest-one year district and intercropping wheat with soybean, wheat with millet and maize with faba bean was the main planting system before 1960. From the 1960s onwards, relay intercropping systems with wheat and maize established more and more due to irrigation and varieties improvement. The improvements also led to a gradual shift to a double cropping system in irrigation farming. Although the Northwest is a typical intercropping region with a long intercropping tradition and with intercropping systems being more differential than in the Northeast and the North, the potential for intercropping changes in direction from strip intercropping to relay intercropping and finally to double cropping. Nowadays, intercropping practice in this region is almost all relay intercropping of wheat and maize or wheat and cotton.

5.2.1 Intercropping Wheat or Maize with Legumes

Intercropping wheat with faba bean or soybean increases the yield of wheat in nearly all studies: yield and nutrient acquisition by intercropped wheat and soybean were all significantly greater than for sole wheat and soybean (Li et al., 2001a). Here, intercropping advantages in yield were between 28% and 30% for wheat/soybean. Wheat/soybean had also significant yield increase of intercropped wheat over sole wheat in the study of Zhang and Li (2003). Intercropping resulted in a yield increase in wheat as well as in faba bean between 7% and 46% (Song et al., 2007).

One reason is the effect intercropping has upon N uptake and availability. Zhang and Li (2003) showed that yield increased of about 53% in wheat/soybean, where aboveground effects contributed 23% and belowground effects contributed 30%. For increased N uptake they measured a contribution of 23% aboveground effects and 19% of belowground effects, resulting in an increased overall N uptake of 42%. In contrast to soybean wheat had a greater capability of acquiring nutrients because of the enhanced aggressivity of wheat over soybean. A greater competitive ability and aggressivity of wheat as well as the better nutrient CR led to a greater capability of wheat to acquire nutrients, concerning not only N, but also P and K (Li et al., 2001a). The N accumulation by wheat was mainly due to increased border row N uptake which shows that intercropping is based upon an edge effect.

Besides nutrient acquisition, additional components like border row effects contribute to the overyielding of intercropped wheat. Yields of wheat in border rows significantly increased compared with yield in the inner rows or in rows of sole. Zhang and Li (2003) pointed out that out of a 64% overall increase in yield in intercropped wheat, about 33% came from inner-row effects and about 67% came from the border-row effects. The higher crop overyields due to extra sunlight that taller crops receive on their borders. But accordingly, the N and P accumulation in the border row were significantly greater than in inner row or in sole wheat. Both, border row and inner row contributed to the increase in yield. Studies of Cruse (1996), Ghaffarzadeh (1999), Leopold Center (1995) and Zhang and Li (2003) mentioned that four to six rows seem to be the optimum. Up to six rows a mixed stand is comparable to a sole stand.

The main advantage of intercropping wheat with a legume like soybean or faba bean is the complementary N use; that means, wheat competes much better for soil-available N than the legume and, conversely, the legume is forced to get nitrogen from atmospheric N fixation. The competition from wheat in acquiring N through intermingling roots enhanced N_2 fixation in faba bean by about 90% (Xiao et al., 2004). In addition, there is a small N transfer from faba bean to wheat between 1.2% and 5.1% of faba bean N, according to the used measuring techniques, the root distance and contact. This supports the hypotheses of N-sparing by faba bean due to increased N_2 fixation and the increased resource-use efficiency by cropping wheat and a legume together.

Nevertheless, the enhanced N availability or uptake of a cereal crop combined with a leguminous plant like faba bean is more investigated by intercropped maize than intercropped wheat. As shown, the belowground interactions between intercropped species can be more important than aboveground interactions. Intermingling of roots makes sure that nutrients can be used more efficiently because of different mobilizing processes, leading to a higher yield (Li et al., 2007; Zhang and Li, 2003; Zhang et al., 2004). Intermingling of maize and faba bean roots increased N uptake by both crop species by about 20% compared with complete or partial separation of the root system (Li et al., 2003b). The N uptake of faba bean was higher than sole cropped faba bean during early growth stages and at maturity, whereas N uptake of maize did not differ from that by sole maize at maturity, except when P fertilization was high. Because organic acids and protons released by faba bean can mobilize P by the acidification of the rhizosphere, both the N and P uptake by intercropped maize was found to be improved compared with corresponding sole maize (Zhang et al., 2004). Hence, the improved N and P nutrition by intercropping could be characterized as a synergistic process.

A mixture of exudates released from two instead of only one species could change rhizosphere conditions being responsible for the enhanced availability of nutrients, e.g. phosphorus. Zhang and Li (2003) showed in a pot experiment that chickpea facilitated P uptake by associated wheat. Wheat prefers inorganic P and is less able to use organic P. In contrast, chickpea is able to use both P resources effectively. As chickpea mobilizes organic P by releasing phosphatase into the soil turning organic P into inorganic P, P gets available for wheat. Because wheat has a greater competitive ability than chickpea, wheat acquires more inorganic P than chickpea so that chickpea is forced to mobilize organic P. Hence, competition turns into facilitation, because both species do not suffer in P supply. According to the N uptake, P uptake is a combination between above- and belowground effects and interactions. Aboveground interactions contributed 26% to a wheat/soybean mixture, whereas 28% of belowground interactions contributed to the grain yield of wheat (Zhang and Li, 2003).

There are various studies dealing with P supply within intercropping systems, especially maize/chickpea (Inal et al., 2007; Li and Zhang, 2006; Li et al., 2004) and maize/faba bean (Li et al., 2003a/2007; Zhang and Li, 2003). But in most cases, those studies were pot experiments in a greenhouse and not field experiments. Experiments with chickpea promote scientific knowledge of plant nutrition being different whether there are inter- or monocropping conditions, but indeed chickpea is not popular in Chinese cropping systems.

Nevertheless, similar to wheat grown together with chickpea, maize intercropped with chickpea too profits by the intermingling of their root systems. Both, faba bean or chickpea and maize accumulated more P in the shoot when intercropped (Li et al., 2003a). The total P uptake by intercropped maize supplied with phytate was twofold greater compared to a monoculture (Li et al., 2004). Li et al. (2004) indicated clearly that the improved growth of maize when intercropped with chickpea was not caused by better N nutrition, but better P uptake. According to Li et al. (2003/2007) four explanations for the increased P uptake by diverse species are plausible:

(1) Greater phosphatase activity in the rhizosphere in intercropping decomposed soil organic P into an inorganic form, which can be used by both species.
(2) Improved P nutrition in maize could have resulted from an increased uptake of P released during the decomposition of root residues after the harvest of e.g. faba bean.
(3) Faba bean, for example, was better nodulated when intercropped, resulting in more fixed N_2. While fixing atmospheric N, legumes take up more cations than anions and release H^+ from the roots. Again, H^+ is important in dissolving P in calcareous soils.
(4) The volume of soil exploited by the maize roots increased and led to a greater ability to absorb P.

But considering intercropped wheat, Song et al. (2007) went beyond the N accumulation in a plant and studied the community composition of ammonia-oxidizing bacteria in the rhizosphere of wheat and faba bean at different growth stages. Autotrophic ammonia oxidizers in the rhizosphere carried out the first and rate-limiting step of nitrification, the oxidation of ammonia to nitrate. The authors concluded that these bacteria could play a key role in N availability to plants and could be important for the interactions between plant species in intercropping. During anthesis the nitrate concentrations in the rhizosphere of wheat intercropped with faba bean were nearly twice as high as in monocropped wheat. Song et al. (2007) suggested that N released from faba bean roots was rapidly mineralized to ammonia and then transformed to nitrate.

Intercropping is known to suppress weeds and pests, because of the higher biodiversity in comparison to monoculture. The soil is covered nearly all the time, and the different plants give home to predators. Most studies dealing with the influence of intercropping on pests and weeds investigated these aspects from the point of view of ecology and less from the point of relationships within a cereal–cereal mixture. Li (2001) reported wheat–cotton relay intercropping being able to control the cotton aphid as well as cotton–rape intercropping that reduced insect damage. The cotton aphid is the main pest of cotton and appears in May. In early May the cotton aphid's natural enemy is the seven-point lady beetle, which is also the natural enemy of the rape aphid. This supports strongly the enemies' hypotheses (Andow, 1991) where the intercropping changes the environmental conditions in such a way that the natural enemy activity is increased. Ma et al. (2007) showed that parasitism of *Allothrombium ovatum* on alate aphids can significantly control the population increase of wheat aphids. Within a strip intercropping of wheat together with alfalfa they examined the possibility to improve the biological control of the wheat aphid by the mite *A. ovatum*. The strip intercropping resulted in higher soil moisture, shadier soil surface and thus a changed microclimate which caused adult female mites to lay more egg pods. In addition, the non-furrow areas provided a more suitable habitat for mites' overwintering, so that the mean number of mites per parasitized aphid was significantly higher in intercrops than in monoculture (Ma et al., 2007).

The different microclimate in intercropping compared to monocropping seems to be substantial for suppressing or enhancing diseases. Chen et al. (2006) showed, that under zero N fertilization, the appearance of powdery mildew in a field was similar in intercropped and monocropped cultivation of wheat and faba bean. They supposed that under deficient N circumstances, plant growth is limited, thus leading to a comparable microclimate within both cropping systems. However, under increased N application rates, the microclimate differs regarding velocity of air movements and correspondingly lower humidity. Chen et al. (2006) concluded that conditions that prevailed in intercropped wheat with faba bean are less conductive to infection by and growth of the powdery mildew compared with sole wheat, because the differences in disease incidence and disease severity due to intercropping between zero and increased N application were significant.

5.3 Type III: Double Cropping with Potential for Relay Intercropping

Cereal – especially maize and wheat – production in the Yellow-Huai River Valley is mostly practiced as relay intercropping and less as strip intercropping. Wheat, maize and cotton are the most important and stable crops grown in parts of Hebei, Shanxi, Shaanxi, Anhui and Jiangsu and in Shandong and Henan province. The region is consequently China's granary. With progress in engineering, breeding and irrigation, relay intercropping within the widespread and current double cropping systems is more and more decreasing. In addition, strip intercropping is the only way practised in vegetable production, agroforestry and in fields along big roads. Single cropping with potential for intercropping is common practice in rainfed upland farming, whereas double cropping in large scale or three harvests in 2 years as a more adjusted and sustainable production are to be found in irrigation farming. For the double cropping system, resistant varieties are needed, because of the narrow crop rotation consisting nearly completely of wheat and maize or cotton. Although, summer maize varieties have to be fast maturing because of the very short growing season between June and September/October. Additionally, for implementing two harvests within one year – wheat in June and maize in September/October – an irrigation in springtime would be necessary. About 60–70% of the rain falls during the hot summer. In contrast, the winters are cold and dry. Within the intercropping region, the climate changes from temperate continental to subtropical-humid with clear-cut seasons and plum rains between spring and summer.

5.3.1 Intercropping Wheat with Maize

Intercropping a cereal–cereal association such as wheat and maize become increasingly popular in irrigated areas and in the North China Plain. Both species grow together for about 70–80 days and yield more than 12,000 kg ha^{-1} (Zhang and Li, 2003). Consequently, both species compete strongly for N and light during their co-growth. Because there is no leguminous plant involved or no facilitation concerning P or Fe supply, the competition seems to be more intrinsic. Nevertheless, scientific research (Li et al., 2001a,b; Zhang and Li, 2003) found that grain yield of both species increased and the N uptake and nutrient accumulation was greater than that by corresponding sole cropping under the same N supply. Indeed, during co-growth biomass yield and nutrient acquisition of the earlier sown wheat increased significantly, whereas at wheat harvest, the biomass of maize in the border row was significantly smaller than in sole maize (Li et al., 2001a). But there is a recovery-compensation growth with the result that at maize maturity the disadvantages disappeared with no significant differences in biomass between border-row maize and inner-row maize or corresponding sole maize. First, the subordinate plant suffers but after harvesting the dominant plant is able to compensate. This competition-recovery production principle rests upon the ecological mechanism of niche differentiation. Li et al. (2001b) suggested that interspecific interactions shifted the peak nutrient requirement of dominated species like maize to after wheat harvest, which was helpful for reducing interspecific competition during co-growth. This principle is suitable to intercrop short-season species together with long-season species.

In addition, the more efficient and temporal distribution of soil nutrient consumption results in a reduced nitrate content in the soil profile (Zhang and Li, 2003). The $NO_3{}^-$ amounts after wheat harvest were greatest under sole wheat and smallest under maize intercropped with wheat (Li et al., 2005). The decrease could be amounting to 30–40% in a wheat/maize association compared to wheat or maize sole (Zhang and Li, 2003). Hence, intercropping can reduce nitrate accumulation and eluviation compared to monocropping.

Like biomass production, grain yield and nutrient accumulation, root growth and root distribution show compatibility of species and niche differentiation of plants. At symmetric interspecific competition, where both species are on an equal footing for acquiring factors of growth, e.g. maize intercropped with faba bean, the spatial root distribution is compatible and similar under inter- as well as monocropped cultivation. Faba bean had a relatively shallow root distribution, maize roots spread underneath them. The shallow root distribution of faba bean results in lower competition for soil resources with the deeper-rooted maize. In contrast, asymmetric competition, where one species dominates over another, e.g. wheat intercropped with maize, results from the greater root proliferation of overyielding species underneath the other. Li et al. (2006) showed that intercropped wheat had a greater root length density compared to sole-cropped wheat, occupied a larger soil volume and extended under maize roots. Roots of intercropped maize were limited laterally to about 20 cm, whereas roots of sole-cropped maize spread laterally about 40 cm. The failure of maize to extend into the soil immediately under wheat may help to explain why maize does not respond positively to intercropping until after the wheat harvest (Li et al., 2006).

5.4 Type IV: Three Cropping Seasons per Year with Rotations Replacing Intercropping

Cultivation spectrum and species diversity is much wider in the southwest region, including parts of Guangxi and Shaanxi as well as the provinces Sichuan, Chongqing, Guizhou and Yunnan. Among others, rice, maize, wheat, sweet potato, sorghum, rapeseed, sugarcane, peanut, tea, sesame, bean, vegetable, cotton, ambary hemp, tobacco, millet, cassava, soybean and pea are cultivated, mostly within a rotation and a double or even triple cropping system. In southern parts, double and triple cropping including paddy fields get more and more common. In medium and high plateau uplands, double cropping as well as single cropping for paddy fields are practiced. The production account of wheat decreases more and more when going southward, hence rice and paddy fields increase. Climate conditions allow more flexible rotations, thus replacing and reducing intercropping potentials. Nearly the whole year is frost free with an average temperature between 15°C and 18°C. From the North of the intercropping region to the South, the climate changes from subtropical, humid, monsoonal with intense sunlight and long, hot summers but low temperatures to a more and more tropical climate with conspicuous dry and rainy seasons. Average precipitation is between 800 mm and 1600 mm per year with Guangxi being one of China's most rainy areas. Rainy season is between May and October with occasional droughts in springtime.

6 Conclusion

In conclusion this chapter shows that intercropping of cereals has a 1000-year old tradition in China and it is still widespread in modern Chinese agriculture. As sustainability is the major challenge for Chinese agriculture, intercropping bears the potential for a more sustainable land use without introducing a new cropping system. Nevertheless, there are still research gaps considering intercropping pattern improvements. This chapter is a first approach to take stock of intercropping history, practice and distribution in China. Mostly, these factors are only worth footnotes in international literature. Intercropping regions, area, cropping conditions and crops are rarely part of statistics or reviews. The evaluation of available data on intercropping in this chapter has shown that a classification of four Chinese cereal intercropping regions may be possible, even if it is, in fact, an interim and general classification. More detailed data and studies are needed for further specification of intercropping regions and patterns. The four intercropping regions are the Northeast and North, the Northwest, the Yellow-Huai River Valley and the Southwest. Going from north to south the cropping systems change from one crop a year with a great potential for intercropping to relay intercropping of especially maize and wheat and double cropping systems and at least three cropping seasons per year with different kinds of rotations and rotations replacing intercropping.

Maize and wheat cultivation is well documented and the main topic of Chinese studies with irrigation and fertilization practice and improvement taking precedence over interspecific competition. Both crops are mostly intercropped with each other or intercropped with legumes and are common all over Chinese intercropping regions. In most studies, maize yields increased when intercropped. Nutrient efficiency increased while N input could be reduced and simultaneously leaching could be reduced because of lower nitrate in the soil. It seems as if maize is a suitable species for intercropping systems in China. Within the double cropping system of wheat and maize, relay intercropping of wheat and maize is common practice. As the region is China's granary the irrigation area and the fertilizer consumption is the highest compared to other intercropping regions, and it is important to reduce input factors and to produce more sustainable output. Improved and adjusted intercropping systems could contribute, e.g. to replace a single maize cycle with an intercropping system, especially in the system of three harvests in 2 years.

The range of species which can be grown in China, especially in the middle and the south of China, is wide, so that there are various possibilities and combinations of intercropping systems. The peanut production in the northeastern region showed as well that intercropping could compensate soil property deficits by reducing Fe chlorosis in peanut plants when intercropped with maize. As intercropping systems offer great opportunities for a more sustainable cultivation, these systems have to be studied more closely in future.

With floods, droughts, landslides and the snow disaster in February 2008, the consequences of environmental pollution will come more and more into mind. In this context, high-input agriculture and monocropping may not be the best-performing systems any more, considering income security, nutritional diversity in rural areas, and possibility of severe impacts to large areas due to pest and disease outbreak in a changing climate.

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Effect of Genetically Modified Bacteria on Ecosystems and Their Potential Benefits for Bioremediation and Biocontrol of Plant Diseases – A Review

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Abstract For centuries, microorganisms have served mankind in many ways. Relatively recent developments include the use of bacterial inoculants for bioremediation and agricultural purposes like biological control of plant diseases. Whereas agricultural applications of bacteria have been successful to some extent, improvement of their efficacy is necessary for commercial applications on a large scale. For instance, the remediation of mixed organic and metal-contaminated sites poses problems that may be overcome by introducing metal resistance in the bacteria used for bioremediation. For biological control of plant diseases the efficacy can be improved by combining several mechanisms of antagonism against pathogens in a biocontrol agent. Genetic modification now enables us to construct microbial strains with such novel and enhanced properties. Large-scale introduction of genetically modified strains into the environment poses some challenging questions.

This chapter provides an overview of studies concerning the application of bacteria and their genetically modified derivatives, with the emphasis on their fate and effects on the ecosystem. We limited this chapter to genetically modified microorganisms (GMMs) for agricultural applications, as biosensors, and for bioremediation purposes. Proliferation and survival of the introduced strains in the environment are discussed, but we have mainly focused on recent studies concerning the possible impact of GMMs on microbial communities and ecosystem functioning. Survival and colonization of GMMs is either equal or less when compared to that of the parental strain. The impact of bacterial inoculants (genetically modified or not) on microbial communities is either negligible or small as compared to effects of general agricultural practices and the effects are transient.

Keywords Agriculture · Biocontrol · Biofertilizer · Biosensor · Bioremediation · Ecosystem effects · Field trial · GMMs · Pseudomonads · Rhizobacteria

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List of Abbreviations

ARDRA	amplified ribosomal DNA restriction analysis
DAPG	2,4-diacetylphloroglucinol
DGGE	denaturing gradient gel electrophoresis
2,4-DNT	2,4-dinitrotoluene
GMM	genetically modified microorganisms
GM	genetically modified
PCA	phenazine-1-carboxylic acid
PCB	polychlorinated biphenyls
PCR	polymerase chain reaction
PGPR	plant growth-promoting rhizobacteria
SSCP	single-strand conformation playmorphism
T-RFLP	terminal restriction fragment length polymorphism

1 Introduction

For more than a century, bacteria have been deliberately introduced into the environment for specific purposes. The efficacy of microbial inoculants for remediation and agricultural purposes is, however, in many cases not sufficient to allow large-scale commercial application. Improved degradation of pollutants or resistance to co-pollutants present in contaminated soils, or increased efficacy of disease suppression by combining different mechanisms of antagonism against pathogens, may lead to increased opportunities for applicability. Current knowledge on bacterial genetics allows us to construct new strains with exciting capabilities. Such genetically modified microorganisms (GMMs) have been postulated to be applicable in agriculture for effective control of plant pathogens (biocontrol), and to improve plant growth (biofertilizers) (Amarger, 2002). Moreover, GMMs have been employed for degradation of polluting compounds in the environment (Dutta et al., 2003), or as biosensors to determine levels of pollutants in soil and water (Belkin, 2003).

However, the success of applications of GMMs also appears to be variable and is dependent on the survival and activity of bacterial species and the prevailing environmental conditions. This situation emphasizes our lack of understanding of the ecology of microorganisms in the environment (Prosser et al., 2007). While a number of investigations have dealt with environmental aspects of genetically modified (GM) plants, little is know on the fate of GM bacteria in the ecosystem (Marchetti et al., 2007). Some constraints that currently prevent large-scale application of GMMs is our lack of knowledge on the effects of the introduced strains on the indigenous microflora (Winding et al., 2004), and public concern regarding gene technology. In this chapter, practical and fundamental aspects of GMMs in agriculture and potential effects on the ecosystem will be discussed.

2 Genetically Modified Bacteria for Agricultural Purposes

Many bacterial genera have members that can suppress plant diseases caused by plant pathogens (biocontrol strains) or that can contribute to increased plant growth by enhancing the availability of nutrients (biofertilizers) (Weller, 1988). These so-called plant growth-promoting rhizobacteria (PGPR) thrive at the interface between soil and plant root (the rhizosphere). The cells can be applied as seed coating or directly to soil. In order to exert their functions, sufficient numbers of the introduced bacteria have to survive in soil and rhizosphere (Raaijmakers et al., 1995). However, the efficacy of PGPR is not always sufficient for commercial applications and strategies to improve their performance include genetic modification (Mark et al., 2006).

2.1 Survival of Genetically Modified Bacteria in Soil

Cells introduced into the environment will encounter a large number of biotic and abiotic factors affecting their survival. The fate of bacteria is determined by an intricate interplay between the environmental conditions and the physiological state of the bacteria. As a reaction to these conditions, bacteria can revert to different physiological states. From being in a "normal" culturable state, cells can become more stress resistant or form dwarf cells (Van Overbeek et al., 1995), they can produce exopolysaccharides for protection (Ophir and Gutnick, 1994), they can enter a viable but non-culturable state (Colwell et al., 1985), and some are able to form spores or associations with plants.

Both biotic and abiotic factors will affect bacteria introduced into soil. Factors such as high clay content, high pH, and relatively high moisture content can have a positive effect on bacterial survival (Da and Deng, 2003; Heijnen et al., 1988; Van Elsas et al., 1986). Factors that may negatively affect numbers of introduced bacteria include dry periods, presence of competing microorganisms, predation by protozoa, and lysis by bacteriophages (Ashelford et al., 2000; Eberl et al., 1997; Heijnen et al., 1988; Johansen et al., 2002; Smit et al., 1996; Stephens et al., 1987; Tan and Reanney, 1976).

An important biotic factor affecting the activity and survival of introduced bacteria is the presence of plant roots that provide nutrients to the microorganisms living in their vicinity (Lugtenberg et al., 2001). Many members of the genera *Agrobacterium, Azospirillum, Azotobacter, Bacillus, Erwinia, Pseudomonas, Rhizobium*, and *Xanthomona*s are microorganisms well adapted to the rhizosphere. Cells of these genera can survive very well or might even increase in numbers in the rhizosphere (Bashan et al., 1995; Liu and Sinclair, 1993; Lugtenberg et al., 2001; Rosado et al., 1996). While most reports on the survival of pseudomonads in soil demonstrated that their numbers decline rapidly (Table 1), some *Pseudomonas* strains have been shown to increase in numbers in the rhizosphere (Bailey et al., 2000; Jäderlund et al., 2008; Mavrodi et al., 2006; Raaijmakers and Weller, 1998). There seems to be a continuous succession of different species or genotypes adapted to a certain growth phase of the roots (Duineveld and van Veen, 1999; Ellis et al., 1999; Rainey et al., 1994). Semenov and co-workers (1999) found wave-like patterns in microbial populations along roots, which could result from subsequent root growth and death. Such wave-like distribution patterns were also reported for a green fluorescent protein-marked *Pseudomonas fluorescens* strain that was introduced into the rhizosphere of wheat plants (Van Bruggen et al., 2008).

Table 1 Decline rates of introduced cells calculated as decrease of cell numbers expressed in Log of cells per week and survival characteristics of bacterial species belonging to different bacterial divisions (mean decline rates for each division is presented)

Taxon/Species	Decline rate	Ecosystem	Detection method [2]	References
Proteobacteria				
Alpha subdivision	(x=0.11)[1]			
Rhizobium leguminosarum	0.21	Soil	Cult.	Heijnen et al. (1988)
Rhizobium leguminosarum	0.15	Soil	IF	Heijnen et al. (1988)
Rhizobium leguminosarum RSM2004	<0.01	Soil*	Cult.	Hirsch (1996)
Azospirillum brasilense	−0.10	Rhiz.	Cult.	Bashan et al. (1995)
Azospirillum brasilense	0.46	Rhiz.	Cult.	Bashan et al. (1995)
Bradyrhizobium japonicum	<0.01	Soil	IF	Brunel et al. (1988)
Sinorhizobium meliloti	0.07	Soil*	Luc	Schwieger et al. (2000)
Gamma subdivision	(x=0.34)			
Pseudomonas stutzeri	0.26	Soil	Cult. + Cat.	Byzov et al. (1996)
Pseudomonas stutzeri	0.22	Soil	Cult. + Cat.	Byzov et al. (1996)
Pseudomonas putida WCS358	0.40	Rhiz.*	Cult.	Glandorf et al. (2001)
Pseudomonas fluorescens	0.20	Rhiz.	Cult.	Frey-Klett et al. (1997)
Pseudomonas fluorescens	1.20	Soil	Cult.	Kozdrój (1997)
Pseudomonas fluorescens R2f	0.18	Rhiz.*	Cult.	Wernars et al. (1996)
Pseudomonas fluorescens Q2-87	−0.06	Rhiz.	Cult.	Raaijmakers et al. (1999)

Table 1 (continued)

Taxon/Species	Decline rate	Ecosystem	Detection method[2]	References
Pseudomonas fluorescens CHA0	0.26	Soil	IF.	Mascher et al. (2000)
Pseudomonas fluorescens CHA0	0.39	Soil	Cult.	Mascher et al. (2000)
Cytophaga-Flexibacter-Bacteroides group				
Flavobacterium sp.	2.45	Soil	Cult.	Thompson et al. (1990)
Firmicutes	(x=0.05)			
Paenibacillus azotofixans	−0.2	Rhiz.	MPN-PCR	Rosado et al. (1996)
Paenibacillus azotofixans	0.5	Soil	MPN-PCR	Rosado et al. (1996)
Bacillus megaterium	−0.3	Rhiz.	Cult.	Liu and Sinclair (1993)
Bacillus thuringiensis	0.12	Soil	Cult.	Byzov et al. (1996)
Arthrobacter globiformis	0.14	Soil	Cult.	Thompson et al. (1990)

*Data obtained from field experiment; [1]average decline rate; [2]Cult. = cultivation-based detection method; Luc = luciferase gene used as marker for confirmation; Cat. = 2,3 dioxygenase gene for degradation of catechol was used as marker; IF = immunofluorescent counts; MPN-PCR = most probable number PCR.

It seems logical to assume that GM bacteria would survive in a similar fashion as their wild-type parents. However, expression of the inserted genes requires an extra amount of energy, which could reduce their environmental fitness (Lenski, 1991); moreover, the insertion could have disrupted unknown functions debilitating the competitiveness of the strains. On the other hand, GMMs could evolve and adapt to the prevailing environmental conditions via natural selection. Velicer (1999) showed that evolutionary adaptation of bacteria to degrade the herbicide 2,4-dichlorophenoxyacetic acid could result in increased competitive fitness to use succinate as a substrate. More recently Kishony and Leibler (2003) reported that environmental stresses could alleviate the debilitating effects of mutations. Their results show that organisms may become more tolerant to genetic perturbations under certain environmental stresses. However, GMMs have not often been shown to acquire increased environmental fitness in microcosm studies. In the study of Timms-Wilson et al. (2000), however, chromosomal insertion of the genes encoding for phenazine-1-carboxylic acid (PCA) production in *P. fluorescens* resulted in enhanced ecological fitness in a microcosm system.

Also in studies with artificial growth conditions, GMMs have been shown to survive better than the wild-type strain (Biel and Hartl, 1983; Bouma and Lenski, 1988; Edlin et al., 1984). However, enhanced survival of GMMs has rarely been observed under field conditions.

Often, numbers of introduced bacterial cells decline rapidly in soil (Table 1), and the survival of GM bacteria is similar to that of non-modified bacteria. There are quite a number of studies in which no difference in survival between GMM and parent strain could be detected (Bailey et al., 1995; Blouin Bankhead et al., 2004; Glandorf et al., 2001; Orvos et al., 1990; Timms-Wilson et al., 2004; Viebahn et al., 2003). Gagliardi et al. (2001) found similar survival rates of the wild-type strain and GM *Pseudomonas chlororaphis* and *P. fluorescens* in five different soils. Schwieger et al. (2000) investigated survival of *rec*A$^-$ and *rec*A$^+$ luc-tagged *Sinorhizobium meliloti* in field lysimeters. Both strains declined from 10^6 to 10^4 colony forming units per gram of soil in lysimeters planted with alfalfa in the first year. However, the *rec*A$^+$ strain survived significantly better.

In other studies GMMs were reported to survive less than their non-modified parent strains (Brockman et al., 1991; Bromfield and Jones, 1979; Da and Deng, 2003; De Leij et al., 1998; Van Elsas et al., 1991; Wang et al., 1991). In the study of Blouin Bankhead et al. (2004) there was no difference in colonization of wheat rhizosphere between *P. fluorescens* Q8r1-96 and its GMM that produces PCA when they were inoculated separately. However, when co-inoculated the GMM was out-competed by the parent strain. Results from a study of De Leij and co-workers (1998) showed that the presence of a number of constitutively expressed marker genes in a GMM had a negative effect on its survival in competition with the wild-type strain. The place of insertion into the chromosome did not affect survival. The evidence suggested that it was purely the metabolic load that was responsible for the decreased fitness, since the study also indicated that this effect did not occur under nutrient-rich conditions.

The method of detection is of crucial importance for interpreting bacterial survival data, since cells that have become non-culturable escape detection with cultivation-based methods.

In a number of studies GMMs introduced into soil were shown to have become non-culturable (England et al., 1995; Kluepfel, 1993). The ecological significance of the presence of viable but non-culturable cells, dead cells, or naked DNA, detected with molecular techniques, remains largely unsolved and definitely requires to be studied further.

Experimental design is of importance to assess the effect of the genetic modification on the fitness of the GMM. Small differences in fitness only become apparent when the GMM and its parental strain are co-inoculated and thus are in direct competition. In most studies in which the GMM was less fit, such a combined inoculation of the parental and GMM strain was used (De Leij et al., 1998; Van Elsas et al., 1991). However, commercial application of GMMs will not include direct competition between GMM and wild-type strain. Therefore, results from such direct competition experiments have to be interpreted with care.

A general conclusion regarding survival of GMMs as compared to their parental strains cannot be drawn. Thus, in each case where colonizing ability and survival of the GMM are of importance, these parameters will have to be determined.

2.2 Ecosystem Effects of Genetically Modified Microorganisms

Possible effects of the introduction of GMMs on natural microbial ecosystems range from the input of organic substrate, displacement of species, changes in population structure, and possible loss of certain functions, to the production of toxic metabolites, which might lead to disturbance of key ecological processes (Smit et al., 1992). However, small changes in community composition are difficult or even impossible to determine, and the relationship between microbial diversity and ecosystem functioning is not well understood (Prosser et al., 2007). Since the number of bacterial species in 1 g of soil is estimated to range between 10,000 and 40,000 (Torsvik et al., 1998), microbial diversity is enormous, and a high redundancy of functions is expected to be present (Degens, 1998; Griffiths et al., 2000). Disappearance of a few species with certain functions will be difficult to detect, since many functions can be performed by a large number of different microbes. Only extreme disturbances might affect soil microbial communities to the extent that certain functions will be reduced (Griffiths et al., 2000).

One of the major problems in microbial ecology is the limited culturability of the indigenous microflora (Amann et al., 1996; Bakken, 1997; Hugenholtz and Pace, 1996; Pace et al., 1986; Schmidt et al., 1991). DNA- and RNA-based techniques, which do not involve cultivation of the microorganisms, are currently used to detect the impact of GMMs on the indigenous microbial community (Torsvik et al., 1998). Methods such as denaturing gradient gel electrophoresis (DGGE) (Fischer and Lerman, 1979; Muyzer and Smalla, 1998), amplified ribosomal DNA restriction

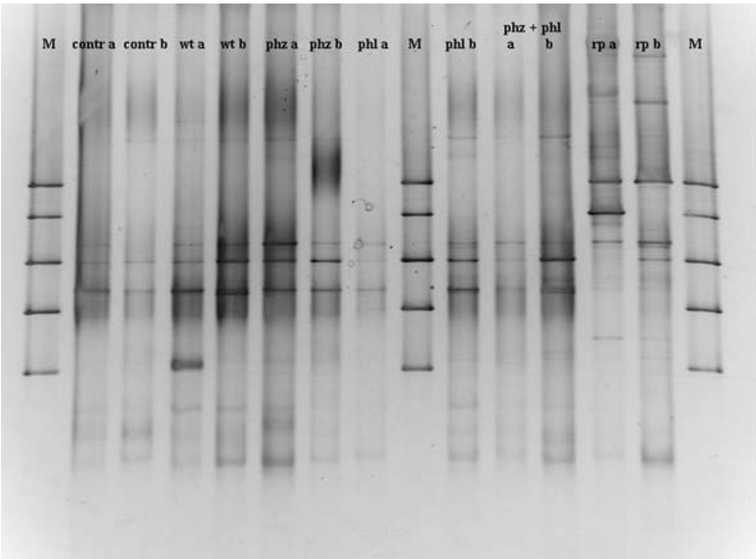

Fig. 1 Denaturing gradient gel electrophoresis (DGGE) profiles showing ascomycete communities of rhizosphere samples of field-grown wheat and potato plants 59 days after sowing. Wheat plants were grown from seeds that were untreated (contr), coated with *P. putida* WSC358r (wt), its phenazine-1-carboxylic acid- or 2,4-diacetylphloroglucinol-producing GMMs (phz, phl), and a combination of both GMMs (phz+phl). rp = samples taken from plots in which potatoes were planted. DNA was extracted from rhizosphere samples and amplified with ascomycete-specific primers. Two replicate plots were analyzed (a, b). Hypothetically, each band in the gel represents one ascomycete species. Note that the potato rhizosphere (rp a, rp b) contains different ascomycete species than the wheat rhizosphere. M = reference marker (Viebahn et al., 2005)

analysis (ARDRA) (Vaneechoutte et al., 1992), terminal restriction fragment length polymorphisms (T-RFLP) (Marsh, 1999), and single-strand conformation polymorphism (SSCP) (Orita et al., 1989) are used to generate banding patterns representing the microbial community in the sample. These methods are more suitable than cultivation-based methods to analyze shifts in community structures. Figure 1 shows DGGE profiles of ascomycete communities of rhizosphere samples from field-grown wheat and potato plants 59 days after sowing, from the study by Viebahn et al. (2005). The wheat plants were grown from control seed and seed treated with wild-type or GM *P. putida* WCS358r. The profiles indicate different ascomycete species in the rhizospheres of potato and wheat and no apparent effects of the bacterial treatments.

2.3 Fate and Effect of Biofertilizer Strains

In this section we will discuss GM derivatives of bacteria that contribute to an enhanced nutrient availability for plants, and thereby increase plant growth.

The most important biofertilizers are bacteria, such as *Azospirillum* and *Rhizobium*, that can fix nitrogen. *Rhizobium, Bradyrhizobium*, and *Sinorhizobium* are plant symbionts, which form root nodules in leguminous plants and fix atmospheric nitrogen. These bacteria have been used widely as plant inoculants to increase yield of leguminous crops. There is a long history of safe use of non-modified rhizobia as inoculants to increase yields of crops (Anon, 1995). However, yield increase is variable (Streeter, 1994), and the success of inoculants seems to be dependent on competition with indigenous strains that are usually less effective (Triplett and Sadowsky, 1992). *Rhizobium, Bradyrhizobium,* and *Sinorhizobium* have been reported to survive in soil for years, in some cases even without the presence of their specific host (Brunel et al., 1988; Diatloff, 1977; Hirsch, 1996, 2004; Schwieger et al., 2000). *Rhizobium* was shown to be able to form nodules when its host plant was planted again after several years (Hirsch, 1996, 2004). This shows that presence of the host plant is not strictly necessary for their survival, but also characteristics of the strain not related to symbiosis play a role in its survival in bulk soil for years. Fast-growing *Rhizobium* species were found to be more susceptible to desiccation than the slower-growing *Bradyrhizobium* (Marshall, 1964). Several *Rhizobium* species have been GM either to improve nitrogen fixation (Birkenhead et al., 1988; Bosworth et al., 1994, Cullen et al. 1998, Hirsch, 2004; Wang et al., 1991), or to study their survival making use of marker genes (Donegan et al., 1999; Hirsch, 2004; Hirsch and Spokes, 1994; Mendum et al., 2001, Van Dillewijn et al., 2002, Watson et al., 1995).

Hirsch and Spokes (1994) studied a Tn*5*-marked *R. leguminosarum* strain introduced into a field as an inoculant for peas and cereals. Cells were enumerated by using a most probable number plant infection test and by direct plate counts. The introduced strain persisted for 5 years in the plots where peas were grown. The persistence of the marked strain was attributed to the soil type, the cultivation of the proper host plants, and the climatic conditions. Potential non-target effects on the microbial ecosystem were not studied (Hirsch, 1996; Hirsch and Spokes, 1994).

Bosworth and co-workers (1994) showed that the use of an improved *R. meliloti* strain, with additional copies of *nifA* and *dctABC*, resulted in an increase of alfalfa yield of 12.9% in a field study. However, at sites with high nitrogen concentrations or native rhizobial populations alfalfa yield did not increase. The plots treated with the recombinant strain target microorganisms were determined. Watson and co-workers (1995) studied the fate of a Tn*903*-marked *R. meliloti* strain introduced into alfalfa-planted field plots. Cell numbers, assessed by polymerase chain reaction (PCR), decreased rapidly after inoculation. One year after introduction, numbers of introduced cells had dropped to below the numbers of indigenous rhizobia.

In a contained field experiment a GM *S. meliloti* strain with enhanced competitiveness for nodule occupancy was released in the rhizosphere of alfalfa (Van Dillewijn et al., 2002). Effects of the GMM and the wild type on the indigenous microbial communities were studied by restriction fragment length polymorphism (RFLP) and temperature gradient gel electrophoresis (TGGE). Inoculation of wild type and GMM had only limited effects. It appeared that alfalfa plants had a greater influence on the microbial community than the inoculated strains.

Schwieger and Tebbe (2000) studied both the fate and ecosystem effects of a *luc*-marked *S. meliloti* in a field experiment with *Medicago sativa*. The bacteria were detected up to 12 weeks after introduction. No effects of the strains on carbon and nitrogen concentrations in the soil could be detected, and there were no differences in the total number of colony forming units of indigenous microorganisms (Schwieger and Tebbe, 2000). Over a thousand bacterial isolates obtained from the plots were further studied by ARDRA, and the dominant groups were identified by 16S rRNA sequencing. In the rhizosphere of *M. sativa* numbers of *Alcaligenes* and *Pseudomonas* were reduced as a result of the inoculation. Molecular analysis by studying SSCP banding profiles revealed shifts confirming the effect of the inoculum on the native microbial population (Schwieger and Tebbe, 2000).

In the south of China both wild type and GM *Alcaligenes faecalis* isolates have been introduced into rice fields at a large scale to improve crop productivity (You et al., 1995; You and Zhou, 1991). *A. faecalis*, a non-nodule-forming nitrogen-fixing isolate, was GM by insertion of a constitutively expressed *nifA* regulatory gene (You and Zhou, 1991). Nitrogen fixation appeared to be 15–20% higher and yield was 5–12% higher compared to the non-treated fields (You et al., 1995). Lin et al. (2000) studied possible ecosystem effects of the introduction of this GMM strain by DGGE of amplified 16S rDNA in a microcosm experiment. The introduced GMM survived well in the rhizosphere with cell numbers of about 10^7 per gram of soil throughout the experiment. DGGE banding profiles of samples treated with the modified strain closely resembled profiles of untreated samples throughout the 40 days of the experiment, suggesting that there are no obvious effects on the bacterial community. Overall, the survival of the strain and the increase in crop yield indicate that this derivative of *A. faecalis* is a good candidate for commercial application, while its ecosystem effects seem very limited.

The use of GM strains as biofertilizers seems promising. So far, non-target effects of GM biofertilizer strains that have been reported are small and insignificant compared to natural variations, such as differences between populations of different plant species (Crawford et al., 1993; Da and Deng, 2003; Lin et al., 2000; Schwieger and Tebbe, 2000; Van Dillewijn et al., 2002; Vázquez et al., 2002). The monitoring of spread and survival of inoculants has been facilitated by use of GM strains and has greatly improved our understanding of rhizobial ecology (Hirsch, 2004).

2.4 Fate and Ecosystem Effects of Modified Biocontrol Bacteria

Many bacterial genera have been described to contain members that can suppress plant diseases caused by soilborne pathogens (Cook et al., 1995; Glick et al., 1999). These PGPR include members of the genera *Azospirillum, Azotobacter, Bacillus, Burkholderia*, and *Pseudomonas* (Weller, 1988). Interest in the application of microorganisms for protection of agricultural crops was stimulated by the public concern regarding environmental effects of agrochemicals. Many biological control agents have been GM to enhance their biocontrol properties including the extension of the metabolic repertoire by the insertion of novel genes and the increase of the levels of active metabolites.

Investigations have mainly focused on fluorescent *Pseudomonas* spp. strains. Pseudomonads are a metabolically diverse group of rhizosphere bacteria, and many strains are able to suppress plant diseases caused by microbial pathogens (Défago and Keel, 1995; Haas and Défago, 2005; Mercado-Blanco and Bakker, 2007). Mechanisms of disease suppression include siderophore-mediated competition for iron (Bakker et al., 1993; Haas et al., 1991), competition for nutrients, induced systemic resistance (Bakker et al., 2007; Van Loon et al., 1998), and antibiosis (Handelsman and Stabb, 1996; Weller et al., 2002). The best-studied antibiotics with respect to biocontrol are 2,4-diacetylphloroglucinol (DAPG) and PCA, which have antibacterial and antifungal properties and play a major role in disease suppression (Raaijmakers and Weller, 1998; Thomashow et al., 1990).

Quite a number of studies describe the potential use, survival or target effects of GM biocontrol strains (Byzov et al., 1996; Choi et al., 2003; Jones et al., 1991; Moënne-Loccoz et al., 1996; Nambiar et al., 1990; Palmer et al., 1997; Sitrit et al., 1993; Van Overbeek et al., 1995; Völksch and May, 2000). However, only a few studies focus on the effects on the indigenous microflora (De Leij et al., 1995; Girlanda et al., 2001), and only some describe field releases of biocontrol agents in the natural environment (Bakker et al., 2002; Glandorf et al. 2001; Jäderlund et al., 2008; Johansen et al., 2002; Schwieger and Tebbe, 2000; Viebahn et al., 2003, 2005, 2006).

Delany et al. (2001) compared the naturally DAPG-producing *P. fluorescens* F113 with its two GMMs modified to produce higher levels of DAPG in a microcosm. All strains inhibited growth of *Pythium*, and the GMMs showed enhanced control of damping-off, comparable to that of commercial fungicides. Natsch et al. (1997) studied the effect of a genetically modified derivative of *P. fluorescens* strain CHA0 on the diversity of resident pseudomonads in the rhizosphere of cucumber. The modification consisted of the insertion of an extra copy of a housekeeping gene, resulting in increased production of the antibiotics DAPG and pyoluteorin and improved disease control. Several days after inoculation both the parent strain and the GMM reduced the number of resident pseudomonads in the rhizosphere, but the impact of the modified strain was more significant. Only the effect of the modified strain persisted for more than a month. However, when considering the composition of the *Pseudomonas* population on different developmental stages of the cucumber roots, the effects of both modified and non-modified strains appeared to be small and transient.

In the Netherlands, a number of field trials with GM biocontrol bacteria have been performed to study their possible impact on the indigenous microflora (Bakker et al., 2002; Glandorf et al., 2001; Viebahn et al., 2003, 2005, 2006). The fields were carefully designed (Fig. 2) to meet permit requirements. They were surrounded by reed mats to reduce spread of possibly contaminated soil particles outside the field, and the fence and netting prevented entry of birds and rabbits. Weed-free buffer

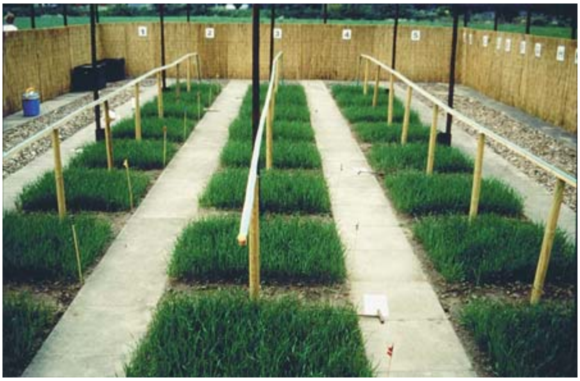

Fig. 2 Experimental field designed to study possible effects of genetically modified biocontrol bacteria on the indigenous microflora of the wheat rhizosphere in Utrecht, The Netherlands. Each treatment was applied to six 1-m^2 plots that were separated by non-planted buffer zones. The fence and netting prevented entry of birds and larger animals, and the reed mats were placed to reduce spread of soil to outside the experimental field (Glandorf et al., 2001)

zones around the experimental plots prevented spread of introduced bacteria by root–root contact.

Possible non-target effects of GM *Pseudomonas* were studied by Glandorf et al. (2001). The biocontrol strain *P. putida* WCS358r was GM with the *phz* locus from *P. fluorescens 2-79*, resulting in constitutive PCA production. Phenazine production in WCS358r resulted in improved biocontrol of take-all of wheat, caused by *Gaeumannomyces graminis* var. *tritici* (Bakker et al., 2002). Two derivatives, one with low and one with high levels of PCA production, were selected for further study in two small-scale field experiments during two subsequent years (Glandorf et al., 2001; Leeflang et al., 2002). Monitoring of various soil ecosystem functions, such as substrate-induced soil respiration, cellulose decomposition, and nitrification potential activity, did not reveal effects of the introduction of any of the strains. Effects of the GMM producing high amounts of PCA on the culturable fungal microflora were transient and were further analyzed using 18S rDNA ARDRA. Introduction of both the wild-type and the GMMs transiently changed the composition of the fungal rhizosphere microflora. However, effects of the GM strains were distinct from those of the parental strain and persisted longer.

In addition to the above-mentioned PCA-producing strain a DAPG-producing GMM was introduced into a wheat field to study long-term effects on the rhizosphere microflora in two consecutive years (Viebahn et al., 2003). In this case, only the DAPG-producing GMM had a transient effect on the structure of the bacterial and fungal microflora, as determined with ARDRA, whereas there was no effect of the PCA-GMM or the wild type. Using DGGE the ascomycete and the bacterial microflora were studied for an additional 2 years (Viebahn et al., 2005, 2006). Whereas the ascomycete communities were not affected by introduction of the GMMs (Viebahn et al., 2005), the bacterial communities were differentially affected by the parental strain and the GMMs, and especially the DAPG producer had an effect in all 4 years of the field trial (Viebahn et al., 2006). In this field trial the impact of the bacterial inoculants was compared to that of crop rotation between wheat and potato, as an example of agricultural practice. In all cases, the effect of the bacterial introductions never exceeded that of changing the crop (Viebahn 2005, 2006).

Two similar studies on the effects of DAPG- and PCA-producing GM *P. fluorescens* strains were conducted by Timms-Wilson et al. (2004) and Blouin Bankhead et al. (2004). The DAPG-producing *P. fluorescens* strain Q8r1-96 was modified with PCA biosynthetic genes of *P. fluorescens* 2-79, and the resulting GMM was compared to the wild type in pot experiments with wheat. T-RFLP profiles of amplified eubacterial ribosomal sequences demonstrated that inoculation with the wild type or the GMM strain resulted in minimal changes of the bacterial population structure in the rhizosphere (Blouin Bankhead et al., 2004). Chromosomal insertion of the PCA biosynthetic genes in *P. fluorescens* SBW25 resulted in enhanced efficacy of damping-off disease of pea seedlings caused by *Pythium ultimum* (Timms-Wilson et al., 2000). Possible impact of this PCA-producing GMM on bacterial and fungal microbial diversity was studied in pot experiments with pea, wheat, and sugarbeet, and with or without *Pythium* disease pressure. In all these experimental conditions, factors like plant species or development of disease had a greater impact on microbial diversity in the rhizosphere than inoculation with the PCA-producing GMM (Timms-Wilson et al., 2004).

In line with these studies a recent paper by Scherwinski et al. (2008) convincingly shows that introduction of active biocontrol agents (*Serratia plymuthica*, *P. trivialis*, and *P. fluorescens*) results in negligible, short-term effects on the indigenous bacterial and endophytic fungal populations.

A different approach is to study the impact of GMMs on specific non-target microbial taxa with an important soil function. Enhanced biocontrol properties of pseudomonads by increased antibiotic production could result in adverse effects on the arbuscular mycorrhizal symbiosis. The hypothesis was tested by Barea et al. (1998) using *P. fluorescens* strain F113 and two GM derivatives: wild-type strain F113, a natural DAPG-producer, and two GM derivatives that either produced no DAPG or had enhanced DAPG-production. Colonization of tomato roots by the arbuscular mycorrhizal fungus *Glomus mossae* was not affected by any of the bacterial strains. Moreover, enhanced DAPG production did not inhibit spore germination and mycelium growth. Apparently, production of DAPG by *Pseudomonas* sp. F113 does not affect the beneficial fungal symbiont *G. mossae*.

From these studies with GM biocontrol strains it can be concluded that effects on non-target microorganisms in soil ecosystems do occur; however, if they occur they are transient and small compared to natural variation. To our knowledge long-term changes in community structure and adverse effects on the functioning of the soil ecosystem after introduction of GM strains have not been reported.

3 Genetically Modified Microorganisms as Biosensors and for Bioremediation

3.1 Genetically Modified Biosensors

An exciting application of GMMs is their use as a sensor for biologically relevant concentrations of agrochemicals, petroleum products, heavy metals, and toxins in environmental samples (Belkin, 2003; Kim et al., 2003; Lei et al., 2006; Shao et al., 2002; Stiner and Halvorson, 2002). GM biosensors are usually not applied *in situ* to soil or water, but samples are taken from polluted sites and incubated in *in vitro* assays. Thus, survival and persistence of the GM biosensor strain is not an issue, since the organism remains contained in the laboratory or in the measuring equipment, and incubation periods are short.

Most of the microorganisms used as biosensors are modified with reporter genes such as *gfp* or *lux* fused to a promoter that induces its expression depending on nature and concentration of the compound(s) of interest. The choice of the promoter determines the applicability of the strain. Some researchers used promoters that respond specifically to certain compounds. For instance, Stiner and Halvorson (2002) used the toluene–benzene transcriptional activator that is only induced by the petroleum products toluene, benzene, ethylbenzene, and trichloroethylene for the detection of contamination in soil and surface water. Miller et al. (2001) used the sucrose repressor gene *scr*R fused to several promoter genes for assessing sugar availability on plant leaves. In order to detect a wider range of toxicants stress-specific promoters such as the SOS and heat shock promoters, *rec*A and *grp*E and *fab*A or *Kat*G, are fused to the reporter gene (Kim et al., 2003; Sagi et al., 2003). Such strains produce signals when they are exposed to substances that exert a stress response. Currently, biosensors are being developed that allow high-throughput monitoring of toxins in waste water (Kim et al., 2003; Philp et al., 2003). Although the future for the application of biosensors is promising (Belkin, 2003), there are a number of problems that have to be solved. Development of highly suitable microbial biosensors is as yet hampered by their long response time, low sensitivity, and poor selectivity (Lei et al., 2006). Non-specific toxic effects of other compounds on the bacteria can influence the measurements, temperature sensitivity of some strains can affect the results, and the need to contain GMMs in the equipment could pose difficulties (Philp et al., 2003, Mirasoli et al., 2002).

3.2 Genetically Modified Microorganisms for Bioremediation

Once the nature and severity of a chemical pollution has been established, GMMs can be applied to clean up the environment by bioremediation. Bioremediation is the reduction or removal of toxic or polluting substances from the environment using microorganisms. Current knowledge on the genetics of metabolic pathways in microorganisms allows the construction of new biochemical pathways to increase their capabilities to degrade environmental pollutants or herbicides (Dutta et al., 2003; Haro and de Lorenzo, 2001; Lange et al., 1998; Mauro and Pazirandeh, 2000; Mitra et al., 2001; Sriprang et al., 2002; Strong et al., 2000). Although a myriad of different GM strains for bioremediation has been constructed, successful applications in the field are rare (Haro and de Lorenzo, 2001; Morrissey et al., 2002; Van Limbergen et al., 1998). Two factors are crucial for successful bioremediation: the microorganisms have to survive in high numbers and the microorganisms have to be metabolically active. Moreover, difficulties to scale up the laboratory experiments, low bioavailability of the compound, and legislative problems with applying GM strains have precluded wide-scale use. Since soil is often an oligotrophic environment cells usually decline after introduction, and they have a low metabolism. Bacterial survival and degradative activity can be enhanced by choosing the right species and by nutrient amendment. Halden and co-workers (1999) studied the degradation of 3-phenoxybenzoic acid in soil and could increase GMM survival and 3-phenoxybenzoic acid degradation six orders of magnitude by adding phosphate and nitrogen.

Microorganisms should be chosen depending on the environmental conditions in which they have to function. Lange and co-workers (1998) constructed a recombinant *Deinococcus radiodurans* expressing toluene dioxygenase to clean up toxic solvents in radioactive waste sites. *D. radiodurans* is extremely radiation resistant, the strain can degrade chlorobenzene in a highly radioactive (60 Gy/h) environment. However, *D. radiodurans* can only grow up to 39°C, and since many radioactive waste sites have high temperatures, there was need for a bacterium that could function at higher temperatures (Brim et al., 2003). *Deinococcus geothermalis* is a remarkable organism since it cannot only thrive in radioactive environments, but it is also resistant to high temperatures. Brim and co-workers (2003) constructed a *D. geothermalis* strain, which is able to reduce ionic Hg(II) to the less toxic elemental Hg at elevated temperatures and in the presence of high radiation levels. Such applications have great value for future remediation, since it is estimated that 40 million cubic meters of soil and 4 trillion liters of groundwater are contaminated with radioactive and toxic waste in the US alone (Brim et al., 2003).

So far, field applications of GM strains for bioremediation are scarce. An example of a field-scale remediation is the study of Strong et al. (2000). *P. fluorescens* HK44 with a *lux* gene fused to a naphthalene-degradative pathway was the first GM strain approved for field release in the US (Ripp et al., 2000; Strong et al., 2000). The strain survived for 660 days, and degradation of hydrocarbons was detected with a fiber optic-based biosensor. Strong and co-workers (2000) also applied chemically killed recombinant *Escherichia coli* to remediate atrazine by bioaugmentation. The addition of phosphate and the chemically killed cells resulted in a 77% reduction of atrazine levels as compared to the controls, in which no significant reduction took place.

A combined inoculation of microorganism and its host plant by providing extra nutrients could enhance microbial survival and activity (Brazil et al., 1995). Such use of a dual microorganism–plant system, in which the plant supports microbial growth and the microorganisms perform the bioremediation, is known as rhizoremediation (Kuiper et al., 2004; Yee et al., 1998). This strategy was adopted by Dutta et al. (2003), who used a GM *S. meliloti* to degrade 2,4-dinitrotoluene (2,4-DNT) in soil. This symbiotic nitrogen-fixing bacterium can nodulate leguminous plants such as alfalfa. Seeds of alfalfa were coated with the engineered strain and seeded in soil contaminated with 2,4-DNT. At moderate pollution levels plant growth was stimulated by the bacterium, and the level of 2,4-DNT was reduced by 60%. At higher levels, plants could not grow, but application of the bacteria to the soil reduced 2,4-DNT levels up to 90%. *P. fluorescens* strain F113 is a good colonizer of rhizospheres of different plant species and could be exploited for rhizoremediation. A polychlorinated biphenyl-degrading derivative of F113, F113rifPCB, was constructed (Brazil et al., 1995). Heavy metals and metalloids are serious co-contaminants of polluted soils (Ryan et al., 2005) and can hamper the success of rhizoremediation by inhibiting both the rhizobacterium and the plant. A recombinant derivative of F113rifPCB containing the arsenic resistance operon *arsRDABC* was constructed, resulting in a strain that degrades biphenyl in the presence of arsenate and that protects the plant to arsenic as well (Ryan et al., 2007). Furthermore, strain F113 was modified with the *bph* operon from *Burkholderia* sp. strain LB400, under the control of a nodbox cassette (Villacieros et al., 2005). This GM derivative of F113 (F113L::1180) had a high level of BphC activity, and in combination with willow plants is expected to degrade a large spectrum of PCB congeners in soil (Rein et al., 2007). In a 168-day microcosm experiment the possible impact of F113L::1180 on bacterial communities in soil and in the rhizosphere of *Salix viminalis*× *schwerinii* was investigated. The modified strain did affect community structure of the eubacteria and specific bacterial populations (α- and β-proteobacteria, acidobacteria, and actinobacteria) in the *Salix* rhizosphere, but not in the bulk soil (de Cárcer et al., 2007).

Bioremediation of pollutants in soil and water using GMMs has been accomplished with success on a laboratory scale; however, field applications have been limited. Long-term field studies are now needed to evaluate their effectiveness. Concerns have been made regarding persistence of GM strains and possible evolutionary adaptation that could increase its environmental fitness (Velicer, 1999). Impact on the natural microbial community by GM bioremediation bacterial strains is a complex issue, since the breakdown of pollutants itself will most likely affect microbial communities.

4 Conclusion

Whereas only a limited number of field trials with GMMs have been conducted, it appears that they can have improved performance in agricultural and bioremediation applications. GM biocontrol bacteria seem to be a suitable alternative for agrochemicals used for crop protection, and modified biofertilizers have been used with success. The success of the introduction is partly related to the survival and activity of the inoculum, which is dependent on the environmental conditions. While the questions related to field introductions of GMMs have helped to develop tools in the area of molecular microbial ecology, our knowledge on the benefits, fate, and effects of GMMs in the environment is still limited and fragmentary. For sensible applications this knowledge needs to increase rapidly, focusing on specific areas. Questions to be addressed include, how and in which physiological state do bacteria survive in soil, and what effects do they exert on the natural microbial community. More knowledge on microbial community structure and functioning is a prerequisite for optimizing the use of GMMs in agriculture.

Only very few studies have focused on ecosystem effects of GMMs, and in most of those cases the GMM used was modified to express certain markers. More recently studies have been performed using GMMs expected to have an impact on non-target organisms, for instance through the production of antimicrobial metabolites with a broad-spectrum activity. Also in this last category of experiments, effects on the microbial ecosystem were found to be small and transient or were not detected.

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Climate Change and Plant Water Balance: The Role of Aquaporins – A Review

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Abstract In the context of global change, attention has been focused on the increases in CO_2 and temperature, as well as a reduction in the global solar irradiance. In this chapter we have explored how components of global change such as CO_2, temperature and radiation will affect water uptake by plants. We focus on how aquaporins will respond to these environmental factors in order to maintain water balance in plants according to the water demand. Plant growth may be stimulated directly by increasing CO_2 concentration, through enhanced photosynthesis, or, indirectly, through induced plant water consumption. However, the fine regulation of aquaporins, also involved in CO_2 transport through membranes, will be crucial in the control of H_2O and CO_2 diffusion. Raised temperatures may benefit some crops but disadvantage others through increased evapotranspiration and thermal damage. However, in general, plants can develop different adaptive mechanisms in order to avoid water-deficit stress and excess transpiration modulating the hydraulic conductance, which involve the expression and activity of aquaporins. In the same way, the response of plants to the amount of perceived radiation affects water balance. Therefore, the study of aquaporin regulation is necessary for establishing future adaptation of plants to global change.

Keywords Climate change · CO_2 · Water · Plant · Aquaporin · Temperature · Radiation

1 Introduction

1.1 A Global Change Scenario

Climate change models suggest an increase in global average temperature, shifts in rainfall pattern and an increase in local climatic extremes. The changes in climate

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and population which are predicted during this century are certain to modify the demands on irrigation and water supply. This also means changes in evaporative demand, irradiance, ultraviolet (UV) irradiance and secondary factors, such as stratospheric ozone concentration, as well as in the prime causative agent, carbon dioxide itself. Therefore, as far as the plant response is concerned, all of these parameters are interlinked and include those with potentially positive, negative, additive and antagonistic effects.

Gases, water vapour and other particles arising from anthropogenic activities are causing changes in the composition of the atmosphere, leading to an increase in the absorption of thermal infrared radiation by the atmosphere, thus over-warming the Earth's surface. However, the complexity and large number of feedbacks in the climate system, including soil composition (Lal, 2007), could modify this prediction. Increasing temperature would be expected to increase the amount of water vapour in the atmosphere, by increasing evaporation and the water-holding capacity of the air; this, in turn, affects the atmospheric absorption of thermal radiation.

Anthropogenic combustion of fossil fuels has caused mean concentrations of CO_2 in the atmosphere to reach and exceed 380 μmol mol^{-1}, a level that is about 0.32 times greater than in pre-industrial times (Keeling and Whorf, 2005). Predictions of atmospheric CO_2concentrations in the year 2100 range between 540 and 970 μmol mol^{-1} (Houghton et al., 2001), unless emissions decrease – for example, through the use of biofuels (Hammond et al., 2008). However, in a recent review, it has been postulated that human use of hydrocarbons has not caused the observed increases in the earth surface temperature (Robinson et al., 2007) and, in addition, atmospheric CO_2 could have beneficial effects, such as fertilisation of plants and enabling plants to grow faster and larger, allowing increases in water uptake.

Also, in the context of the global change, attention has been focused on stratospheric ozone depletion, which is leading to increased UV radiation reaching the biosphere and accumulation of atmospheric greenhouse gases, producing a reduction in the global solar irradiance (Morton, 2007), principally due to blockage of sunlight. Roderick and Farquhar (2002) mentioned that considerable scepticism exists about the reported large declines in global solar irradiance. However, this effect on solar radiation is very important in the context of global climate change, since the increased cloud coverage and/or aerosol concentration should result in a decrease in the diurnal temperature range (DTR) (Ramanathan et al., 2001), affecting not only water uptake, but whole-plant physiology (Roderick et al., 2001).

1.2 Plant Water Relations

Water absorption from the soil into the root tissue is how plants maintain their water status in a suitable range. The water is then distributed in plant tissues, and approximately 95% evaporates from the leaves through the stomata. The movement of water through plant tissues may occur by the following coexisting pathways: apoplastic, symplastic and transcellular (Steudle and Peterson, 1998). The apoplastic pathway involves water flux around the cells, in the symplastic pathway the water is transported across cells involving plasma membrane, and in the transcellular pathway water is transported also through vacuoles involving tonoplast. The symplastic and transcellular, together comprising the cell-to-cell pathway, are under fine regulation, since they involve transport through plasma membranes (Fig. 1). In the root cortex, the flow of water is largely apoplastic, but at the endodermis, the Casparian band, a deposit of suberin and/or lignin, restricts the radial water movement in the apoplast and cell-to-cell transport is carried out (Zimmermann and Steudle, 1998). Thus, there are different pathways for water movement and they are used with different intensities depending on the species, the plasticity of the root and the nature of the driving forces. Therefore, depending on the environmental conditions and transpiration demand, plants can modify the apoplastic and "cell-to-cell" pathway contributions. For instance, in the presence of hydrostatic pressure gradients (such as in transpiring plants), water flow is largely apoplastic, since this route represents a low hydraulic resistance. However, under osmotic gradients (in the absence of

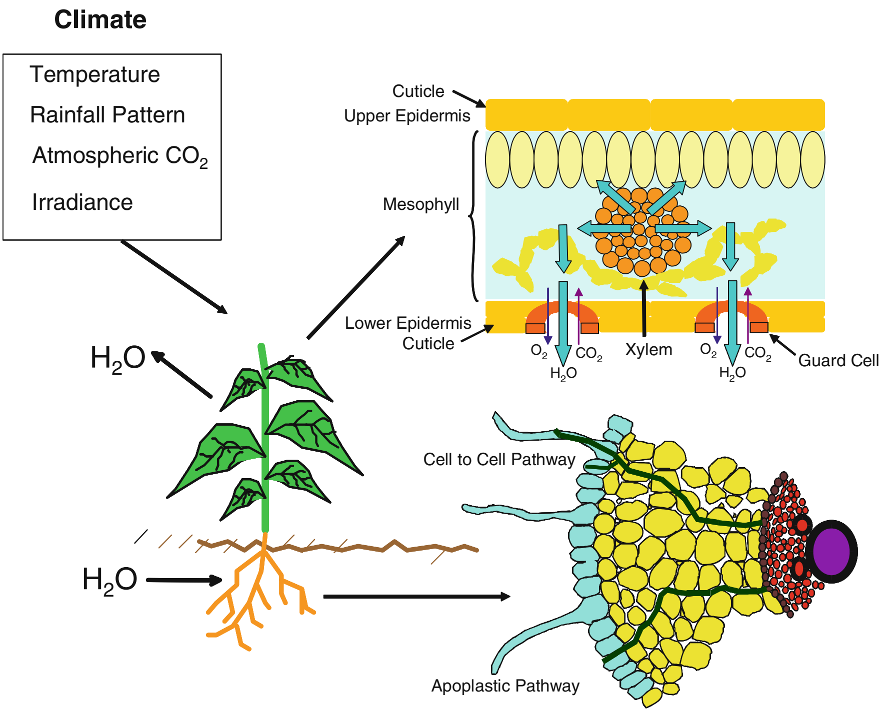

Fig. 1 In plants, water is transferred from the roots, in response to hydrostatic and osmotic gradients, to the aerial parts, where it evaporates in the transpiration stream through the stomatal pores. The movement of water through plant tissues may occur by three coexisting pathways: the apoplastic pathway involves water flux around the cells, in the symplastic pathway the water is transported across plasmodesmata and the transcellular pathway involves water transport across the plasma membrane and vacuolar membrane. There are no approaches to separate the symplastic and transcellular pathways and they are summarised as the "cell-to-cell" pathway

transpiration, i.e. during the night when stomata are closed, in water-stress conditions or during an exudation phenomenon of an excised root), water flow is low and occurs mainly through membranes predominating a fine regulation of water uptake by a group of membrane proteins called water channels or aquaporins. The aquaporins belong to the major intrinsic protein (MIP) family and facilitate the flow of water across cellular membranes (plasma and vacuolar membranes), following osmotic or hydrostatic pressure gradients (Chrispeels and Maurel, 1994). These proteins give the plant the possibility of accelerating water movement through membranes, but diffusion will still occur in parallel (Fig. 2). Thus, the biological significance of aquaporins in plants is their ability to modulate transmembrane water transport in situations where adjustment of water flow is physiologically critical (for reviews see Baiges et al., 2002; Luu and Maurel, 2005, Martínez-Ballesta et al., 2006). Furthermore, the ability to increase or decrease the water permeability of a cell justifies the very high diversity of MIP homologues in plants: the complete genome of *Arabidopsis thaliana* has 35 full-length MIP genes (Johanson et al., 2001; Quigley et al., 2002) and similar diversity was found in other plant species such as maize (Chaumont et al., 2001) and rice (Sakurai et al., 2005). The two major sub-groups are the plasma membrane intrinsic protein (PIP) and the tonoplast intrinsic protein (TIP), localised in the plasma membrane and tonoplast, respectively (Maurel et al., 1993; Kammerloher et al., 1994). PIPs play an important role in controlling transcellular water transport whereas TIPs seem to be involved in the water exchange between the cytosolic and vacuolar compartments (Maurel et al., 1997; Tyerman et al., 1999). The highly regulated expression of aquaporins in plants suggests that transmembrane water transport may be important not only in root water uptake but also in many other processes in addition to those related to transpiration. Furthermore, the recent localisation of the *Nicotiana tabacum* AQP1 in chloroplast

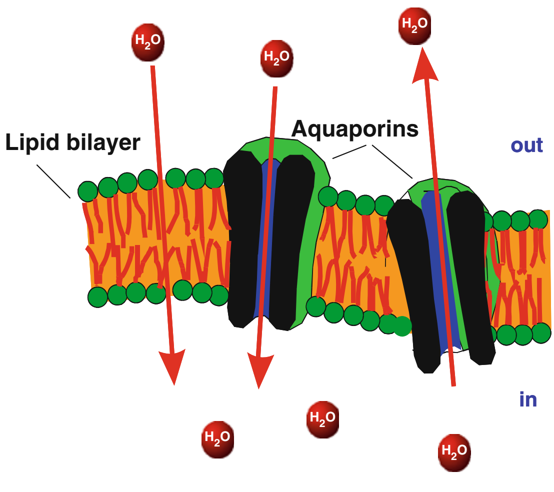

Fig. 2 Aquaporins are membrane proteins that were originally characterised as being selective to H_2O, so can facilitate water permeability through biological membranes

membrane, in addition to the plasma membrane, suggests that its classification as PIP is misleading (Uelhein et al., 2008).

In this review, we will explore how components of global change such as CO_2, temperature and radiation will affect water uptake by plants. We will focus on how aquaporins will respond to these environmental factors in order to maintain water balance in the plants according to the water demand, enabling plants' adaptation to future changes in the environment.

2 Plant Water Relations at Elevated CO_2

Recent reviews confirm and extend previous observations that elevated CO_2 concentrations stimulate photosynthesis, leading to increased plant productivity and modified water and nutrient cycles (Nowak et al., 2004, Soussana and Lüschert, 2007). Plant growth may be stimulated directly, through enhanced photosynthesis, or indirectly, through induced plant water consumption. This includes the effects on root mass and whole-plant water transport, the consequences for water-use efficiency (WUE) and soil water content and, finally, the reduction in stomatal conductance and its impact on leaf water potential. As it has been reported that some aquaporins also transport CO_2 (Uehlein et al., 2003), the involvement of aquaporins is crucial for studying the response of plants to increased atmospheric CO_2.

2.1 Effect of CO_2 on Root Proliferation

For a long time, it has been known that root systems become larger when plants are grown at elevated CO_2, but the capacity of the root system to take up water depends not on root mass but on rooting volume (or rooting depth) and fine-root area and activity (Wullschleger et al., 2002). Previous work in intact, native grassland systems has revealed that significant stimulation of the size of root systems (biomass, length, density or root number) is not a universal response to elevated CO_2 (Arnone et al., 2000). Some studies showed little or no change in root-system size under elevated CO_2 (Handa et al., 2008), while others showed marked increases (Norby et al., 2004) or a slight decrease (Brown et al., 2007). High concentrations of CO_2 can also modify the distribution of roots in the soil, stimulating the production of larger roots, highly branched at shallow depths, which may improve resource acquisition in annual herbaceous plants (Pritchard et al., 2006) or stimulating root proliferation into deeper soils in sweetgum (Norby et al., 2004) and loblolly pine (Pritchard et al., 2008). Although greater root volume is related to higher root hydraulic conductivity (Fernandez-García et al., 2002), other results have shown that an increase in the fine roots of a standing crop was not related to a higher water uptake (Matamala and Schlesinger, 2000). This was in agreement with the results obtained by Huxman et al. (1999), which showed that root hydraulic conductivity decreased under elevated CO_2. Therefore, the functionality of aquaporins is of extreme importance, since it is involved directly in the water conductivity of the root independent of root volume (Martínez-Ballesta et al., 2003a).

A way to relate more closely the size of the root system to the water balance is to consider the ratio of fine-root mass to leaf area (FR/LA), which corresponds to the balance between the supply organ and the demand organ. High concentrations of CO_2 have been shown to have contrasting effects on this ratio. In some experiments, this ratio increased (Norby et al., 1999), whereas in other reports no differences were observed (Centritto et al., 2002). Therefore, the assumption that an increase in root growth in a CO_2-enriched atmosphere may increase water uptake must be evaluated under the influences of other, interacting environmental effects (Wullschleger et al., 2002), and the role of aquaporins has to be taken into account.

2.2 Water-Use Efficiency at High CO_2 Concentration

Although an increase in instantaneous transpiration efficiency (ITE) for leaves at high concentrations of CO_2 has been observed as a result of reduced stomatal conductance and/or improved photosynthesis (Johnson et al., 2002), it is difficult to relate this parameter to water relations. It has been recognised that, in water-limiting environments, the primary productivity of crops may be estimated simply from the amount of water available for transpiration and the WUE for biomass production (Fischer and Turner, 1978). Thus, WUE conveys a more integrated measure of plant responses to high levels of CO_2. Several studies have suggested that whole-plant WUE increases significantly with elevated CO_2 (Centritto et al., 1999; Wullschleger and Norby, 2001). In general, this effect appears greater in plants and ecosystems under drought conditions (Centritto et al., 2002; Robredo et al., 2007). However, this increase in WUE may result from an increased biomass accumulation in plants grown in a CO_2-enriched atmosphere (Centritto et al., 1999). On the other hand, increased soil moisture under elevated CO_2 has been widely reported in grassland systems (Moore and Field, 2006), with similar results in soybean (Bernacchi et al., 2007). Nevertheless, an increase in WUE at elevated concentrations of CO_2 does not ensure that water uptake will decrease, to increase soil moisture (Moore and Field, 2006).

2.3 Reduction in Stomatal Conductance Under Elevated CO_2

The response of plants to elevated CO_2 is to reduce stomatal conductance (Medlyn et al., 2001; Ainsworth and Long, 2005). Stomatal responses to CO_2 involve direct responses, resulting in stomatal closure, and more indirect responses, via long-term changes in soil water availability. Stomatal closure is generally associated with reduced leaf-level transpiration. Bernacchi et al. (2007) showed that elevated CO_2 resulted in a highly significant decrease in ecosystem evapotranspiration (ET), closely correlated with a decrease in upper-canopy stomatal conductance.

In addition, Gedney et al. (2006) suggested that decreasing g_s, with rising CO_2, will lower water transfer to the atmosphere, so altering regional hydrology and climate. The reduced leaf conductance and transpiration occurring under elevated CO_2 often lead to more negative leaf or stem xylem water potentials (Morgan et al., 2004), but it is not clear if this impact on leaf water potentials is of sufficient magnitude for plant survival during periods of drought stress. The close relationship between stomatal conductance and soil water status (Tardieu and Davies, 1993) may explain the lack of a significant reduction in stomatal conductance in water-stressed seedlings of *Prunus persica* grown at elevated CO_2 concentrations (Centritto et al., 2002). Therefore, this is in agreement with the results obtained by Martínez-Ballesta et al. (2003b), where the control of water transport through the plant was suggested to be in the root, indicating their involvement in maintenance of plant homeostasis.

Aquaporins were characterised originally as being selective for H_2O, so facilitating water permeability through biological membranes (Kammerloher et al., 1994). However, the possible role of aquaporins in CO_2 diffusion in higher plants was first examined by Terashima and Ono (2002) and confirmed by Uehlein et al. (2003). These latter authors speculated that increased CO_2 permeability through aquaporins is related to the signals regulating stomatal guard cells, resulting in high stomatal conductance g_s in transgenic tobacco plants over-expressing NtAQP1 (Uehlein et al., 2003). However, Hanba et al. (2004) showed that the increase in g_s in transgenic rice leaves over-expressing the barley aquaporin HvPIP2;1 caused a loss of water from the leaves. Thus, intrinsic WUE was slightly decreased, indicating that the increase of the aquaporin HvPIP2;1 in the leaves enhanced water loss from the leaves to a greater extent than the CO_2 assimilation rate. Therefore, if the level of aquaporins exceeds a certain threshold, the plants might have higher growth rates, as a consequence of the increased CO_2 diffusion and photosynthesis, but they could be more susceptible to water stress. Recently, it has been reported that mesophyll conductance to CO_2 (g_m) changes in response to varying CO_2 even faster than g_s, suggesting also the possible role of aquaporins in mediating the CO_2 responsiveness of g_m (Flexas et al., 2006). Therefore, if they are expressed in stomatal guard cells, the influence on photosynthesis could be due to an effect on H_2O transport across the guard cell plasma membrane, resulting in a decreased or increased stomatal aperture. Moreover, when considering the different concentrations of H_2O and CO_2 in the water phase surrounding photosynthesising cells, as well as the differences in the concentration gradients of H_2O and CO_2 across the plasma membrane of these cells, the involvement of aquaporins is crucial for studying the response of plants to increased atmospheric CO_2.

3 Plant Water Relations and Increase in Temperature

The air and surface temperatures are two of the main factors affecting crop productivity. In the past century, the net global change in the mean temperature of the terrestrial surface was $0.6 \pm 0.2°C$ (Folland et al., 2001), and there is a high probability that both atmospheric CO_2 and global mean temperatures will continue increasing substantially during this century (Albritton et al., 2001). Although there are a lot of studies on the effects of the increased temperatures on plant water relations, it is still not known clearly whether the primary response is to avoid direct thermal damage or to minimise transpiration water loss. However, the clearest observation is that water conservation tends to have higher priority than minimising leaf temperature.

3.1 Leaf and Root Responses to Increasing Temperature

Temperature is one of the main factors influencing ET in plants. Thus, Goyal (2004) reported an increase of 14% in total ET demand with an increase in temperature by 20% (maximum 8°C) along 32 years (1971–2002) in a case study performed at an arid zone of Rajasthan (India). It is known that the driving force for transpiration is the water vapour pressure gradient between the air spaces within the leaf and the surrounding air ($\Delta\omega$). Because the amount of water needed to saturate a body of air rises exponentially with temperature, increased air temperature commonly results in an increase in the capacity of air to hold water vapour, increasing also the potential for transpiration, and decreasing WUE. The increasing rate of ET with temperature reaches a critical point, which varies, depending on the species, from 30°C to 40°C, at which stomata close abruptly in order to avoid water loss. This closure, although reducing transpiration water loss, disturbs gas exchange and photosynthesis (Jones, 1998) and affects negatively plant growth and production. However, when more environmental factors are taken into account, responses can be more complex. In this respect, Matzner and Comstock (2001) observed in *Phaseolus vulgaris* that when temperature was raised without raising absolute humidity there was stomatal closure, but if $\Delta\omega$ was kept constant and temperature was raised, there was an opening response.

Temperature-induced changes in stomatal conductance have been found to show a linear correlation with temperature-induced changes in plant hydraulic conductance, which could be, in part, explained by larger tracheid lumen diameters in the xylem at elevated temperatures (Maherali and DeLucia, 2000). Hence, a question to be answered is: do stomata respond primarily to changes in temperature, with their responses inducing changes in hydraulic conductance, or do changes in hydraulic conductance due to increases in temperature provoke stomatal responses? According to Whitehead (1998), rapid and reversible short-term changes in stomatal conductance following a perturbation of the water potential gradient in the flow pathway suggest that stomata respond directly to hydrostatic signals. Ripullone et al. (2007) manipulated plant hydraulic conductance in *Pinus pinaster* to study its effects on regulation of stomatal conductance, finding a strong and positive relationship between the two parameters. Their results were consistent with the hypothesis that two parallel modes of stomatal regulation explain the rapid responses of stomata to changes in hydraulic conductance: one involving leaf water status, and another involving ABA as a root-to-shoot signal.

The soil-root system can also be influenced by an increase in the environmental temperatures, affecting root temperatures and growth. Roots play critical roles in plant survival in environments with high soil temperatures, due, in part, to their lower optimum temperature range for growth and higher sensitivity to fluctuations of environmental conditions (Nielsen, 1974). Moreover, most of the functions of roots, like uptake of water and nutrients, are very sensitive to heat stress (Urrestarazu et al., 2008); normal root growth, root dry weight and root/shoot ratio are also affected, specially at early vegetative stages (Tahir et al., 2008). Root temperature is reported to affect both water and ion transport (BassiriRad et al., 1991) and either of these changes could affect shoot growth by limiting the supply of water and nutrients to the expanding tissue. Supra-optimal root zone temperatures can induce shoot water deficit in pepper, by altering the balance between water uptake by the root system and water loss from the shoots (Dodd et al., 2000). Cochard et al. (2000) observed that increased soil temperature induced a great increase in root and shoot hydraulic conductance for *Quercus robur*, and, as a consequence, plant transpiration responded linearly to these temperature-induced changes in whole-plant conductance. Cabañero et al. (2004) observed an increase in root hydraulic conductance dependent of aquaporins in response to a temperature increase. However, previous changes in root hydraulic conductance with temperature have been attributed to changes in membrane fluidity and permeability (Carvajal et al., 1996), changes in water viscosity (Hertel and Steudle, 1997) or a combination of both (Sellin and Kupper, 2007).

3.2 The Role of Aquaporins in Plant Water Status Under High Temperature

Changes and adaptations in root physiology can be developed, also, in order to avoid water deficit without suffering losses in productivity. An alternative mechanism of water-regime regulation is the modulation of the amount of water coming from the roots, due to a reduced resistance to water flow. Following the discovery of aquaporins as specialised water-channel proteins, it was elucidated that plants could increase rapidly water flux from the roots, in response to environmental changes, by changing aquaporin abundance and activity (Luu and Maurel, 2005). Aquaporin gene expression is activated during the daytime, accelerating water inflow as the transpiration rate increases (Henzler et al., 1999). According to aquaporin gene expression, root hydraulic conductance could be changed as well during the daytime (Henzler et al., 1999; Martínez-Ballesta et al., 2003a). In this case, an increase in the rate of transpiration with increasing air temperature is compensated by a reduction in the hydraulic resistance, mediated by the regulation of aquaporin expression or functionality, which provides for the maintenance of water content in the leaves without stomatal closure. Thus, an increase in root hydraulic conductance permits the stomata to remain open in spite of increased water deficit in the air (Farkhutdinov et al., 2003). In this study, when the air temperature was elevated by 3–4°C, transpiration was accelerated and remained high. However, it did not result in a decrease of water content in the shoot, due to an increased hydraulic conductance. It has been postulated that stomata could respond directly to changes in root hydraulic conductance, via an ABA-mediated mechanism (Steudle, 2000). Kamaluddin and Zwiazek (2004) also proposed that ABA could have a direct effect on root water channels. The effect of ABA in aquaporins has been widely studied by several authors, and a clear conclusion could not still be established. Beaudette et al. (2007), for example, using ABA in *Pisum sativum* roots in order to decrease hydraulic conductivity, observed variable changes in PsPIP2-1 gene expression, and that the direction of the response was strongly dependent on the dose of ABA that was applied. Also, the interesting analysis of the effect of ABA on the expression of the 13 PIP genes in *Arabidopsis*, performed by Jang et al. (2004), revealed that the responsiveness of each aquaporin to ABA was different, implying that the regulation of aquaporin expression involves both ABA-dependent and ABA-independent signalling pathways. When plants experience any biotic or abiotic stress, including heat stress, ABA accumulates rapidly, leading to active plant response to these stresses (Xiong et al., 2002). Heat treatments (38ºC) performed by Wang et al. in 2005 in *Vitis vinifera* plants observed a rapid increase in ABA contents as a response to heating, and an improved thermotolerance in treated plants, indicating that both plant regulators could be involved in thermotolerance regulation. At a molecular level, Zhang et al. (2008) analysed heat shock and ABA content in ABP9 transgenic *Arabidopsis*(constitutively expressing a transcription factor for the ABA response element), concluding that expression of ABP9 might be enhanced by abiotic stresses like heat, and it has an important role in the increase of ABA content and expression of other stress-defensive genes.

Recently, some authors have highlighted the importance of aquaporins, not only in water transport in roots, but also in leaf hydraulic conductance. Cochard et al. (2007) found in *Juglans regia* a positive relationship between leaf conductance and the abundance of two PIP2 aquaporin isoforms, in response to light and temperature increases. Some earlier physiological studies seem to support this idea; for instance, in 2005, Nardini et al., using $HgCl_2$ as an aquaporin blocker, showed a reduction in leaf hydraulic conductance in sunflower (*Helianthus annuus*).

4 Plant Water Relations and Solar Radiation

In recent decades, the natural UV-B distribution has been affected significantly by anthropogenic activities, primarily through the release of man-made chlorine and bromine compounds, which result in stratospheric O_3 depletion (Giorgi et al., 2003; Forsher and Joshi, 2005). A small decrease in ozone levels may cause a large relative increase in biologically effective UV radiation, with significant consequences for plant biochemistry, physiology and productivity (Searles et al., 2001; Robson et al., 2003; Glenn et al., 2008; Kristian et al., 2008). The use of aerosols also alters, indirectly, the amount of solar irradiance incident on the earth's surface, by modifying the frequency and lifetime of clouds (Hobbs, 1993).

4.1 Light Intensity and UV Radiation Effects on Plant Water Status

One of the most important aspects of global change is that of stratospheric ozone depletion resulting from air pollution, and the resulting increase in UV radiation reaching the surface of the Earth (Blumthaler and Ambach, 1990; Gleason et al., 1993). Adaxial stomatal density has been found to be depressed by the direct action of UV upon the developing epidermal structures (Gitz et al., 2005); stomatal conductance generally exhibited a response pattern similar to that of the reductions in stomatal density. However, Nogués et al. (1999) found that reductions of the conductance in pea (*Pisum sativum*) plants exposed to high levels of UV-B were largely the result of altered stomatal (guard cell) functioning rather than of stomatal density. Water uptake and transport in the plant can be affected by changes in the stomatal conductance and density. Thus, alteration of stomatal conductance in the absence of reduced CO_2 assimilation could enhance instantaneous WUE or increase the stomatal limitation to photosynthesis. In addition, it has been hypothesised that UV signal perception can lead to photomorphogenic responses in plants that may confer adaptive advantages under conditions associated with high-light environments, such as water stress (Gitz and Liu-Gitz, 2003). Also, other researchers have reported anatomical changes that could lead to improved water relations upon exposure to UV-B radiation under drought conditions (Manetas et al., 1997; Laakso et al., 2000). However, Nogués and Baker (2000) found no effect of UV-B on the drought response in three Mediterranean species grown under enhanced UV-B radiation before and during the imposition of drought treatments. Also, reductions in transpiration and conductance with UV-B have been reported in soybean, but altered carbon isotope ratios were attributed to photosynthetic disruption (Feng et al., 2003).

High light intensity may tend to intensify the water flow across living tissue (Sack et al., 2003; Lo Gullo et al., 2004), but as soon as the water status (turgor/water potential) declines, cell hydraulic conductivity (Lp) is reduced, most likely via a closure of aquaporins (Kim and Steudle, 2007). This seems to be a reasonable response to minimise water loss and to keep cell turgidity, but there may be other variables in the system. Thus, changes in symplastic or apoplastic pH or pCa, which are known to affect aquaporin activity (Johansson et al., 1996, 1998; Tournaire-Roux et al., 2003; Alleva et al., 2006), must be considered, since light can change the pumping of protons and Ca^{2+} (Shabala and Newman, 1999). In addition, light irradiation has been shown to cause a significant influx of ions such as K^+, Cl^-, Ca^{2+} and NO_3^-, and this could originate changes in the plasma membrane polarisation (Weisenseel and Ruppert, 1977; Takagi and Nagai, 1988; Stoelzle et al., 2003; Stiles and Van Volkenburgh, 2004; Zivanovic et al., 2005 and 2007) that may affect the functionality of plasma membrane proteins.

The attenuation of solar radiation by atmospheric aerosols simultaneously decreases the amount of radiation reaching the surface and increases the fraction of radiation which is diffuse. Decreasing the total amount of photosynthetically active radiation (PAR, 400–700 nm) tends to decrease the amount of photosynthesis occurring in plant leaves. In addition, the variations of hydraulic conductance in the whole plant with the irradiance may depend greatly on the amount of the solar radiation.

4.2 Leaf and Root Responses to Light Availability: Involvement of Aquaporins

It has been proposed that leaf hydraulic conductance (K_{leaf}) increases during the day in response to the increased water demand, due to stomatal opening (Tsuda and Tyree, 2000). The higher K_{leaf} in the light period might be due to light-dependent, new expression/activation of aquaporins (Nardini et al., 2005). Recently, Cochard et al. (2007) showed that the K_{leaf} of walnut leaves (*Juglans regia*) was low under dark conditions, increasing by 400% upon exposure to light. The authors associated this low K_{leaf} in the dark with down-regulation of *Jr*PIP2 aquaporin, whereas high K_{leaf} in the light was associated with up-regulation of*Jr*PIP2. Thus, these walnut leaves could rapidly change their hydraulic conductance during the dark-light cycle by regulation of the expression of plasma membrane aquaporins. Similar results were observed by Tyree et al. (2005). These authors showed that the increase in K_{leaf} mediated by irradiance could be due to the de novo expression of aquaporins in addition to the activation of pre-existing aquaporins and the consequent enhancement of water transport.

In addition, the responses of leaf conductance to irradiance may vary depending on the leaf position. The water flow to the shade foliage has to overcome a greater resistance than that to the sun foliage. Thus, Sellin and Kupper (2005) found that silver birch trees adjust their water relations to the prevailing environment by coordinating hydraulic capacity with changes in stomatal conductance, to prevent leaf water potential from reaching critical values. These results support the idea that stomatal conductance at the base of the live canopy is limited not only by low light availability but also by the liquid-phase transport capacity.

Other studies have shown a correlation between diurnal variations in root hydraulic conductivity and the expression of aquaporins. Thus, it has been reported that water uptake and aquaporin transcript levels increased during the light cycle in *Lotus japonicus* (Henzler et al., 1999) and *Arabidopsis* (Martínez-Ballesta et al., 2003a), in the dark in barley (Katsuhara et al., 2003) and in both light and dark in maize (Lopez et al., 2003). These data indicated that roots can alter their water permeability within a few hours depending on light, and the rapid changes in water permeability are most likely mediated by aquaporins.

5 Conclusion

The components of global change reviewed here, elevated CO_2, high temperature and low radiation, will affect plant water uptake and transport involving aquaporins. However, if the effect of each component is difficult to quantify and interpret, the global effect is really unpredictable. Therefore, a better knowledge of the mechanisms implicated in these processes, including the role of aquaporins in CO_2 and water diffusion, is required to predict the responses of terrestrial plants. Moreover, the degree to which changing environmental conditions produce direct or indirect responses and the relationships between variables need to be assessed. Furthermore, the climate change is expected to cause a net increase in the proportion of land classed as semi-arid, which will affect plant production as well as obviously plant water relations due to frequency of drought conditions. This outlook complicates even more the whole picture.

Temperature affects a great number of physiological and metabolic processes in plants, and therefore it is difficult to establish whether future increases of temperature will affect whole-plant physiology negatively or positively. Raised temperatures may benefit some crops but disadvantage others through increased ET and thermal damage. The way in which recent increases in temperature due to global change could affect plant water relations is still not clear. Many studies with different plant types, including trees, grasslands, crops and ornamental plants, show that they are developing different adaptive mechanisms in order to avoid water-deficit stress and excess transpiration, like increases in WUE and hydraulic conductance – involving the expression and activity of aquaporins.

The use of halocarbons causes other components of global change, such as the stratospheric ozone depletion that makes plants vulnerable to the increased UV-B radiation. However, the response of plants to UV-B radiation can be altered by concurrent changes in other micro-climatic factors such as PAR (Mirecki and Teramura, 1984; Cen and Bornman, 1990), nutrient status (Murali and Teramura, 1985) and water stress (Sullivan and Teramura, 1990; Nogués et al., 1998). The response of plants to the amount of perceived radiation has been studied extensively and there are numerous reports about the effect of light and shade on water relations. However, the combined effect of different environmental factors must be considered in order to elucidate the effect on plant water balance (Sellin and Kupper, 2007, Cochard et al., 2007; Maherali and DeLucia, 2000). In addition, it is now established that aquaporins are expressed widely, in all organs of the plant, allowing fine physiological regulation of water transport. Also, their involvement in CO_2 conductance has been recently identified, which emphasises the central role of aquaporins in plants – for modulation of water and CO_2 uptake by cells.

All the results summarised in this chapter reinforce the importance of aquaporin regulation under environmental changes, such as those that will produce climate change. However, the large number of aquaporins present in each species and the different types of protein regulation make a complicated picture. Therefore, a complete knowledge of aquaporin function in plants is necessary before interpretation of their key role in the response to environmental changes.

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Responses of Cereal Plants to Environmental and Climate Changes – A Review

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Abstract The projections of the global climate changes on the Earth expect a rise in the concentration of greenhouse gases, increase in temperature and aridisation of the environment. By the middle of the twenty-first century, the concentration of CO_2 will probably rise up to 500 $\mu l\ l^{-1}$ of air. Already now, 61% of the area of land on the Earth has precipitation lower than 500 mm. One-sixth of the world's population can be affected by an acute shortage of water. A total of 35–50% of inhabitants of the Earth are struggling with salinity of soil. All this currently has and will have consequences for the agricultural production. The areas between 15° and 30° of the north and south longitude and the deep inland areas are endangered the most. Cereals are major crops with respect to human nutrition. In order to ensure permanently sustainable production of cereals, it is important to study the diversity of their production under the influence of natural and climate changes. Based on this analysis, it is necessary to design measures to stabilise yields. This is the purpose of this chapter. Based on the study of a number of literature sources, we presume that the increased concentration of CO_2 will only partly compensate for the losses of the yields of cereals resulting from the increase in temperature and aridisation of the environment taking place on a global scale. However, cereals have a number of adaptation mechanisms to maintain turgor and to improve water management in dry and salinised habitats. With a view of ensuring permanent sustainability of agricultural production under the changing natural and climatic conditions, we present two options of using the diversity of adaptation mechanisms: (1) to adapt the composition of the cereals grown to changing conditions; (2) to breed varieties more resistant to changing conditions. Breeding resistant genotypes is the least costly solution to ensure sustainable development of agriculture in arid areas. We believe that the choice of suitable selection criteria is most important. For the screening of genotypes resistant to drought and salinisation, it is important to use more parameters: physiological and biochemical indicators at the cellular level and the genes of resistance

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to drought and salinisation. The suitable selection criteria and the important features of drought and salinisation resistance are a high level of osmotic adjustment, low stomatal conductivity and good growth of roots. Breeding aimed at achieving a higher degree of drought resistance should be focused on (1) improvement of the availability of water through the root system; (2) the limitation of water loss through transpiration and higher water use efficiency for production of biomass; (3) prolongation of the activity and increase of the power of the sink. In the selection of the genetic sources of resistance to abiotic stresses, we recommend paying an even greater attention to wild species and region-specific and primitive varieties of cereals, originating from worse natural and climatic areas. Another promising path is to use gene transfers to improve the photosynthetic and growth capacity of cereals during the presence of stress factors. In order to improve the water management in cereals under dry conditions, we also suggest using growth regulators to a greater extent. Abscisic acid applied on plants can serve as an antitranspirant. It can increase water use efficiency.

Keywords Cereals · Climate changes · Environmental stress factors · Elevated CO_2 concentration · Drought · Salinisation

1 Introduction

The growing number of the Earth's inhabitants places constantly increasing demands on the environment. One of the risks, which could substantially influence human society's life with its long-term character, is the climate change. Prognoses of global climate changes on the Earth expect the increase of greenhouse gases (CO_2, CH_4 and CF_X, etc.) concentration in the air, natural global warming and the aridisation of plant environment with consequences for the agricultural production. From the middle of the nineteenth century until now, the concentration of CO_2 in the air increased from 270 μl CO_2 l^{-1} nearly up to the present 400 μl $CO_2 l^{-1}$. It is expected, that it will rise up to 500 μl $CO_2 l^{-1}$in the following decades (Natr, 1998; Kimball et al., 2002; Rizza et al., 2004; Ainsworth and Long, 2005; Long et al., 2006). On a worldwide basis, drought is the most serious factor limiting yields of the main crops. It influences the natural ecological systems as well as the production of agricultural systems (Solomon and Labuschagne, 2003). Sixty-one percent of the areas on the Earth have precipitation lower than 500 mm (Deng et al., 2005). The most endangered parts of the planet are the regions between 15° and 30° of the north latitude and south latitude, the localities on the lee side of mountains and areas situated in the deep inland. Up to one-sixth of the world's population can be affected by an acute shortage of water (Larcher, 2003). The annual average temperatures are showing an upward trend and evapotranspiration requirements for the water are growing. Periods without water are becoming longer; thus, plants often suffer from water shortage (Rizza et al., 2004). The result is instability of the quantity and quality of production. The path leading to better stability leads through limitation of the effect of the risk, stress and other reduction factors. This is important for further development of not only intensive systems but also the environment-friendly land management systems based on the concept of permanently sustainable development of plant production (Marcelis, 1996).

Cereals are probably the oldest and most important crops with respect to human nutrition. They are grown in both warm and cold areas, dry and humid areas, and lowlands and higher mountainous areas. The total area of agricultural land on the Earth amounts to approximately 19,824,000 km^2, of which cereals occupy around 35%. The expected growth of the total population on the Earth, the increasing requirements for the quality of life as well as the requirement for production of energy biomass will bring about increased pressure on the global plant production, particularly on the production of cereals. In addition to that, this desirable increase of production must be accompanied by a high degree of sustainability (Spiertz et al., 2007). This is why the purpose of this study is to analyse the diversity of the physiological reactions and the production processes of cereals in the changing conditions of the environment. The output should consist of proposals for limiting the effect of stress factors of the environment and for ensuring steady sustainable production of cereals even under the changing natural and climatic conditions.

2 Responses of Cereals to Elevated CO_2 Concentration in Air

Professor Natr (1998) examined the influence of the changes in the concentration of CO_2 in the atmosphere on the dissemination of plant species in the publication titled "Plants, People and Sustainable Life of Man on the Earth". Significant dissemination of C_4 plant species occurred at the end of the Miocene Period. This was related to the decrease of the concentration of CO_2 in the atmosphere down to approximately 180 $\mu l\ CO_2 l^{-1}$ of air. This favoured the C_4 plants where the enzyme PEP-carboxylase has a higher affinity to CO_2 than the RUBISCO enzyme in the C_3 species. During the last 160,000 years the CO_2 concentration in our atmosphere varied from 0.020% to 0.030% (200 and 300 $\mu l\ CO_2 l^{-1}$ of the air). In the last 2,000 years until the middle of the nineteenth century the CO_2 concentration was generally constant at the level of 270 $\mu l\ CO_2 l^{-1}$. Since that time, that is, from the beginning of what is referred to as the Industrial Revolution, it has been continuously increasing. The average CO_2 content in the air currently hovers around 0.038% and is constantly increasing. This amount varies moderately in the course of the year. On the land hemisphere, the highest CO_2 concentration is present towards the end of the winter period and the lowest concentration is present at the end of the summer, that is, in the period of maximum photosynthetic CO_2 fixation by the plant communities (Natr, 1998).

Photosynthesis starts approximately at the level of 0.01% of CO_2 in the air, but even at the concentration level of 0.38% the photosynthesis of C_3 plants is not fully saturated. It is expected that the CO_2 content in the atmosphere will be increasing gradually up to 500 $\mu l\ CO_2 l^{-1}$ of air, possibly even higher, in the following decades and centuries. In combination with a higher temperature, this can significantly influence the water regimen of the plants. This currently has and probably will continue to have a great effect on plants: on the production of biomass and the dissemination of the C_3 and C_4 species (Natr, 1998; Kimball et al., 2002; Rizza et al., 2004; Ainsworth and Long, 2005; Long et al., 2006).

In recent years, there has been an increase in the number of experimental works which bring information into this area – on the supposed influence of the global climate changes on the Earth, on the phylogenesis and the dissemination of various plant species. While increasing temperatures and altered soil moisture arising from climate change in the next 50 years are presumed to decrease yield of food crops, elevated CO_2 concentration is predicted to enhance yield and offset these detrimental factors. However, C_4 photosynthesis is usually saturated at current CO_2 and theoretically should not be stimulated under elevated concentration of CO_2 (Leakey et al., 2006). However, the highest quantity of results about reactions of plants to the increased CO_2 concentration was achieved on young individuals during the short-term application of an increased CO_2 in an assimilation chamber, particularly with herbs or agricultural crops. The overwhelming majority of these trials were carried out on plants not adapted to a higher CO_2 concentration. The informative value of such experiments can be disputable on the global environmental scale. In general, there is very little data on the influence of the long-term effects of the increased CO_2 concentration on the photosynthesis, growth and other physiological characteristics of, for example, cereals.

It is presented in the literature that an increase of the CO_2 concentration above its common level in the atmosphere (approximately 400 μmol mol^{-1}) or on the contrary its decrease directly influences, for example, the stomata density per leaf or the water use efficiency (WUE) levels. In the experiments of Ulman et al. (2000) with wheat plants the decreased CO_2 concentration (150 μmol mol^{-1}) manifested itself on the leaves of cultivated plants by a higher stomata density and a higher stomatal conductivity. Conversely, an increased CO_2 concentration (800 μmol mol^{-1}) caused reduction of these parameters. The photosynthesis rate of the trial plants was inversely proportional and on the contrary their leaf area was directly proportional to the CO_2 concentration under which they were cultivated. The changes in the physiological traits induced at a very early ontogenetic phase persist also after the termination of the effect of the increased or decreased CO_2 concentration. It is interesting that certain effects manifested themselves not only on the directly affected leaves but also on the leaves that emerged from the plant after the termination of the effect of the increased or decreased CO_2 concentration. In the experiments of Mitchell et al. (1999) with spring wheat plants, the increased CO_2 concentration at the level of 650 mmol mol^{-1} CO_2 in comparison with 350 mmol mol^{-1} CO_2 was manifested on flag leaves by an average decrease of the stomatal conductivity by 10%. Del Pozo et al. (2007) identified a significantly lower chlorophyll and RUBISCO enzyme content in wheat under an increased CO_2 concentration for the upper sun-exposed leaves as well as the lower shaded leaves in comparison with plants which grew under the standard CO_2 concentration. Kurasova et al. (2003) studied the response of spring barley to elevated CO_2 (700 mmol mol^{-1}). In comparison with the barley grown under ambient CO_2 (350 mmol mol^{-1}) the elevated CO_2 acclimation resulted in a decrease in photosynthetic capacity, reduced stomatal conductance and decreased total chlorophyll content.

The growth "sink" also has an important effect on the photosynthetic efficiency of the plants under an increased CO_2 concentration. The beginning of the vegetation period is generally associated with the positive effect of the increased CO_2 concentration on the photosynthetic assimilation. In the leaves of plants growing under the effect of an increased CO_2 concentration, it was found out that there was a significantly higher starch accumulation (De Lucia et al., 1985; Wong, 1990; Wullschleger et al., 1992). The relation between the accumulation of the reserve form of the assimilates (starch) and the photosynthesis rate (P_N) is inverse; that is, the accumulation of starch is connected with a decline in the photosynthesis rate (Johnsen, 1993). The starch accumulation thus indicates a situation in which the photosynthetic production of the assimilates in chloroplasts exceeds their transport from chloroplasts and their subsequent consumption. The transport of assimilates from chloroplasts then becomes the limiting factor. This means that it is only possible to maintain a high photosynthesis rate under the effect of an increased CO_2 concentration if there is a permanently active and strong "sink" (Stitt and Quick, 1989; Stitt, 1991). If such "sink" is not present, then a feedback limitation of the photosynthetic activity occurs and therefore a decline in the photosynthesis rate (Slafer and Savin, 1994; Slafer et al., 1999).

Brestic and Olsovska (2001) state that the key issue of the plant resistance to the water stress is the solution of the conflict between biomass production on one hand and water loss prevention on the other. The plant "strives" for an ideal compromise between a degree of the stomatal openness when a high water loss through the transpiration does not occur and when, at the same time, there is a sufficient CO_2 diffusion through the stomata into mesophyll for the photosynthesis requirements. Marek (1998) shows that the general response of the guard cells of the stoma to an increase of CO_2 concentration in the ambient air around the leaf is a partial closing of the stomatal aperture and a perceptible decrease of the stomatal conductivity (g_s). In this connection, hypotheses have been raised that the increasing CO_2 concentration on the global scale enables the same photosynthesis rate with less open stomata and therefore with a lower transpiration loss. It would mean a better plant potential for the growth in drier environment than up until now in connection with the growth of the WUE level (Brestic and Olsovska, 2001). The relation between the WUE level and CO_2 concentration in the atmosphere is linear (Tolley and Strain, 1984). But Natr (1998) presents that a higher dry matter production under the higher CO_2 concentration will lead, in spite of the more favourable WUE, to a total higher water consumption for the biomass production. This would mean a faster depletion of the water reserves and the aridisation of many habitats still before the end of the vegetation period. In addition to that, a number of studies from the recent years show that the yield of field crops, and consequently the yield of cereals, too, is favourable influenced by an increased CO_2 concentration far less than it had been presumed (for example, Ainsworth and Long, 2005; Leakey et al., 2006; Long et al., 2006; Ainsworth, 2008). This provokes doubts, not only on our part, about the capability of an increased CO_2 concentration to compensate, in the first half of the 21st century, for the losses resulting from the continuous temperature increase and aridisation of the environment taking place on a global scale.

3 Responses of Cereals to Drought and Salinisation

3.1 Limitation by Water Supply

Drought globally ranks among the most serious problems, which stems from the fact that 61% of the areas on the Earth have precipitation lower than 500 mm (Deng et al., 2005). Drought influences natural ecosystems as well as cultural ecosystems' production (agri-environmental systems). The term "drought" includes also irregularities in the distribution of natural precipitation over time during the main vegetation period of plants. Maracchi et al. (2005) state that the agriculture in the northern areas of Europe may be positively influenced by the climate changes by introducing new crops and varieties, by increased crop production and enlargement of the areas suitable for cultivation. In the southern areas where there already is the typical temporary soil water deficit, the negative phenomena, such as extreme weather fluctuations, increase of water deficit and high fluctuations in the yield of agricultural crops, including cereals, will be predominant.

For plants in the moderate zone it is decisive whether drought occurred during the vegetation period or whether the plant grows in a relative drought from the very beginning. As Lewitt (1980) explains, if drought had occurred not until in the course of the vegetation period, the effect of water stress on metabolism is stronger; whereas during the growth in a relative drought from the beginning of the vegetation period, the plant manages to adapt to these conditions better – it has a root system reaching to a greater depth, thicker cuticle, lower number of stomata as well as a relatively smaller leaf area.

If there is not enough precipitation in the humid areas, this disrupts the water balance and the result is a water deficit. The cereal plants limit the opening of stomata and also shorten the period for which they are open. At the beginning of the stress the transpiration decreases during noon hours, latter on it again restores. When a water shortage continues, a subsequent afternoon increase of the transpiration will cease to occur and still later stomata are opening only in the morning when there is a relative drop of the temperature. In the end, but when the plants still have sufficient water content, the stomatal transpiration completely stops and the plants transpire only through cuticle. Under extreme conditions, if the plant is unable to adapt, irreversible wilting and dying of plants takes place (Jones, 1998; Orcutt and Nielsen, 2000; Larcher, 2003).

The significant role of water is to maintain turgor. This is important for the growth of the plants, that is, speaking precisely, during the elongation growth of the cells and during the opening of the stomata. This is why the elongation growth of cells is the process that is most sensitive to a water shortage from all physiological processes (Hsiao, 1973). From a certain threshold level, the growth rate is dependent linearly on the turgor pressure. A measurable decrease in the growth occurs even when there is a very small water loss when turgor decreases only by 0.1 MPa through 0.2 MPa. A decrease of the water potential down to approximately −0.1 MPa through −0.2 MPa corresponds to this. The growth comes to a complete halt when turgor decreases to the threshold level, which is usually between 0.3 MPa and 0.4 MPa. A halt in the growth occurs before the leaf wilting is evident or before the main metabolic processes, including photosynthesis, are affected evidently. If there is further decrease of the water potential of cells, approximately to the levels between −0.4 MPa and −1.2 MPa, a very substantial (up to a 40-fold) increase of concentration of the abscisic acid (ABA) occurs in the cells. Its content is increased through synthesis or through release of the reserves in chloroplasts. The consequence of this is particularly the closing of stomata of the leaves. The change in the opening of the stomata leads to decreasing the photosynthesis and transpiration rate. Within this range of the levels of water potential fast changes in enzymatic activity occur as well: enzymatic activity of nitrate reductase decreases, on the contrary alpha-amylase, ribonuclease and hydrolase activities increase. The synthesis of proteins and cytokinins decreases and the cell division slows down (Hsiao, 1973; Orcutt and Nielsen, 2000; Larcher, 2003; Hejnak et al., 2004; Taiz and Zeiger, 2006). Dehydrins are a special group of approximately 20 stress proteins, which are newly generated during shortage of water. The generation of dehydrins depends on the increased concentration of ABA. For survival of a high dehydration, they are absolutely necessary (Close, 1996). Dehydrins are exclusively intracellular proteins. They are hydrophilic and they bind a considerable quantity of water in their molecules. They were identified in virtually all intracellular organelles in all organisms using photosynthesis (Saavedra et al., 2006). The decrease of a water potential down to the levels around −1.0 MPa and lower is linked to the creation of amino acid proline, carbohydrates, alcohols and other compounds. During a longer water deficit other metabolic changes begin to manifest, particularly in photosynthesis and transport processes in a cell (Cornic, 2000; Griffiths and Parry, 2002).

The majority of crop plants are classified as mesophytes, meaning that they are not specially adapted to survival in dry conditions. In contrast to xerophytes that can survive leaf water potentials (ψ)as low as −10 MPa, most crop plants start to exhibit signs of physiological damage and leafwilting at about −1.5 MPa (Hsiao et al., 1976; Fitter and Hay, 2002; Hay and Porter, 2006). Leaf and cell water potential are used as indices of drought or water stress in plant physiology, with the convention that ψ is said to drop and drought increase as water potential becomes more negative. Water flows from regions of higher to lower water potential (less to more negative); thus water flows from a soil at field capacity where ψ_{soil} is zero to the leaves where ψ_{leaf} can be −0.5 MPa to −1.0 MPa on a day when leaves are freely transpiring. Water stress for a typical mesophyte has been characterised into three classes (Hsiao, 1973): mild stress, ψ between 0 and –0.5 MPa; moderate stress, ψ between −0.5 and −1.2 to 1.5 MPa; severe stress, ψ below −1.5 MPa.

An alternative method for expressing the water status of the plants is using the relative water content (RWC). This expresses the ratio between the total water content and the water content in full turgescence. There is a mild water stress if RWC around 90% and lower results in a decreased intensity of cell elongation and organ growth, increasing ABA accumulation in the leaves and the beginning of the stomata closing. The level of 80% and lower represents a strong water stress. This level is accompanied by changes in tissue structure, metabolic changes, proline accumulation, possibly changes of other osmotically active substances. The level below 70% represents an intervention into the mechanism of the basic physiological processes, irreversible metabolic disorders occur and leaf apparatus damage occurs, etc. (for example, Rulcova and Pospisilova, 2001; Brestic and Olsovska, 2001; Pospisilova et al., 2005).

The majority effect of a mild water stress in leaves consists primarily in the closing of stomata. Disorders of the photosynthetic apparatus usually do not occur. The result of the stomata closing during a drought is a reduction of CO_2 internal pressure and subsequently reduction of photosynthetic assimilation. In the relation between O_2 and CO_2, priority is given to photorespiration (Sharkey and Seemann, 1989; Ghashghaie and Cornic, 1994). Decreased CO_2 diffusion through cell walls, membranes, cytoplasm and chloroplast coats of mesophyll cells leads to the decrease of CO_2 concentration in chloroplasts and to photosynthesis rate reduction (Flexas et al., 2002, 2004a,b, 2006). The initial effect related to the stomata closing and the depletion of CO_2in intracellular spaces is referred to as stomatal inhibition of photosynthesis (Cornic et al., 1992). However, this mechanism does not explain all the effects of drought. Under a strong water stress, it is presumed that there is also non-stomatal inhibition of photosynthesis. A strong water stress results in a decrease of carboxylation and the activity of the electron transport chain. It also induces ultra-structural changes in chloroplasts, which concern the oxidative damage to lipids, proteins and pigments (Tezara et al., 1999; Foyer and Noctor, 2000; Maroco et al., 2002; Johnson et al., 2003; Flexas et al., 2004a,b; Selote and Khanna-Chopra, 2004; Hura et al., 2006).

The ratio of net photosynthetic rate (P_N) and transpiration rate (E) labelled as water use efficiency (WUE) indicates whether photosynthesis is limited only with respect to stomata or whether it is also limited in ways other than by stomata. A notable decrease of this indicator during the course of water stress shows that the photosynthesis is limited also non-stomatally. Under a mild water stress the stomatal limitation of the photosynthesis markedly predominates over non-stomatal limitation (for example, Escalona et al., 1999; Rulcova and Pospisilova, 2001; Maroco et al., 2002).

The atmospheric drought affects the leaves and the stomatal apparatus directly. Soil drought develops gradually. When a plant is exposed to a gradual dehydration of the soil substrate, it maintains the relatively high water content for a longer time. This leads to hyperproduction of ABA, formation of proteins, dehydrins and osmotins, etc. A plant can gradually adapt osmotically and redistribute water in organs, if the water is not too strong and if the integrity of the physiological systems of the plant is not affected (Hay and Porter, 2006).

It can be summarised that agricultural crops have a number of physiological adaptation mechanisms to maintain turgor and to improve water management in dry conditions. The time factor and the intensity of the water stress are important. Most cereals are able to adapt if the water potential of the soil solution does not drop below −1.5 MPa for a longer time (Larcher, 2003; Hay and Porter, 2006).

3.2 Limitation by Salinisation

Soil salinity is an important worldwide problem mainly in arid and semiarid regions. El-Hendawy et al. (2007) show that the issue of soil salinity is addressed by 35–50% of the world's population from approximately 80 countries.

A high Na^+ and Cl^- ion concentration in the soil solution affects the balance in the nutrient uptake and reduces the soil's osmotic potential. The plants respond to the reduced water availability by the stomata closing and restricting the water release. As a result of salinity restricting the water uptake into the plant, it also adversely influences the plant elongation growth (Mc Kersie and Leshem, 1994; Fricke and Peters, 2002; Munns, 2002; Koyro, 2006). The elimination of CO_2 availability for photosynthesis occurs simultaneously with the partial closing of stomata, which is one of the causes of the productivity reduction under the stress conditions (Hasegawa et al., 2000; Flexas et al., 2004b; Abdelkader et al., 2007; Balogh et al., 2007).

The capability of plants to resist salinisation is determined by a number of biochemical and physiological changes. These facilitate retention or reception of water by the plant, regulate the concentration of ions in the protoplast and protect the cellular organelles, particularly chloroplasts, from damage. The synthesis of osmotically active substances, specific proteins, the activation of enzymes regulating the reception and output of ions of salts and the activation of the enzymatic antioxidants for detoxication of the active forms of oxygen are most important (Parvaiz and Satyawati, 2008).

It was found out that the genotypes tolerant to salination have a capability to eliminate the undesirable ions. Garthwaite et al. (2005) identified this ability in wild barleys that have, in comparison with the cultivated species, significantly higher tolerance to salination. A similar potential for ion elimination was established as well for rice. Jiang et al. (2006) state that barley is the agricultural crop that has the highest tolerance to salination. While wheat is able to grow in soils containing up to 5% of salt, barley tolerates even a double of that amount of salt in soil (Santrucek, 2003; Colmer et al., 2005, 2006; Munns et al., 2006).

High nutrient and salt concentrations in the soil solution generally affect adversely the plant mineral nutrition. At the same time they adversely influence the plant water regime, because they reduce the osmotic potential of the soil solution. Thus a high salination is often a source of water stress. Piterkova et al. (2005) state that it is possible to reduce the effect of salinity on the productivity of plants by adding Ca^{2+} ions into the irrigation water.

We can say that the stress resulting from salinisation is causing great damage to agricultural production all over the world. Therefore, fast implementation of the results of biological research in practice is necessary. The main intention of the eco-physiologists, production physiologists, biochemists and geneticists is to choose the suitable selection criteria. For the screening of the genotypes resistant to drought and salinisation, it is important to use more parameters: physiological and biochemical indicators at the cellular level and the genes of resistance to drought and salinisation. For the sake of permanently sustainable agriculture, breeding the resistant genotypes is the least costly solution.

3.3 Osmotic Adjustment

Osmotic adjustment of the tissues to a water deficit maintains the gradients of the water flow and water intake from soil during the period of low water potential levels. It also makes stomatal adjustment and continuation of transpiration and photosynthesis possible (Subbarao et al., 1995; Gonzalez et al., 2008). Osmotic adjustment takes place very slowly, sometimes even several days. In the majority of cases, the concentrations of substances such as carbohydrates (sucrose), organic acids (malic and citric acids), amino acids and inorganic substances (K^+) increase during osmotic adjustment (Moinuddin et al., 2005). During the stress, the concentration of highly soluble (osmotically active) substances as well as the metabolically still unused low-molecular organic substances further increases. The said substances include for example the proline amino acid (Sanchez-Urdaneta et al., 2005). Its accumulation is almost a general response of plants to a water stress, while the capability to accumulate other groups of components is more or less species specific (Meloni et al., 2004). To carry out the osmotic adjustment, the plant needs to expend extra energy, which negatively influences its further development. Therefore the breeders' work is aimed at breeding cereal varieties with a higher osmotic adjustment because suitable genotypes are the least expensive solution to the entire problem (Muramoto et al., 1999). In this respect Liao et al. (2005) point out that in their experiments with six wheat cultivars, the cultivars with a medium drought resistance featured a significantly higher P_N and grain yield than the cultivars with a high or, conversely, decreased resistance to drought. Breeding wheat only to achieve increased drought resistance could thus result in a yield depression, unless a strong water stress occurs. In trials with spring barleys, Arnau et al. (1997) found out that the variety with the highest potential for osmotic adjustment can also maintain the highest level of RWC. Such a variety also shows the highest photosynthetic activity under stress conditions. As Morgan (2000) presents, wheat plants with a higher osmotic adjustment provide a yield higher by up to 40% when in dry conditions. Also, according to Gonzalez et al. (1999), the resistance to drought is characterised by a high osmotic adjustment, low stomatal conductivity and good growth of the roots. Thus, osmotic adjustment is a suitable characteristic for screening genotypes for a higher drought resistance. A higher capability of osmotic adjustment to water stress was found in the roots rather than in the shoots. The moderate osmotic stress leads to a fast growth inhibition of the leaves and stems, while the roots can usually continue their growth (Spollen et al., 1993, 2008). As a result of the halt of the plant's growth, carbohydrates are preserved in the cells for the basic metabolism of the plants and for fast establishment of balance in the cells when the effect of the stressor disappears. The growth inhibition of the shoots during the influence of water stress contributes to the accumulation of osmotically active substances inside the cells and thereby to their adaptation, too. On the contrary, root growth is the plant's adaptation mechanism which contributes to water absorption from lower soil layers. With this, it helps to thin the substances accumulated inside the cells and to restore the plant into a state without stress (Osorio et al., 1998). For barley the osmotic stress is manifested by the fast growth of the root part, faster ontogenetic development and earlier flowering (Munns et al., 2000).

The osmotic adjustment capability varies according to the drought resistance of the species. In wild cereals coming from areas with low availability of water, a higher capability of osmotic adjustment was identified than in the cultural species (Oukarroum et al., 2007; Suprunova et al., 2007). In barley, the osmotic adjustment capability is very good; in wheat, it tends to be lower. This is why barley is a significant cereal in dry areas. In comparison with the other C_3 cereals, barley has a better strategy for water management. Of course a short vegetation period and a weaker root system increase the agro-technological requirements for its cultivation (Samarah, 2005).

It follows from the above text that one of the most important properties of cereals for adaptation to drought is their ability to tolerate a temporarily reduced water content in tissues, without growth and development processes being disrupted by this. Osmotic adjustment is a significant feature for drought resistance. In our opinion, it is therefore very important to breed new varieties of cereals with a higher level of osmotic adaptation. The genetic properties of the wild plants can be the basis of the breeding process. The new varieties obtained in this way would apparently be the least costly input with respect to permanently sustainable cultivation of cereals in drier areas.

3.4 Effect of Phytohormones

The stomata openness is generally in closer correlation with the soil water content than with water content in the leaves. This indicates the existence of a chemical signal between roots and leaves, which can, independently of the hydraulic effect, regulate the opening and closing of stomata. In many species of plants, the chemical signals are produced before the hydraulic signals and they give the plants "an early warning" about the water deficit in the soil (Goodger et al., 2005). Although the stomata can respond to all classic groups of phytohormones, the most attention is paid to the ability of ABA to induce the closing of stomata. ABAis synthesised mainly in the mature leaves. Roots are also important places of ABA synthesis. It was found that roots exposed to water stress generate more ABA. ABA is transported to the leaves via xylem and it induces the closing of stomata in the leaves (Liu et al., 2005). In this case, ABA plays the role of the said correlation signal between a root and a stem (Liang et al., 1997; Wittenmyer and Merbach, 2005; Jiang and Hartung, 2008). The stomatal sensibility to ABA varies across various species and cultivars depending on the nutritional condition of plants, ion composition of the solution in xylem, time of day, temperature, radiation, air humidity, concentration of CO_2 in the environment, leaf age and its water status (Auge et al., 2000).

Exogenously applied ABA also induces stomata closing. ABA reduces the gas exchange rate, and consequently also the photosynthesis and transpiration rates. This happens without any radical change in the water status of the leaf. ABA thus favourably influences the drought resistance of plants. The plant responds to ABA application very quickly by closing the stomata. For example, the stomata of barley start to close already 5 min after its application (Mc Ainsh et al., 1992). In the experiments of Stuchlikova et al. (2007) with maize and Safrankova et al. (2007) with barley, the strong effect of ABA on limitation of the parameters of gas exchange during a moderate water stress was observed. The mean levels measured on the seventh and eighth days after interruption of irrigation in the examined group of barley genotypes are shown in Fig. 1 (the photosynthesis rate) and Fig. 2 (the transpiration rate). The stronger effect of ABA applied through irrigation at the roots than the

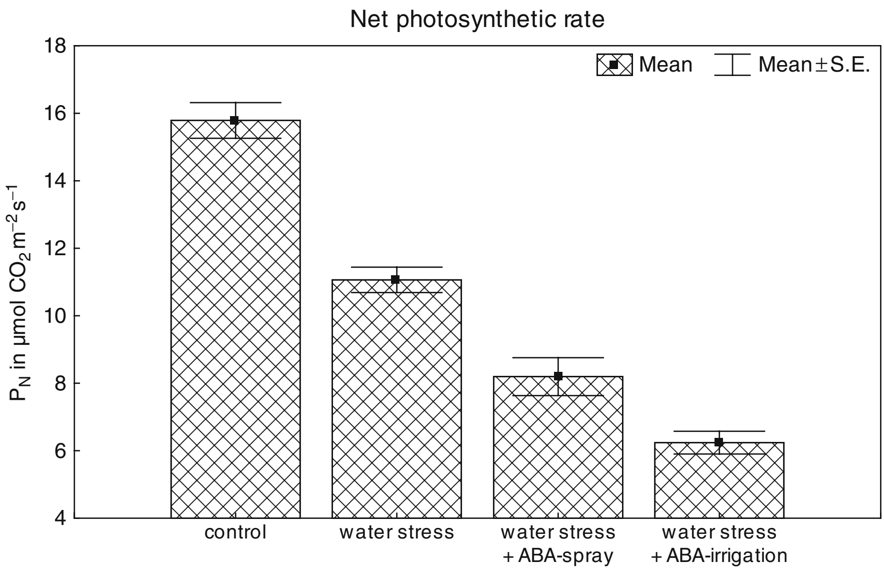

Fig. 1 The effect of water stress and abscisic acid (ABA) on the net photosynthetic rate (P_N) of spring barley. Expressed in μmol $CO_2 m^{-2} s^{-1}$. The average levels measured on the seventh and eighth day after interruption of irrigation in the group of genotypes of various proveniences are shown here. S.E. = standard error. Genotypes: Namoi from Mexico, Adagio from France, Novosadski 420 from Yugoslavia, Braemar from United Kingdom, Maridol from the Czech Republic and a wild barley *Hordeum spontaneum* Koch genotype W154 from Israel. (Adapted from-Safrankova et al., 2007)

Note: Notice the strong, statistically provable effect of ABA on reduction of the photosynthetic rate. The stronger effect of the ABA applied through irrigation at the roots is apparent, as compared with the effect of the ABA supplied to the plants by being sprayed on their leaves.

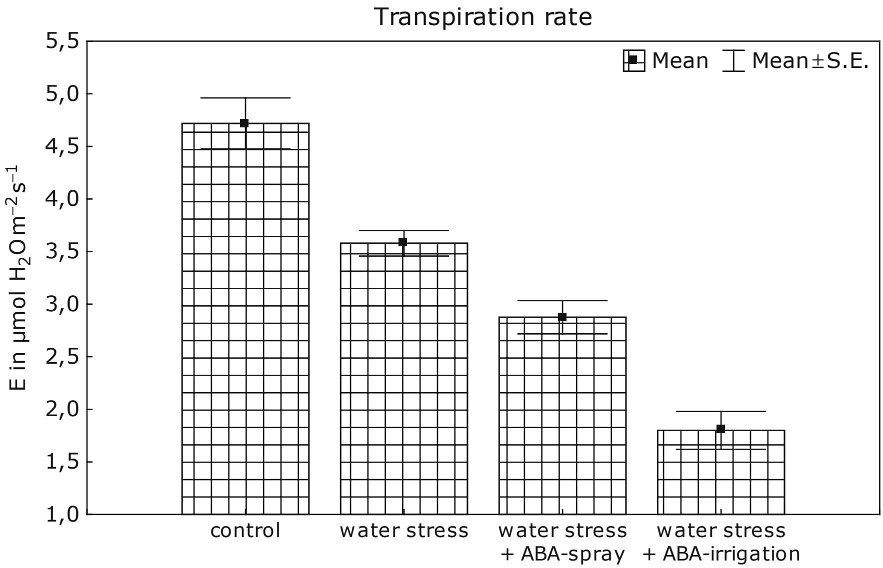

Fig. 2 The effect of water stress and abscisic acid (ABA) on the transpiration rate (E) of spring barley. Expressed in mmol H_2Om^{-2} s^{-1}. The average levels measured on the seventh and eighth day after interruption of irrigation in the group of genotypes of various proveniences are shown here. S.E. = standard error. Genotypes: Namoi from Mexico, Adagio from France, Novosadski 420 from Yugoslavia, Braemar from United Kingdom, Maridol from the Czech Republic and a wild barley *Hordeum spontaneum* Koch genotype W154 from Israel. (Adapted fromSafrankova et al., 2007)
Note: Pay attention particularly to the statistically provable effect of ABA on the reduction of the transpiration rate. The ABA applied through irrigation at the roots has a stronger effect than the ABA supplied to the plants by being sprayed on their leaves.

effect of ABA supplied to the plants by spraying is apparent. Table 1 show that the provenience of the examined barleys is related to their capability of managing water during a moderate water stress.

Since ABA supports the growth of roots and limits the growth of the aboveground parts of a plant, the plants exposed to drought can have a reduced ratio between the shoots and the roots (LeNoble et al., 2004). Under the water deficit, ABA is also among the significant mediators of the gene expression for what is referred to as stress proteins. Its increased concentration is necessary for the generation of dehydrins. In this way, a part of the existing proteins is replaced with new proteins with more suitable properties under stress conditions. Accumulation of stress proteins in plants during a drought fairly well correlates with the degree of the plants' resistance to water stress. The evidence is that stress proteins are not synthesised in the plant mutants, which have a limited capability to synthesise this phytohormone (Bray, 1993).

The content of endogenous ABA is a significant criterion of the plants' drought resistance. Brestic and Olsovska (2001) state that the high content of endogenous

Table 1 The influence of the country of origin on the water use efficiency (WUE) in various genotypes of spring barley. WUE is expressed as net photosynthetic rate/transpiration rate (P_N/E) in μmol $CO_2 m^{-2} s^{-1}$/mmol $H_2O m^{-2} s^{-1}$. The averages (± standard error) of the levels measured on the seventh and eighth days after interruption of the irrigation are shown here. The effect of water stress and abscisic acid (ABA) is examined. (Adapted from Safrankova et al., 2007)

Country/Variants	Control	Water stress	Water stress + ABA-spray	Water stress + ABA-irrigation
Israel/'W 154'	3,435 ± 0,20	2,987 ± 0,18	2,856 ± 0,03	3,057 ± 0,20
Mexico/'Namoi'	3,355 ± 0,06	3,535 ± 0,31	2,892 ± 0,16	2,984 ± 0,25
France/'Adagio'	**4,543 ± 0,11***	3,391 ± 0,30	3,471 ± 0,20	3,079 ± 0,06
Yugoslavia/'Novosadski'	2,710 ± 0,24	2,679 ± 0,14	2,235 ± 0,36	3,012 ± 0,28
United Kingdom/'Braemar'	2,993 ± 0,17	3,146 ± 0,03	2,857 ± 0,18	**5,322 ± 0,03***
Czech Republic/'Maridol'	3,482 ± 0,17	2,923 ± 0,06	2,858 ± 0,22	**4,946 ± 0,07***

*bold font – significant differences.

Note: Notice especially the high level of WUE in the control variant of the Adagio variety (France). This is probably the genotype with the highest photosynthetic performance from among the examined genotypes. Also, notice that from among all the examined genotypes the Adagio variety manifested the greatest decrease of WUE in the variant stressed by drought in comparison with the control variant. This can be regarded as a proof of the sensitivity of the Adagio variety to water stress. In most of the genotypes, decrease of WUE occurred in the variant stressed by drought as compared with the control variant. Only in the Namoi variety (Mexico), a slight increase occurred. This indicates its higher resistance to drought as compared with the other genotypes. In the Braemar (United Kingdom) and Maridol (Czech Republic) genotypes, notice the strong effect of the ABA supplied through irrigation on the increase of WUE. It is probably a case of ABA significantly improving water management in a sensitive genotype.

ABA prevents disruption of the ultrastructure of chloroplasts in some maize cultivars during drought. It is also known that maintaining the volume of chloroplasts prevents non-stomatal inhibition. The reason is primarily the accumulation of K^+ and an increase of the osmotic potential of chloroplasts. This increases the overall resistance to drought. Potassium is often discussed as a factor increasing the drought resistance of plants.

Other phytohormones can also influence the water management of plants, particularly through interaction with ABA. Cytokinins and auxins can reduce or defer the closing of stomata induced by ABA or inhibit the ABA accumulation induced by the water stress (Pospisilova et al., 2000, 2005; Tanaka et al., 2005; Veselova et al., 2006; Stuchlikova et al., 2007). Jasmonates can stimulate this effect (Stoll et al., 2000). Non-stomatal effects of phytohormones on the photosynthetic apparatus were also observed. However, most effects are concentrated on influencing the photosynthesis by changing the stomatal conductivity and consequently the inner concentration of carbon dioxide. However, the extent of this influence depends on the plant's uptake and degradation of the applied substance and its interaction with the endogenous phytohormones. A different method is to use transgenic plants with a modified content of endogenous phytohormones. Application of ABA can help the plants survive for a certain period by closing the stomata.

As a result of the ABA accumulation and redistribution in the leaves, stomatal activity, effective osmoregulation, and as a result of the cell compartment volume being maintained, the photosynthetic structures remain relatively undamaged until the medium levels of water deficit in the leaves are reached. A high content of the endogenous ABA and particularly the WUE (Blum, 2005; Safrankova et al., 2007; Stuchlikova et al., 2007; Duskova et al., 2008) are the significant criteria for the plant drought resistance assessment.

4 Ensuring Yield of Cereals under Environmental Stress Factors

For the production process of cereals, the photosynthesis rate, the size of the assimilation apparatus and the duration of the process are the decisive aspects (Petr et al., 2002; Hejnak, 2003a,b). These characteristics can be unfavourably influenced by stress factors. The resulting effect of the stress is determined by its depth, duration and the development stage, at which the plant is found (Hafsi et al., 2007). In cultural plants, periods referred to as critical periods have been described with respect to the requirements for water supply. Water stress during these periods can fundamentally influence the final yield. In most plants, these are the development stages, at which sexual organs are differentiating and the growth of vegetative organs is most intense (Orcutt and Nielsen, 2000). Cereals have little resistance to drought at the stage of tillering and shooting (Brestic and Olsovska, 2001). In this period, there is a fast growth and enlargement of the biomass of the plants and the demands on their photosynthetic performance rise. This is related to the intensive growth of the assimilation apparatus and the photosynthesis rate between the tillering stage and the flowering stage. Subsequently, in the period after earing and after termination of the flowering period, the number of photosynthetically active leaves is quite rapidly decreasing. The ageing of the photosynthetic apparatus is also gradually manifested by significant decline in the stomatal conductivity and by decrease of the photosynthesis rate (Hejnak, 2003a; Hejnak and Krizkova, 2004). The photosynthesis rate (P_N) during the ontogenesis of spring barley is shown in Fig. 3 on the Krona variety, as an example. A similar process was observed in the plants of winter wheat by Hnilicka et al. (2000) and Hnilicka et al. (2004). As mentioned above, the photosynthesis rate achieves the highest levels at the beginning of the flowering stage. The research of Sanchez-Diaz et al. (2002) and Hejnak and Krizkova (2004) documents that water stress significantly limits the photosynthesis rate during this period. Water stress resulting from a decrease of soil moisture from 70% to 35% of the maximum capillary water capacity reduced the photosynthesis rate in the Krona variety by 39% at the beginning of the flowering stage as compared with the non-stressed control group (Fig. 3). Such a significant decrease of the photosynthesis rate leads to the presumption that non-stomatal factors might have also participated in the inhibition of the photosynthesis in this case. These factors block the use of CO_2 by the photosynthetic apparatus of the stressed plants. For example, the regeneration of RuBP can be affected. This is stated by Escalona et al. (1999) as one of the possible

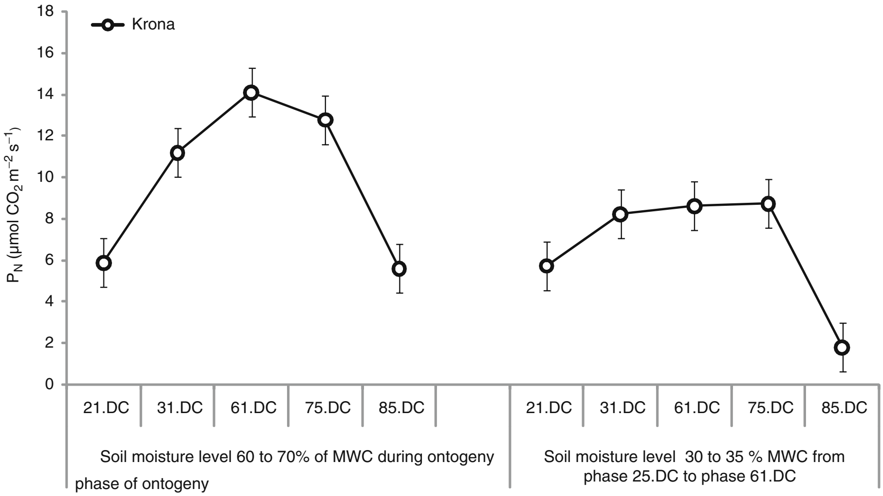

Fig. 3 Net photosynthetic rate (P_N) during ontogeny of spring barley cv. Krona (Germany). Expressed in µmol $CO_2 m^{-2} s^{-1}$ ± standard error. MWC = maximum capillary water capacity. DC = the ontogenesis stage according to Zadoks (Zadoks decimal code): 21 – Tillering (main shoot and one tiller); 25 – Tillering (main shoot and five tillers); 31 – Stem elongation (first node detectable); 61 – Flowering (10% of spikes are flowering); 75 – Milk development (mid milk, increase in solids); 85 – Dough development (soft dough – kernels firm but finger nail impression not held). *Left*: P_N under soil moisture at the level of 70% MWC throughout the ontogenesis. *Right*: P_N influenced by soil moisture decreased from 70% to 35% MWC in the period from 25th DC to 61st DC. (Adapted fromHejnak and Krizkova, 2004)
Note: *Left*: Notice that the photosynthesis rate in the spring barley gradually increases during the ontogenesis. It reaches the maximum level during the period of flowering. Subsequently, P_N decreases in connection with the ageing of the assimilation apparatus. *Right*: Compare the course of P_N with the levels shown on the picture on the left. Notice the statistically significant decrease of P_N induced by water stress. It decreased by up to 39% at the stage of the 61st DC.

general explanations in field conditions. Nevertheless, Bota et al. (2004) state that the RuBP content and the activity of the Rubisco enzyme are only influenced under a very strong water stress. However, the possibility of a direct influence on the photosynthetic apparatus of plants under a medium-strong water stress is also indicated by the results of Gorny (2001a,b) or Loboda (2000). When the soil moisture was decreased from 60% to 35% of the maximum capillary water capacity, he identified twice as lower photosynthesis rate in four genotypes of spring barley and wheat as compared with the non-stressed plants. And yet the availability of CO_2 from the intercellular spaces was not influenced by the reduction of stomatal conductivity in a statistically provable manner.

The quantity of organic matter created and translocated in a plant can be expressed in grams of dry matter or carbon. We can assume that 1 g of dry matter corresponds to approximately 0.5 g of carbon. Also, the energy-based expression in kJ can be used. Then we speak about the energy content and flow (Duvigneaud, 1988). The energy value of dry matter is determined by its chemical composition.

Carbohydrates have a lower energy content than proteins and lipids. In general, carbohydrates have 17.2 kJ, proteins 23.7 kJ and lipids 39.6 kJ of energy per gram (Hejnak, 2003a).

From among plant organs of cereals, the reproduction organs usually have the highest energy content and the straws have the lowest energy content. Kumar (1994) arrived at this conclusion during experiments on spring barley. Krizkova and Hejnak (2007) also identified the lowest levels in straws (13.3 kJ of net energy in 1 g of dry matter on the average) and the highest levels in spikes (14.5 kJ of net energy per gram of dry matter on the average). This is given by the fact that in the post-floral period, a significant translocation of assimilates to the generative organs takes place. This is manifested by an increase of the energy content in spikes. In most cases, straws primarily serve as the transport routes for assimilates.

Stress is manifested by a decrease of the energy content in plants. In the experiment of Krizkova and Hejnak (2007), water stress in spring barleys was manifested by a decrease of the energy content particularly in the leaves (by up to approximately 1 kJ g^{-1} of dry matter). This significantly limits the production performance of stressed plants in the following stages of ontogenesis, which are important for translocation of assimilates and for transfer of the energy from the leaves to the generative organs.

The relation between the source of assimilates and the point of their consumption or accumulation (sink) is often a discussed issue. We speak about a sink–source relation. If the external conditions are not limiting photosynthesis, the primary role in regulation of the creation and transport of assimilates is played by the sink. In the work of King et al. (1967), the photosynthesis rate of the flag leaf decreased in wheat after the spike had been cut off. A decrease of the photosynthesis rate was also observed in the period shortly after anthesis. At that time, the growth of the straw and the shoots is tiny and, at the same time, intensive growth of caryopses is not taking place. The photosynthesis of the flag leaf again started to increase when the caryopses started to grow. Abbad et al. (2004) state that in the past, the flag leaf in wheat used to be regarded as the main source of assimilates for filling the grain. Araus et al. (1993) had already accepted spike as a significant producer of photosynthetic assimilates. They emphasise its contribution to the final yield particularly during the times of water stress. The experiments of Tambussi et al. (2005) on wheat show that if there is water deficit during the period when the grain is being filled, the spike is affected to a minimum degree. The drought resistance of a spike is ensured by its higher ability to keep a sufficient amount of water, a high RWC, during the water deficit as compared with the flag leaf. Wang et al. (1997) state that in the wheat plants, during the stage of filling grains, the leaf blade accounts for the highest amount of produced assimilates (more than 60%), the spike accounts for 15% and the leaf sheath accounts for around 11% and the straw also accounts for ca. 11%.

It follows from the work of Hejnak (2003a,b) and Hejnak and Krizkova (2004) that in cereals there is no temporal accordance between the production of assimilates and their accumulation in caryopses. The maximum production is shortly before and after flowering. At that time, the plants and the whole vegetation have the maximum assimilation area. However, the growth of caryopses at this time is very gradual. As the speed of growth of grains gradually increases in the post-floral period, the production of assimilates decreases. It can be inferred from this that the dry matter in caryopses particularly consists of assimilates, which were accumulated in some other organs of plants for a variously long time. With regard to the morphological and anatomic structure of cereals, it is clear that straw has the highest storage capacity. It follows from the work of Takohashi et al. (1993) that the period of production and filling of grains can be divided into four stages. The first stage is the stage of initial filling of the grains. It takes place during the period from anthesis until the end of shooting. At this stage, assimilates are used primarily for the growth of the straw. For the growth of the generative organs, their use is negligible. The second stage is characterised by frequent filling of the grain. It takes places from the beginning of heading until the stage of milk ripeness. In this period, assimilates are used evenly for the growth of the grain and they are also deposited as reserve materials in straws. After this stage, the late filling of the grain starts. This begins at the stage of milk ripeness and ends at the time of termination of photosynthesis. At this stage, all assimilates produced by the photosynthesising leaves are used to fill the grain. At the same time, reserves from the straws are translocated to the grain. The last stage is defined as the final filling of the grain. It takes place from the termination of photosynthesis until full ripeness. The growth of the grain at this stage only takes place through translocation of assimilates from the straws because the photosynthetic apparatus is not operational any more. This concept of the filling of the grain with assimilates is also supported by the work of Przulj and Momcilovic (2003). They state that during the growth of spike in barley, the grain is filled predominantly with the assimilates deposited in the vegetative parts of the plant or possibly with the assimilates produced during the process of filling the grain.

In the experiments of Hejnak (2003a,b) with the old and modern varieties of spring barley, the leaves accumulate the maximum quantity of dry matter mostly at the beginning of the flowering period. The straws usually achieve the maximum weight of dry matter later, at the stage of milk ripeness. Spikes show an intensive growth of dry matter in the period from milk ripeness to full ripeness. Thus, that is the period in which the dry matter of vegetative organs already stagnates or decreases. The dynamics of this process is higher in modern varieties than in the older varieties of spring barley. This is documented by comparison of the production of dry matter in the Krona variety (Fig. 4) and the historically oldest preserved Nürnberg variety (Fig. 5) from the year 1832. The results of Petr et al. (2002) were confirmed with the old (Nürnberg) varieties of spring barley and oat.

For the yield of cereal grains, higher production of dry matter in the post-floral period is more advantageous and the modern varieties are better in this respect. One of the main successes in the breeding is the increased distribution of assimilates in favour of spikes, that is, in favour of grains in the modern varieties. This is also connected with the breeding efforts aimed at achieving short straws, which means that with overall identical yield of dry matter, the modern varieties invest more into grains and less into straws (Petr et al., 2002; Hejnak, 2003a,b). This corresponds to the results of Mattsson et al. (1992) with a regional variety of spring barley called

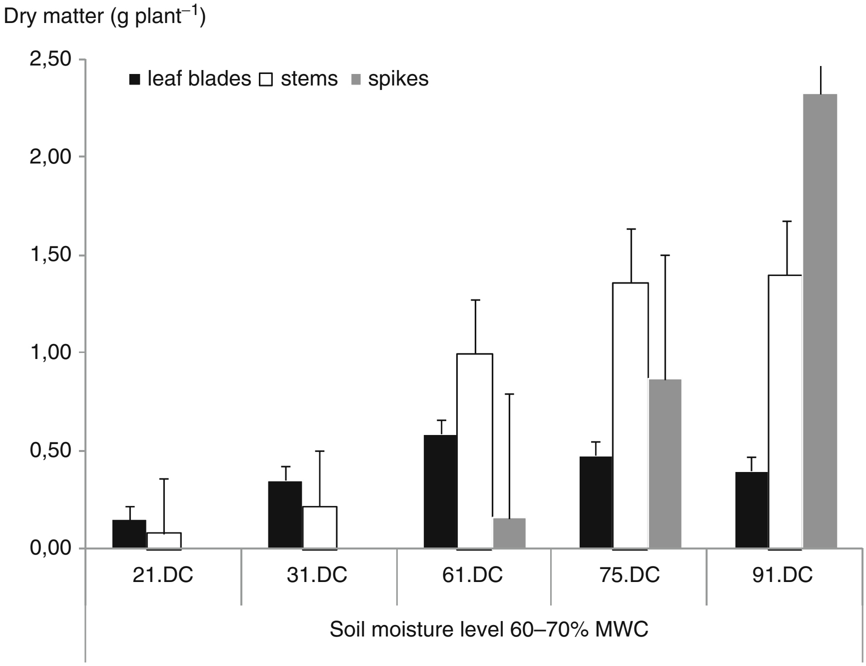

Fig. 4 Dry matter production during ontogeny of modern spring barley cv. Krona (Germany) under normal moisture conditions. Expressed in g plant^{-1} ± standard error. MWC = maximum capillary water capacity. DC = the ontogenesis stage according to Zadoks (Zadoks decimal code): 21 – Tillering (main shoot and one tiller); 31 – Stem elongation (first node detectable); 61– Flowering (10% of spikes are flowering); 75 – Milk development (mid milk, increase in solids); 91 – Ripening (harvest ripe – can no longer be dented by thumb nail).(Adapted fromHejnak 2003b) Note: Notice that the highest amount of dry matter is accumulated in the leaves at the beginning of the flowering. Straws reach the maximum dry matter weight at the stage of milk ripeness. Spikes show intensive growth of dry matter in the period of milk ripeness to full ripeness. The dynamic translocation and accumulation of dry matter in spikes in the post-floral period is higher in comparison with the historical Nürnberg variety (see Fig. 5).

Laevigatum, which is region-specific and has low yield, and with two modern high-yield Golf and Mette cultivars. Similarly, Uzik and Zofajova (2006, 2007) attribute the increase of the share of the grain in the total biological yield of modern varieties of cereals to the reduction of the height of the straw and to the increased distribution of biomass to the grain. Liang et al. (2001) also identified fast accumulation of assimilates in the caryopses of the modern varieties of rice.

It is presumed that the actual process of transporting assimilates between the source and the sink is regulated by endogenous phytohormones, which play the role of a correlation signal. The mutual relations between the source and the sink are also strongly influenced by the environment.

Stress resulting from drought is a significant factor limiting the yield of cereals. In the period before the filling of the grain, it strongly reduces the number of

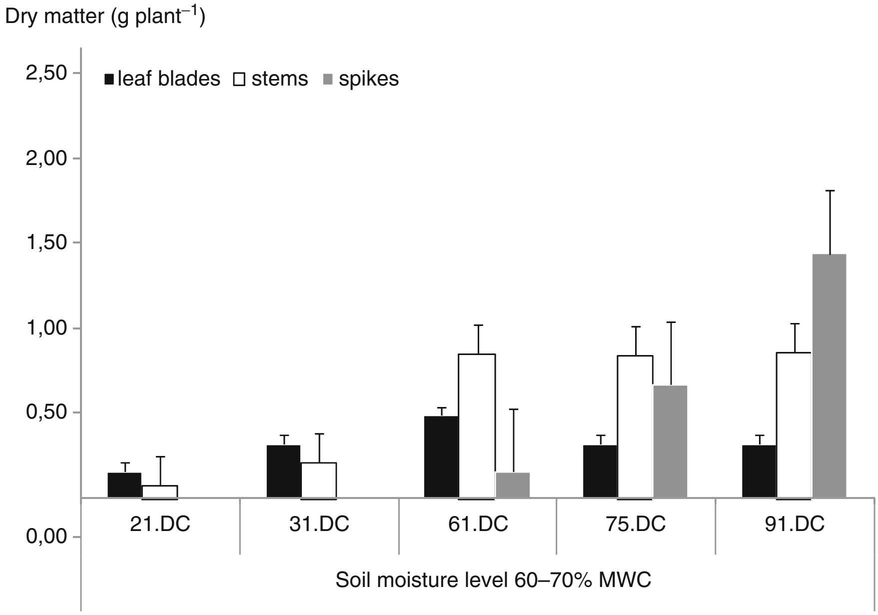

Fig. 5 Dry matter production during ontogeny of historical spring barley cv. Nürnberg (Germany) under normal moisture conditions. Expressed in g plant^{-1} ± standard error. MWC = maximum capillary water capacity. DC = the ontogenesis stage according to Zadoks (Zadoks decimal code): 21 – Tillering (main shoot and one tiller); 31 – Stem elongation (first node detectable); 61 – Flowering (10% of spikes are flowering); 75 – Milk development (mid-milk, increase in solids); 91 –Ripening (harvest ripe – can no longer be dented by thumb nail).(Adapted fromHejnak 2003b)
Note: Notice the significantly lower production of dry matter in the post-floral period in comparison with the Krona variety. The distribution of assimilates for the benefit of spikes is slower and the accumulation of dry matter in spikes is lower in comparison with this modern variety (see Fig. 4).

offshoots and accelerates the transition of the cereals from the vegetation stage to the generative stage. This was proved by Samarah (2005). Ebadi et al. (2007) state that under water stress, a higher quantity of assimilates are remobilised and re-transported from other organs of the plant to the swelling caryopses. Thus, their growth could be taking place without disruption, even if photosynthesis is temporarily limited due to unfavourable conditions. This is also confirmed by Hejnak (2003a,b) for a moderate water stress. However, he also documents, similarly as Svihra and Talapka (1995), Brestic (1996), Gulli et al. (2005), Fageria et al. (2006) and Barnabas et al. (2008) that under a strong water stress, transport processes are limited in cereals during the post-floral period. Consequently, assimilates are accumulated in straws and do not get to grains. At the same time, the growth of the assimilation apparatus is usually limited even after the end of drought. In the stressed plants, this results in a decrease of the production of assimilates necessary for filling the grains in spikes. Similarly, Ehdaie et al. (2008) state that the growth of wheat grains depends on photosynthesis and on the soluble sugars accumulated in straws. Under a strong water stress, these carbohydrates are not sufficiently utilised for filling the grain.

The reduction of yield of cereals under a water stress is attributed to the shortened period of the direct filling of grains with assimilates. The plants of cereals stressed by drought and high temperatures during flowering and maturity stages have shorter duration of grain filling than well-watered plants. Grain dry weight for severe drought stress plants reach a maximum value earlier than grains from mild drought stress and well-watered plants. Drought stress treatments reduce grain yield by reducing the number of tillers, spikes and grain per plant and individual grain weight (Hoffmann and Burucs, 2005; Samarah, 2005; Gonzalez et al., 2007).

The detailed knowledge of the physiological mechanisms of cereals yield production is important for the analysis of their reproduction capabilities. It is necessary to define the measures, which will make sustainable growing of cereals in various agricultural areas on the Earth possible. One of the main reasons for the growth of yield of cereals was that cereals were bred to achieve a higher harvest index. Through targeted selection, the share of grains in the total dry matter of a wheat, barley and maize plant increased from ca. 25% to nearly 50% (Natr, 1998; Hejnak, 2003a). Through breeding activity, the size of caryopses, the leaf area and the duration of its existence was increased in cereals. However, at the same time, the photosynthesis rate was strongly reduced (Zhang and Gao, 1993). We presume that breeding with a focus on this important feature of productivity can be an important step towards further increase of the yield of cereals.

We also think that another necessary breeding step is to increase resistance of cereals to the stress factors related to the climate change. For example, for arid areas, breeders are looking for genotypes with a good potential yield and phenological and physiological characteristics guaranteeing resistance to drought (Annicchiarico and Pecetti, 1995; Campos et al., 2006). Particularly the capability to use water efficiently for production of dry matter and thus to ensure sufficient yield in arid areas is important (Brestic, 1996). Breeding aimed at achieving higher resistance to drought can be focused on three key areas (Hamdy et al., 2003; Condon et al., 2004): (1) to improve the availability of water through the root system; (2) to limit the loss of water through transpiration and to gain more biomass for the otherwise transpired water and (3) to use a larger part of the produced biomass for production of the harvested product.

In the selection of the genetic sources of resistance to abiotic stresses, attention is also concentrated on wild species and on the region-specific and primitive varieties of cereals originating from areas with worse conditions. For example, in the wild barleys growing in arid areas, a higher capability of osmotic adjustment was identified. They are the basis for breeding varieties resistant to water stress. In the wild form of barley (*Hordeum spontaneum* KOCH), a gene of drought resistance was found, called Hsdr4 or Hordeum spontaneum dehydration response (Suprunova et al., 2007).

Among cereals, there are significant differences between various species in the physiological mechanisms of resistance to stress factors, including water stress. Lu and Neumann (1998) discovered this during experiments with various genotypes of maize, rice and barley. Thus, we can say that the promising path to go is to use gene transfers in order to improve the photosynthetic and growth capacity of cereals during the presence of stress factors.

5 Conclusion

Based on the study of a number of literature sources, we presume that the increased concentration of CO_2 will only partly compensate for the losses of the yields of cereals resulting from the increase of temperature and aridisation of the environment taking place on a global scale. However, cereals have a number of adaptation mechanisms to maintain turgor and to improve water management in dry and salinised habitats. With a view of ensuring permanent sustainability of agricultural production under the changing natural and climatic conditions, we present two options of using the diversity of adaptation mechanisms: (1) to adapt the composition of the grown cereals to the changed conditions; (2) to breed varieties more resistant to the changing conditions. Breeding resistant genotypes is the least costly solution to ensure sustainable development of agriculture in arid areas. We believe that the choice of the suitable selection criteria is most important. For the screening of genotypes resistant to drought and salinisation, it is important to use more parameters: physiological and biochemical indicators at the cellular level and the genes of resistance to drought and salinisation. The suitable selection criteria and the important features of drought and salinisation resistance are a high level of osmotic adjustment, low stomatal conductivity and good growth of roots. Breeding aimed at achieving a higher degree of drought resistance should be focused on (1) improvement of the availability of water through the root system, (2) the limitation of water loss through transpiration and higher WUE for production of biomass, and (3) prolongation of the activity and increase of the power of the sink. In the selection of the genetic sources of resistance to abiotic stresses, we recommend paying an even greater attention to wild species and region-specific and primitive varieties of cereals, originating from worse natural and climatic areas. Another promising path is to use gene transfers to improve the photosynthetic and growth capacity of cereals during the presence of stress factors. In order to improve the water management in cereals under dry conditions, we also suggest using growth regulators to a greater extent. ABA applied on plants can serve as an antitranspirant. It can increase WUE.

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Induction of Plant Tolerance to Semi-arid Environments by Beneficial Soil Microorganisms – A Review

R. Aroca and J.M. Ruiz-Lozano

Abstract Currently arid or semi-arid land areas are increasing worldwide due to global warming and the soil is becoming saline because of the use of intensive irrigation in the crop fields. Consequently, the proportion of plants living under water shortage conditions is increasing. This phenomenon is limiting seriously crop production in such areas. In many cases, the fields are being abandoned and become uncultivable again in a period of time due to erosion. Although plants have their own mechanisms to cope with drought stress, they become more tolerant to drought when associated with different soil microorganisms. Among these soil microorganisms, the most abundant and effective are rhizobia, plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF). Rhizobia fix atmospheric nitrogen and transport this fixed nitrogen to the legume host plant. PGPR promote plant growth by means of several mechanisms. They are present in almost all ecosystems across the world. AMF were vital to plants when starting to colonize dry land surface, and hence for improving plant mineral nutrition, especially uptake of phosphorous, among other factors. Here we detail the most recent advances about how these microorganisms enhance plant drought tolerance at physiological and molecular levels, including decreased oxidative stress, improved water status or regulation of aquaporins. It has been found that legume plants inoculated with rhizobia grow faster under drought conditions than non-inoculated ones. However, how rhizobial symbiosis affects root water transport has not been addressed yet. At the same time, it seems that there is a correlation between drought tolerance in rhizobial bacteria and rhizobia-induced plant drought tolerance, at least in terms of reducing plant oxidative stress. Under drought conditions, PGPR regulate the levels of stress-related hormones, i.e. abscisic acid and ethylene. The regulation of these hormones could be the cause of an enhancement of plant drought tolerance mediated by PGPR. However, a more detailed molecular approach is still needed to fully understand this process. Arbuscular mycorrhizal symbiosis improves almost every

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physiological parameter of the host plant under drought stress, i.e. water status, leaf transpiration, photosynthesis or root water uptake. However, the molecular basis for this improvement is far to be understood. At the same time, AMF in combination with rhizobia or PGPR usually have an accumulative beneficial effect on plant drought tolerance, although this depends on the specific pair of strains inoculated. Therefore, although there are many studies in order to understand at the physiological level how beneficial soil microorganisms induce plant drought tolerance, there is still a lack of knowledge about the molecular basis behind this improvement.

Keywords Aquaporins · Arbuscular mycorrhizal fungi · Drought · Oxidative stress · Plant growth promoting rhizobacteria · Rhizobia

1 Introduction

In the last decades the dry land surface becoming arid or semi-arid is rising progressively, increasing concomitantly, vegetative surface areas subjected to drought (Herrmann and Hutchinson, 2005). Plants have developed several mechanisms to cope with drought episodes during their evolution, ranging from morphological characteristics to molecular functions (Fig. 1). Morphological mechanisms

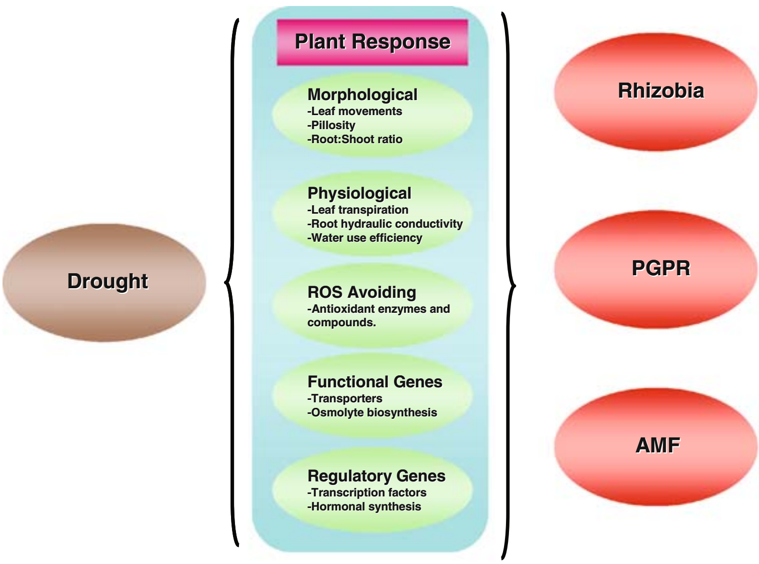

Fig. 1 Scheme summarizing the modulation of several plant processes by some beneficial soil microorganisms (rhizobia, plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi) in response to drought stress. ROS: reactive oxygen species, PGPR: plant growth promoting rhizobacteria, AMF: arbuscular mycorrhizal fungi

include, among others, leaf movements and abscission, increased leaf pillosity or greater root:shoot ratio (Gu et al., 2007; Susiluoto and Berninger, 2007). The above-cited mechanisms are aimed to avoid water loss from the leaves and to increase the area of soil explored by the roots in order to take up more water and nutrients.

The most common physiological mechanisms plants have to cope with drought episodes are changes in leaf transpiration and root hydraulic properties. Thus, leaf transpiration and leaf conductance decrease under water stress conditions, and therefore, in some circumstances the water-use efficiency rises (Tambussi et al., 2007). As the water-use efficiency rises, the amount of water needed to produce the same amount of plant biomass decreases. This mechanism is important to keep plant growth under water-limited environments (Tambussi et al., 2007). At the same time, plant root hydraulic conductivity (L) usually decreases under drought conditions, although the contrary has also been reported (Siemens and Zwiazek, 2004). Under severe drought conditions L decreases in order to avoid water lost from root tissues. However, when the water deficit is moderate, plants can increase their L in order to take up more water from soil (Siemens and Zwiazek, 2004). These changes in L are related to changes in the abundance of aquaporins (Aroca et al., 2006). Aquaporins are proteinaceous channels present in the membranes of all living organisms that facilitate the passage of water following an osmotic gradient (Maurel, 2007).

Under water-deficit conditions the production of reactive oxygen species (ROS) increases in plant tissues, but plants have several compounds and enzyme systems capable of removing ROS efficiently (Shvaleva et al., 2006) (ROS: reactive oxygen species). Although there are several antioxidant compounds in plants, the most important and better studied are ascorbate and glutathione (Noctor, 2006). Plants have several antioxidant enzymes that, acting in synchrony, are able to remove ROS generated during drought. These enzymes include those from the ascorbate-glutathione cycle, catalases and superoxide dismutases (Wu et al., 2006a).

The above-cited mechanisms that allow plants to cope with drought stress are regulated by changes in gene expression. Drought-regulated genes can be divided into two groups: functional or regulatory. Functional genes include those encoding for transporters, detoxification enzymes, chaperones or enzymes involved in osmolyte biosynthesis. On the other hand, regulatory genes encode for transcription factors, protein kinases or phosphatases, or enzymes involved in hormone biosynthesis (Shinozaki and Yamaguchi-Shinozaki, 2007).

It must be considered, however, that in nature, plants usually interact with several soil microorganisms that make the plants more efficient to cope with environmental stresses like drought. The most important soil microorganisms that associate with plants are nitrogen-fixing bacteria in the case of legumes (rhizobia), plant growth promoting rhizobacteria (PGPR) and mycorrhizal fungi (Barea et al., 2005). In the present review we intend to describe the most recent physiological and molecular advances about how the above-cited soil microorganisms allow plants to be more tolerant to drought stress.

2 Rhizobial Symbiosis

2.1 Background

The term rhizobia include collectively all nitrogen-fixing bacteria that form nodules in legume plants, including several genera (reviewed by Willems, 2006). It has been estimated that approximately 53 million tonnes of nitrogen are harvested globally in food crops each year, and most of this nitrogen is coming from inorganic fertilizers (Garg and Geetanjali, 2007). Thus, the biological nitrogen fixation carried out by rhizobia offers an opportunity for reducing nitrogen inputs, and also diminishes nitrogen loss to the atmosphere. In the past recent years, a substantial progress of how rhizobial bacteria and legume plants start the symbiosis and which signals and genes are involved has been made (Kinkema et al., 2006; Garg and Geetanjali, 2007). Here, we focus on how this symbiosis improves legume plant tolerance to drought stress.

2.2 Rhizobia-Induced Plant Drought Tolerance

A beneficial effect by nodulation on plant growth under drought stress conditions has been usually observed in legume plants (Goicoechea et al., 1997; Figueiredo et al., 1999, Shisanya, 2002; Mnasri et al., 2007). However, there are few examples in the literature where it has been proven that nodulated plants had better water status than non-nodulated ones as evidenced by their leaf water potential (Ψ_W; Mrema et al., 1997). Plant water status depends on the balance between water loss from the leaves and water uptake by roots. In fact, no differences in leaf transpiration between nodulated and non-nodulated plants under drought conditions have been observed so far (Goicoechea et al., 1997; Figueiredo et al., 1999). Surprisingly, there is no information about how rhizobial symbiosis may affect root water uptake or root hydraulic properties. This point should be checked in the near future. The only insight about the possible regulation of root hydraulic properties by rhizobial symbiosis was found by Porcel et al. (2006). In this work, Porcel and co-workers found a diminution of the expression of a plasma membrane aquaporin gene in the roots of soybean plants inoculated with the nitrogen-fixing bacteria *Bradyrhizobium japonicum* under both control and water-deprived conditions (Fig. 2). However, since aquaporin family in plants is composed of about 30 different genes, a more comprehensive study is needed to elucidate the significance of these changes. At the same time, it is known that some aquaporins may transport ammonia or ammonium, and hence they could be involved in the transport of nitrogen compounds between the nodules and the host plant (Tyerman et al., 2002; Uehlein et al., 2007). In fact, some plant aquaporins have been detected by immunogold labelling on different nodule tissues of soybean plants (Fleurat-Lessard et al., 2005).

It is known that plant nitrogen status regulates root hydraulic properties. In fact, when plants are deprived of nitrogen, root hydraulic conductivity is lower

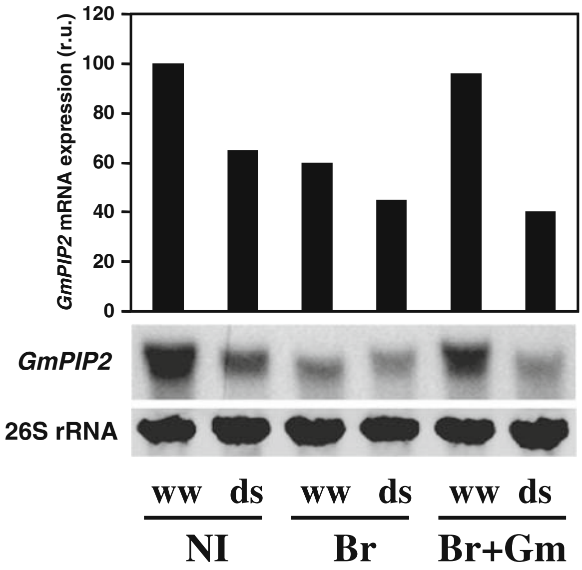

Fig. 2 Expression analysis of the *Glycine max* aquaporin *GmPIP2* gene in roots of non-inoculated plants (NI), inoculated with *Bradyrhizobium japonicum* (Br) or double-inoculated with Br and the arbuscular mycorrhizal fungi *Glomus mosseae* (Br+Gm), under well-watered (ww) or drought stress (ds) conditions. r.u. means relative units. Data adapted from Porcel et al. (2006)

than when enough amount of nitrogen is available (Gloser et al., 2007). Therefore, since nodulated plants have usually more nitrogen content than non-nodulated ones (Figueiredo et al., 1999; Shisanya, 2002), it is possible that this difference in nitrogen content also accounts for differences in root hydraulic properties. However, this hypothesis needs to be checked empirically.

The beneficial effects of rhizobial symbiosis on plant growth and yield under water stress conditions are bacterial-genotype specific. Thus, Mnasri et al. (2007) found that *Phaseolus vulgaris* plants inoculated with a salt-tolerant nitrogen-fixing bacterial strain (*Ensifer meliloti*) were more tolerant to drought than those inoculated with a salt-sensitive bacterial strain (*Rhizobium tropici*). These data clearly illustrate the relationship between the osmotic stress tolerance of the bacterial strain and the efficiency of the symbiosis. Similar results were reported by Swaine et al. (2007), since they found that a strain of *Bradyrhizobium elkanii* isolated from a drought environment was more tolerant to an in vitro osmotic stress than strains isolated from wet environments.

It is known that drought stress causes an oxidative stress in nodules (Porcel et al., 2003), and it is possible that the different efficiency of the symbiosis under drought conditions among different bacterial strains could be related to their different tolerance to drought-induced oxidative stress. However, again, this hypothesis needs to be checked empirically.

As summary, nodulation confers plant drought tolerance in terms of growth, improvement of water status and diminution of oxidative stress. However, this beneficial effect depends on the origin of the rhizobial strain, with the strains more tolerant to osmotic stresses also conferring more tolerance to such stresses to plants.

2.3 Perspectives

There is increasing information about how the nitrogen-fixing bacteria and the plant recognize each other (Kinkema et al., 2006; Garg and Geetanjali, 2007) and how the nodule per se suffers an oxidative stress under drought conditions (Ruiz-Lozano et al., 2001b; Porcel et al., 2003; Gunther et al., 2007). However, there is still lack of information about how rhizobial symbiosis confers plant drought tolerance. Experiments dealing with the effects of nodulation on root hydraulic properties and aquaporin expression and with the different antioxidant capacities among different nitrogen-fixing bacterial strains are needed.

3 Plant Growth Promoting Rhizobacteria (PGPR)

3.1 Background

The term plant growth promoting rhizobacteria (PGPR) refers to soil bacteria which are able to colonize root systems and promote plant growth. In the broadest sense PGPR include rhizobia, but the term PGPR is usually referred to free-living bacteria present either in the rhizosphere, in the root surface, or inhabiting spaces between cortical cells (Gray and Smith, 2005). The mechanisms by which the PGPR enhance plant growth are diverse and include non-symbiotic nitrogen fixation, phosphate solubilization, counteraction of plant pathogen microorganisms, or regulation of different plant hormone levels (Gray and Smith, 2005; Tilak et al., 2005; Cohen et al., 2008). Here, we intend to highlight the mechanism by which PGPR induce plant drought tolerance.

3.2 PGPR-Induced Plant Drought Tolerance

Several free-living soil bacteria induce plant drought tolerance in terms of plant growth promotion (Arkhipova et al., 2007; Jaleel et al., 2007; Sziderics et al., 2007). However, the mechanism involved could be very different. For example, Arkhipova et al. (2007) found that the beneficial effect on lettuce plant growth under water-limited conditions caused by the inoculation with a *Bacillus* sp. strain was related to an increased level of cytokinins. Another mechanism involved in plant drought tolerance induction by PGPR is the diminution of ethylene production. Thus, PGPR containing1-aminocyclopropane-1-carboxylate deaminase enzyme, which catabolizes the ethylene precursor, are able to diminish the ethylene contents in plant tissues under drought stress, favouring the plant growth and a better water status (Mayak et al., 2004). Abscisic acid (ABA) could also be involved in the enhancement of plant drought tolerance by PGPR. Arkhipova et al. (2007) found that *Bacillus* sp.-inoculated lettuce plants had also increased amounts of ABA when compared to non-inoculated plants. Since ABA is necessary for plant drought tolerance (Davies et al., 2005), alterations of ABA levels could be another mechanism for tolerance enhancement. Also, Cohen et al. (2008) recently found that the PGPR *Azospirillum brasilense* is able to synthesize ABA in vitro and to increase its production in presence of NaCl. *Arabidopsis* plants inoculated with *Azospirillum* had more ABA content than non-inoculated ones (Cohen et al., 2008).

One of the mechanisms by which ABA enhances plant drought tolerance is via regulation of leaf transpiration and root hydraulic conductivity (Aroca et al., 2006). However, although Arkhipova et al. (2007) found an increase of ABA levels in lettuce plants inoculated with *Bacillus* sp., authors did not find any difference in stomatal aperture between inoculated and non-inoculated plants. This behaviour could be caused by the counterbalance of the higher levels of cytokinins, since the ratio between ABA and cytokinins determines stomatal aperture (Goicoechea et al., 1997; Dodd, 2003; Davies et al., 2005; Arkhipova et al., 2007). Unfortunately, there is only one report in the literature showing a positive effect of a PGPR (*Azospirillum brasilense*) inoculation on root hydraulic conductivity under control and osmotic stress conditions (Sarig et al., 1992). It is possible that this positive effect described by Sarig et al. (1992) could be caused by an up-regulation of aquaporins. Marulanda et al. (2006) found that under drought conditions, *Retama sphaerocarpa* plants inoculated with *Bacillus thuringiensis* took up more water than non-inoculated plants. However, in this experiment, inoculated plants also had more root biomass than non-inoculated ones, and the water uptake calculated on the basis of root biomass was lower in inoculated plants. From these experiments we can see the necessity of studying how PGPR modify aquaporin expression.

In 1999, Timmusk and Wagner (1999) found that prior to drought stress *Arabidopsis* plants inoculated with the PGPR *Paenibacillus polymyxa* had an elevation of copies of mRNA encoding for ABA-related genes (*ERD15* and *RAB18*), and moreover, these plants were more tolerant to drought than non-inoculated ones. The authors said that the inoculation with the bacteria could be causing a mild biotic stress and preparing the plants to cope with subsequent drought stress. More recently, Sziderics et al. (2007) have found that pepper plants inoculated with several PGPRs showed, after a mild osmotic stress, lower expression of genes related with abiotic stresses than non-inoculated plants. Authors explained this different behaviour considering that inoculated plants suffered less from the stress and also had less expression of stress-related genes. Although here we report some of the experiments where a gene expression analysis has been made in order to clarify which mechanisms could be involved in the induction of plant drought tolerance by PGPRs, a more wide and comprehensive approach is necessary. In this way, we could understand which genes (regulatory or functional) are behind this drought tolerance enhancement.

Taken together, it seems that PGPR are able to modify the hormonal contents of plants and, in this way, improve their drought tolerance (Fig. 3). At the same time, this growth promotion increases the capacity of the plants to take up more water during drought episodes.

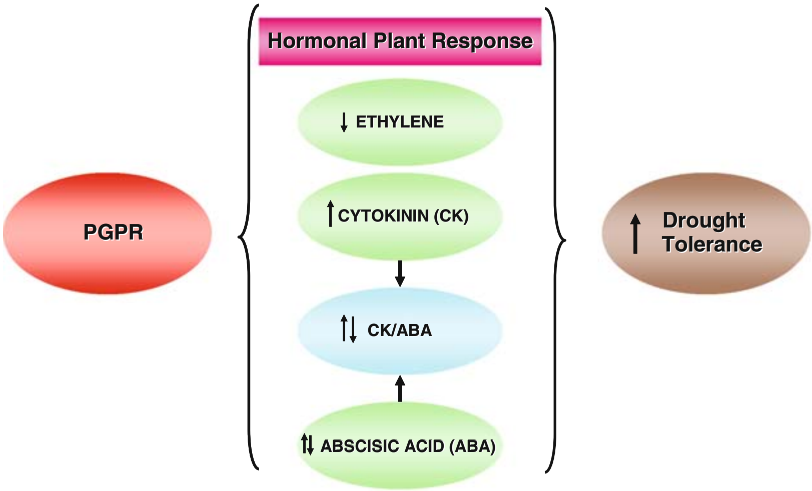

Fig. 3 Scheme showing how plant growth promoting rhizobacteria (PGPR) modify plant hormonal contents in order to increase their drought tolerance

4 Mycorrhizal Fungi

4.1 Background

The transition of plants from aquatic environment to land was possible through the association of plants with mycorrhizal fungi (Strullu-Derrien and Strullu, 2007). By the symbiosis betweenplants and mycorrhizal fungi, the plants get more nutrients and water resources from the soil, and the fungi get a protected niche and carbon skeletons from the plant, since the fungi are obligate symbionts (Entry et al., 2002). Among the different kinds of mycorrhizal symbiosis, arbuscular mycorrhizal one has the widest spread. About 90% of vascular plants establish a symbiosis with arbuscular mycorrhizal fungi (AMF; Gai et al., 2006). At the same time, by the symbiosis plants become more tolerant to environmental stresses with an osmotic component like drought (Augé, 2001; Ruiz-Lozano, 2003). Here we will describe the most recent advances on how this drought tolerance improvement is achieved from physiological and molecular points of view.

4.2 Arbuscular Mycorrhizal Symbiosis-Induced Plant Drought Tolerance

In most of the cases studied, the association between an AMF and a plant makes the host plant more tolerant to drought in terms of plant growth (Wu and Xia, 2006; Bolandnazar et al., 2007). However, this positive effect on plant growth depends on the AMF species involved (Marulanda et al., 2003, 2007; Wu et al., 2007) and on the drought intensity (Aliasgharzad et al., 2006). One of the mechanisms involved in this induction of drought tolerance is the regulation of plant transpiration. In general, under both well-watered and drought conditions, AMF-colonized plants have higher transpiration rate than non-inoculated plants (Wu and Xia, 2006; Bolandnazar et al., 2007; Wu et al., 2007). By this mechanism, AMF-colonized plants are able to fix more CO_2 than non-inoculated plants and hence their growth is improved (Querejeta et al., 2007). In addition, in some cases, also their water-use efficiency is stimulated independent of changes in transpiration rate (Bolandnazar et al., 2007). These changes described in transpiration rate by AM symbiosis are correlated with changes in the ABA:cytokinins ratio (Goicoechea et al., 1997).

At the same time, AMF-colonized plants, by the action of the fungal hyphae, are able to explore more soil and therefore to take up more water from it than non-inoculated plants (Marulanda et al., 2003; Khalvati et al., 2005; Bolandnazar et al., 2007). AMF colonization induces an increase in root hydraulic conductivity of the host plants under osmotic stress conditions (Sánchez-Blanco et al., 2004; Aroca et al., 2007). However, when aquaporin expression was analyzed in AM and non-AM roots under drought stress, a lower expression has been found in AM ones (Porcel et al., 2006; Aroca et al., 2007). These results could indicate a water conservative mechanism in AM plants, decreasing the amount of water lost from the roots to the soil, following a water potential gradient. The above-cited works did not cover the expression of the full aquaporin gene family of the host plants, and this kind of work is absolutely needed. Also, the possible involvement of the AMF aquaporins on the greatest water uptake capacity of the host plants has not been addressed because no aquaporins from any AMF have been described yet.

The beneficial effects of the AM symbiosis on plant drought tolerance have not been only assayed by measuring plant growth or plant water status. At the same time, these beneficial effects have been addressed by measuring the expression of some genes related to drought stress. These genes include late embryogenesis abundant proteins (LEA) or Δ^1-pyrroline-5-carboxylate synthetase (P5CS) enzyme. LEA proteins are involved in acquiring desiccation tolerance (Tunnacliffe and Wise, 2007), and P5CS enzyme catalyzes the limiting step in the synthesis of the osmolyte proline (Aral and Kamoun, 1997). Porcel et al. (2004, 2005) found that both kinds of genes increased their expression under drought conditions more in non-AM plants than in AM ones. Thus, the use of these two genes as stress markers can be used in studies involving AM symbiosis and osmotic stresses (Aroca et al., 2008; Jahromi et al., 2008).

Other beneficial effect of the AM symbiosis on plant drought tolerance is the diminution of the oxidative stress generated during drought periods (Porcel and Ruiz-Lozano, 2004; Wu et al., 2006a,b). In general, AMF-colonized plants had highest activities of several antioxidant enzymes than non-colonized ones (Wu et al., 2006a), but it depends on the enzyme activity, plant organ and the AMF genotype involved (Lambais et al., 2003; Wu et al., 2006b). At the same time, some superoxide dismutase isoforms from lettuce are specifically up-regulated by drought conditions in AMF-colonized plants (Ruiz-Lozano et al., 2001a). However, Porcel and Ruiz-Lozano (2004) found some evidence supporting the idea that the lower

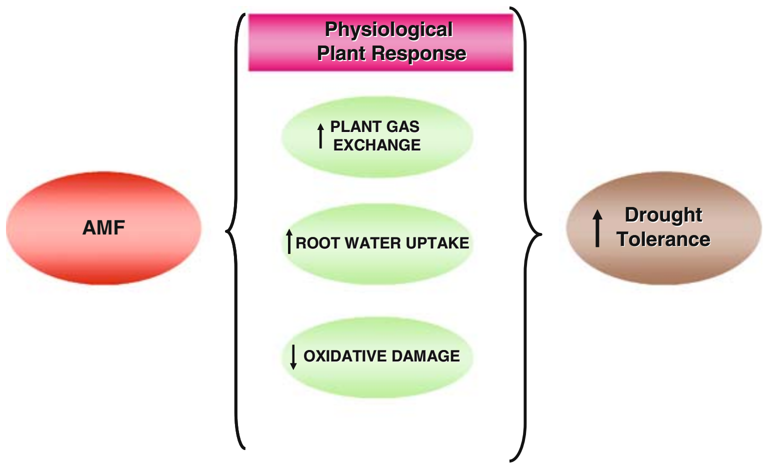

Fig. 4 Scheme showing how arbuscular mycorrhizal fungi (AMF) modify plant physiology in order to increase their drought tolerance

oxidative damage found in AMF-colonized plants under drought stress could be a consequence of their better water status. These results, as commented before, highlight the importance of studying at molecular level how AM symbiosis improves the water status of the host plants under drought conditions.

To summarize, AM symbiosis improves almost all the physiological aspects of plants during drought, like growth rate, leaf transpiration, root water uptake or diminishing the oxidative damage associated to drought (Fig. 4). However, the molecular basis behind this improvement is still far from being known.

4.3 Interaction Between Arbuscular Mycorrhizal Fungi and Other Beneficial Soil Microorganisms

In the literature there are several reports showing a positive effect on plant drought tolerance when AMF and either PGPR or rhizobia are inoculated together (Tarafdar and Rao, 2007; Valdenegro et al., 2001; Behl et al., 2007). Thus, Valdenegro et al. (2001) found that the positive effects of three different AMF isolates on plant growth in *Medicago arborea* plants under drought conditions were stimulated by the co-inoculation with the PGPR *Enterobacter* sp., depending on the rhizobial strain inoculated and on the AMF isolate. The same authors also found that some combinations of AMF, PGPR and rhizobia increased the nodule number present in the roots. This dependency on the pair of symbionts co-inoculated was also found for lettuce plants inoculated with the PGPR *Bacillus megaterium* and three different isolates of AMF, being one of the combinations harmful in terms of plant growth (Marulanda-Aguirre et al., 2008; Fig. 5). It would be very interesting to elucidate which molecular signals are involved in this different behaviour of plant growth depending on the counterpart microorganisms inoculated.

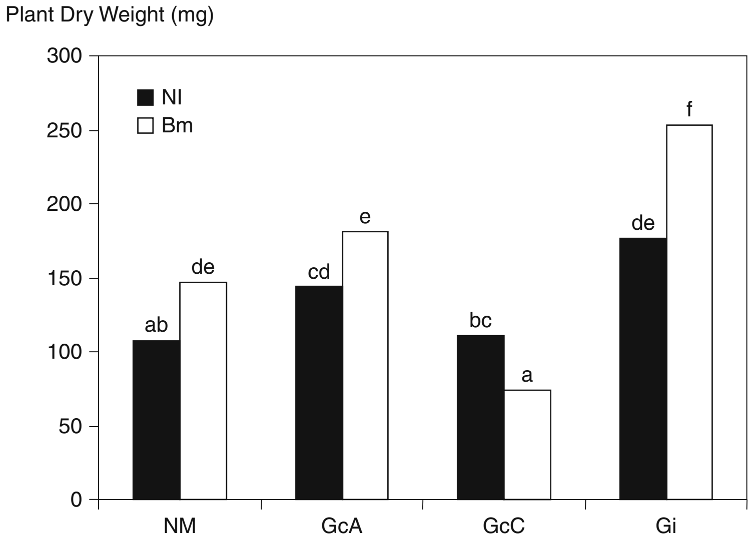

Fig. 5 Total dry weight of lettuce non-inoculated plants (NI) or inoculated with a *Bacillus megaterium* strain (Bm), alone (NM) or with different arbuscular mycorrhizal fungal isolates. GcA: *Glomus constrictum* autochthonous; GcC: *Glomus constrictum* from collection; Gi: *Glomus intraradices*. Different letters mean significant differences ($p<0.05$) after ANOVA and LSD tests. Data adapted from Marulanda-Aguirre et al. (2008)

One of the most common beneficial effects of AMF is the diminution of the oxidative stress occurring in the nodules of the legumes under drought stress (Ruiz-Lozano et al., 2001b; Porcel et al., 2003). This diminution is caused in part by a higher activity of the antioxidant enzyme glutathione reductase in the nodules of the AMF-colonized roots (Porcel et al., 2003). However, this beneficial effect could also be related to the lower water deficit suffered by the nodules of the AMF-colonized plants (Porcel et al., 2003). Specific experiments to resolve this question are needed in the near future. On the other hand, carbon metabolism of nodules from roots of *Anthyllis cytisoides* is improved under drought conditions by AM symbiosis (Goicoechea et al., 2005). How this improvement on nodule carbon metabolism is taking place is still unknown.

Therefore, although in general there is a positive interaction between AMF and other beneficial soil microorganisms, sometimes a negative effect is found. Which molecular signals are behind this beneficial or negative interactions need to be elucidated.

5 Conclusion

It is clear that the symbiosis between the microorganisms cited here and plants confers an enhancement of plant drought tolerance. However, in the case of rhizobial symbiosis, there are still basic physiological studies to be done. These studies would include analysis of the behaviour of root hydraulic conductivity under drought conditions in nodulated and non-nodulated plants. Also, studies on how nodulation affects plant aquaporin gene expression would complement the above physiological studies. Regarding PGPR and AMF symbiosis, studies at molecular level focusing on which signals are involved in both the drought tolerance enhancement per se and in the different interrelationships between these two kinds of symbionts are needed.

As conclusion we can summarize that rhizobia improve plant drought tolerance in terms of growth, water status and diminution of oxidative damage, but these beneficial effects depend on the origin of the strain inoculated. At the same time, it seems from the literature that the beneficial effect of PGPR on plant drought tolerance is caused by changes in hormonal contents, mainly that of ABA, ethylene and cytokinins. Moreover, PGPR could also improve the capacity of plants to take up more water under drought conditions. Finally, at physiological level it is clear that AM symbiosis makes the plants more tolerant to drought stress, and that this is enhanced by the combination of other soil beneficial microorganims. However, this interaction is adverse to the plant in some circumstances.

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Essential Oil Crops for Sustainable Agriculture – A Review

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Abstract Multifunctionality and diversification of farming systems, integration of agricultural practices with the non-agricultural productive systems operating on the territory, biodiversity safeguards, and reduction in off-farm inputs, are key factors for all modern development strategies in agricultural areas. Such issues are valid worldwide, but are especially true in areas in which the cultivation of the more widespread and "classical" crops is constrained by factors of varying degree and importance. In Mediterranean areas, where many environmental and economic factors often reduce rural areas to marginal conditions, the search for new crop opportunities has become one of the newest topics in agricultural research. In this review, we focus on the state-of-the-art cultivation of essential oil crops, in Mediterranean environments with a special interest in herbs. The following are the major points of our analysis. (1) Growing such crops as specialized cultivations, especially for species native to the selected environments, is the only practical and sustainable way to obtain naturally derived raw matter for both industrial and domestic purposes. (2) Most essential oil crops are suitable for many different uses, and fully adaptable for transformation even by small, local manufacturers. (3) In many cases, they may be grown with environmentally friendly or organic techniques; this enhances their environmental compatibility and also gives them an additional economical advantage, raising their chances to be addressed in the emerging market sector of "natural" products. Our conclusion is that crops grown for the extraction of economically valuable essential oils may be a strategic resource for many environments, even marginal, and that there is scope for farmers to improve the cultivation of such species on arable land. There is room, however, for many agronomic and economic questions to be studied in future experimentation and research.

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1 Introduction

In recent times, a deep interest has been addressed worldwide to the search for "new" crops, to be allocated to farming systems in which traditional crops are losing competitiveness, and also meant as a diversification option for farmers who want to increase their income (Prohens et al., 2003). To be successful, a "new" crop must meet a number of conditions: first, it must be economically reliable; second, it must be grown using the minimum amount of off-farm technical inputs as possible; and third, it should find a place in an "integrated" scheme, i.e., a planning strategy including both agricultural and external commodities, with a special interest in diversified production opportunities such as cottage industries, on-farm processing, agribusiness, recreation, tourism, and so on (UN-ESC, 2008).

The advantages of crop diversification, both in space and time, are many, and they include a better exploitation of land resources, lower risks from pests and diseases, and a higher stability in yields and income (Altieri, 2004; Prohens et al., 2003). In areas where environmental constraints set a limit to agricultural management, this issue takes a special importance. In many Mediterranean areas, a number of climatic limiting factors may be of concern. Prolonged dry periods in summer and spring with a high seasonal evapotranspiration demand, and lack and poor quality of irrigation water, bring as a consequence a growing tendency to soil salinization; rainfall mostly occurring in winter, often as intense rainstorms, may cause the breakdown of soil structure and lead to soil erosion (Arnon, 1992). In recent times, attention was called on the expected overall worsening of this scenario due to anthropogenic climate warming, whose effects would be more severe for farming systems currently located in marginal areas (Olesen and Bindi, 2002). Under these conditions, the options for farmers are often limited, and the growing difficulties for agricultural entrepreneurs have led in many cases to the abandonment of the territory.

Our basic idea is that the cultivation of crops for the extraction of economically valuable essential oils may be, in this context, an interesting option. Here we review some of the major points related to the introduction of essential oil crops as alternative cropping opportunities, with a special emphasis on their potential role for sustainable agriculture in Mediterranean environments. The major advantages and constraints to cultivation are discussed, together with the genetic, technical, and environmental factors that exert some effect on essential oil yield and quality. Some examples are given about the technical solution that are, or could be, used in Mediterranean environments and for Mediterranean essential oil crops, in order to optimize yields and quality features.

1.1 Producing Essential Oils

Essential oils are volatile mixtures of different liposoluble organic substances, most of which are aromatic, and include alcohols, aldehydes, ketones, and so on. They are produced by plants in variable quantities and may be easily extracted by means of simple distillation processes. Although the major use of essential oils worldwide is for flavoring purposes, they also have a number of important industrial and domestic uses linked to their specific actions. As an example, rosemary oil is claimed to have simultaneous insecticidal (Katerinopoulos et al., 2005), antioxidant (Etter, 2004), fungicidal (Pauli and Knobloch, 1987), antimicrobial (Pintore et al., 2002), and even hypoglycemic (Mentreddy et al., 2005) activity. Therefore, there would be interest in sectors in which one, or more, of such activities are of use.

The European chemical industry annually imports a great deal of essential oils: the FAO statistic bulletins report that more than 52,000 tons of essential oils (terpeneless or not) were imported in 2005 by France, Germany, the United Kingdom, Netherlands, and Italy (FAO, 2007). The major sources of this massive amount of essential oils were the USA, Brazil, China, and India, accounting for over 50% of the total imports. However, it is worth noting that this partition of supply does not match the monetary exchange. For example, the USA produced 10.7% of the sold oil and received 16% of the corresponding value in dollars, whereas Brazil produced 26.9% of the oil, but only received 5% of the related monetary value.

There are basically three ways to produce essential oils: the collection of plants from the wild, their cultivation ad hoc, and,recently, their extraction from callus or tissue cultures. The first method, largely used in earlier times, is obviously limited to small supplies and local uses. First, collecting plants from the wild does not guarantee the quantitative and qualitative uniformity requested by industry (Ruta et al., 2006; Shetty et al., 1996). Second, in many cases, overharvesting caused severe environmental impacts, resulting in a loss of biodiversity (Schippmann et al., 2002; Skoula, 2006; WHO, 2003) and in the depletion of spontaneous populations, such as reported for rosemary in Sardinia (Mulas and Mulas, 2005).

The production of essential oils by means of biotechnology seems to be the most promising way to meet the exigencies of industry. Genetic engineering, micropropagation, tissue culture, and in vitro regeneration could reach the objective to produce, stabilize, and quickly propagate plant materials containing oils with a given composition or flavor, or even to produce selected secondary metabolites (Weiss, 1997). Much effort has been invested in these areas, and the results are often satisfactory, such as for mint (Tariqul Islam et al., 2003), some *Salvia* species (Olszowska and Furmanowa, 1990; Savona et al., 2003; Santos-Gomes and Fernandes-Ferreira, 2003; Scarpa et al., 2006), thyme (Iapichino et al., 2006; Shetty et al., 1996), rosemary (Gatti and Predieri, 2006; Misra and Chaturvedi, 1984), lavender (Lucchesini et al., 2003) alkanet (*Anchusa officinalis* L.; Su et al., 1994), periwinkle (*Catharanthus roseus* L.; Hirata et al., 1994), *Coleus* spp. (Petersen, 1994), myrtle (*Myrtus communis* L.; Rigoldi and Satta, 2006), anise (*Pimpinella anisum* L.; Santos et al., 1998), and many others. Notwithstanding, such technologies are still at an experimental stage and many technical problems remain to be solved. These include the

Fig. 1 Many essential oil crops are native to Mediterranean environments, where they grow as significant landscape components. In the photo, *Rosmarinus officinalis* L. (at flowering) in association with *Erica multiflora* L. (at the end of flowering) in a dry riverbed in the Nebrodi mountain (NE Sicily). (*Photo: R. Bontempo*)

stability of production, the occurrence of autotoxicity phenomena in the production of tissue cultures, and the productivity level, which is often very low (Collin, 2001).

Hence, the easiest and quickest way to obtain essential oils so far is the specialized cultivation of starting plant material. Many of the essential oils traded worldwide are obtained from plants that are native to Mediterranean environments (Fig. 1), and their wide trade opens new crop opportunities to farmers with arable land. At this point, a question immediately arises, and it relates to the choice of the plant-growing method. An overview of the world trade situation for spices, medicinal plant extracts, and essential oils (ITC, 2006), allows the observation that nowadays, and differently from the past, much attention is paid to organic production methods. This is even in the flavor and fragrance industries, which traditionally did not care about production methods, being generally more interested in obtaining a constant qualitative level of used raw material. In fact, some manufacturers have started setting special organic production lines (ITC, 2006a). The implications of such a tendency are many for crops strictly connected to the "organic" and "natural" market sector. Many European buyers, for example, tend to associate the production of herbs and related items to an idea of "naturality", and expressly require the herbs to be cultivated with organic methods in the belief that such methods confer to the products a higher healthiness value. When their "naturality" features are enhanced by means of organic labeling, it is possible for essential oil crops to meet the requests of more cautious and exacting consumers who are willing to pay more for a "natural" and "healthy" product (Bianco and Santoprete, 1996; Thomas and Dorko, 2006).

It is still debatable whether natural products are safer than other products. Although it is certain, for example, that pesticide residues in herbs may harm the consumer, it is still uncertain whether the use of chemicals may influence other traits, such as the essential oil composition. Some preliminary studies performed in such a direction on coriander oil composition (Carrubba et al., 2002) and peppermint oil yield (Gruszczyk, 2004) did not stress any difference between materials obtained with organic or conventional production methods, but of course this topic requires further experimentation. Until now, it was only possible to conclude that the higher prices that consumers are willing to pay for a certified organic product should compensate the higher production costs linked to organic management (Pank, 1993). In our case, it is true that many essential oil crops are suitable for cultivation with a reduced use of energy and technological inputs (Demarco et al., 1999), and the growing trend in Mediterranean cropping systems towards organic production techniques offers many new possibilities for such crops.

2 Essential Oil Crops and Development Strategies for Marginal Mediterranean Lands

Many definitions of "marginality" have been suggested (Gurung and Kollmair, 2005). According to that offered by the FAO Consultative Group on International Agricultural Research (FAO-CGIAR, 1999), "marginal lands" are those "having limitations which in aggregate are severe for sustained application of a given use". In such lands, increased inputs are required to maintain productivity, and without them, options for diversification are often limited. Because of their special configuration, marginal lands cannot be cultivated like other lands, simply because their resources cannot sustain the weight of ordinarily managed agriculture. Hence, it is necessary to find some agroecosystem able to guarantee the optimization of the use of resources and their correct maintenance over time, under the assumption of the maximum economy of off-farm inputs.

For a number of reasons, many Mediterranean lands, including large areas in the inner part of Sicily, cope with severe conditions of marginality, sometimes leading to the interruption of all agricultural activities and to the abandonment of the land. Some of these constraints are linked to special environmental features of the area that may be characterized, as an example, by extreme levels of temperature and/or moisture, pedological anomalies regarding soil depth, pH level, texture, salinity, toxic substances, and orography. Some Authors (Olesen and Bindi, 2002; Thomas et al., 2004) call attention on that a further worsening of these environmental constraints would be expected in future due to global climate warming. This could bring as direct consequences habitat losses and environment unsuitability for many species, starting from those areas having a higher fragility level. Such issues are expected to have a strong impact on agronomical practices, as e.g. the choice of genotypes to be included in cropping systems (Ventrella et al., 2007). A search in the literature offers many examples of essential oil crops finding suitable cropping conditions even under such special environmental conditions as drought (thyme, oregano, and milk thistle), extreme pH soil conditions (chamomile > 9.2 and Erica spp. < 4.0), or very high soil salinity levels (chamomile and liquorice) (Fig. 2). A few essential oil crops (vetiver, rosemary, and thyme) have even been successfully used to consolidate soils at risk of erosion (Bagarello et al., 2004; Durán Zuazo et al., 2004).

Fig. 2 Many essential oil crops may grow and produce under erratic climatic conditions. In the photo: sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) after a rare snowfall in western Sicily. (*Photo: A. Carrubba*)

The features above allow to suggest the introduction of selected essential oil crops in marginal farming systems as a proper and sustainable exploitation strategy (Carrubba and Catalano, 2007). Moreover, looking at the overall question from a wider point of view, some further remark is possible. The key concepts of the leading strategies used for the sustainable development of marginal lands are basically two: integration and diversification. First, all the intervention methods feasible for the development and exploitation of environmental resources of rural lands, especially when "marginal", must pay great attention to the integration of economic development, social development, and environmental protection as "interdependent and mutually reinforcing pillars of sustainable development" (UN, 2002). One of the main goals is to promote all economical activities that fit in unitary production pathways, as well as the production of raw material, including the first transformation, and, whenever possible, the packaging and marketing processes. A tighter linkage between production, transformation, and services for distribution and marketing of the products themselves is encouraged.

Second, the aspect of economic diversification of such areas must be considered. In a context in which the small and medium concerns are mostly represented by family farms, and very often the production relies on one cash crop with a secure albeit low market income, diversification could reduce the risks linked to agricultural practice. This seems to be one of the most concrete and quickest ways practicable for farmers to enhance their income level. Economic diversification, in this context, takes two different forms: diversification of crops and enhancement of the multifunctional role of agriculture. Crop diversification is considered the integration of new species, varieties, and gene pools inside existing agricultural systems, and, in such a sense, it is also encouraged as a useful way to promote biodiversity (COM, 2006; SAN, 2004). The aspect of multifunctional agriculture, on its turn, recalls the new role that is today assigned to agriculture, which is also the satisfaction of different needs, not only from the agricultural community, but also from society as a whole. According to its new role, besides ensuring food and fiber production, agriculture should also contribute to environmental safeguards, to the supply of recreational services, to the creation of alternative opportunities for income and employment for the farmers, etc.

Do essential oil crops fit into such a framework? An answer must first take into consideration the basic property of these crops, that is, their aptitude to be transformed. Interest in crops having good industrial potential, capable of producing valuable chemicals to address most industrial sectors' needs, known as "botanochemicals" (Buchanan et al., 1980) – a term that did not receive the diffusion and spread it deserved – is growing worldwide. Although essential oil crops are mostly used for the direct seasoning of foods, a major interest is nowadays coming from their potential as raw material for the production of food flavorings, additives, or industrial raw materials. This entails a higher degree of transformation (and therefore a higher market price) compared with fresh herbs. The use of low-cost on-farm equipment could help farmers increase their income by retaining on-farm the added value of the transformation process, developing in this way small, local, agrofood industries. Interest in this area has already been seen in the USA (Quinn et al., 1998), the West Asian and North African drylands (Amri et al., 2006), and in Europe (Cristóbal et al., 2005). Here, many "minor", "alternative", or "uncommon" crops, including essential oil crops, have been suggested to small farmers seeking to diversify their income source.

Furthermore, they represent a good opportunity for agrotourist concerns, helping to attract people from urban areas by means of the development of herb-based commercial items (handicrafts, oils, extracts, and honey) (Fig. 3) besides representing a further source of aesthetic land valorization (Deidda and Mulas, 2004; Devecchi,

Fig. 3 Coriander (*Coriandrum sativum* L.), being greatly attractive to insects, has a potential as a significant honey plant. (*Photo: R. la Torre*)

Fig. 4 The esthetic value of many essential oil crops may play a role in rehabilitative or healing gardens. In the photo: Clary sage (*Salvia sclarea* L.) at full bloom. (*Photo: A. Carrubba*)

2006; Domizi et al., 2006). It is worth noting that many essential oil crops, due to their special sensory attractiveness, are listed among the species to be utilized in rehabilitative or healing gardens suggested in the therapeutic programs of the newest "horticultural therapy" (Cooper Marcus and Barnes, 1999; Ferrini, 2003) (Fig. 4).

Of course, the first thing is to improve the economic value of the crops by maximizing their yield and reducing the cost of production. Regarding the first goal, research data show that it is not difficult, nowadays, to obtain good yields from many herbs. In Mediterranean environments, especially when marginal, special attention must be paid to the choice of the species to cultivate and on the cropping technique to apply; however, many such possibilities are available to farmers. Table 1 shows some examples of essential oil crops that could prove useful as crop species in Mediterranean marginal environments.

Some problems arise when the economic feasibility of production processes is considered. Most cropping techniques traditionally used for essential oil crops rely heavily on manpower. Because underdeveloped countries mostly have low labor costs, it is difficult for developed countries to compete because of their relatively

Table 1 Products, active ingredients, and chemotypes identified by literature of some selected essential oil crops grown in Mediterranean areas, according to botanical family

Plant	Utilized part	E.O. content (%)	Main active ingredients of E.O.	Identified chemotypes	References
Apiaceae (ex Umbelliferae)					
Anise (*Pimpinella anisum* L.)	Fruits (“seeds”)	1.5–5	Trans-anethole (80–95%), methyl chavicol, anis aldehyde.		Babulka (2004); Santos et al. (1998)
Caraway (*Carum carvi* L.)	Fruits (“Seeds”)	1.0–9.0	Carvone (45–62%), limonene (35–50%)		Lawrence (1996); Sedláková et al. (2003) and (2003a).
Coriander (*Coriandrum sativum* L.)	Fruits (“Seeds”)	0.5–2.5	Linalool (60–70%)		Carrubba et al. (2002); Diederichsen (1996)
Cumin (*Cuminum cyminum* L.)	Fruits (“seeds”)	2.1–2.7	γ-terpinene (11.4–18.5%), p-cymene (8.8–14.7%), cumin aldehyde (25.1–34.4%)		Bandoni et al. (1991)
Dill (*Anethum graveolens* L.)	Fruits (“Seeds), herb	2.3–3.5	Carvone (40–60%)	(i) limonene 40–51%, carvone 44–58%; (ii) limonene 31–41%, carvone 25–47%, dillapiole 6–32%; (iii) limonene 37–47%, carvone 18–46%, myristicin 0.2–20%, dillapiole 6–32%	Lawrence (1994); Simon (1993)

Table 1 (continued)

Plant	Utilized part	E.O. content (%)	Main active ingredients of E.O.	Identified chemotypes	References
Fennel (*Foeniculum vulgare* Mill.)	Seed, herb[1]	1–6	Anethole Fenchone (bitter fennel only)	(i) anethole >60% a) anethole >66.5%, estragole < 7%; b) anethole >63.5%, estragole 12.5–15%; c) anethole >60%, estragole <7% d) anethole >62%, estragole 8–15%, fenchone 16–25%; (ii) fenchone >30%; (iii) estragole >30%.	Bernath et al. (1996); Carrubba et al. (2005)
Asteraceae (ex Compositae)					
German chamomile (*Chamomilla recutita* (L.) Rausch.)	Flowers	0.2–0.4	α- and β-bisabolol, bisabololoxyde A and B, (pro)chamazulene.	(i) > bisabololoxyde A (chem. "A") (ii) > bisabololoxyde B (chem. "B") (iii) > α–bisabolol (chem. "C")	Franz (1992); Dellacecca (1996a)
French tarragon (*Artemisia dracunculus* L.)	Leaves, herb.	0.3–3.0	methyl chavicol (70–80%), anethol (10%), trans-β-ocimene (up to 22%), cis-β-ocimene (up to 15%), γ-terpineol (up to 17%), limonene (2–6%).		Arabhosseini et al. (2007); Bruneton (1995); Catizone et al. (1986)

Table 1 (continued)

Plant	Utilized part	E.O. content (%)	Main active ingredients of E.O.	Identified chemotypes	References
Lamiaceae (ex Labiatae)					
Basil (*Ocimum basilicum* L.)	Leaves	0.04–0.7	Linalool (15–60%), methyl chavicol (0–37%), eugenol (4–40%), 1,8-cineol (1–17%), cynnamic acid, anethole.	(i) linalool; (ii) methyl chavicol; (iii) both linalool and methyl chavicol; (iv) both linalool and eugenol; (v) both methyl chavicol and methyl eugenol.	Ceruti et al. (1993); Elementi et al. (2006); Grayer et al. (1996); Marotti et al. (1996); Sifola and Barberi (2006); Simon et al. (1990)
Calamintha (*Calamintha nepeta* subsp. *nepeta* (N), *C. nepeta* subsp. *glandulosa* (G)	Leaves, inflorescence	0.4–1.2	Pulegone, menthone, piperitone, piperitone oxide.	(i) high menthone (>40%); pulegone 19%, piperitone oxide *trans* 8%, limonene 5%. piperitenone oxide <1%; (ii) high piperitone oxide *trans*(≅ 30%); limonene 13%, piperitenone oxide 12–13%, menthone 9%, pulegone 12%; (iii) high pulegone (≅ 50–56%); menthone 20%, limonene 6%, piperitone oxide *trans*1%; piperitenone oxide <1%, .	Ristorcelli et al. (1996); Baldovini et al. (2000)
Clary sage (*Salvia sclarea* L.)	Leaves, inflorescence	0.19–0.52	Linalool (15–70%) linalyl acetate (14–77%)		Carrubba et al. (2002a)
Hyssop (*Hyssopus officinalis* L.)	Flower heads	0.3–0.9	α-pinene (50%)		Ceruti et al. (1993)

Table 1 (continued)

Plant	Utilized part	E.O. content (%)	Main active ingredients of E.O.	Identified chemotypes	References
Lavender and hybrids (*Lavandula* spp.)	Leaves, inflorescence	1.4–1.6	In *L. vera* DC: linalool (25–38%), linalyl acetate (25–45%), cineole (0.3–1.5%, camphor (0.2–0.5%). In *L. spica* auct.non L.: linalool (25–50%), linalyl acetate (< 3%), cineole (30–40%, camphor (8–20%) In *L. x intermedia* Emeric ex Loisiel: linalyl acetate (28–38%), cineole (4–7%, camphor (6–8%).	(i) linalool >30%; (ii) linalyl-acetate >30%; (iii) lavandulyl-acetate >25%	Bruneton (1995); Ceruti et al. (1993); Tucker et al. (1984)
Lemon balm (*Melissa officinalis* L.)	Leaves	0.05–0.2	Neral, geranial, germacrene-D		Ceruti et al. (1993)
Mint (*Mentha* spp.)	Herb, leaves	In *M. x piperita* L. var. *citrata* 1.2–1.4 on the whole plant, 2.5–2.8 on leaves.	Carvone, menthol, menthyl acetate, pulegone, linalool, linalyl acetate, 1,8-cineole	in *M. x piperita* L. var. *citrata* (Ehrh.) Briq.: (i) >linalool, <linalyl-acetate; (ii) >linalyl-acetate, <linalool in *M. pulegium* L.: (i) pulegone; (ii) pulegone-menthol; (iii) carvone-pulegone.	Ben Fadhel et al. (2006); Bruneton (1995); Diaz-Maroto et al. (2003); Malizia et al. (1996); Simon (1993); Paris et al. (1974)

Table 1 (continued)

Plant	Utilized part	E.O. content (%)	Main active ingredients of E.O.	Identified chemotypes	References
Oregano (*Origanum* sp.)	Inflorescence	0.5–4.0	Thymole Carvacrole	(i) high thymole; (ii) high carvacrole	De Mastro et al. (2004); Melegari et al. (1995)
Rosemary (*Rosmarinus officinale* L.)	Leaves, inflorescence	1.5–3.5	α–pinene, camphor, 1,8-cineole, borneol, bornyl acetate, verbenone	(i) cineoliferum (high 1,8-cineole); (ii) camphoriferum (camphor >20%); (iii) verbenoniferum (verbenone >15%).	Angioni et al. (2004); Carrubba et al. (2006a); Ceruti et al. (1993); Cioni et al. (2006); De Mastro et al. (2004a)
Sage (*Salvia officinale* L.)	Leaves, inflorescence	0.3–0.6	α– and β–thujone, 1,8-cineole, eucalyptol, linalyl acetate	*α- thujone/β- thujone ratio:* (i) 10:1 α/β, (ii) 1.5:1 α/β, (iii) 1:10 α/β.	Ceruti et al. (1993); Dudai et al. (1999); Perry et al. (1999)
Savory (winter) (*Satureja montana* L.) Savory (summer) (*S. hortensis* L.)	Inflorescence	0.5–1.0	Carvacrole Thymole	In *S. montana*: (i) high thymole (ii) high carvacrole	Bruneton (1995)
Thyme (*Thymus vulgaris* L.; *Thymus capitatus* (L.) Hoffm. & Link = *Thymbra capitata* L. (Cav.))	Leaves, inflorescence	0.5–1.5	Carvacrole Thymole	According to the prevailing occurrence of: (i) thymole (ii) carvacrole (iii) geraniol (iv) linalool (v) α-terpineol (vi) *trans*-4-thujanol and *cis*-8-myrcenol (vii) cineol	Bruneton (1995); Catizone et al. (1986); Granger and Passet (1973); Rodrigues et al. (2006)
Rosaceae					
Rose (*Rosa damascena* L., *R. centifolia, R. Gallica*)	Flowers	0.03–0.04	(-)-β-citronellol (38%), geraniol (14%), nerol (7%), eugenol (1%)		Retamar (1993)

[1]Only used for domestic purposes.

higher labor costs. For this reason, intensive cultivation with irrigation, adoption of improved varieties, and more effective cropping techniques and postharvest technologies (including better methods of dehydration) could lead to improvements in the productivity of such crops and also minimize the cost of production processes.

Another important concern, especially when the products are expected to be used for industrial transformation, is improving their quality. It is likely that in the near future, greater market penetration will be achieved by selling a higher-quality product, with control of microflora contamination, an improved shelf life, a guaranteed level of active ingredients, and produced according to set guidelines (with few or no pesticides). Growing such crops with organic cropping techniques (governed by the EU) that may offer a substantial safety guarantee to buyers and consumers is an important opportunity for farmers.

3 Essential Oil Production in Plants

In plants, essential oils are generally recognized as secondary metabolites. Chemically, their primary components are terpenes (mono- and sesquiterpenes, and to a lesser extent diterpenes) and aromatic polypropanoids, synthesized via the shikimate and mevalonate pathways (Croteau et al., 1986; Lamarti et al., 1994; Sangwan et al., 2001; Simon, 1990). The shikimate pathway intermediates and aromatic amino acids are precursors of a large number of secondary plant products (Herrmann, 1995; Kutchan, 1995). Each essential oil generally retains the organoleptic characters (taste and flavor) of the parent plant, and the special and unique aroma pattern of each plant species is provided by the characteristic blending of its aromatic components. This explains the huge number of fragrances that are available in nature and the fact that, in practice, industry considers each a whole raw material rather than a mixture of different chemical principles (Salvatore and Tateo, 1992).

Minimal variations in the ratio among components may generate important modifications in the aromatic profile of the essential oil. These are sometimes too small to be instrumentally detected, but large enough to be perceived by human senses. Many techniques have been developed to characterize the essential oils obtained from plants, and much effort has been devoted to studying their biological activities. Being secondary plant metabolites, their production in plants could vary with the environment (Sangwan et al., 2001; Bruni, 1999) and with the ability of the plants to allocate their resources. For example, crops that produce mainly primary metabolites would have high seed yields in favorable environments, and crops that produce high quantities of secondary metabolites would have high essential oil yields in unfavorable environments (de la Fuente et al., 2003). A further complication is the existing dynamic relationships between primary and secondary plant metabolism, where when there is a demand, the secondary compounds may be recycled back into primary metabolites (Collin, 2001).

Considerable research has been undertaken into essential oil production in plants, utilizing a large number of plants and many different technical and scientific approaches. Attention has also been paid to the simultaneous formation of different compounds within the plants that might affect the results of this research. Bouwmeester et al. (1995) argued that the formation of carbohydrates in seeds would result in an apparent increase of essential oils, even if essential oil formation itself has not been affected. The authors suggested that to avoid confusion, the absolute amount of essential oil in each seed should be referred to. However, the available literature indicates that the classical volume/weight percentage is by far the most often used method worldwide.

Essential oils are produced by plants for many reasons, including the attraction of pollinating insects (Lodi, 1986), repelling noxious insects by means of toxic, repellant, and antifeedant activities (Van Beek and de Groot, 1986; Simmonds, 1997; Bottega and Corsi, 2000), improving plant disease resistance (Goidanich, 1981), allelopathic effects that could be involved in interspecific competition mechanisms (Raven et al., 1979), or increasing drought resistance in semi-arid environments (Fluck, 1955; Munné-Bosch and Alegre, 2001). They are produced and stored in specialized plant structures that are distributed over the plant's entire epidermis or in special organs. These include the sacs or ducts of the epidermis in citrus peels or *Eucalyptus* leaves, or the glands and glandular trichomes originating from epidermal cells in *Labiatae* (D'Andrea, 2006; D'Andrea and Circella, 2006; Maleci Bini and Giuliani, 2006; Sangwan et al., 2001; Weiss, 1997) (Fig. 5).

Essential oils are processed from plants using distillation or extraction. Steam distillation is the most common method used by commercial-scale producers, and uses heat from steam or water to break the oil glands in plants and vaporize the oil, which is then condensed and separated from the wastewater. Distillation can be undertaken using on-site facilities, mobile units that come to the farm, or on-farm equipment that requires a significant, but not impossible, capital investment. Sometimes the waste, which retains many of the organoleptic traits of the herb, finds some market opportunity. For example, the wastewater from oregano distillation is

Fig. 5 *Thymus longicaulis* Presl. In evidence the essential oil glands in leaves. (*Photo: R. Bontempo*)

usually sold in Turkey as “Kekik suyu”, which is claimed to have positive digestive effects. The possibility has also been suggested that distillation wastewaters could be submitted to a further extraction process to recover a greater amount of essential oil (Rajeswara Rao et al., 2005). Several new processing facilities for oil extraction have been reported in the literature. For example, supercritical carbon dioxide, microwave-assisted hydrodistillation, or novel solvent extraction techniques (Joy et al., 2001; Kosar et al., 2005; Platin et al., 1994; Riela et al., 2008; Sedláková et al., 2003) have been suggested, but in many cases their costs are prohibitive for on-farm realization.

4 Cultivation of Essential Oil Crops: Goals and Constraints

In order to improve the economic competitiveness of essential oil crops, the first important step is to state the goals for such cultivation. There is a considerable difference between cultivation for producing herbs, essential oils, or secondary metabolites dealing with some biological activity. Generally speaking, these may be thought of as subsequent steps, with each product representing the raw material for the following industrial pathways. Consequently, the income level that may be obtained from passing one type of product to the following processing step will vary (Carrubba et al., 2006c). As an example, dried rosemary is a commercial herb *per se*, but it may also be considered the starting material for the production of an essential oil, which may further represent the raw material for the extraction of some active principles dealing with antioxidant properties. Each single step:

(1) requires a higher technical refinement than the preceding one,
(2) confers to the obtained product a higher degree of economic value, and
(3) possesses very specific quality standards to which each product must conform.

Usually, the income derived from producing the raw material for one step varies from that of another. This is because of the different levels of expertise required when shifting from an agricultural to a more industrial process. Retaining as many production steps as possible on-farm would enable farmers to obtain higher added value, which could prove a great advantage.

The first economic interest in essential oils is oriented towards the industrial exploitation of their naturally occurring actions, that is, the activity due to their aromatic, insecticidal, antioxidant, and antimicrobial compounds. These would be mostly used as natural products in food, cosmetics manufacturing, and preservation. In fact, many papers have been published worldwide regarding the numerous properties of essential oils. For example, in 2003, Kalemba and Kunicka estimated that more than 500 works had been published just concerning their antimicrobial activity. It is likely that this number has now been greatly surpassed.

The scientific finding that an essential oil possesses some specific activity (antimicrobial, antioxidant, etc.) does not represent per sea certainty of its suitability to industrial use. In order to find a suitable use, every plant extract must retain very specific characteristics, roughly summarized by the triple constraint “quality–security–effectiveness” (Franz, 1996), i.e., it is necessary that the products derived from it must fulfill adequate and constant quality standards, address consumers’ concerns regarding the safety of their use, and be satisfactorily effective. Regarding the last requirement, some concern is related to the amount that needs to be added to the various industrial items to show a good effectiveness of use. As an example, when essential oils are intended as antioxidants for food manufacturing, in order to have a satisfactory effectiveness they must be added to foodstuffs in very large amounts. This inevitably leads to a modification of the taste and flavor of the products to which they are added. For this reason, they may only be added to a limited number of suitable food items (Roller, 1995; Brul and Coote, 1999). In cases in which the typical scent of a given herb is unpleasant, a recent possibility is offered by deodorized extracts, endowed with technical properties identical to those of the starting plant material, but absolutely free from its typical odor. As an example, starting from rosemary extracts, some antioxidant mixtures have already been patented, produced, and sold, such as GUARDIAN™ (Danisco Co. Ltd., Denmark) (Fig. 6).

Fig. 6 Rosemary (*Rosmarinus officinalis* L.) has been largely studied for its antioxidant properties. (*Photo: A. Carrubba*)

5 Factors Affecting Essential Oils Yield and Composition

Because the first goal of cultivating essential oil crops, whatever their final use, is to obtain adequate amounts of plant material, biomass productivity is obviously the first target. Many studies have been performed around the world to improve various aspects of productivity of essential oil crops and the role of agronomic practices on yield (Pank, 1993; Carrubba et al., 2006; 2006c). It must be considered, however, that when quality aspects are concerned, conclusions may be dramatically different from those concerning bare quantitative aspects. In some cases, the same factors positively affecting the biomass yield of one herb might exert a negative action on its quality features. This is the reason why a decision about the goal of cultivation should be taken as the first priority. In so doing, the same species could be cultivated according to different cropping protocols that would depend on the kind of product to be obtained.

Many factors are claimed to exert an influence on the yield and chemical composition of essential oils. Generally, these factors are classed as "endogenous" and "exogenous". The first group includes all characteristics natural to the plant, such as its genetic constitution, but also other nongenetic factors such as its age or development stage. The second group includes all external factors that plants may experience during their growth cycles (Bruni, 1999). Both groups have extremely variable effects on essential oil quantity and quality. First, it has been ascertained that certain essential oil components are more sensitive than others to variations in plant characteristics and environmental conditions. This is the case with some terpenic compounds such as α-pinene, p-cimene, α-terpinene, and linalool in coriander (Carrubba et al., 2002), or α-thujene, α-terpinene, β-phellandrene, and camphor in fennel (Carrubba et al., 2005). These were of the greatest importance in the assessment of the variability of essential oil composition, whatever its source (geographic provenance, crop management, and age of the samples).

5.1 Endogenous Factors: The "Inner" Sources of Variability

Because the essential oil composition is governed by the biosynthetic pathways (which are under enzymatic control) that act in plant metabolism, it is undoubtedly genetically determined. Studies performed on the heritability of qualitative essential oil traits have ascertained that the biosynthesis of certain compounds (such as prochamazulene or bisaboloids in chamomile) underlies a simple Mendelian behavior (Franz, 1992). The studies found that an "aut/aut" law applies (they may or may not be there) and allowed the deduction that the compounds are determined by only one (or few) gene(s). Other compounds are, instead, under polygenic control, and the continuous conversion from one compound to another (such as the shifts among the various bisaboloid forms in chamomile) may explain the occurrence of intermediate chemotypes dealing with different amounts of the above compounds (Franz, 1992; Wagner et al., 2005). On this genetic basis, however, all the other factors play

Fig. 7 Many essential oil crops reach their maximum essential oil content at flowering time. In the photo: Oregano (*Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart) at full blooming. (*Photo: A. Carrubba*)

some role, and a great deal of experimental work has been carried out on this subject. Concerning the nongenetic endogenous factors, it is generally acknowledged that most aromatic plants gain their maximum levels both in yield and in the quality of their essential oils when they are close to the blooming stage (sometimes at the beginning, sometimes at the end) (Fig. 7). A higher essential oil content near flowering was noted for oregano (Ietswaart, 1980; Putievsky et al., 1988), peppermint (Dellacecca, 1996), *Artemisia annua* (Chalchat et al., 1994), *Thymbra spicata* (Müller-Riebau et al., 1997), *Thymbra capitata* (Rodrigues et al., 2006), and *Ocimum basilicum* (Macchia et al., 2006). In some cases, it was also confirmed when the commercial product was formed by parts of the plants other than the flowers (Shultz and Stahl-Biskup, 1991). This general feature is not always so definite, and a different tendency was observed in *Salvia officinalis*, in which oil obtained from shoots collected in May (i.e., at flowering) was 1.6 mL kg^{-1}, much less than the 4.7 mL kg^{-1} achieved when harvested in September (Scartezzini et al., 2006). Similarly, in clary sage (*Salvia sclarea* L.) an increase in the essential oil content of inflorescences was found when they were passing from the full blooming stage to the seed ripening stage (Carrubba et al., 2002b). In trials carried out on basil, maximum oil yield was obtained at the 50% seed set stage (Sangwan et al., 2001).

Ontogenetic development may also influence the biosynthetic pathways of oil constituents (Piccaglia et al., 1991), and therefore their relative quantities in the essential oils. In peppermint (*Mentha* × *piperita* L.), the (-)-menthone content was found to decrease and (-)-menthol content was found to increase during the vegetative cycle (Bruneton, 1995). *Thymbra spicata* showed an increase in the concentration of phenols, especially carvacrol, from spring to mid-summer (June–July), when the plants had reached full blooming (Müller-Riebau et al., 1997). In clary sage, moving from full blooming to seed ripening resulted in a significant increase

Fig. 8 In sage (*Salvia officinalis* L.), the essential oil obtained from younger leaves has a different composition with respect to older leaves. (*Photo: A. Carrubba*)

in some important oil compounds, e.g., linalyl acetate, which was found to vary from 35% to 53% (Carrubba et al., 2002b). In common sage (*Salvia officinalis* L.), a delay in the collection of shoots from May to September resulted in a decrease of α- and β-thujone and camphor in the oil, and therefore to an improvement in oil quality (Scartezzini et al., 2006). Significant variations in essential oil components with the growth stages of plants were also assessed in leaves of both *Ocimum basilicum* (Macchia et al., 2006) and *Coriandrum sativum* (Smallfield et al., 1994).

Irrespective of development stage, in perennials, the age of the plant seems to play some role as well: oils extracted from *Lavandula spica* Vill. (Carrasco, 1980) and peppermint (Dellacecca, 1996; Gruszczyk, 2004; Piccaglia et al., 1993) have shown important variations both in yield and composition from one year to the next. Probably due to a similar mechanism, several trials on sage found significant differences between oil yield and composition in lower (older) leaves compared with upper (younger) leaves (Bezzi et al., 1992; Dudai et al., 1999) (Fig. 8).

5.2 Exogenous Factors: Variability Due to the Environment

The second group of factors ("exogenous") includes all growing and environmental conditions (e.g., temperature, daylength, quality of light, soil and air moisture, wind patterns, and nutrient levels) that may exert some direct or indirect influence on essential oil production and accumulation in plants. Such an effect may be more or less intense, e.g., being more important in species having a more superficial location of oil storage structures, such as the glandular trichomes in *Labiatae* (Bruneton, 1995). Studies regarding the effect of climate on yield and composition of secondary metabolites are many, and have often led to interesting results. For example, the common belief that aromatic plants possess a stronger aroma when grown under arid and sunny climates seems to find a scientific basis from the demonstrated increased activity of the phenylalanine ammonia lyase (PAL) enzyme under these prevailing climatic conditions. This is because PAL causes protein synthesis to shift towards the production of phenols, which are the major compounds responsible for the aromatic features of essential oils (Landi, 1994). A relationship between various climatic indexes and the occurrence of certain chemotypes was noted for *Thymus piperella* L. (Boira and Blanquer, 1998).

However, if studies concerning climatic conditions as a whole are interesting in the assessment of the distribution of a certain genotype, great scientific interest is linked to ascertaining the environmental trait responsible for a given action on essential oil biosynthesis. This is quite a difficult task, especially in Mediterranean areas where chemical polymorphism is important (Boira and Blanquer, 1998) and its relation with environmental factors is the subject of a deep debate.

Temperature surely plays a crucial role, and since all secondary metabolites are the result of a series of biochemical steps, each with its own optimal temperature, it is possible that the best temperature for obtaining a specific compound is the one resulting from the optimal temperature levels for the single reactions (Catizone et al., 1986). High (but not excessive) temperatures are considered, as a whole, to be best for producing essential oils, a result validated by much experimental data on *Pelargonium* spp. (Motsa et al., 2006) and chamomile (Bettray and Vömel, 1992). The composition of essential oils in relation to temperature has been studied as well, and, for example, in chamomile, the (-) α-bisabolol, pro-chamazulene, and apigenin content in flowers increased significantly when the temperatures were raised from 16 °C to 20 °C to 26°C (Bettray and Vömel, 1992) (Fig. 9).

Because secondary metabolites are a side effect of photosynthetic activity, it may be expected that variations in light duration, intensity, and quality can affect their production in plants. Generally speaking, plants growing under good illumination

Fig. 9 In essential oil from Chamomile (*Chamomilla recutita* Rausch.) some compounds show an increase with temperature. (*Photo: A. Carrubba*)

exhibit an increase in oil yield with respect to the same plants grown in shade. This feature was demonstrated in *Pelargonium*, both in whole plants (Kaul et al., 1997) and in tissue cultures (Brown and Charlwood, 1986), where the oil obtained from tissue cultures of *Pelargonium fragrans* exhibited 50% limonene in dark-grown tissue cultures compared with 5% limonene in the oil extracted from the parent plants. A general biochemical explanation of the higher amount of esters (highly aromatic substances) in plants grown in sunny areas suggests that the photolysis reaction, by eliminating the water molecules obtained from the esterification processes inside plants, would stabilize esters in the plants themselves (Catizone et al., 1986). Photoperiod was also noted as a crucial factor in oil production and composition, and a long photoperiodic treatment was responsible for an increased amount of *cis*-sabinene hydrate in marjoram oil (Circella et al., 1995) and higher menthol content in some mint species (Fahlén et al., 1997), probably due to a favored conversion of menthone to menthol in the leaves (Voirin et al., 1990).

The effect of light quality was taken into account by Maffei et al. (1999), whose data demonstrate that UV-A radiation on peppermint during the day generates an increase in total leaf area and total essential oil content, menthofuran, and menthol.

Many other examples could be considered to demonstrate the importance of environmental factors in assessing (alone, in interactions with themselves, or in genotypic interactions) the various quality aspects of essential oil crops. It is not incorrect to note that cropping techniques are also an important source of variation in the growth of plants, and that a difference in crop management may therefore generate important variations in plant biochemistry and quality.

6 Breeding Activity

Research into the breeding and genetic improvement of essential oil crops has been sparser than the efforts expended on other crops such as cereals, and much work remains to be done: genetic variability in essential oil crops is considerable and scarcely explored, and a great possibility exists to use this variability for future breeding programs. Notwithstanding, the literature shows many examples of screening, selection, and breeding processes of essential oil crops, utilizing various techniques ranging from traditional crossing and selection methods (Dudai et al., 1999; Landi, 1994; Landi and Bertone, 1996) to the most advanced biotechnology programs (Shetty et al., 1996; Novak, 2006).

Efforts into breeding essential oil crops may take one of two different approaches (sometimes both): genetic improvement for crude yield and genetic improvement for one, or more, selected qualitative features. In many cases, unfortunately, it seems that most of the results of breeding and selection efforts are still unavailable to farmers; very few essential oil crops may show satisfactory availability of certified reproduction material, and most are cultivated using locally grown ecotypes, devoting little interest to the choice of the best genotype (Fig. 10).

Fig. 10 Few essential oil crops have a satisfactory number of commercially available improved varieties. (*Photo: A. Carrubba*)

6.1 Breeding for Biomass Yield

The approach oriented to the obtainment of high crude yield involves selection for enhancing the biomass yield of a certain plant, or its marketable part (seeds, roots, or flowers). That means, the plant's growth mechanisms should be pushed to the highest efficiency in exploiting environmental resources. Some authors (McConnell and Anderson, 2002) call attention to the generally low environmental plasticity of some of the crops above, in that behaving as "weedy" species does not enable them to succeed in exploiting environmental resources, and they improve performance under low fertility conditions. This is the case for some species grown for fruit ("seed"), such as coriander and dill, where higher fertility conditions push towards an enhancement of biomass production, consequently reducing seed yield.

It is likely that much work remains to be done to develop genotypes more capable of "capitalizing" on environmental resources, and therefore to react more positively to technical inputs such as fertilization.

In fact, many of the most common essential oil crops bear morphobiological traits originating from their adaptation to growing in the wild, and that retaining such traits often sets limits to their agronomic suitability. Breeding was, therefore, also addressed to solve some agronomic problems that may arise in cultivation, such as seed dormancy, indeterminate growth habit, or lodging tendency (Holm and Slinkard, 2002; Langbehn et al., 2002). Seed dormancy may be an important concern when planning and managing sowing operations, and strategies to cope with this inconvenience may vary depending on whether it is caused by physical (such as thickness or special traits of the outer seed layer, which is easily fixed by seed scarification) or physiological mechanisms. A few studies have been oriented towards the study of the mechanisms underlying seed germination, and, although information is far from complete, some interesting conclusions may be drawn. For example, it has been ascertained that in some species, especially those bearing small seeds, the germination process is tightly dependent upon light, showing a strong inverse correlation with the depth of planting (Benvenuti et al., 2006). In other studies, the use of hormones such as gibberellic acid has shown good effects on breaking dormancy in seeds of *Lavandula angustifolia* Mill. (Macchia et al., 1996).

An indeterminate growth habit is considered an important adaptive trait to extend the reproductive period and ensure reproductive success for plants in environments in which the availability of water in the soil is variable and unpredictable (Arnon, 1992). Similar to seed dormancy, this is a very common trait correlated to the lack of genetic amelioration in many essential oil species, especially in the *Apiaceae*,

Fig. 11 Indeterminate growth habit may be a problem in many essential oil crops. In the photo: Coriander (*Coriandrum sativum* L.) plants bearing flowers and seeds at different ripening stages. (*Photo: A. Carrubba*)

but also in other families. It is mostly considered unwelcome because the presence of reproductive organs at various stages of development in plants may be a serious constraint to the mechanization of harvest (Fig. 11).

In some species, the results of breeding activity addressed to solve these agronomical problems and enhance plants productivity have been rather satisfactory: due to the efforts of research centers, e.g. from Canada (Blade and Slinkard, 2002) and India (Kallupurackal and Ravindran, 2005), in a few annuals such as coriander, caraway, fennel, and dill, some improved material is already available. Similarly, high-yielding clones of essential oil perennial species such as rosemary, lavender, or thyme have been selected (Catizone et al., 1986; Mulas et al., 2002; Rey, 1992; Verlet, 1992). Additional efforts should be oriented to a wider diffusion of such improved genotypes.

6.2 Breeding for Qualitative Traits

Concerning breeding for essential oil yield and chemical characteristics, intense research has been conducted worldwide, and many plants have been studied to investigate the composition of their oils to detect the occurrence of valuable and stable chemotypes. Chemotypes, or "chemical breeds" (Bruneton, 1995) are groups of individuals within each species that even while bearing the same morphological structure may be distinguished according to special characteristics of their chemical traits. Studies in this direction have led to the establishment of a new discipline: the study of plant classification called chemotaxonomy (Granger and Passet, 1973; Granger et al., 1973; Weiss, 1997).

Of course, the choice of the most proper chemotype, suitable for a selected market sector or industry, could be crucial for the commercial success of the species to be cultivated. Table 1 shows some of the chemotypes found in various essential oil crops from the available literature; examples are given for thyme, oregano, and lavender, which are targeted to the many studies characterizing and exploiting their essential oils (Verlet, 1992).

Breeding activity regarding chemical oil characteristics has been primarily directed to the selection of genotypes having high quantities of special compounds with a particular economic value or considered primarily responsible for the aromatic properties of the essential oil, such as *cis*-sabinene hydrate in marjoram (Langbehn et al., 2002), and chamazulene and bisabolol in chamomile (Franz, 1992). Otherwise, it was directed towards obtaining genotypes with a low content of unwanted compounds, such as thujone in sage or elemicin in tarragon (Catizone et al., 1986). Also in this case, a further diffusion of these improved genotypes would be of great practical interest.

7 Cropping Technique and Quality Traits

Because they act to modify the growth environment of plants, cropping techniques are often crucial in assessing many quality traits of essential oil crops; a search of the literature shows a major influence of agronomic factors on their yields and essential oil composition. Hence, the choice of cropping technique must be straightforward and fit into the rotations and mechanization of the farm. However, it appears that much effort must still be applied to the development of seed selection, breeding, harvesting technology, distillation technology, and organic production.

7.1 Propagation and Planting Management

Many essential oil crops (such as hybrid peppermint) are sterile; hence, they must be propagated vegetatively by rhizomes, stolons, or plant parts. When seed propagation is possible, it could present an interesting opportunity for farmers. However, the choice of propagation method is claimed to exert a significant effect on the quality traits of many essential oil species. When the crops are open-pollinated, their seeds often produce plants that are not homogeneous for growth or aroma. Bruneton (1995) reports, as an example, significant variations in the chemical composition of essential oil obtained from lavender plants propagated by seed or vegetative multiplication, and he concludes that the second method is more suitable for cultivating plants bearing constant selected morphological, biological, and qualitative characteristics. The use of direct seeding in the field is a rather difficult practice for perennials, because, as experienced for sage (Caligani and Adamo, 1987), their generally slow establishment in the field causes many problems concerning competition with weeds (Fig. 12).

In addition, planting methods (population density, arrangement in space, time of sowing, or planting) may be crucial for obtaining the best cultivation results

Fig. 12 Emergence may be a crucial factor in the establishment of essential oil crops. In the photo: a germinating fennel (*Foeniculum vulgare* Mill.) plantlet. (*Photo: R. la Torre*)

Fig. 13 A proper settlement of rows and inter-row distances has a major importance in crop management. In the photo: 1-year-old Oregano (*Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart) at full blooming. (*Photo: M. Militello*)

(Fig. 13). Plant population is important because essential oil crops may have varying responses to increases in intraspecific competition. In sage, a higher plant density was accompanied by a lower unitary plant biomass, because of a lower number of leaves per plant and a smaller leaf size, but the higher number of plants per unit area seemed to compensate for this feature (Bezzi et al., 1992). Similarly, many species cultivated only for plant biomass (such as sage, oregano, rosemary, and thyme) seem to react positively to an enhancement of plant population provided there is a satisfactory level of water and nutrients in the soil (De Mastro et al., 2006). It is not clear, however, if such a positive response in terms of aerial biomass is accompanied or not (and if it is, to what extent) by modifications in essential oil composition. Some results regarding this may have been obtained for peppermint (Dellacecca, 1996), in which variations both in yield and content of essential oils were assessed with varying plant populations. However, in oregano, a decrease in essential oil yield with higher plant density seemed to be mostly due to a higher percentage of stems and woody parts (having a lower essential oil content) in the harvested material (Scarpa et al., 2004).

Concerning planting date, many annual essential oil species (anise, cumin, and coriander), being fairly sensitive to frost and cold, are usually sown in spring. Notwithstanding, in Mediterranean environments, in which climatic patterns are characterized by mild winter temperatures, a prevailing distribution of rainfall in autumn and winter, and severe drought periods in spring and summer, an earlier sowing date is claimed to exert a major effect on the establishment of crops. This is because it allows plants to grow when the water content in the soil is still satisfactory, i.e., before drought occurs. In any case, an earlier intervention allows, generally speaking, a higher stand uniformity and plant population, and therefore higher biomass yields (Catizone et al., 1986).

This general statement seems especially important in annual crops, since sowing date may influence the timing of harvest. This, in turn, may be very important both for quantitative and qualitative aspects of production. The advantage of an earlier sowing date, well assessed in coriander (Carrubba et al., 2006b; Luayza et al. 1996), fennel (Leto et al., 1996; Masood et al., 2004), anise (Zehtab-Salmasi et al., 2004), and cumin (Mirshekari, 2004), could be due to the progressive lengthening of the vegetative growth stages, especially those immediately preceding the onset of flowering, the duration of which has shown a high direct correlation with yield in some experiments (Carrubba et al., 2006b).

In perennials, experimental findings seem to give the same results, and under semi-arid climatic conditions, there is a substantial agreement on the necessity to make the planting date close to rainy periods, even when the crop is managed under irrigation (Rajeswara Rao, 1999). Such a choice allows a better establishment of crops, e.g., in peppermint it allowed the formation of many runners and an earlier canopy closure, which resulted in higher biomass yields when compared to a spring-planted crop (Piccaglia et al., 1993). A similar result may be found concerning essential oil yields, which clearly showed a decrease with a postponement in sowing date for peppermint (Piccaglia et al., 1993), dill (Hornok, 1980), anise (Zehtab-Salmasi et al., 2004), and cumin (Mirshekari, 2004). In coriander, sowing date seemed to have a slight influence on oil composition (Carrubba et al., 2006b).

7.2 Weed Management

This is one of the major constraints on the cultivation of essential oil crops. Weeds exert an effect as crop competitors, are responsible for problems of harvest mechanization, and when mixed with the harvested product may alter its end quality (Fig. 14). Competition with weeds, which are involved in the sharing and consequent allocation of environmental resources, may reasonably be considered a factor affecting essential oil yield and composition. In an Argentinean coriander landrace

Fig. 14 Weeds may be a major concern for essential oil crops cultivation. In the photo, a heavily infested fennel (*Foeniculum vulgare* Mill.) plot. (*Photo: A. Carrubba*)

(Gil et al., 2002), weeds were a significant factor in determining the geraniol and geranyl acetate content in the oil, but it was difficult to ascertain their real effect because it acted in interaction with location and year.

In some cases, the use of herbicides has been studied with good results. For example, the production of aromatic oils seems unaffected by herbicide application (provided the crop is tolerant to its active ingredients), and the quality of oil may even be improved (Pank, 1992; Zheljazkov and Topalov, 1992). It is true, however, that many active ingredients in herbicides have not been expressly tested on essential oil crops. Furthermore, the choice of organic production method (which, as previously stated, is often a precise choice for essential oil crop growers) sets a limit to the possibility of intervention with chemical products; in this case, the choice is restricted to a few allowed techniques. Many nonchemical solutions suitable for use under organic management have been suggested (Bond et al., 2003; Kristiansen, 2003), with results that varied according to plant species, timing of intervention, and expected results.

First, the use of transplantation instead of direct sowing may be useful for planting larger, and therefore more competitive, individuals in the field. The adoption of double instead of single rows, successfully tried for oregano (Carrubba et al., 2002a), could allow a more satisfactory execution of mechanical weed control.

Mechanical weeding is, by far, the most immediately applicable method for weed management when the use of chemicals is undesirable (Chicouene, 2007). It must be applied taking into consideration the growth stages of the crop and weeds, as well as the biology and characteristics of the weeds, but in most cases, one or two treatments are enough.

In fact, the greatest difficulty in mechanical weed control is the necessity of planning crop settlement and taking into account, from initiation, the kind of equipment used for weeding, and therefore properly setting inter-row distances. Many of the failures of mechanical weeding are linked to this lack of management.

Mulching (Fig. 15) has been successfully tried, and many growers have obtained good results using polyethylene mulch or black porous plastic (Galambosi and Szebeni-Galambosi, 1992). In cultivation trials of *Artemisia absinthium,* mulching resulted in a 5% increase in average plant weight (Giorgi et al., 2006).

Alternatively, an environmentally friendly technique is flame control, performed with special equipment that when passed over and around weeds, quickly boils the water in their cells, causing wilting of the apex and death. Flaming was tried on some essential oil crops such as coriander and fennel (Carrubba and la Torre, 2006), and sage and lavender (Martini, 1996), and the results seemed to depend upon the seasonal climatic patterns and the competition between the crop and the weeds. The low labor required represents an important advantage of flaming, but for effective weed control, an exact timing of the intervention is crucial, since flamers should be used when weeds are still young and tender. Flaming kills annual weeds completely (although more will reappear), but it does not kill the roots of perennial weeds. These will send up new shoots within a week or so after flaming; therefore, additional treatments are often required.

Fig. 15 Mulching may help in managing weeds, but the more resistant weeds may pass through the plastic film. In the photo: plastic mulch on coriander (*Coriandrum sativum* L.). (*Photo: A. Carrubba*)

7.3 Soil Nutrients and Fertilization

The nutrient level in the soil is one of the most investigated aspects of agricultural research, also including research into essential oil crops. The effect of N fertilization has been studied in detail for peppermint (Dellacecca, 1996; Piccaglia et al., 1993), sage (Bezzi et al., 1992), and marjoram (Trivino and Johnson, 2000). In general, N fertilization seems to promote plant development, but without any enhancement in essential oil. In some cases, it even seemed to negatively affect crop results, for example, allowing leaves to develop instead of other desired plant parts, delaying flowering, or interfering with the production of essential oils, such as in *Lavandula spica* (Catizone et al., 1986). An interesting hypothesis (Mirshekari, 2004) suggests that there is an inverse correlation between protein and essential oils in plants; hence, all factors that promote protein synthesis (such as N fertilization) would have a depressive effect on essential oil yield. Apparently, contrasting data come from experiments on peppermint (Piccaglia et al., 1993) and geranium (Araya et al., 2006), in which N fertilization increased not only plant biomass but also the essential oil yield per unit area. This increase could possibly be a consequence of the increased biomass level rather than an effect of the enhancement in oil percentage. In coriander, significant variations were found both in essential oil content and composition when N fertilization was enhanced from 0 kg ha^{-1} to 135 kg ha^{-1}, but the direction and amplitude of these variations were also affected by growing conditions (year and cultivation site) and genotype (Gil et al., 2002).

Much less abundant (and less conclusive) is the literature about the effects of N fertilization on essential oil quality traits. Gil et al. (2002) found that it affected the linalool content of coriander, but this was noted only in a European landrace; an Argentinean landrace was not affected at all. Piccaglia et al. (1993) found an increase in pulegone content in peppermint oil by increasing the N fertilization rate, but such an effect was detected only in one of two years.

Some interesting findings concern the effect on yield of organic N fertilization: yield enhancements have been claimed for biomass and oil in *Pelargonium* spp. (Araya et al., 2006) and in coriander seeds (Ursulino Alves et al., 2005). In both cases, the authors suggest that, besides the bare nutritional effect, some influence should be attributed to the positive action of organic fertilizers towards some soil characteristics, namely the water holding capacity, cation exchange capacity, and microbial activity.

Concerning other nutrients, there are few reference papers: generally speaking, P is claimed to have a positive influence on the development of reproductive organs and to stimulate flowering, whereas K has positive effects on root development (Radanović et al., 2004). However, to our knowledge, few experiments have been performed regarding the effect of such elements on yield and quality traits of essential oil crops. An experiment on P fertilization in peppermint (Piccaglia et al., 1993) recorded an increase in menthol content with increasing P dose, but this was only noted in one of two years. As such, no definite conclusions can be drawn.

7.4 Irrigation

Water deficiency has a major role in the growth and yield of crops, and this has been studied in depth for many plants that are native to, or cultivated in, Mediterranean environments (Fig. 16). The effects of water shortage may vary according to the duration and severity of stress, the resistance and/or tolerance features of the plant,

Fig. 16 Watering has a major effect on biomass yield, especially in dry and semi-arid climates. In the photo: sage (*Salvia officinalis* L.) under irrigation. (*Photo: A. Carrubba*)

and the plant material to be harvested (whole aerial biomass, leaves, roots, flowers, or seeds). In myrtle (Vicente et al., 2006) and *Mentha arvensis* (Misra and Srivastava, 2000) water stress exerted significant reductions in plant biomass, including plant height and leaf area, but it did not have, at least in the latter species, any significant effect on oil yield and composition. However, in *Artemisia annua*, it resulted in a shortening of plant height, but only when induced in the two weeks before harvest (Charles et al., 1993).

When yield is represented by seeds and fruits, it is very often mainly determined by photosynthesis occurring after flowering (Arnon, 1992), and water stress should therefore have more negative effects when experienced at that phase than at any other growth stage. A direct consequence is that all annual species that finish their growth cycles in summer could be seriously affected by water shortage at the seed-filling stage, a feature not rare in Mediterranean environments. Under such situations, emergency watering could be a great help to production.

In perennials grown under dry or semi-arid climates, the recourse to irrigation (better if coupled with N fertilization) should push production towards its highest levels, and therefore represent an effective technical choice to obtain abundant and homogeneous yields. This is, for example, the choice of many Mediterranean farmers who want to cultivate sage or oregano in open irrigated fields. In this case, the costs needed for such an operation (water supply and the setting of watering lines) must be justified by the higher prices obtained from the sale of the product.

Frequently, water is not readily available and the recourse to irrigation is too expensive; here, essential oil crops are grown under a dry regime. In this case, annual plants can only use the water stored in the soil after the autumn–winter rainfall, and perennials are restricted to one cutting, normally taken at flowering time. In intermediate cases, farmers let the crop grow without irrigation for most of its cycle, and perform an irrigation after the main cutting is taken (at flowering time); in this way, the plants are allowed to bear a second harvest during the year, formed by the leaves that have regrown after watering.

When the water supply is limited, irrigation may be used occasionally as an emergency intervention if water stress threatens intolerable injury to plants. Above all, it is used following little or no rainfall, or as a planned intervention scheduled for the more critical development stages of crops, namely, the phases in which a water deficit could exert the worst effects on yield. Because good crop establishment is crucial for the success of almost all perennial crops, including lavender, oregano, thyme, mint, and rosemary, watering soon after transplantation is considered an essential practice.

If in Mediterranean semi-arid environments irrigation exerts a strong positive effect on biomass yield, under different climatic conditions crop responses are not always so positive: a field trial in Saskatchewan on German chamomile, as an example, gave a surprisingly much higher herbage yield under dryland conditions than following recourse to irrigation (Wahab and Larson, 2002).

An overall increment in oil yield per area unit, due to the highest leaf production, was observed under irrigation in sage (Bezzi et al., 1992), even when the essential oil percentage was not affected. In mint, it has been noted that a controlled induction of water stress could even increase oil accumulation, without having any effect on oil composition (Simon, 1993). Many authors, however, call attention to a negative effect that irrigation could have on essential oil content, stressing the importance of proper water management on oil yields.

7.5 Mechanization and Harvest

Mechanization is one of the areas in which research is lacking, but in which there are the strongest opportunities to develop new techniques and facilities that may significantly reduce production costs. Studies regarding the mechanization of essential oil crops may be divided into three main sectors: seeding/transplanting operations, weeding, and harvest.

The first group of operations differs in importance according to the species grown; it is generally a crucial aspect for many annual species, especially those with smaller seeds, in which setting seed distribution to desired values is more difficult, and it is generally difficult to achieve the planned plant population. Furthermore, smaller seeds require more care in soil preparation, because an uneven distribution will generate inhomogeneity in stands and operational difficulties at harvest. An interesting experience comes from southern Italy, where chamomile was mechanically sown by distributing the seeds on the tractor tires; it was also performed in this way to benefit simultaneous soil compression. In perennials, specific studies have addressed the mechanical transplantation of sage and lavender (young rooted plants), *Iris pallida* (the rhizomes), and saffron (the bulbs) by means of different equipment obtained with small modifications to normally adopted machines (Caligani and Adamo, 1987).

Along with mechanical weeding, that has been discussed already in a previous section, harvest exerts a strong effect on yield, both from quantitative and qualitative points of view. In many cases, and for many essential oil crops, manual harvesting allows a more careful operation, and therefore a higher yield and quality level. However, it is a time-consuming and labor-intensive practice, and its costs may be prohibitive when cultivation is performed over large areas.

The scheduling and management of harvest are operations in which farmers must make crucial decisions that may dramatically alter the productive and qualitative results of essential oil crops, and many aspects of harvest management must be considered in order to achieve a satisfactory result. First, proper timing of such an operation is crucial, since it determines the age and the development stage of the harvested material. In oregano (Jerkovic et al., 2001) and rosemary (Nevo, 1998), it has been proven to significantly modify qualitative and quantitative traits, since a delay in harvesting may cause the plants to become woody, which would reduce their active ingredients.

Second, the intensity of cutting is especially important in essential oil crops harvested for their foliage: the maximum biomass yield is reached when the stems are cut as close as possible to the soil. However, if excessive, this cutting may injure axillary buds, limiting the plants' capability to regrow for further harvests (Fig. 17).

Fig. 17 Cutting should spare the regrowth capacity of plants. In the photo, the restarting of vegetation after cutting in *Artemisia abrotanum* L. (*Photo: A. Carrubba*)

Furthermore, too severe a cutting, which would result in a higher percentage of stems (that usually have a poorer quantity and quality of oil) with respect to leaves and shoots in the harvested material, may alter the chemical characteristics of the essential oil. In many perennials (such as mint, and sometimes sage or oregano), the crop is managed with the intention of executing more than one cutting. In cornmint, cases of six to seven cuttings taken throughout a cropping cycle 17–18 months long have been reported (Rajeswara Rao, 1999). Even if the subsequent harvests do not allow the same essential oil yield obtainable from the first (Piccaglia et al., 1993), and the chemical profile of the essential oil obtained from the different cuttings varies (Omer et al., 1994), the economical success of the cultivation may rely on the possibility of making more than one harvest. For this reason, the ability of the plant to regrow must be considered.

There are many examples of the machinery used to harvest essential oil crops, such as for oregano (Leto et al., 2002; Verlet, 1992), sage, lavender (Caligani and Adamo, 1987), and chamomile (Wahab and Larson, 2002) (Fig. 18). Results seem to vary with the dryness of the herb to be collected and the destination of the product: the herb picked up mechanically may tend to brown, which is unwanted if the product is destined for the herbal market, but less so if it is destined for the extraction of essential oils. Some simple adjustments may help when using mechanical equipment and solve many of the technical problems that may arise, e.g., choosing fast-growing genotypes, such as achieved in some selected *Salvia* hybrids (Dudai et al., 1999), or setting a proper arrangement of rows for plant populations (Caligani and Adamo, 1987).

Different methods are required for essential oil crops where specific parts such as seeds are harvested, rather than the leaves or entire plants. Coriander, fennel, anise, and dill are examples of such production. Here, the tendency of growers and breeders is oriented to the mechanization of harvest, by means of the equipment

Fig. 18 Mechanization is a necessity for modern essential oil crops growers. In the photo, a prototype of mower modified for harvesting of oregano (*Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart). (*Photo: A. Carrubba*)

normally used on farms. Difficulty may result from the indeterminate growth habit of many such herbs, which may cause the contemporary appearance of flowers, ripe and unripe seed on the same plant. In this case, the best recourse should be hand harvesting, picking up the seeds (or umbels) as soon as they are marketable. Obviously, this is time and labor intensive, and many techniques and facilities for mechanical harvesting have been developed. Coriander and fennel, for example, may be cut to ground level and after some hours of open-air drying, be threshed mechanically.

7.6 Diseases and Pest Control

Pathogens and pests may cause considerable losses to the yield and quality of essential oil crops. Generally speaking, this topic has not been debated in depth in terms of essential oil crops, and until a few years ago, a widespread idea was that most such crops had no serious pests or diseases (Simon et al., 1984). More probably, the lack of information regarding this issue was mostly due to the limited cropping area of essential oil crops. In fact, the sources of information related to the diseases of essential oil crops were mostly limited to areas in which their cultivation reached appreciable levels. For example, in the 1980s, an infestation of *Ramularia coriandri* was found in coriander cultivations in the former Soviet Union (Gabler, 2002), and it forced growers and researchers to concentrate (successfully) their efforts towards the breeding and selection of resistant genotypes. Similar histories may be found in the literature for other crops and other pathogens, such as stem necrosis in fennel caused by *Phomopsis foeniculi* (Anzidei et al., 1996; Mugnai and Anzidei, 1994), sweet basil wilt caused by *Fusarium oxysporum* (Dudai et al., 2002), coriander and caraway foliar necrosis caused by *Ascochyta* and *Aureobasidium* sp. (Anonymous, 2002), rosemary root rot caused by *Rhizoctonia solani* and *Sclerotinia* sp. (Conway et al., 1997; Mohan, 1994), laurel leaf blight caused by *Glomerella cingulata* (Constantinescu and Jonsson, 1987), and mint leaf rust caused by *Puccinia menthae* (Joy et al., 2001).

Research concerning insects is also scarce in this context: red scale (*Aonidiella aurantii*) and several mealybugs have been reported to be common insect pests of jasmine, whereas hairy caterpillars, cut worms, semi-loopers, red pumpkin beetle, and termites have been observed in cultivations of Mint (*Mentha arvensis* L.) and controlled by means of suitable insecticides (Joy et al., 2001) (Fig. 19). However, the belief in the insecticidal effectiveness of many essential oil crops is so strong that some, such as *Artemisia vulgaris*, *Ocimum basilicum*, and *Mentha cordifolia,* have been suggested as intercrop species to repel insects from the main crop (IIRR, 1993).

A growing presence of insects on crops is a source of risk. Other than their direct damage to crops, they have a well-known ability to transmit dangerous viruses and viroids that in the specific case of essential oil crops could injure plants both from a quantitative and qualitative point of view. A survey of the virus diseases of some essential oil crops was carried out in Italy by Bellardi and Rubies-Autonell (2003), where 12 different virus strains affecting about 40 species were detected. According to the authors, damage was not only on plant biomass, but also on essential oil production and composition. For example, oil production from clary sage infected by Broad Bean Wilt Virus serotype I (BBWV-1) was one-third of that obtained from healthy plants, with an increased content in α-terpineol, germacrene D, and sclareol, and a lower percentage of myrcene and limonene.

An increase in pathogens and pests in essential oil crops, however, may be expected to be a direct consequence of the growing rate of cultivation (Gabler,

Fig. 19 Pests may have some impact on essential oil crops cultivation. In the photo: a young plant of clary sage (*Salvia sclarea* L.) with evident symptoms of attack by mites (*Acari*). (*Photo: A. Carrubba*)

2002), and it is possible to foresee that if essential oil monocultures spread over wider areas, such problems will become a major concern in the future. Experiments aimed at evaluating insecticides and pesticides useful for essential oil crop cultivation could certainly be performed, but the specific orientation of markets and the high "naturality" content of such products implies special care in their field management. The general tendency in cultivation of such crops is therefore oriented to their organic or integrated management, as also expressly suggested for extracts destined for the pharmaceutical industry or human therapy by the WHO guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants. Such guidelines imply the use of agrochemicals at the minimum possible level and "only when no alternative measures are available" (WHO, 2003).

With the purpose of improving general plant health conditions, many strategies have been recently developed. These include the employment of, and increase in, natural mycorrhization under many different agricultural environments (Kothari et al., 1999). Many essential oil crops have proven highly suitable to vesicular arbuscular mycorrhizal (VAM) fungal colonization, achieving a high percentage of internal infection (Camprubi et al., 1990), and some species (*Salvia officinalis, Lavandula officinalis*, and *Thymus vulgaris*) have been successfully used as indirect inoculation media to increase VAM root colonization of other tree species (Camprubi et al., 1992) (Fig. 20).

Another relevant concern is tied to the occurrence of pathogens, such as molds, on harvested and stored plant material. Usually, the use of proper storage conditions in clean and well-aerated places, and avoiding any possible retention of moisture in containers and contamination with insects, rodents, or other pests, should be enough to ensure the long-term conservation of plant material.

7.7 Postharvest Treatments

For quality features, postharvest treatments also play a crucial role. Depending on the species, the herb part used, the harvest timing and conditions, the water amount in herbs ranges from 40% to 80%. Some differences in essential oil characteristics have been claimed between dry and fresh plant material (Cioni et al., 1991; Shalaby et al., 1995), but drying is often necessary to increase the shelf life of the final product, to allow proper conditions for storage, and for the long-distance transport of the harvested product. Drying acts by slowing the growth of microorganisms and preventing some biochemical reactions that may alter the organoleptic characteristics of the herb (Díaz-Maroto et al., 2003).

In earlier times, most research papers available on this topic referred mostly to drying methods. This was in relation to the kind of material to be dried (tubers, roots, leaves, and bark), rather than to the different species (Chiumenti and Da Borso, 1996). More recently, many trials have been performed to find the best drying technique for most herbs, and it is certainly possible to find among them the best technique for the chosen environment and species.

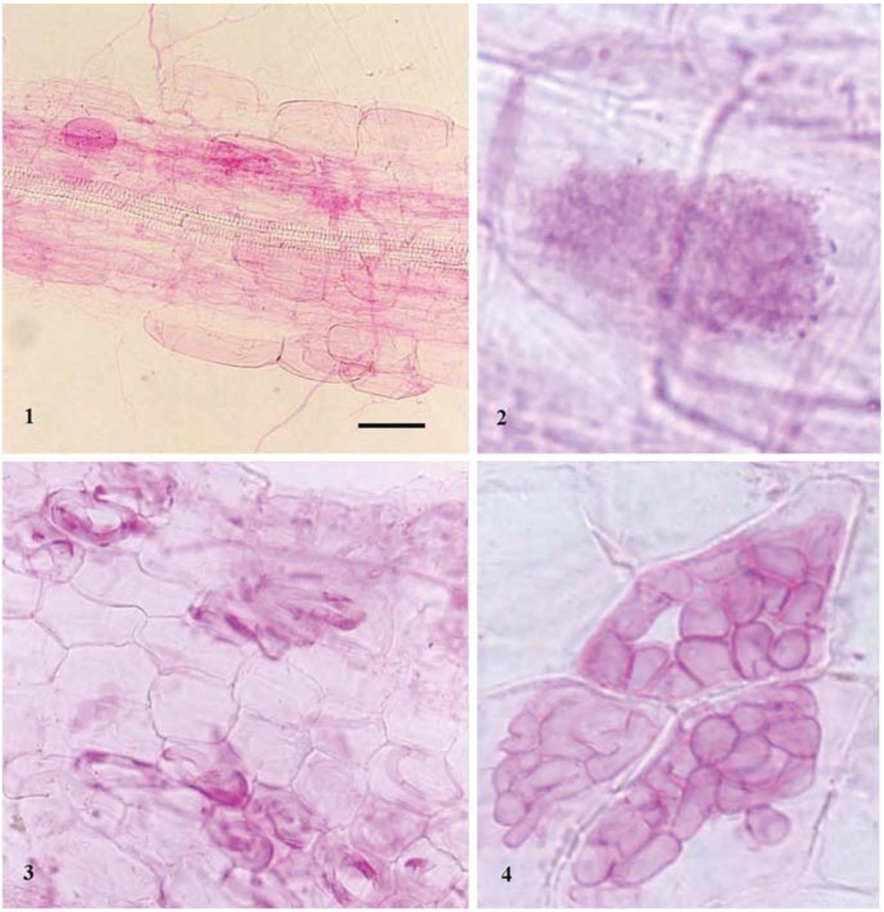

Fig. 20 Mycorrhization has been studied for many essential oil plants. In the photo, from left to right, examples of AM association in essential oil plants: (1) intra- and extramatrical structures of AM fungi and (2) arbuscule in basil root; (3) hyphal coils in cortical cells of lavander root; (4) myceliar structure in cortical cells of laurel root. Bar: 1, 3 = 30 μm; 2, 4 = 10 μm. (*Photo: L. Torta*)

There is no standard method for drying herbs, and each individual grower has often developed his own system, choosing from among the numerous methods that have been suggested, from air-drying to oven, microwave, or freezing systems. Each method has advantages and disadvantages, and the choice (besides the economic aspects) mostly relies on the desired result (texture, color, or scent) of the final product, and, of course, the requirements of its market destination. Method, duration, and temperature of drying, moreover, can affect the volatile oil content of the herb, which is a crucial factor in its quality. Such effects may vary according to the chosen plant material: in dill and parsley, oven- and freeze-drying lead to significant losses of volatiles with respect to fresh herbs, whereas such techniques exert a lower effect in sage and thyme (Díaz-Maroto et al., 2003). In the latter species, freeze-drying was found to produce oil yields about 10% higher than after a flow-through method (Lawrence, 1998).

An important target of research activity is to find, for each species, the maximum temperature for drying, in order to maximize volatile oil yield and quality. Generally, the higher the drying temperature, the greater the killing effect on microorganisms. However, a thermal excess could seriously damage the quality of essential oil; in thyme and sage, oven-drying induced lower losses of volatiles at 30°C and higher losses at 60°C (43% in thyme and 31% in sage with respect to the fresh herb) (Díaz-Maroto et al., 2003), whereas in French tarragon, the best oil retention was obtained at a working temperature of 45°C (Arabhosseini et al., 2007). In some cases, more attention is required on the duration of heat exposure than on the final temperature. For example, Charles et al. (1993) found that a treatment at 80°C for 12h had approximately the same effect on the artemisinin content of *Artemisia annua* as exposure to a much lower temperature (50°C) for a longer time (48 h).

The most ancient and traditional drying system is air-drying, which is performed outdoor (in warmer environments) or indoor (when external climatic conditions are not optimal and adequate structures are available). Many crops, such as mint and sage, may be successfully dried in plastic-covered greenhouses. Usually, the process involves stacking flat trays of herbs in the shadows (direct light may often alter the color of the product) and in aired places, sometimes with the help of dehumidifiers or forced circulating air equipment. Air-drying is a slow and labor-intensive process (there is the need to often move the mass to exsiccate so as to avoid brownish, fermentations and microbial attacks), but many experiments (Charles et al., 1993) have demonstrated that it allows the best results in terms of product quality.

Solar drying has been successfully tried in many environments (Buckenhüskes et al., 1996; Charles et al., 1993; Garg et al., 1998), and it has proven to give better color, texture, and content of active ingredients than conventional stove driers. Much equipment is available, is easy to set up, and can quickly dry large amounts of different plant material. Traditionally, such equipment has always been cheap, but nowadays problems have arisen due to quality control requirements and the need to reduce the bacterial count. When more sophisticated machinery (such as dehydration machines) is necessary, herb drying starts to become more capital intensive and the cost of equipment is often too high for many herb growers.

8 Conclusion

Plants producing aromatic oils have been used for flavoring throughout history. Many of them have formed part of the economy of countries with growing populations where there is an inevitable pressure on agricultural land as a resource for food and fuel crops. Many species for essential oil production might have direct interest as crop species for Mediterranean areas. Although some are native to Mediterranean

Fig. 21 Mixed cropping systems including essential oil crops may enhance farm productivity and biodiversity level. In the photo: oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart) in association with young olive trees. (*Photo: M. Militello*)

environments, others are from different areas of the world, yet targeted to a growing interest as food or flavoring items. Most of the aforementioned herbs, especially those that are native, are easily grown and adapted to a wide variety of soil and climatic conditions. Therefore, they have already been cultivated by many farmers at a small scale, mostly for domestic or local use.

Currently, problems with essential oil crop production are above all commercial, linked to the establishment of market channels, to their high investment costs, and to the rapid expansion of competitive production from developing countries. In Mediterranean environments, such problems often add to general marginality conditions, requiring appropriate and well-constructed land management (Fig. 21).

Notwithstanding, today, a considerable pressure is exerted by consumers worldwide, to use perceived natural compounds in edible and personal products, and many opportunities seem therefore to be open for such crops. It is essential that producers are able to service this growing demand efficiently, economically, and above all, reliably. It is therefore important to understand and develop ways of ensuring maximum return on the investments made in establishing and growing these crops (Weiss, 1997). The great amount of experimental research carried out worldwide has brought important advances to cropping techniques. In some cases, it has improved the plant material available for cultivation, although its availability to growers is far from satisfactory. Much work still remains to be done to further advance the techniques required for the special environmental conditions of Mediterranean environments, and for the wider application of such techniques and improvement of genetic materials available to farmers.

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Sugarcane and Precision Agriculture: Quantifying Variability Is Only Half the Story – A Review

Daniel Zamykal and Yvette L. Everingham

Abstract The world's population is expected to reach 13 billion people by 2065. Increasing food production and sustaining the environmental resource base on which agriculture depends will prove a significant challenge for humankind. Precision agriculture is about putting the right input, in the right amount, at the right place, in the right manner at the right time. Precision agriculture is a tool which manages the variability in crop and growing conditions for improved economic and environmental returns. For precision agriculture to be successful, industry must collect, analyse, process and synthesise large amounts of information from a range of integrated enabling technologies. However, quantifying variability alone will not constitute successful precision agriculture in sugarcane production. Success will be measured by the extent to which these technologies are adopted by industry. This review broadens the popular within-field definition of precision agriculture to encompass higher levels of variability present at the farm, mill and regional scale. We propose that managing all these levels of variability is important, although, many of the technologies available for within-field management require further research prior to operationalisation. While a discussion on the range of enabling technologies such as the global positioning system, global information system, proximal sensing, remote sensing and variable rate technology is essential, we emphasise the need to develop a participatory action research environment to facilitate the adoption of precision agriculture for the benefit of whole of the industry.

Keywords Sustainability · Variability · Technology · Data mining · Modelling capability · Adoption

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1 Introduction

The world's population of approximately 6.5 billion people is currently growing at 1.2% annually and is expected to reach 13 billion people by 2065 (Pimentel et al., 2007). The evolution of agricultural production towards increased mechanisation has contributed to this growth rate and has continued to feed and more recently fuel our booming population. However, this success has been achieved at an environmental cost with the degradation of soils, lowering of water tables and the impairment of ecosystem services (Daily et al., 1998). The immanent need to double farm production to match population growth over the next quarter of a century with diminishing land and water resources through further intensification will only exacerbate these problems (Srinivasan, 2006). It has become increasingly important to develop tools that will enhance productivity and economic profits while simultaneously conserving the environment (Srinivasan, 2006). Precision agriculture is one of many potential solutions to overcome these problems and has grown and evolved to incorporate the best of multidisciplinary science and technology to solve the riddle posed by spatial and temporal variability. For this to be achieved, industry must collect, analyse, process and synthesise large volumes of data from a number of technologies, so better, more informed management decisions can be made. By managing the variability inherent in agricultural production, precision agriculture has the ability to match input application to deficiencies on the ground, potentially translating into better, more efficient use of inputs, reduction in off-site losses, improved yield through targeted management and increased profits. However, collecting data via different technologies, analysing the data and describing the variability (although important) does not constitute successful precision agriculture. Often overlooked are the problems associated with the adoption of complex technologies (i.e. precision agriculture) essential for wider industry acceptance. When assessing the success of precision agriculture in the sugarcane industry, it will not be the ability to produce detailed management plans from cutting-edge technologies but will be the extent to which growers participate in the evolving science of precision agriculture. With 153.5 million tonnes of sugar consumed by the world in 2006/2007 (FAO, 2007) it is important that the industry takes steps towards facing the challenge of increasing production sustainably. This review aims to describe the process and philosophy of precision agriculture, the technologies and quantitative tools essential for modern implementation, the obstacles for adoption pathways and how these elements must be integrated to achieve sustainability.

2 Principles and Concepts of Precision Agriculture

Across the cities of the world many of us look out onto our balconies and notice that our potted herb gardens are in desperate need of water. We march out with our watering cans and proceed to water our poor wilted herbs, giving more water to those plants that look the closest to death. At the same time we may pick the ripest-looking tomato, remove a few leaf-eating insects and continue inside to eat our breakfast. Does this morning ritual performed by many each day constitute a form of precision agriculture? Well, yes, if the definition of precision agriculture encompasses the implementation of management practices to match the variability in growing and crop conditions (Srinivasan, 2006). Indeed, precision agriculture is about putting the right input, in the right amount, at the right place, in the right manner at the right time (Raj Khosla, personal communication). Therefore, many of us practice a simple form of precision agriculture because we have an intimate knowledge of our gardens and the plants that grow within them. The principles of precision agriculture can be extended across a range of scales in sugarcane-growing communities to include the region, mill, farm, paddock and sub-paddock level. Irrespective of the scale, precision agriculture encompasses all attempts to address the heterogeneity of an agricultural system. However, the trend towards increased mechanisation in commercial agriculture has contributed to increased economic pressure to treat large crop areas with a uniform or one-size-fits-all management approach (Bongiovanni and Lowenberg-Deboer, 2004). As farming enterprises become larger, the need for information about the variability in growing and crop conditions will become essential to facilitate management actions. This collected information can then be used to divide the once homogenous growing space into zones where a tailored management program can be implemented (Whelan and McBratney, 2000).

Modern precision agriculture depends heavily on a range of enabling technologies to gather as much information about the variability in growing conditions as possible. Key enabling technologies for medium/large-scale precision agriculture broadly include crop yield monitors, global positioning systems (GPSs), remote sensing, proximal sensing and geographical information systems (GISs). These tools allow a producer to acquire detailed spatial information about crop performance and to tailor management actions to match the expected goals of yield, quality and environmental objectives (Bramely and Quabba, 2002). However, precision agriculture is not just an umbrella term for a suite of technologies used to gather data but a cyclic process of data collection, knowledge extraction, planning, management and evaluation (Fig. 1). Each component adds to the refinement of the production process and hence the final product through a continual cycle of incremental development over time.

Srinivasan (2006) describes three criteria that are required for the implementation of precision agriculture. First, there must be significant spatial and/or temporal variability in the soil and crop conditions within a field and among fields within a region. We emphasise that this variability should also occur at the region and mill levels as well within and among fields. This first criterion is almost always fulfilled due to the heterogeneous nature of soil irrespective of the scale (Machado et al., 2002; McBratney and Pringle, 1999). Second, the industry must be able to identify and quantify the observed field variability. Describing variability depends on uniting the best of farmer’s knowledge on field variability, positioning and information technology and quantitative expertise. Third, it is the ability and willingness to reallocate inputs and change management practices to improve productivity and profitability while improving environmental stewardship. Not only does the information about variability need to be made available, but responding appropriately

Fig. 1 The process of modern precision agriculture is cyclic in nature and begins with collecting large amounts of data over time. This data must be analysed so the essential information is extracted. This information can then be used to enhance planning and collaboration between stakeholders. A new or modified management regime is then implemented and the results are observed and evaluated in response to the treatment

to the observed variability is essential to achieve the desired outcome. These three criteria provide a foundation for the implementation of precision agriculture and are essential for any industry to address when embarking on the process of managing variability at any scale. At its most simplistic level, precision agriculture treats a heterogeneous space differentially. However as production systems become larger and the variability in the growing conditions become more complex the multidisciplinary partnership between information technology, farmers and scientists becomes integral for describing and managing this variability.

2.1 Sustainability and Precision Agriculture

Can agricultural production remain sustainable using current practices and technologies? The answer is probably not; however, precision agriculture and sustainability are fundamentally linked and provide an avenue for increased production. Precision agriculture promises sustainable benefits in the form of profitability, productivity, crop quality, on-farm quality of life, food safety and rural economic development, essential for the longevity of any industry (Srinivasan, 2006). Intuitively, by applying inputs when and where they are needed precision agriculture will potentially lessen the environmental loading of inputs, reduce the variability of crop performance, increase yield and shrink immediate input costs (Bongiovanni and Lowenberg-Deboer, 2004; Whelan and McBratney, 2000). This in turn should minimise the opportunity for off-site losses and hence detrimental effects to the surrounding environment. The large amount of data collected for the purpose of quantifying within-field variability could be further used in bio-physical models to understand the pattern of input leaching (Delgado and Bausch, 2005). Sugarcane has a relatively high use of fertiliser when compared to other crop production systems and may represent an important tool for managing input application by better understanding the dynamics of nitrate loss into the surrounding environment (Bramely, 2007; Delgado and Bausch, 2005). This alludes to a greater concept of precision conservation, in which the site-specific approach is expanded to a three-dimensional scale that assesses inflows and outflows from fields to watersheds at a regional scale (Berry et al., 2003). Therefore, the individual farm is no longer defined by property boundaries but is part of the wider agro-ecological system which has a responsibility to the sustainability of the greater region. Although, the word “sustainability” is often associated with environment, it is important to address the intrinsic link between environment and economics. Without the “sustainability” of environment and economics considered together, agricultural enterprise will struggle in the evolving agro-economic climate. All too often the economic rationalists forget to consider the costs or benefits of environmental effects when assessing precision agriculture. One such reason is the difficulty in assessing an unknown quantity and the lack of economic literature valuing damage from agricultural pollution (McBratney et al., 2005). Another aspect is in relation to the environmental regulation associated with agriculture which directly affects input choice and profitability (McBratney et al., 2005). McBratney et al. (2005) refers to nitrogen fertiliser regulation and taxes in Europe which are constrained for environmental purposes. This type of system provides “incentive” to farmers to adopt practices that efficiently use the inputs and hence increase their profits. The availability of environmental impact information available to the farmer as part of the precision agriculture process provides an additional opportunity to understand the impact of management actions and permits an assessment of the practice in relation to the environment. Precision agriculture promises a framework to unite both the economic and environmental facets of sustainability while simultaneously addressing the challenges associated with an increase in production. This framework is central for a discussion on precision agriculture and embodies the philosophy of a precision agricultural regime.

3 Sugarcane Production

The majority of the world’s sugarcane is grown across the tropical and subtropical cline where rainfall or irrigation is well distributed during the growing season (Fig. 2). Sugarcane is a commercially valuable crop with the majority of developed countries adopting a highly mechanised approach to production. The most

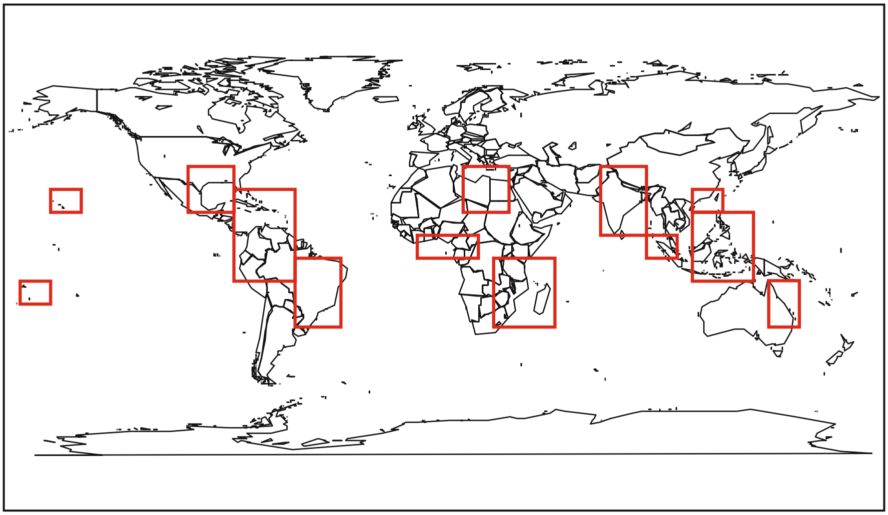

Fig. 2 Sugarcane is generally grown in the tropical and subtropical cline between 31.0° south and 36.7° north. This region has well distributed rainfall throughout the year and experiences high amounts of solar radiation

common system of cultivation is row cropping where cane is planted in rows. This method is tailored for mechanical planting and harvesting and increased efficiency. The cropping cycle of sugarcane is somewhat unusual compared with other crops in that it involves a series of ratoons. Unlike other crops, once the cane has been harvested the bud and root primordia of the stool develop and produce a stubble or ratoon crop with each ratoon grown for approximately 12 months (longer in cooler climates) before being harvested again. Most sugarcane cropping systems allow the crop to go through three or more ratoon cycles before the crop needs to be ploughed out and replanted. This is usually in response to a decline in sugar yield with each successive ratoon and an increase in exposed risk to the crop (e.g. loss of soil structure with time) (James, 2004). Once the new crop has been planted, soil moisture may be low as a result of preparing new rows and furrows and germination may be enhanced by irrigation. Further integration of herbicide and fertiliser application, pest control and the timing of supplementary irrigation are important aspects of the production system. Chemical ripeness can be further applied to the crop before harvest to improve sucrose yield across the block and harvested during the window of maximum advantage (Irvine, 2004). Just like many other crops, the application of inputs in a timely and integrated fashion onto a spatially variable growing space makes sugarcane production well suited to precision agriculture practices. In sugarcane, probably more than most other crops, production is rapidly increasing (mainly by expansion into new areas e.g. India, Brazil, Africa), there is an increased pressure on many traditional producers with protected markets to become more (globally) competitive and an industry focus to become more environmentally friendly (e.g. Better Sugarcane Initiative). These combined forces together with the suitability of the production system make sugarcane cultivation a prime candidate for the adoption of precision agriculture.

4 Technology and Precision Agriculture

The advent of information technology has accelerated our ability to acquire large volumes of spatial data. It is widely acknowledged that key technologies facilitating modern precision agriculture are yield monitors, remote and proximal sensing, GPS and GIS (Stafford, 2006). Although these technologies may be advanced in cereal cropping systems (Srinivasan, 2006), the majority of these technologies require more research and development before they can become operationalised as part of the sugarcane production system. Although technology is portrayed as the most important component of precision agriculture it is important to understand the limitations of the current technologies with respect to the application. For example, soil sampling at 1 sample per 1.5 ha may be inappropriate for the delineation of fertility zones that require 20–30 m sampling grids (Mallarino and Wittry, 2004; McBratney and Pringle, 1999). Or conversely, remotely sensed data with a pixel size of 30 m may be appropriate for the classification of variety or an outbreak of disease at the block level (Apan et al., 2004; Everingham et al., 2007). Furthermore, precision agriculture may simply be inappropriate under particular circumstances. Variable rate spraying of pesticides against a highly mobile pest may prove ineffective with the pest moving to other parts of the field. In this case homogenous treatment of the growing space may be a better option for managing such a threat (Srinivasan, 2006). Technology certainly plays a significant part in the precision agriculture scheme, but understanding the appropriateness of the different forms of technology in response to a management problem is an essential element for successful precision agriculture implementation. Many of the enabling technologies discussed are at varying stages of development with many having restricted application and usefulness when applied at the within-paddock level. However, these technologies can still contribute to larger-scale forms of precision agriculture at the region, mill, farm or paddock level.

4.1 The Global Positioning System

GPS is a key enabling technology for the implementation of precision agriculture. Since the advent of GPS in the late 1980s a new era of positioning and time information became available 24 h a day, provided a line of sight to sufficient satellites was maintained (Auernhammer, 2001; Stafford, 2006). A constellation of 24 satellites orbit the earth in 6 different planes to provide global coverage. Each satellite transmits two or three signals via a spread spectrum within two carrier waves (Kruger et al., 1994). One wave transmits navigation information, while the other two carry positioning information. The positioning waves are designed to be used by either military or civilian users, with the military having access to higher position resolution. During these early years, civilian users experienced an artificial error introduced by the United States government of 100 m known as selective availability. However, a decision to remove such bias effective May 1, 2000 improved GPS accuracy to about 20 m. This simple form of GPS technology is unreliable for dynamic positioning within the field and other forms of GPS technology are available to the precision agriculture community.

A solution for improving the accuracy of GPS was the advent of differential GPS (DGPS). Simply, DGPS requires that two GPS receivers acquire the GPS signal simultaneously. One receiver must be on a point that has previously been surveyed (depends heavily on accuracy of this reference station), and the other receiver at an unknown location (usually at the point of interest) (Kruger et al., 1994). At the known point, the instantaneous GPS total error can be found by comparing the difference between the observed and known position. DGPS also compensates for errors due to refraction in the ionosphere and troposphere. This system assumes that the source of error is external to the GPS receiver and does not fully take into account tectonic plate movement or accuracy degradation from using a single reference station (Denham et al., 2006). The use of DGPS is necessary for users to get a position accuracy of 1–3 m and is a minimum resolution to enable other precision agriculture technologies such as yield mapping, crop scouting, variable rate technology, auto guidance technologies and map building (Srinivasan, 2006).

Kinematic GPS is another positioning system that measures the phase shift in the satellite carrier signal between transmission and reception and has the potential for centimetre accuracy. In particular, Real Time Kinematic (RTK) solutions may achieve positional accuracy of 20 mm with consistent and repeatable positions (Dijksterhuis et al., 1998). A study of the application of RTK GPS in the Mauritian sugarcane industry consisted of a modular hardware package suitable for short range control up to 10 km with on-the-spot real-time accuracy eliminating post-processing of data (Jhoty, 1999). When used in "fine-mode" this system was accurate horizontally to 10 mm and vertically to 20 mm. However, a major drawback is that the total cost of setup is carried by the individual land holder. A solution to this problem can be achieved through Continuously Operating References Stations (CORSs) with Networked RTK (NRTK) capability (Denham et al., 2006). The main advantage of networked CORS is an increase in the distance between base stations from 20 km to 70 km, representing a reduction in capital investment and providing a network that can be used by a region or cooperative. Additional advantages of such a system include the use of mobile communications which overcome the limitations of radio range, multiple networked reference stations which are more reliable than a single station, 1–2 min initialisation time, consistent and repeatable positions and no requirement to buy or rent a reference receiver or monitor a receiver each day (Neale, 2007). Alarmingly, half of the base stations currently owned by all Australian farmers would be able to service all national cropping areas if they were used in a network. The Australian sugar industry in particular could have experienced a fivefold reduction in base station cost if networks had been established initially (Neale, 2007).

GPS is an essential precision agriculture technology integral for enabling controlled traffic systems (Cox, 1997), auto-steer technology, soil mapping, yield mapping, GIS, digital elevation models, designed paddock and farm layouts to name a few; in fact, GPS could be considered the trunk of the precision agriculture tree with the majority of precision agriculture innovations emanating from it. GPS technology is commonly used in the global sugarcane industry as a coupled technology in controlled traffic systems (Braunack and McGarry, 2006; Cox, 1997), yield mapping (Saraiva et al., 2000), yield monitoring (Magalhaes and Cerri, 2007) and for supplying precise location data for topographic and contour maps (Jhoty, 1999). With the potential availability of networked RTK CORS GPS, the sugarcane industry would benefit immeasurably from increased positioning accuracy at the within-paddock level.

4.2 Geographic Information Systems

GIS is a software application that is used to manage and analyse spatial data. GIS is mainly used to store, retrieve and transform spatial information relating to productivity and agronomy (Blackmer and White, 1998). This information can be derived from a number of data sources including digital maps, digitised maps and photographs, soil and crop surveys, sensor data with positioning information, point analytical data and/or yield maps. The graphical interface of this software reproduces the analysed data as maps for the specific field. These maps are then used to understand the interaction between yield, soil fertility, pest and disease, weeds, landscape changes and other factors of interest (Ulbricht and Heckendorff, 1998; Zaizhi, 2000). These spatial relationships can then be used to make management decisions. For use in precision agriculture, GIS can create primary information maps of soil type, distribution of N, P, K and other nutrients, topography, soil moisture, pH and crop cover. The strength of GIS lies in its capability to graphically overlay and display different data layers (Jhoty, 1995). However, GIS alone is unable to statistically validate these relationships and must be used in conjunction with spatial statistical techniques to draw valid conclusions.

Like GPS, GIS is a key enabling technology in the sugarcane industry and is used in conjunction with other precision agriculture technologies to relate spatial information to the crop. GIS is a powerful tool that has a demonstrated ability to enhance decision-making through the visual interface (Campbell, 1994) and has been explored in the sugarcane industry (Fuelling and Wright, 1997; Jhoty, 1995; Johnson and Walker, 1996; Markley et al., 2003; Schmidt et al., 2001). The usefulness of linking and integrating GIS with other precision agriculture technologies such as remote sensing (Markley et al., 2003; Schmidt et al., 2001), GPS (Fuelling and Wright, 1997) and base maps (soil, topography, rainfall, etc.) with agronomic data (Jhoty, 1995) makes GIS an attractive precision agriculture technology. However, the main concern with linking GIS with other spatial data is the difference in spatial resolutions between technologies. For example, when combining data derived from accurate paddock and coarse remote-sensed data the placement of paddock boundaries can only be within the order of the pixel resolution of the remote-sensed data (Markley et al., 2003), which in turn influences the resolution of management recommendations. Although these limitations pose a significant challenge when overlaying multiple data sources at different resolutions, the GIS is an essential precision agriculture technology for linking spatial data to crop characteristics on a graphical interface.

4.3 Proximal Sensing

Proximal sensors are used in precision agriculture to quantify in-field crop and soil variability or to monitor yield at the point of harvest, with the majority of technologies still in development. Soil sensors for testing pH, electrical conductivity, N content, organic matter, sodicity and salinity are in use. The most commonly used method takes measurements using electromagnetic induction (EM38) soil sampling with the data correlated or ground-truthed to chemically analysed soil samples (Nelson and Ham, 2000; Wong and Asseng, 2006). These relationships can then be used to produce maps of the variable of interest. Other on-the-go soil sensors using optical, radiometric, mechanical, acoustic, pneumatic and electrochemical measurement concepts have been trialled; however, only electromagnetic sensors are widely used at this time (Adamchuk et al., 2004). Crop sensors for determining a wide range of crop attributes including N status and water stress levels are in use throughout the industry. Examples of such proximal sensors are the "Yarra N Sensor", "Greenseeker" and the "Crop Circle" (Neale, 2007; Srinivasan, 2006). These convert red and near-infrared spectral bands to normalised difference vegetation index (NDVI). This data can be used to recommend a dosage rate of nitrogen fertiliser to the crop (Neale, 2007). Proximal sensors are particularly useful when integrated with variable rate technology to enable variable rate application of inputs on-the-go and are currently being used in other industries such as cotton and wheat. The current generation of proximal sensors provides a quick and cost-effective alternative for identifying spatial variability not afforded to the grower using laborious physical sampling and laboratory analysis.

Yield monitoring and mapping is another family of proximal technology used to gain insight into the variability of crop performance. Currently, yield monitors are the most widely used precision agriculture technology (Batte and Arnholt, 2003; Magalhaes and Cerri, 2007; Pelletier and Upadhyaya, 1999; Simbahan et al., 2004), possibly because farmers perceive a high degree of benefit from the information-gathering tool (Batte and Arnholt, 2003) with the data representing actual rather than surrogate measures. Due to the highly mechanised harvesting of the majority of the world's sugarcane, this crop is well suited to yield monitor technology to produce yield maps. With the cost of a yield monitor making up only a small fraction of the total harvester cost it is an attractive add-on technology. During the later part of the 1990s the development of a gravimetric yield monitor by the University of Southern Queensland in Australia spurred a yield mapping trial over two seasons with the prototype system used to evaluate the presence of management zones in the Herbert river district (Bramely and Quabba, 2002). The research showed marked spatial structure of cane yield with a coefficient of variation of 30–45% within a

12 ha block (Bramely and Quabba, 2002) which was similar in range to the majority of crops where yield monitoring was available at the time (Pringle et al., 2003). Additional testing of the University of Southern Queensland yield monitor system for accuracy in Mauritian conditions reported accuracies of 97% and a range of yield variation similar to that seen under Australian conditions (Jhoty, 2003). This study highlighted the correlation between high yield and high water-holding capacity of the soil and demonstrated the application of yield monitors as an intermediate technology for understanding drivers that effect yield. However, innovation within this area is strongly dependent on the country of origin and the type of harvesting commonly employed. Within Brazil where both mechanical and hand harvesting are in use the focus has been on three main systems; these include a harvest-mounted yield monitor (Cerri and Magalhaes, 2005; Pagnano and Magalhaes, 2001), a weighing system for grab loaders specifically designed for measuring hand-cut cane (Saraiva et al., 2000) and a system that is based on load cells that measures the weight of the material in the haulout bins (Molin and Menegatti, 2004). A new yield monitor system incorporating a mass flow sensor, GPS and a data acquisition system was field-tested during the 2004–2005 Brazilian harvest season (Magalhaes and Cerri, 2007). The results showed that the system was capable of producing accurate yield maps with a mean error of 4.3%.

Although the failure to commercialise the University of Southern Queensland yield monitoring systems has caused frustration to some within the Australian industry, other systems have become available, with the Cuban TechAgro^{TM} system being field-tested and displayed to growers during the 2006 harvest season with promising results. At the same time the competing Australian AgGuide^{TM} yield monitor designed by Jaisaben Enterprise^{TM} revealed that field trials conducted in 2006 were accurate to ±5% on a row-on-row basis (Bramely, 2007). However, gravimetric-based monitors like the University of Southern Queensland system and the recently developed Brazilian yield monitor (Magalhaes and Cerri, 2007) should also be considered in the race to accurately map sugarcane yield. Currently a number of Australian cane-growing districts are conducting large-scale trials with the TechAgro^{TM} and the AgGuide^{TM} yield monitoring system to test the robustness of the technology and to quantify biomass variability within the block.

The yield monitoring approach adopted by the United States is that of an electronic load cell situated in the field transport wagon instead of on the harvester (Johnson and Richard, 2005a,b). The focus of this work differs from the majority of yield monitoring researches in that sugar production in conjunction with cane yield is measured. Current research and development of an optical yield monitor placed on the floor of the harvester conveyor system has delivered several benefits in the form of accuracy and robustness under normal operation (Price et al., 2007). However, the main advantage of this system is the ease of installation and the lack of maintenance due to the self-cleaning nature of the design.

Although a great deal of research effort has gone into yield monitor technology the usefulness of the current information is certainly questionable and will depend if the crop is being used for food or fuel. Unlike other crops where the biomass measurement is directly related to the commodity, the spatial relationship between sugarcane biomass and commercial cane sugar within the field is not well understood. An evaluation of the spatial and temporal relationship between yield and commercial cane sugar at the farm level concluded that there was no significant spatial relationship between the two (Lawes et al., 2004). Significantly, the same study revealed that the relationship of yield and commercial cane sugar between farms was stable over time. Therefore, future research must focus on determining if these spatial relationships occur at the field or within-field scale. In its current form, the use of yield maps may certainly improve the ability of the grower to improve biomass yields across the field. However, a possible reduction in commercial cane sugar as a result will only reduce his/her bottom line negating any economic benefits derived from the technology. Undeniably yield monitoring is central to understanding variability at the point of harvest, but further research needs to be done to relate this information to commercial cane sugar variability. Moreover, yield maps alone cannot uncover the drivers of variability inherent in the system. Combining yield maps with nutrient distribution, soil type, water-holding capacity and other attribute maps will prove essential to uncover those drivers that underpin the observed variability across time.

4.4 Remote Sensing

Civil remote sensing took birth in 1972 with the launch of LANDSAT-1 and revealed an excellent tool for monitoring the bio-geo-physical processes at a regional and global scale (Dorigo et al., 2007; Goward and Williams, 1997). Subsequently, the North American Large Area Crop Inventory Experiment (LACIE) demonstrated that remote-sensed data could assist in crop identification, estimation of canopy properties and crop forecasting (Dorigo et al., 2007; Moran et al., 1997). Since these early days, a plethora of canopy state and driving variables have been investigated using remote-sensing data with the goal of understanding crop indicators such as photosynthesis (Clevers, 1997; Gobron et al., 2000), phenology (Everingham et al., 2007; Karnieli, 2003; Xin et al., 2002), plant function (Doraiswamy et al., 2004; Mo et al., 2005), plant development (Bouman, 1995; Haboudane et al., 2002), nitrogen stress (Haboudane et al., 2002; Zhao et al., 2005), drought stress (Hurtado et al., 1994), crop quality (Mutanga et al., 2004) and crop yield (Mo et al., 2005). Imagery can then be integrated with other layers of spatial data in a GIS, with GPS receivers used to locate positions of interest. Management actions can then be taken to apply corrective measures to those zones of concern. In recent times, commercial availability of higher resolution imagery (0.6 m pixel) has propelled the usefulness of remote sensing for agronomic purposes and precision agriculture. With an increase in band width technology from blue, green, red, near-infrared (NIR), mid-infrared (MIR) and far-infrared (FIR) to the new generation hyperspectral sensors that operate over 256 bands, an increase in information definition is possible. Historically, this level of information could only be obtained from expensive laboratory analysis at point-specific locations. With a planned increase in the number of satellites, remotely sensed data should become cheaper, with better resolution, and have faster delivery time. It is unsurprising that remote sensing has become a major information-gathering tool for the advancement of precision agriculture.

At the regional level remote sensing can play an important role in monitoring the harvest and planting of small-scale farms where current information is difficult to obtain (Lebourgeois et al., 2007). Remote sensing using the SPOT satellite system was used to successfully update information about paddock boundaries at a regional level and to produce thematic maps to be integrated into a GIS package (Lebourgeois et al., 2007). The building of a spectral library in India for a number of crops including sugarcane and the classification of those crops at the cultivar level showed considerable skill with 86.5% and 88.8% correct classification at the canopy and pixel scale, respectively, further proving the usefulness of remote sensing for discerning cropping systems (Rao et al., 2007). At the paddock scale, preliminary work showed the importance of high-resolution IKNOS 1 m pixel data for obtaining good information for understanding crop variability, which was undiscernible at lower resolution (25 m pixel) (Neale, 2007) and highlighted the importance of resolution when describing within-field variability. In countries where mechanical harvesting is limited, remote sensing technology has been proposed as a surrogate for on-the-go yield mapping (Roloff and Focht, 2006). The South African sugarcane industry has placed considerable emphasis on satellite-based remote sensing (Schmidt et al., 2001) for quantifying yield and to target the use of fertiliser and water inputs. More mechanised countries such as Australia and Brazil have also explored the use of remote sensing. Within the Australian sugar industry, early research centered on crop estimation and evaluation using the SPOT and LANDSAT technologies which have an on-ground resolution of 20–25 m (Markley et al., 2003). The resolution of such technology for the application of within-field precision agriculture has been questioned given that the information of 1 pixel is a composite of 16 rows (Bramely, 2007). However, yield estimation accuracy in the order of 10% has been achieved using these systems and could be considered for higher resolution paddock-scale applications. A solution to the problems posed by these technologies is the use of airborne digital multi-spectral video (DMSV) remote sensing that has a pixel resolution of 50 cm and has been used successfully in South Africa to distinguish variety, age and water stress (Schmidt et al., 2001) and is a solution for finer-resolution applications. Another avenue of remote sensing is the use of EO-1 Hyperion hyperspectral imagery. The benefit of this type of imagery is the coverage of the entire spectrum as opposed to LANDSAT and SPOT that cover only 4–8 windows across the spectrum. The first use of hyperspectral imagery in the Australian sugarcane industry was used to detect "orange rust" disease (Apan et al., 2004). The disease produced a set of unique spectral bands that could be used to identify areas within the 30 m pixel. The subsequent development of a disease water stress index could then be used to produce classification accuracies of 96.6% for pixels with disease. Armed with this spatial information, growers could potentially control outbreaks in a timely fashion before major crop devastation occurs. Another study revealed that hyperspectral imagery captured prior to harvest could be used to classify nine sugarcane varieties 100% of the time using an innovative statistical method (support vector machines) (Everingham et al., 2007). Similar work using hyperspectral imagery in Brazil focused on discriminating sugarcane varieties (Galvao et al., 2005, 2006), assessment of the area under production and yield estimation (Almeida et al., 2006). Indeed, remote sensing may circumvent or solve the problems associated with understanding the spatial relationship of yield gathered from the yield monitor and commercial cane sugar of the paddock. Regardless of the use of remote sensing technology, the major challenge facing the industry is the process of streamlining interpretation. Research should be directed towards automated, generic and mechanistic solutions for interpreting and analysing remote-sensed data for whole of industry benefit (Davis et al., 2007).

4.5 Variable Rate Technology

The concept of variable rate technology and application is the embodiment of precision agriculture. The ability to variably apply inputs on-the-go makes this technology the “ultimate challenge” for industry and researchers. The basic concept draws together several enabling technologies in one package. A GPS must be positioned on the machinery used within the field; an on-board computer with input recommendation maps controls the distribution of inputs onto the field by matching position and input recommendations. Alternatively, these systems can be sensor based to segment the growing space into different target areas. For instance, segmentation of weeds, crop and soil in real time could be used as part of an on-the-go variable rate application of herbicide targeting weeds while sparing the unaffected soil and crop areas (Marchant et al., 1998, 2001). An example of a commercially available on-the-go proximal sensor based real-time nitrogen management tool is the GreenSeeker RT200^{TM} developed by NTech Industries^{TM}. This technology is used on crops such as cotton and epitomizes the potential of variable rate technology and robotics in agriculture. Currently there are no commercial examples of variable rate technologies that have been specifically designed for sugarcane farming. This is unsurprising as variable rate technology is the culmination of a number of enabling technologies that must be operating at a high level of consistency and accuracy. Additionally, baseline data collected over many seasons and the cause and effect nature of the production system must be well understood before this technology will become commercially available. Given that many of these technologies are still being specifically developed and tested for use in sugarcane production it will be a number of years before the industry has a choice of variable rate tools. However, steps are being taken to gather the baseline data and evaluate the benefits of variable rate technology. Research in the United States emanating from yield monitoring trials investigated the relationship between yield and soil variability and found that a large number of weak correlations existed between soil chemical properties and yield (Johnson and Richard, 2005b). These parameters were then shown to be spatially correlated, and an experiment to variably apply lime to cane blocks that varied in the amount of pH (4.9–6.4) was undertaken (Johnson and Richard, 2005a,b). This work highlighted the reduction in lime costs when applied variably to those parts of the block where it was needed and exemplifies the benefits of variable rate technology when an input is matched to field variability. In Thailand, a field test of a real-time inter-row variable rate tractor-mounted herbicide applicator performed satisfactorily with a measured reduction of at least 20% in herbicide when compared to the uniform management approach (Tangwongkit et al., 2006). However, this technology is still in development and needs considerable investment for full-scale commercialisation. With many of these technologies still in their infancy, variable rate technology is still an ongoing research area that may be close to fruition in some parts of the world. With the speed of technological development, variable rate technology will have a big impact on sugarcane production in the medium to longer term and offers a range of environmental and economic benefits derived from more efficient use of inputs.

5 Knowledge Extraction

With the rapid surge in spatial data acquisition methods, it is obvious to the precision agriculture community that we are drowning in data but thirsty for knowledge (Kitchen, 2008). Managing and synthesising information embedded in these large and complicated spatial datasets will become a major challenge for farmers and researches alike. As part of a participatory environment, data-driven statistical methods, innovative data mining techniques, process-orientated crop models and decision support systems can contest this challenge. With an increase in the amount of data collected by industry, modelling capability is becoming an increasingly recognised component of the precision agriculture process and must be addressed by the global sugarcane industry.

5.1 Statistical Analysis

Within the broad field of precision agriculture many different approaches to analysing spatial data have been explored. Conventional statistics does not take into account the spatial location of objects; however, if these methods are used to test for attributes of spatially located objects the user can test if these are geographically separate (Burrough, 2001). Two approaches have been developed under the umbrella of statistical data analysis. These are exploratory data analysis methods where the accent is on descriptive univariate and multivariate statistics. Here the operator can visually search for outliers or patterns across a spatial domain of interest (Burrough, 2001; Tukey, 1977). The second centres on confirmatory spatial analysis where the attention is turned towards defining relationships by building regression models and/or testing hypotheses (Burrough, 2001). In many applications it is sensible to consider the variation of an attribute in terms of a continuous albeit noisy surface which is interpolated from sets of data which are collected with spatial information. There are three broad categories of spatial data: geostatistics, where the response is measured at a point location (e.g. soil Phosphorus at points within the paddock); regional data, where the response is obtained from a spatial region (e.g. tonnes of cane crushed at each mill); and point patterns, where the response is the location of an event (e.g. locations of a disease outbreak within a farm). These methods use the stochastic theory of spatial correlation for apportioning uncertainty and for interpolation. Primarily, spatial statistics is about making predictions under limited information and uncertainty using the spatial auto-covariance structures of points in space. It is important to note that GIS ignores statistical variation while spatial data analysis uses the understanding of variation as an important source of information for predicting un-sampled points. Although there are a number of ways to analyse spatial data sets using conventional or spatial statistics, the importance of incorporating spatial information is essential for precision agriculture. Therefore, statistical analysis plays an important role in drawing together field observations so that management decisions can be applied to the observed variability. Maximum benefit to the grower will be realised when the industry develops generic tools for collecting accurate and timely data, analyses it in an appropriate manner and uses that data to effect on-farm management decisions.

5.2 Data Mining

With the ever-increasing availability of data collected as part of the precision agriculture process, industry is in need of a rigorous tool for modelling and analysis. Data mining is a methodology that draws on innovations from the fields of machine learning and multivariate and computational statistics. Data mining is the specific process of selection, exploration and modelling of large and complicated data sets for the extraction of patterns and models with the aim of obtaining clear and useful results (Giudici, 2004). This makes data mining a powerful tool for extracting patterns from the plethora of precision agriculture information. This review does not aim to discuss the myriad of methodologies associated with data mining, but draws attention to a possible solution for extracting meaning from the abundance of spatial data collected by industry. Indeed, the application of data mining to precision agricultural problems has been successfully used in the past. A neural network model was used to set realistic yield goals for parts of the field and predicted corn yields with 80% accuracy (Liu et al., 2001). The ability of the model to integrate historical and measured input data to build a knowledge discovery environment that captures the interaction between inputs and yield shows considerable promise for understanding complex agricultural systems. Other examples of the application of data mining includes soil map modelling with the use of induction rules based on decision trees (Moran and Bui, 2002) and the identification of hyperspectral bands with minimum redundant information (Bajawa et al., 2004). A specific example relating to sugarcane production includes the classification of variety and crop characteristics (Everingham et al., 2007) using modern data mining techniques. It is clear that data mining tools are beginning to show considerable value for analysing large data sets derived from complicated systems and may provide high-quality information for decision makers. This is certainly one tool available to the precision agriculture community for extracting information and should be explored further by sugarcane industries.

5.3 Crop Models

Statistical methods alone however are not sufficient. The field of agro-statistics is multidisciplinary and requires a systems understanding. Crop modelling is a process-oriented approach that can form part of a framework for assessing and understanding agronomic systems. Crop models can be used to understand yield variability in space and time from which optimisation and prediction outcomes can be achieved (Boote et al., 1996; Van den Berg et al., 2000). Therefore, crop models are particularly useful for managing temporal variability (Singels, 2007), as opposed to most of the other statistical techniques described which focus on managing spatial variability. Understanding variability across space and time is essential because the factors that affect crop yield exhibit different spatial and temporal behaviour. By combining a number of enabling technologies, such as GIS, GPS and remote sensing, the means to asses the spatial variability within the field has become increasingly available. Traditional static analytical techniques have failed to explain the reasons behind yield variability due to the dynamic and temporal interactions of stresses on crop growth and development (Basso et al., 2001). Process-oriented simulation models have the ability to integrate the effects of time and multiple stress interactions on crop growth under different hypothesised environmental and management conditions (Basso et al., 2001). Examples of such agronomic models are the CERES, DSSAT (Jones et al., 2003), APSIM (Keating et al., 2003; McCown et al., 1996), STICS (Brisson et al., 2003), CropSyst (Stöckle et al., 2003) and CROPGRO family that have specific applications for a broad range of crops. Specific sugarcane models are the Australian APSIM-Sugarcane model (Keating et al., 1999; Lisson et al., 2000), the lesser known Australian model QCANE (Liu and Kingston, 1995), the South African CaneSim (Singels and Donaldson, 2000) and CANEGRO model (Inman-Bamber, 1991) that is distributed in the DSSAT crop modelling package and the Brazilian MOSICAS (Martiné and Todoroff, 2002) to name a few. Since inception, refinement to the models has continued to improve our understanding of variability across time and space and has emerged as a powerful tool available to the sugarcane industry. Keating et al. (1999) describes how the APSIM-Sugarcane model can simulate a number of physiological responses such as growth, water use efficiency, N accumulation, yield for plant and ratoon crops in response to external influences such as soil, climate, genetic and management influences. When directly comparing the model predictions against observed data, the coefficients of determination in some cases were as high as 0.93 for biomass or 0.86 for N accumulation which suggest a high degree of reliability. Similar results were obtained for a stalk water content module developed for the integration into the dry matter sugarcane simulation model QCANE for the explicit simulation of fresh weight yield (Liu and Helyar, 2003). Comparison of observed and simulated yields returned an average coefficient of determination of 0.95 which clearly demonstrates a high degree of reliability (Liu and Helyar, 2003). Another example of the application of crop models in sugarcane is the use of APSIM-Residue to evaluate the decomposition of surface residues over time (Thorburn et al., 2001). The rate of decomposition can significantly affect soil hydrology and nitrogen cycling further influencing crop growth. This study compared predictions to measured residue decomposition at different sites across contrasting climates. APSIM-Residue could produce more accurate predictions of the decomposition of large (>10 t DM ha^{-1}) residue masses which are common in sugarcane production and highlights the need for a crop-specific modelling approach (Thorburn et al., 2001). Crop modelling research in sugarcane has also focused on simulating dry matter partitioning. Sugar production depends on stalk biomass where sucrose is stored in stalk parenchyma with various dry matter components. Particularly important is the sucrose concentration used to calculate cane consignment delivered to the mill (Inman-Bamber et al., 2002). A detailed evaluation of this body of work is beyond the scope of this review and the authors point the reader to the following articles and references therein: Inman-Bamber et al. (2002), O′Leary (2000), and Singels and Bezuidenhout (2002). Specific examples of crop modelling and precision agriculture for sugarcane cropping systems are difficult to source in the scientific literature to date. However, an example using the crop model CROPGRO for soybeans and remote sensing for the identification and cause of yield variability within paddocks (Basso et al., 2001) highlights a potential avenue for exploration in sugarcane. A better understanding of site-specific variability using other enabling technologies such as remote sensing and the potential to build that knowledge into crop models will only enhance our ability to manage variability both spatially and temporally.

5.4 Decision Support Systems

Decision support systems complete the precision agriculture information-gathering process. There is no use gathering information about variability if there is no decision support system to make site, farm or regionally specific recommendations to the grower. This service integrates data sources with expert knowledge of the production system with crop models to derive strategic short- and long-term decisions. Specifically, they operate by combining crop models that contain information on crop growth, with peripheral economic and environmental constraints. As a strategic tool, decision support systems should supply the user with a range of possible management strategies in response to a set of hypothesised scenarios. Alternatively for day-to-day use in precision agriculture users may prefer a set of guidelines in response to a particular situation. For example, within the Australian sugarcane industry the development of integrated cane harvest management systems have been developed within some sugarcane-growing regions. This system targets the marketing and logistics, mill start times, transport arrangements and harvest schedules of the production system (Crossley and Dines, 2004; Davis et al., 2007; Markley et al., 2003). A further study involving the global industry identified the possibility of incorporating seasonal climate forecasts into key industry decisions of growing, harvesting, milling and marketing and presented a number of case studies (Everingham et al., 2002). These studies are examples of precision agriculture working across farm and mill levels to assist industry decision making to support a whole of industry strategy (Davis et al., 2007). Ultimately the information provided could potentially direct a grower, miller or marketer to perform a set of actions in response to the forecast or recommendation. The realisation that the challenge for decision support systems is to link the science of technology with the "needs" of industry is central to the adoption of any decision support system (Everingham et al., 2002). Indeed, to turn variability into decisions the global sugar industry must understand how to integrate precision agriculture into an industry that has such a diversity of decision makers. The solution to these problems extends beyond agricultural science and into the realms of social science. Nonetheless, decision support systems are important precision agriculture tools for bridging the gap between the technology and the recommendations derived for enhanced on-farm decision making.

6 Adoption

Advancement in data acquiring technology essential for precision agriculture has grown steadily over the past decade. However, the adoption rate of precision agriculture has not advanced at the speed predicted at the turn of the century (Fountas et al., 2005; McBratney et al., 2005). Reasons for the proposed lack of adoption are the lack of appropriate decision support systems (McBratney et al., 2005), time requirements, data handling costs (Fountas et al., 2005), lack of integration among precision agriculture technologies, perceived complexity (Pierce and Nowak, 1999), lack of benefit, and lack of demonstrated effects on yield, input use and environmental performance (Batte and Arnholt, 2003). Furthermore, our ability to acquire large amounts of data has in many cases blinded our ability to analyse, understand and apply knowledge embedded in these data sources further exacerbating the problem of fast adoption arising from complexity issues (Lamb et al., 2008).

The sugarcane cropping system is composed of many individual components that act synergistically to determine yield. These components may include crop rotation, tillage, fertilizer efficiency, water stress, competition from weeds, predation from pests, disease, nutrient availability, climate and other factors. The complexity associated with each component, and how the different components interact with one another poses many challenges (Clay et al., 2006). Consequently, it is unsurprising that the complexity of managing such a system and the costs associated with acquiring new technologies overwhelms many growers. Furthermore, critics of precision agriculture argue that the actual benefits (both economic and environmental) have not been clearly demonstrated to the scientific community. The basis of this argument centres on the lack of published data that explicitly demonstrates an economic benefit from the adoption of precision agriculture (Bramely, 2007). However, Bramely (2007) correctly points out that producers do not generally publish analysis of their own economic performance. Economic benefits derived from the implementation of precision agriculture are also complex in nature and are dependent on the interaction between farm size, expense of equipment and the increase in yield required to offset these costs (Godwin et al., 2003). If the view of precision agriculture to sugarcane production is confined to economic rationalisation it appears likely that benefits will accrue to some producers and not others. This will depend on the ease of which variation can be identified and the ease of managing this variation on each individual farm (Bramely, 2007; Griffin and Lowenberg-DeBoer, 2005). The cost of diagnosing this variability and the cost of time associated with managing this variability will also be a major consideration. The resultant cost/benefit trade-off will take into account the likely size of the benefit in relation to these and other perceived costs. If the perceived benefits are large enough to motivate change then the likelihood of adoption will be increased (Davis et al., 2007).

Sugarcane production is well suited to the adoption of precision agriculture with many exploring a myriad of technologies for quantifying variability (Bramely, 2007; Bramely and Quabba, 2002; Cook and Bramley, 1998; Cox, 1997; Everingham et al., 2003; Everingham et al., 2007; Galvao et al., 2005, 2006; Higgins et al., 1998; Johnson and Richard, 2005b; Magalhaes and Cerri, 2007; Mehrotra and Siesler, 2003; Muchow et al., 1998; Saraiva et al., 2000; Sparovek and Schnug, 2001). However, the ability to successfully quantify variability will not drive the adoption of precision agriculture. It will be essential to demonstrate and link the economic, environmental and yield/quality benefits of precision agriculture to the individual grower. As an example, the knowledge of spatial variability of commercial cane sugar may enable more sugar to be produced at the district and from the farm as a whole. A number of studies in Australia have explored this issue and concluded that a potential for significant increases in profitability could be obtained if the spatial structure of commercial cane sugar at the mill level is taken into consideration when planning harvesting times (Higgins et al., 1998; Lawes et al., 2004; Muchow et al., 1998; Wood et al., 2005). This low-cost example of precision agriculture operating at a mill level has the ability to improve sucrose yield and profits for individual land holders and illustrates the benefits of managing spatial variability. It is also important to have a long-term vision when assessing the benefits of precision agriculture and look beyond the immediate benefits to the grower and towards the longevity of the industry. For example, depending on market pressure and expectation, the grower may not see an immediate economic return from the implementation of precision agriculture but will improve the environmental and/or yield/quality objectives on the farm. These traceable improvements to the environment may in turn open new markets which will increase the demand and profitability of the commodity otherwise unexplored under the old management regime. Nonetheless, it is all well and good to provide examples of the usefulness of precision agriculture but understanding the "how's" and "why's" of adoption are critical for wide-scale acceptance. The slow adoption rate of precision agriculture is a general phenomenon exhibited by all agricultural sectors involved in precision agriculture and is strongly linked to perception and adoption dynamics of innovative technologies.

6.1 Adoption of Innovations

The dynamics of adoption can be examined under the theory of diffusion of innovations inherent in any group of adopters (Fig. 3) (Lamb et al., 2008; Rogers, 2003). The dynamics of the group vary from the initial appearance of an innovative product. The *innovative minority* (∼3%) who take up the technology are proactive information seekers and are often risk takers. The next group of adopters are the *early adopters* (∼13%) who by definition are highly educated and may be local or regional leaders. The next to follow are the *early majority* and *late majority* (∼68%). The *early majority* are generally more deliberate in their analysis of the risk/benefits of the innovation with the *late majority* tending to be more sceptical. Finally, the *laggards* make up the remaining 16% and rely on informal contact with neighbours about developments. It has been argued that precision agriculture has not progressed beyond the early adopter level (Lamb et al., 2008). The reason proposed by the author is that the *early adopters* tend to rely on primary sources of information direct from the developer and are willing to piece the product together and often assume roles in the research and development phases of the product. However, the majority (∼68%) rely on word of mouth which contains the hard lessons learnt by others. If the hard lessons learnt are many, the uptake is stalled (Lamb et al., 2008). Central to overcoming this adoption barrier are the perceived usefulness and perceived ease of use any new technology (Davis, 1989). Growers will only continue to use and recommend a new technology if they believe it will help them perform

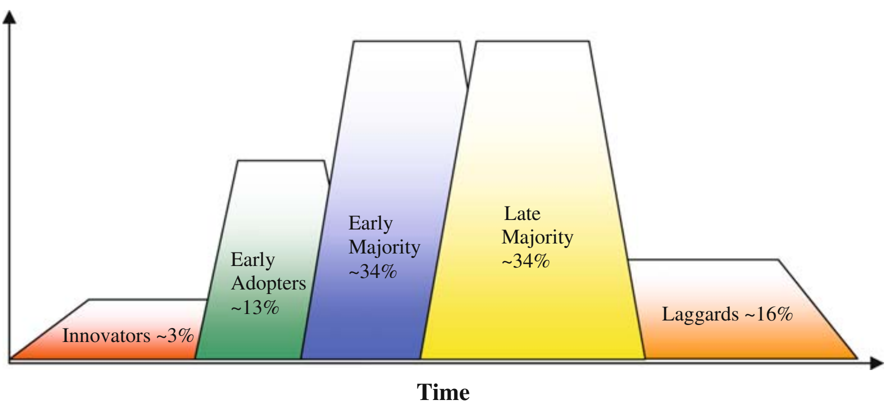

Fig. 3 Distribution of adopter cycle based on innovativeness. As an innovation moves through the adopter cycle from left to right the piece of technology encounters different groups which have different needs and perceptions (Adapted from (Lamb et al., 2008; Rogers, 2003))

their job better and perceive it to be useful and easy to use. However, a grower may perceive the technology to be useful but believe the system is difficult to use and the benefits of using the system are outweighed by the extra time and effort required. An example of this dynamic was highlighted in a case study of six leading edge adopters of precision farming technology (Batte and Arnholt, 2003). These technology-capable farmers expressed frustration with hardware, software and procedures of the systems (Batte and Arnholt, 2003). However, these farmers all agreed they would continue to adopt new technologies as they became available. Clearly the usefulness of the products outweighed the ease-of-use issues associated with the technologies; however, this mixed message may dissuade others from adopting the technologies. The key lesson for technologists is the inherent difference among the *innovators*, *early adopters* and the *majority*. An unrealistic view of the needs of the *majority* may be formed from dealings with the *innovators* and *early adopters* with the *majority* mostly expecting "plug and play" solutions (Lamb et al., 2008). In order to overcome these problems precision agriculture technologists need to understand the changing expectations of the groups as the technology goes through the adoption cycle (Fig. 3). Evaluation programs which engage a broad cross-section of users and a feedback loop from users to technologists in response to how the technology is being used in the field is essential for improving the compatibility with the next in line on the adopter cycle (Lamb et al., 2008).

6.2 Adoption Pathways

One solution for improving the adoption of any complex technology is the concept of participatory action research. Participatory action research ensures that the technology is developed in a way that involves end users. Specifically, researchers and collaborators work together in cycles of action and research to identify and address problems as they arise (Parkes and Panelli, 2001). Participatory action research is a bottom-up approach which focuses on locally defined objectives and involves local people in the research and planning phases (Cornwall and Jewkes, 1995). However, the perception of the technology and how each group and individuals within each group interacts with that piece of technology can be vastly different between and among the two groups. These differences in perception can cause a disjunction between farmers and researches and can lead to a breakdown in the action research environment. A proposed framework for facilitating the process of participatory action research is the concepts of technology frames, interpretive flexibility and boundary objects (Jakku et al., 2007). Within the immediate social context, technology frames are the mental constructs that people build about a particular technology while interpretive flexibility is the idea that an object/technology can mean different things to different people (Jakku et al., 2007). An example of these concepts can be illustrated in the development of a hypothesised GPS technology for within-field use. Growers might think that the GPS is only useful on a tractor for controlled traffic applications for minimising soil compaction, while researchers may perceive GPS to be integral for providing precise locations of within-paddock management zones that can be used for the variable rate application of fertiliser. Both the growers and researchers have built a mental construct of what they think GPS is useful for within the field (interpretive flexibility). Both the growers and researchers have interpreted the meaning and value of the GPS, they have formed an opinion on whether it should be implemented, why it should be implemented and how it can be used (technology frame) (Jakku et al., 2007). At this stage both the growers and researchers have differing views of the GPS technology; however, through the process of participatory action research the technology can be refined to meet the expectations of both groups. The characterisation of a boundary object can be used to describe the situation when the technology can be considered plastic enough to adapt to local needs and constraints, but robust enough to maintain a common identity or point of reference (Jakku et al., 2007; Star and Griesemer, 1989). Therefore, a boundary object means different things to different people yet still maintains a common identity between the researchers and the growers and facilitates the process of negotiation, cooperation and learning among developers (Jakku et al., 2007). The boundary object facilitates the cycle of action learning (Parkes and Panelli, 2001) integral for participatory action research development and increases the shared understanding among the different groups involved. As the product and research evolves in response to the action and learning environment the product is likely to fulfil the needs of both groups creating a perception of usefulness. Therefore, the likelihood of the technology being adopted by the majority of growers would improve as a result. Although the global sugarcane industry is in the early stages of precision agriculture reform, it would be prudent for those involved in developing the technologies to plan and understand how precision agriculture will become adopted. An understanding of the sociology of science and technology may prove a useful vehicle for overcoming problems associated with the adoption of complex technologies. Without attention to these aspects of precision agriculture the promised benefits will not be realised by the majority of growers and the technology will be left languishing at the early adopter level, failing to fulfil its commercial potential.

7 Conclusion

Precision agriculture is often thought of as a within-paddock management system; however, we propose that precision agriculture can operate at the region, mill, farm, paddock and sub-paddock scale and encompasses all attempts to manage the cropping system in response to variability. An understanding of how the different components perform individually and how these interact with each other is integral for assessing this variability. Regardless of scale, the process begins with collecting and extracting information from large amounts of data so that growers can plan, manage and evaluate actions in response to the observed variability. This process must be performed over time so that the temporal and spatial relationships are taken into consideration. As production systems become larger and the variability in the growing conditions become more complex, the multidisciplinary partnership between farmers, scientists and information technologists becomes integral for actuating the precision agricultural process. By managing the variability within the sugarcane production system, precision agriculture can potentially lessen the environmental loading of inputs, reduce the variability of crop performance, increase yield and shrink immediate input costs. In sugarcane, production is rapidly increasing, markets are forced to become more globally competitive and the production system is expected to become more environmentally friendly. In response to these pressures, precision agriculture promises a framework to address the economics associated with an increase in production while conserving the environmental resource base on which agriculture depends.

As part of the information-gathering process, technologies such as the GPS, GIS, remote sensing, proximal sensing and variable rate technology play an important role in ascertaining and managing variability. The majority of these technologies are still in the research and development stage in sugarcane for application at the within-paddock level. The non-commercial readiness of these technologies exemplifies the need for the sugarcane industry to "catch up" to other more progressive agricultural sectors. However, the majority of these technologies can be used for larger-scale forms of precision agriculture and are being used successfully to manage variability at a regional, mill and farm level. With the commercialisation of precision agricultural technologies, the amount of data collected by industry will rapidly expand. As part of the precision agriculture process, statistical methods, data mining techniques, crop modelling and decision support systems are a number of methods for turning data into decisions. These components of the precision agricultural cycle are often underestimated but will become increasingly important with an increase in data availability. Nonetheless, quantifying variability will not lead to the widespread and successful adoption of precision agricultural technologies. The adoption rate of precision agriculture has not progressed as quickly as many in the field expected.

Unlike many discussions on precision agriculture, we have examined the adoption of innovative technologies integral for wide-scale implementation. Particularly important are the changing expectations of each of the adopter groups as the technology goes through the adopter cycle. These technologies must be perceived to be both useful and easy to use by the majority of growers for wide-scale implementation. Participatory action research is proposed as one solution for improving the adoption rate of innovative technologies. For this framework to be effective, researchers and growers must work together in cycles of action and research to achieve a common goal. When both the developers and end users are involved in the development of a technology the likelihood that the technology will progress beyond the early adopter level can potentially be improved. As we struggle to increase productivity to meet the needs of an ever-increasing population we must change our agricultural practices to become more efficient and environmentally sustainable. Not only do we need to understand and quantify our agricultural systems but we need to have a pathway that facilitates the progression of technologies from development to wide-scale commercialisation.

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Fungal Disease Management in Environmentally Friendly Apple Production – A Review

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Abstract Many pesticides are used very effectively against fungal diseases in crop protection. However, the widespread use of synthetic pesticides in conventional fruit production clearly indicates that pesticides have several limitations and serious harmful effects on the environment and on human health. This prompted a serious need for a more environmentally benign view in the practice of fruit growing and particularly in plant protection, which also strengthened the concept of environment-friendly approach for apple. In this review article, the present status, possibilities and approaches towards fungal disease management for organic and integrated apple production systems, which are the most prominent environmentally friendly production systems of apple, are reviewed. The review focuses on the control of five important apple diseases: apple scab (*Venturia inaequalis*), apple powdery mildew (*Podosphaera leucotricha*), European canker (*Nectria galligena*), brown rot (*Monilinia* spp.) and the disease complex of flyspeck and sooty blotch. The first section of this study provides background information and basic features of current disease control in both apple production systems. Then, in the second section of this study, details of novel aspects of non-chemical control approaches against apple fungal diseases, including agronomic measures, mechanical, physical and biological control options as well as essential features of apple cultivar resistance to fungal diseases are given. The overview on five groups of agronomic measures: (1) cropping system and cover crop, (2) plant material and planting, (3) pruning and canopy management, (4) orchard floor management and (5) nutrient supply and harvest, and another five groups of mechanical and physical control methods: (1) pruning, (2) removal of inoculum sources, (3) shredding of leaf litter, (4) burying of inoculum sources and (5) flaming of leaf litter, showed that these non-chemical control measures are one of the most essential approaches for reducing the infection potential of inoculum sources in apple orchards. However, most of these methods

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are not widely spread in the apple-growing practice due to their high labour costs and/or time limits during the season. We showed that expert-system-based automatisation in the future may greatly enhance the effective integration of these methods into apple growing. We also described almost 30 biological control options, including antagonists, extracts/oils of plants and composts, which were explored recently against fungal diseases of apple, though only few of them are commercially available for the apple-growing practice. Most of these biological control options are suitable only for organic apple growing, as their effectiveness against the key fungal diseases is not able to fulfil the requirements for integrated apple orchards or they are not substantially cost-effective. Developing an effective biological control against polycyclic fungal diseases of apple will be a great challenge in the future for preharvest disease management programmes. In our literature analyses, host resistance, based on breeding programmes for multiple disease resistance, was evaluated as the greatest potential in the effective disease management of environmentally friendly apple production systems. Theoretically, aiming for complete host disease resistance would result in eliminating one of the basic elements of the epidemic triangle and omission of chemical control approaches from disease management of apple.

In the third section of this study, developments in chemical control options for individual diseases are described presenting recently explored knowledge on approved fungicidal products in integrated and organic disease management. Efficacy evaluations of fungicidal products coupled with recent developments on disease-warning systems as well as season-long spray schedules for each disease are discussed for both integrated and organic apple orchards. In addition, the main features of six inorganic chemical compounds, copper, lime sulphur, elemental sulphur, bicarbonates, hydrated lime and kaolin, are described for organic apple production. Then in the fourth section of this study, non-chemical and chemical control approaches are integrated into a multiple management tactic across all fungal diseases and are specified for integrated and for organic apple production systems. Here it was shown that in the past 20 years continued developments of disease-warning systems and host resistance to fungal pathogens, as well as incorporation of some non-chemical control options into fungal disease management of apple resulted in a considerable reduction in the number of fungicide sprays of the season-long disease management programmes. In the final section, suggestions and future trends are given for further improvements in fungal disease management for the two environmentally friendly apple production systems. Finally, it was concluded that the challenge for apple integrated pest management (IPM) programmes in the twenty-first century is to complete the fourth, final IPM level, which supplements IPM level 3 with cultural, social and political realms. While in organic apple orchards, the most essential task is to develop effective non-chemical control options that are practically feasible and can be incorporated easily into the orchard management practices.

Keywords Apple scab · Brown rot · Copper · Sulphur · DMI · QoI · Disease control · European canker · Flyspeck · Integrated · *Malus* × *domestica* · Organic · Apple powdery mildew · Sooty blotch · Sustainable agriculture

Abbreviations

AUDPC	=	area under the disease progress curve
BCA	=	biological control agent
CLW	=	cumulative leaf wetting
cv.	=	cultivars
CWE	=	compost water extract
DMI	=	demethylation inhibitors
EBI	=	ergosterol-biosynthesis inhibitors
FRAC	=	Fungicide Resistance Action Committee
GSE	=	grapefruit seed extract
IFOAM	=	International Federation of Organic Agriculture Movements
IPM	=	integrated pest management
LWD	=	leaf wetness duration
MPH	=	mono-potassium phosphate
PAD	=	potential ascospore dose
PBC	=	potassium bicarbonate
PF	=	petal fall
QoI	=	quinone outside inhibitors
SBC	=	sodium bicarbonate
SMS	=	spent mushroom substrate

1 Introduction

In the 1960s, after not more than a decade of the widespread use of the first non-phytotoxic and highly effective synthetic pesticides in conventional agriculture, it became obvious that they have several limitations and serious harmful effects on the environment and on human health. This prompted a serious need for a more environmentally benign view in the practice of agriculture and particularly in plant protection which strengthened the concept of environmentally friendly approach for agriculture. By the end of the 1970s after a long period of development (started long before the introduction of the above-mentioned synthetic pesticides), environmentally friendly production systems emerged in apple production and later two directions became known worldwide: the integrated and organic production systems (e.g. Sansavini, 1990, 1997; Sansavini and Wollesen, 1992; Reganold et al., 2001; Bellon et al., 2001; Ferron and Deguine, 2005; Lancon et al., 2007).

By now, the rules and several tools for fungal disease management are well-defined and most of them are successfully implemented for the two environmentally friendly production systems in apple (e.g. Anonymous, 1989; Cross and Dickler, 1994; Zalom, 1993). Disease management practices in integrated and organic apple production differ markedly from those in conventional production. Synthetic products are restricted in integrated and banned in organic apple production. In organic apple growing, only natural products such as compost, suspendable rock powder, sulphur and copper compounds, fungicidal and botanical soaps, traps and biological methods are permitted against fungal diseases according to IFOAM (International Federation of Organic Agriculture Movements) standards (Anonymous, 2000), while many synthetic pesticides can be used in conventional apple production. Via the application of these management options, disease management may be less effective in integrated, and especially in organic, apple production than in conventional production with the consequence that production risks are likely to be higher in such systems.

Integrated pest management (IPM) was introduced in apple production in the second half of the twentieth century with the aim of integrating pest management tactics in order to reduce pesticide use. The general acceptance of the apple IPM concept in disease management was the result of some highly forcing elements in fungicide use. First, in apple disease management programmes, narrow-spectrum systemic fungicides are usually combined with broad-spectrum protectant fungicides in order to increase efficacy and minimise fungicide resistance of pathogens. However, the registration of broad-spectrum fungicides is jeopardised due to the zero-risk standard (NRC, 1987; Merwin et al., 1994) with the result that less and less broad-spectrum protectant fungicides are/will be available for disease management. Second, in spite of the fungicide combination or rotation tactics, some apple diseases, mainly apple scab (*Venturia inaequalis*) and apple powdery mildew (*Podosphaera leucotricha*), became resistant to several highly important narrow-spectrum fungicides. For instance, resistance to dodine of the apple scab fungus, *V. inaequalis*, has been known since the late 1960s, to benzimidazoles since the early 1970s, to demethylation inhibitor (DMI) fungicides since the mid-1980s, to anilinopyrimidines since the late 1990s, and such resistant strains frequently occur all over the world (e.g. Szkolnik and Gilpatrick, 1973; Wicks, 1974, 1976; Stanis and Jones, 1985; Köller, 1988; de Waard, 1993; Köller et al., 1997, 2005; Küng et al., 1999; Köller and Wilcox, 2000). *V. inaequalis* also showed reduced sensitivity recently to kresoxim-methyl, a member of the more recently introduced quinone outside inhibitor (QoI) fungicides (Sallato and Latorre, 2006; Jobin and Carisse, 2007) though this has yet only local importance. The really damaging effect on apple disease management programmes is caused by resistance to DMI, anilinopyrimidine and QoI fungicides, as they are the principal classes of fungicides used in the post-infection control of apple scab and other less important fungal diseases. Furthermore (i) the high cost of developing new classes of fungicides, (ii) strict rules of registration, and (iii) the risk of resistance to these new site-specific fungicides have been directing on the way that the future availability of synthetic fungicides for tree-fruit diseases has become increasingly uncertain (e.g. Merwin et al., 1994; Holb et al., 2006). All the above reasons have created a great interest in and priority of other, non-chemical disease management strategies in spite of the fact that these are more expensive and biologically not so effective against the fungal pathogens as compared to synthetic fungicides.

Organic production has its origins in Germany starting at the beginning of the twentieth century (Vogt, 2000) though its worldwide establishment and regulation started in 1977 when the first IFOAM congress was held in Sissach, Switzerland (Weibel, 2002). Industrialised organic apple production started only in the late 1980s in Europe (Weibel and Häseli, 2003). Presently, the organic apple production area is still small (a few thousand hectares in Europe) compared to integrated production but it is continuously growing year by year. The major problem of fungal disease management in organic apple production is the lack of effective fungicides or natural products against the most damaging apple diseases such as apple scab and European canker. Therefore, organic apple growers have to rely strongly on integration of direct and indirect non-chemical control options, which often result in 15–50% yield loss caused by fungal diseases (e.g. Weibel, 2002; Holb, 2005a, 2008b).

More than a hundred pathogens cause diseases of apples (Biggs, 1990) but only three of them (apple scab, apple powdery mildew and fire blight) have worldwide importance in disease management of environmentally friendly apple production. Out of several other diseases, cankers, fruit rot, flyspeck, sooty blotch and rust can also be highlighted with different levels of importance in a regional scale. In this review, an attempt is made to review the recent fungal disease management options in the two environmentally friendly apple production systems: integrated and organic. More specifically the aims of this review were first, to evaluate each non-chemical management approach for fungal diseases of apple with emphasis on apple scab, apple powdery mildew, European canker, brown rot, flyspeck and sooty blotch; second, to show recent developments in chemical control options for individual diseases; and third, to integrate non-chemical and chemical control approaches into a multiple management tactic across all fungal diseases separately for integrated and organic apple production. This study reviews only preharvest, not post-harvest, disease management of apple.

2 Non-chemical Control Approaches Against Fungal Diseases of Apple

Non-chemical control options are of basic interest for both integrated and organic apple production, which include indirect (orchard management practices) and direct (e.g. physical and biological) control measures. In this section, recent developments in non-chemical control options are listed and then their efficacy is evaluated on fungal diseases of apple, focusing on apple scab, apple powdery mildew, European canker, brown rot, flyspeck and sooty blotch (Table 1).

2.1 Orchard Management Practices

Orchard management practices in apple production include several options (e.g. cropping system, planting, pruning, orchard floor management, nutrition supply and harvest) that affect fungal disease management. Orchard management practices are applied in order to provide the best conditions for tree growth as well as to improve yield and fruit quality. This indicates that orchard management has more general aims than just to protect the crop from fungal diseases. Thus, an impact of a particular orchard management practice may have a more indirect

Table 1 Some reviewed aspects of non-chemical control approaches used against scab, powdery mildew, European canker, brown rot, flyspeck and sooty blotch in preharvest disease management of apple

	Fungal disease				
Non-chemical control approaches	Apple scab	Apple powdery mildew	European canker	Brown rot	Flyspeck and sootyblotch
Orchard management practices					
Cropping system and cover crop	Tall grass (reduces ascospore escape)	–*	–	Tall grass (increases sporulation on dropped infected fruit)	–
Plant material and planting	–	–	Infected young nursery tree	–	–
Pruning and canopy management	Effect on canopy microclimate and fungicide coverage	–	–	–	Effect on canopy microclimate and fungicide coverage
Orchard floor management	Mulching, tillage	–	–	–	–
Nutrient supply and harvest	Effect of harvest in rainy days on storage scab	Effect of nitrogen supply on shoot infection	–	Effect of late harvest on post-harvest damage	–
Mechanical and physical control					
Dormant pruning	Woody-shoot, bud	Woody-shoot, bud	Infested twig, leaf scars	Mummified fruit	–
Removal of alternate host	Wild apple, hawthorn, mountain ash, firethorn, locquat	–	–	Rosaceous hosts	Blackberry

Table 1 (continued)

Non-chemical control approaches	Fungal disease: Apple scab	Apple powdery mildew	European canker	Brown rot	Flyspeck and sooty blotch
Removal of crop debris	Fallen leaf; against primary sexual inoculum	–	–	Dropped fruit; clustered fruit against asexual inoculum	Clustered fruit; against asexual inoculum
Shredding of leaf litter	Against primary sexual inoculum	–	–	–	–
Burying of inoculum	Against primary sexual inoculum	–	–	–	–
Flaming of leaf litter	Against primary sexual inoculum	–	–	–	–
Biological control					
Antagonists	Against leaf and fruit infection as well as ascocarp in leaf litter	Against shoot infection	Against leaf scar and pruning wound infection	Against fruit infection	Against fruit infection
Extracts of plants	Against leaf and fruit infection	Against shoot infection	–	–	Against leaf and fruit infection
Plant oils	Against leaf and fruit infection	Against shoot infection	–	–	–
Composts	Against leaf and fruit infection	–	–	–	–
Host resistance	Genetic resistance of cultivars	Genetic resistance of cultivars	Age-related wound resistance, host physiology	Dependent on insect damage and growth cracking	Dependent on fruit skin colour, maturity date, epicuticular wax

*– not investigated for the pathogen in detail in scientific studies.

effect on pathogen populations and, subsequently, on disease severity by affecting the host or modifying the environment. However, these effects on fungal diseases cannot be simply classified and in most cases it is difficult to establish a straightforward cause-and-effect relationship between orchard management practices and disease development. On the other hand, several orchard management practices also have direct effects on the pathogen such as providing an increased food base or physically placing the fungal spores nearer to an infection court. Some orchard management practices have received more attention and are used as direct control measures against fungal diseases of apple such as pruning, nitrogen fertilisation in autumn or host resistance. Some of these specific measures, therefore, will be described in the appropriate section for mechanical, physical control or host resistance.

2.1.1 Cropping System and Cover Crop

Cropping system is not a widely used approach in fruit production due to the long establishment of the crop; therefore, there are limited possibilities for using crop rotation or a multiple cropping system, such as are widely used in arable crops (e.g. Bernoux et al., 2006; Anderson, 2007). However, cover crops are used in apple production, especially in organic orchards; some leguminous crops in a mix with grass are suggested to cover orchard floor space within and between rows in order to protect soil from erosion, loss of nutrients and water, and to support beneficial insects (e.g. Haynes, 1980; Anonymous, 2000; Cross and Dickler, 1994). The effect of cover crop on apple disease management has received little attention and only limited information is available on both the negative and positive effects of cover crops in apple disease management. Aylor (1998) showed that wind speed near the ground of an apple orchard with a tall *Festuca arundinacea* grass alley was only 11% of that in an orchard without a grass alley. Consequently, the presence of a grass alley significantly reduced the escape of *V. inaequalis* ascospores from infected leaf litter at ground level. This might also suppress the escape of other pathogenic fungal spores from the alley which are produced on the ground-level of infected leaf litter. On the other hand, ground cover could also increase the incidence of Phytophthora crown and root rots due to enhanced moist microclimatic conditions at ground-level and in the soil (Merwin et al., 1992). These ground-level moisture conditions can also help the sporulation of *Monilinia* spp. on dropped infected apple fruit, which can be an early summer source of inoculum for infection of fruit on the tree (Holb and Scherm, 2008). From another aspect, an increased population of antagonistic fungi, bacteria and actinomycetes resulting from the application of cover crops may suppress indirectly the parasitic activity of plant pathogens resulting in less disease (e.g. Sumner et al., 1981; Rickerl et al., 1992; Sumner et al., 1995), although this has not yet been proven experimentally for tree-fruit disease management. It is also necessary to emphasise that the advantages of cover crops against fungal diseases have to be viewed with respect to the insect and weed management practices of apple orchards.

2.1.2 Plant Material and Planting

Certified and disease-free plant material is a basic issue for both integrated and organic apple production systems. Vigorous, healthy plant materials grow faster and have more resistance to environmental stresses and less susceptibility to fungal pathogens. However, infected young plant materials can have a large negative effect on the productivity of the young orchards. For instance, McCracken et al. (2003) demonstrated that canker development in young apple orchards, that can cause death of the young trees, could partly be associated with infected nursery material. Also, root and trunk wounds can enhance Phytophthora collar rot development in young trees, especially in humid growing regions (Merwin et al., 1994). Inappropriate planting time, planting rate and planting depth have been shown to increase susceptibility of crops to fungal diseases (Palti, 1981), though this has not been widely studied in apple orchards.

2.1.3 Pruning and Canopy Management

Pruning of apple trees enables management of tree shape, an increased growth of fruiting spurs and improved fruit colouration. Selective removal of branches also increases air movement within the tree canopy, which facilitates quicker drying of plant surfaces and a more uniform application of pesticides (e.g. Latham and Hollingsworth, 1973; Sutton and Unrath, 1984; Childers et al., 1995). Pruning was shown to reduce the abundance of tree canopy, which resulted in a lower incidence of sooty blotch (e.g. Ocamb Basu et al., 1988; Williamson and Sutton, 2000) and apple scab (Holb, 2005a).

An indirect, positive control effect of pruning on flyspeck and sooty blotch has been known for a long time (Brooks, 1912; Colby, 1920) and has a long tradition among recommendations for managing these summer diseases (Williamson and Sutton, 2000). Hickey (1960) clearly demonstrated that pruning created an environment less favourable for the diseases and also allowed improved fungicide coverage. Latham and Hollingsworth (1973), Ocamb-Basu et al. (1988) and Cooley et al. (1997) provided more quantitative data to support the value of pruning. Latham and Hollingsworth (1973) showed that severe pruning could reduce the incidence of sooty blotch and flyspeck by up to 30%. Ocamb-Basu et al. (1988) demonstrated a significant reduction in incidence and severity of sooty blotch after dormant pruning in a non-sprayed orchard, but flyspeck could not be reduced consistently by pruning. They found that improved fungicide coverage could be associated with the improved sooty blotch control. Cooley et al. (1997) found a strong positive correlation between summer pruning (early July) and reduced incidence of flyspeck. The reduction was up to 50% during a 2-year study in which fungicides were not applied. The authors concluded that at least two mechanisms contribute to decreased flyspeck incidence and severity in summer-pruned apple trees: summer pruning decreased the number of hours of relative humidity in the canopy by 63%; and led to increased spray deposition in the upper two-thirds of the tree canopy. A more recent study on apple scab showed that cultivar susceptibility can affect the efficacy of dormant pruning on disease incidence; therefore, a combination of both measures was studied (Holb, 2005a). The results showed that if the cultivar was scab-susceptible and the pruning was severe then pruning had a significant suppressing effect on disease development during summer in organic apple orchards. The pruning-cultivar effect was more consistent on the foliage than on the fruit. The author concluded that improvement in spray penetration in the tree and modification of in-canopy microclimate are the main mechanisms of the indirect control effect of dormant pruning on apple scab.

Finally, it should be emphasised that pruning of apple trees enables the management of fungal diseases not only indirectly by modifying microclimate and/or spray deposition, but also directly by the removal of diseased shoots, stems or dead wood that can harbour pathogens (more details in Section 2.2.1).

2.1.4 Orchard Floor Management

Orchard floor management can include cover crops (mentioned above), mulching and tillage systems. Mulching can have similar positive and negative effects on fungal diseases as was shown for cover crops, though it has the great advantage that it can be used both temporarily and permanently; therefore, the negative effect of live cover cropping can be reduced by removal of, for example, overmoist mulch. Tillage can have a large effect on the compaction of soil, which can occur during any technological operation such as cultivation, pesticide application and harvest. Soil compaction restricts for instance plant rooting and soil aeration, which may be stressful for the tree causing it to become more susceptible to soilborne diseases. Tillage changes the soil environment, which has a great effect on population dynamics of plant pathogens and may affect infection of trees (e.g. Sumner et al., 1981; Boosalis et al., 1986). Tillage can also directly affect sources of inoculum of fungal diseases by cutting and/or burying diseased plant debris, which is described in detail in Section 2.2.4.

2.1.5 Nutrient Supply and Harvest

Properly balanced nutrition is a critical factor for realising the full yield potential. Macro- and micronutrients have long been recognised as being associated with changes in the level of fungal diseases. Here, the effects of each element on apple diseases are not detailed; it is noted only that there are two major objectives of nutrient applications to crops for protection from fungal pathogens. First, nutrition should be applied to satisfy apple requirements and, second, nutrients should be manipulated to be advantageous for plants and disadvantageous for diseases (e.g. Palti, 1981; Nesme et al., 2006). It is known that an overdose of nitrogen supply can increase apple shoot growth during the season and, as a consequence, these shoots will be more susceptible to infections of powdery mildew and/or apple scab.

Harvest also has an impact on fungal diseases of apple and especially timing of harvest can have a great influence on post-harvest diseases such as on Monilinia fruit rot. By delaying harvest until the crop is fully ripe, the fruit may become more susceptible to post-harvest diseases. Mature fruit also becomes more susceptible to mechanical damage, which in turn also predisposes them to post-harvest diseases. In addition, unharvested fruit may become potential inoculum sources for the following years (Byrde and Willetts, 1977). Environmental conditions may also influence storability of fruits; for instance, frequent rain events a few weeks before and during harvest will significantly increase pinpoint scab symptoms on stored fruit caused by *V. inaequalis*.

2.2 Mechanical and Physical Control

Mechanical and physical methods of control of apple diseases is aimed to reduce or eliminate inoculum sources and to suppress disease spread. Mechanical and physical control can be achieved by several means including pruning of infected plant parts; removal, shredding, burying and flaming of inoculum sources located in above-ground parts (Table 2).

For any of these methods, some general rules need to be followed in the field to avoid disease spread. First, treatments should be applied to less severely infected fields first, followed by more severely infected ones; second, treatments should not be made in wet foliage; third, all used equipment should be cleaned of soil, debris and disinfected; and fourth, all removed plant material and crop debris need to be removed from the orchard and destroyed, or if it is not possible then cut into as small pieces as possible and plough into the soil as deep as possible (Palti, 1981). Though, it needs to be emphasised that the handling of removed plant material depends on the pathogen. For example twigs infested with primary powdery mildew do not have to be removed from the orchard or to be ploughed.

2.2.1 Pruning

Pruning of apple trees enables direct management of several fungal diseases of apple by the removal of diseased shoots, fruit, stems or dead wood that can harbour pathogens. Dormant pruning has been shown to reduce the number of overwintered conidial inoculum of *apple scab* that overwintered in association with budscales (Holb et al., 2004, 2005a). A threshold of 40% autumn scab incidence was determined, which predicted the need for control against overwintered conidial inoculum in the following spring. The authors showed that most conidia overwintered in buds could be found in the upper two-thirds of terminals in early spring. Based on these, a control strategy was suggested: if autumn scab incidence on leaves was above 40%, winter pruning of the upper two-third of terminals may need to be performed before bud break to eliminate overwintered conidia associated with buds. This control measure was able to suppress the contribution of asexual inoculum to early scab epidemic significantly (Holb et al., 2005a).

Dormant season pruning was also shown to reduce primary inoculum of *apple powdery mildew* as the fungus mycelium overwinters in buds (e.g. Csorba, 1962;

Table 2 Summary of some mechanical and physical control methods against scab, powdery mildew, European canker, brown rot, flyspeck and sooty blotch in preharvest disease management of apple

Target organism	Studied plant part	Reasons for control	Control method	References
Pruning				
V. inaequalis	1-year-old woody shoots	Overwintering conidia	Dormant pruning	Holb et al. (2004, 2005a)
P. leucotricha	Terminal shoots	Overwintering mycelia	Dormant and seasonal pruning	Csorba (1962); Yoder and Hickey (1983); Hickey and Yoder (1990); Holb (2005b)
N. galligena	Terminal shoots and twigs	Overwintering canker	Dormant pruning	Kennel (1963); Swinburne (1971)
N. galligena	Pruning cuts and leaf scars	Infection site	Modified pruning shears fungicide treatment, canker paint	Seaby and Swinburne (1976); Cooke (1999)
M. fructigena	Mummified fruit	Overwintering stromata	Dormant and seasonal pruning	Wormald (1954); Leeuwen et al. (2002)
Removal of inoculum sources				
Monilinia spp.	Wild rosaceous hosts	Inoculum reservoir	Removal of alternate host	Byrde and Willetts (1977)
*Zygophiala jamaicensis**	*Rubus* spp.	Inoculum reservoir	Removal of alternate host	Sutton (1990a)
V. inaequalis	Fallen leaves in autumn	Overwintering of pseudothecia	Raking of leaf litter	Curtis (1924); Louw, (1948)
V. inaequalis	Fallen leaves in autumn	Overwintering of pseudothecia	Collection of leaf litter by flail mower	Holb (2006, 2007b)
V. inaequalis	Fallen leaves in autumn	Overwintering of pseudothecia	Leaf sweeping	Gomez et al. (2007)
M. fructigena	Summer-dropped fruit, naturally or by hand thinning	Summer inoculum source	Drop-removal	Holb and Scherm (2007)

Table 2 (continued)

Target organism	Studied plant part	Reasons for control	Control method	References
*Peltaster fructicola, Geastrumia polystigmatus, Leptodontium elatus, Z. jamaicensis**	Clustered fruit	Favourable microclimate for disease development	Removal of clustered fruit by hand thinning	Sutton (1990b)
V. inaequalis	Clustered fruit	Incomplete fungicide coverage	Removal of clustered fruit by hand thinning	Holb (this review)
M. fructigena	Clustered fruit	*M. fructigena* infection via fruit-to-fruit contact	Removal of clustered fruit by hand thinning	Leeuwen et al. (2000); Xu et al. (2001)
M. fructigena	Clustered fruit	Codling moth damage via fruit-to-fruit contact	Removal of clustered fruit by hand thinning	Holb and Scherm (2008)
Shredding of leaf litter				
V. inaequalis	Fallen leaves in autumn	Overwintering of pseudothecia	Leaf shredding	Sutton and MacHardy (1993); Sutton et al. (2000); Vincent et al. (2004); Holb (2007b)
Burying of inoculum sources				
V. inaequalis	Fallen leaves in autumn	Overwintering of pseudothecia	Ploughing of leaf litter	Curtis (1924); Louw (1948)
V. inaequalis	Fallen leaves in autumn	Overwintering of pseudothecia	Disc cultivation for reducing leaf litter	Holb (2007b)
Flaming of leaf litter				
V. inaequalis	Fallen leaves in autumn	Overwintering of pseudothecia	Flaming of leaf litter	Earles et al. (1999)

**Peltaster fructicola, Geastrumia polystigmatus* and *Leptodontium elatus* are the causative agents of sooty blotch, and *Zygophiala jamaicensis* is the causative agent of flyspeck.

Yoder and Hickey, 1983; Hickey and Yoder, 1990; Holb, 2005b). Removal of infected terminal shoots during winter pruning is a generally recommended control practice which enhances the efficacy of chemical control measures (Hickey and Yoder, 1990). In Central Europe, removal of mildew-infected terminals reduced primary mildew incidence from 60 to 13% on the highly mildew-susceptible cultivar 'Jonathan' (Csorba, 1962). In the United States, research revealed that the removal of powdery mildew primary inoculum may be valuable and economically feasible in orchards with moderate-to-low numbers of primary infections per tree and might lead to less need for fungicides (Yoder and Hickey, 1983; Hickey and Yoder, 1990). A recent study in integrated and organic apple orchards showed that dormant pruning had no significant effect on primary mildew incidence on slightly susceptible cultivars such as 'Gala', 'Rewena' and 'Liberty'. However, primary mildew incidence was significantly lower in moderatelysusceptible cultivars 'Elstar', 'Pilot' and 'Jonica' in both production systems, but the severity of pruning did not cause significant differences in mildew incidence (Holb, 2005b). Studies also emphasise that seasonal disease development of powdery mildew can greatly be reduced by removal of infected shoot not only in dormant bud stage but during the season when primary infestation is manifested on unfolded young shoots (e.g. Csorba, 1962; Yoder and Hickey, 1983; Hickey and Yoder, 1990).

Dormant pruning also successfully reduces *European canker* (*Nectria galligena*). Removal of infested twigs reduced the spread of twig death within the tree and also reduced the inoculum that could spread and infect new twigs (Kennel, 1963; Swinburne, 1971). On the other hand, pruning cuts coupled with leaf scars are important sites for infection, which need to be protected against infection (Kennel, 1963; Swinburne, 1971). Heinrich (1982) showed that pruning wounds are more susceptible to *Nectria* than leaf scars. The incidence of canker lesions caused by *N. galligena* was shown to be greater following the inoculation of fresh pruning cuts than older cuts (Marsh, 1939; Saure, 1962; Seaby and Swinburne, 1976). High inoculum dose and young pruning wounds resulted in short incubation periods as well as a high incidence of canker (Xu and Butt, 1996). Wounds on woody trees become increasingly resistant to infection by *N. galligena* as they age (Krähmer, 1980; Doster and Bostock, 1988a,b; El-Hamalawi and Menge, 1994). This type of resistance is related to the wound healing process, which leads to the formation of boundary zone tissue and wounds periderm (Mullick, 1975, 1977; Krähmer, 1980). Therefore, all cuts needs to be treated by using infection-suppressing methods such as using modified pruning shears (Seaby and Swinburne, 1976), fungicide treatment and wound-treatment with an effective canker paint (Cooke, 1999).

Removal of mummified fruits by pruning is also a generally recommended practice to reduce primary inoculum sources of *brown rot* (Wormald, 1954; Leeuwen et al., 2002). This practice will disrupt the primary inoculum source in spring, which is released from these mummified fruits attached to the trees. During the season, this practice needs to be followed with all infected fruit, which will reduce sporulation and the number of airborne conidia in the orchard.

2.2.2 Removal of Inoculum Sources

Removal can be performed for at least two purposes: one is removal of alternate hosts of the pathogens and the other is removal of infected crop debris such as infected leaves, dropped fruit and clustered fruit.

Most apple diseases have numerous reservoirs or *alternate hosts*. Removal of these hosts would aid in reducing the inoculum. In the case of apple scab, wild *Malus* spp., hawthorn (*Crataegus* spp.), mountain ash (*Sorbus* spp.), firethorn (*Pyracantha* spp.) and loquat (*Eriobotrya japonica*) should be removed in a 5 km radius from the orchard. This option is rarely followed in practice because most of the inoculum comes from the neighbouring apple orchards and alternate hosts have little importance in most regions. *Monilinia* spp. also have several rosaceous hosts; therefore, it is recommended to remove wild hosts near the orchard and to keep ornamental bushes under surveillance to prevent introduction of inoculum from outside (Byrde and Willetts, 1977). Sutton (1990a) demonstrated that patches of blackberry (*Rubus argutus*) have provided large numbers of inoculum for flyspeck infection on apple trees. He recommended mowing *Rubus* spp. in ditch banks near the orchard or along orchard borders to reduce inoculum levels.

Removal of *crop debris* is of primary value when the inoculum is located on the above-ground parts. For instance, the sexual stage of the apple scab pathogen (*V. inaequalis*) overwinters on leaf debris and produces abundant ascosporic inoculum in the spring. The earliest scab studies (Curtis, 1924; Keitt, 1936; Louw, 1948) already demonstrated that leaf removal greatly reduced the primary inoculum source of apple scab in the following spring. Curtis (1924) and Louw (1948) suggested 62–73 and 83% reduction of scab, respectively, in spring by raking of leaf litter. Recent studies (Holb, 2006, 2007b) also demonstrated that leaves collected by flail mower in orchards reduced ascospore production by 56–79% but reduction in spur-leaf scab incidence in spring was lower (18–57%) in integrated and organic apple orchards compared to earlier studies. The variation in the scab-reduction effect of leaf removal might be associated with differences in scab-susceptibility of the cultivars, disease pressure in spring, spray schedules applied during the season and environmental conditions of the orchards. As leaf removal is generally a good sanitation method against apple scab, this method is strongly advised in both integrated and organic orchards in order to reduce scab risk potential in the following spring. Leaf removal in autumn can be performed in combination with other orchard management activities, using a leaf collector adapter, which is commercially available for most tractors or using a flail mower. An alternative to leaf removal, leaf sweeping, is also successfully used to reduce scab primary inoculum sources in organic apple orchards (Gomez et al., 2007).

Removal of dropped fruit has been shown to be an effective approach to reduce summer inoculum of apple diseases such as *M. fructigena*. Recently Holb and Scherm (2007) demonstrated that the early summer fruit dropped either naturally or by hand thinning serves as a bridge between sporulation from overwintered fruit mummies in the spring and the first fruit with sporulating lesions in the tree in mid-summer. The authors showed that removal of these dropped fruits from the orchard floor resulted in a significantly lower disease incidence on fruits in the tree. Though the treatment was more effective in organic orchards than in integrated ones, drop-removal was suggested as a useful brown rot management practice in both types of environmentally friendly apple orchard.

Removal of clustered fruits was shown to be an effective control option against *sooty blotch and flyspeck*, because clustered fruits provide a more favourable micro-climate for disease development than single fruit, and tightly clustered fruits are difficult to cover thoroughly with sprays (Sutton, 1990b). Incomplete fungicide coverage on clustered fruit can affect negatively the control of other airborne apple diseases such as apple scab and brown rot. Brown rot management in particular showed a relationship with fruit-to-fruit contact among fruits in a cluster. A positive significant correlation was demonstrated between brown rot development and clustered patterns of fruit (Leeuwen et al., 2000; Xu et al., 2001). The disease spread within a fruit-cluster is probably due to physical contact and/or insect damage among the members of the cluster (Leeuwen et al., 2000; Xu et al., 2001, Holb and Scherm, 2008). Within the insect pest group, codling moth showed the strongest correlation with brown rot development (Holb and Scherm, 2008). In a fruit-cluster, codling moth larvae could easily infest all members of the fruit-cluster throughout the contact points. Moth larvae could carry fungal spores and avoid insecticide spray as well, as spray coverage in the contact points of the fruit-cluster can be incomplete (I.J. Holb unpublished). Due to this process, brown rot development can frequently occur in organic orchards where neither codling moth nor brown rot control are sufficient; therefore, a more effective moth control, combining several control methods such as removal of infected fruit and clustered fruit is suggested (Holb and Scherm, 2008).

2.2.3 Shredding of Leaf Litter

Leaf shredding has been widely studied since the 1990s in several apple orchards, and because of its high (from 45 to 85%) efficacy in reducing primary scab infection, it is a widely recommended sanitation method in integrated orchard management practices (Sutton and MacHardy, 1993; Sutton et al., 2000; Vincent et al., 2004). Based on this, Sutton et al. (2000) developed a sanitation action threshold method in order to reduce early-spring application of fungicides in integrated apple orchards. In a recent study, Holb (2007b) showed that leaf shredding applied alone resulted in less reduction of spur-leaf scab incidence (from 25 to 36%) in organic apple orchards compared to those in integrated orchards. He concluded that although large disease pressure in spring and the low efficacy of approved fungicides in organic apple production can greatly lower the efficacy of leaf shredding, it could be a useful sanitation method against apple scab in organic orchards.

2.2.4 Burying of Inoculum Sources

Burying can be a useful element of crop debris removal if the orchard floor is not covered by grass, where leaf removal by flail mower can be difficult to perform.

In these orchards, covering crop debris by soil can help in degradation of infested leaf litter and in reducing inoculum sources. Ploughing and disc cultivation can be used for this purpose. In early studies, Curtis (1924) and Louw (1948) showed that ploughing the leaf litter into the soil resulted in a 66% reduction of scab incidence in the following spring. However, a recent study by Holb (2007b) showed that disc cultivation resulted in only a 7–26% reduction in scab incidence in spring in organic apple orchards. This study also demonstrated that disc cultivation buried the fallen leaves with 50–58% efficacy. The author concluded that this leaf-burying proportion is not sufficient for a significant reduction of spur-leaf scab incidence in commercial organic apple orchards; therefore, the leaf-burying efficacy of disc cultivation needs to be improved. This is likely to be done by soil cultivation deeper than 200 mm, by selecting suitable soil moisture conditions for disc cultivation and by adjusting the disc-tiller to greater rotating ability. Holb (2007b) summarised that applying improved disc cultivation would be an advantageous indirect control option against apple scab for organic growers as it is a general soil-maintaining practice in orchards with bare soil between rows, and therefore it does not represent additional costs. However, the author also indicated that efficacy of disc cultivation in reducing scab incidence would probably be greatly increased by combining it with other non-chemical control options.

2.2.5 Flaming of Leaf Litter

Flaming is generally used against weeds in orchards though it also has good effect against ascocarps of *V. inaequalis* in leaf litter (MacHardy, 1996; Anderson, 2007). A torch-directed flame works not by burning the leaf litter but rather by searing it and causing cells to rupture. For efficacy, leaf litter on the orchard floor should reach a minimum of 70°C during the treatment; otherwise, it does not have a detrimental effect on the initials of *V. inaequalis* ascocarps. The autumn flaming treatment can reduce ascospore production by 70% in the next spring (Earles et al., 1999). Flaming is not widespread in orchard management, neither as a weed management nor as a sanitation practice against *V. inaequalis*, due to its cost which is two to four times higher than other sanitation or chemical control methods.

In summary, although mechanical and physical control methods are one of the most basic approaches for reducing infection potential of inoculum sources in apple orchards, most of these methods are not widely spread in the apple-growing practice due to their high labour costs and/or time limits during the season. In addition, it is hard to justify the cost-effectiveness of some mechanical and physical control treatments compared to chemical fungicide use. In the future, expert-system-based automatisation may greatly enhance the effective integration of mechanical and physical control methods into apple growing.

2.3 Biological Control

Although field application of biological control agents (BCAs) has received great attention, there are only a few commercially applied biological control products against fungal diseases of apple. There are several reasons which do not allow the widespread use of biological control but the two major ones are first, biological products against phylloplane pathogens reduce diseases rather than completely control them and second, market potential of commercialised bioproducts is lower compared with that of conventional fungicides (Andrews, 1990, 1992). Here, research which has revealed promising biologically important antagonists and plant extracts against key fungal pathogens of apple is described (Table 3).

2.3.1 Antagonists

Apple scab biocontrol related to antagonists has a three-decade history. Field success of apple scab antagonism was achieved, first, by spray application during preharvest season, second, by reducing ascocarp development of the fungus on infected leaf litter during off-season and, third, by decomposing leaf litter.

Chaetonium globosum was the antagonist of *V. inaequalis*, which showed promising efficacy by spray application during the preharvest season against the asexual phase of the pathogen's life cycle (Andrews et al., 1983; Cullen et al., 1984). Studies demonstrated that spray application of ascospores of *C. globosum* reduced significantly the number of lesions and lesion sizes of apple scab in the field. *C. globosum* also reduced conidial production of *V. inaequalis* effectively. The antagonistic effect was due to antibiosis, nutrient competition and high colonisation activity. In spite of the successful in vitro and in vivo studies with *C. globosum*, the antagonist has never become a commercial product due to the commercially unfeasible field application.

Suppressing the ascocarp development of *V. inaequalis* on infected leaf litter received the largest attention and has been investigated most extensively. Zuck et al. (1982) found that *Cladosporium* spp. sporulated on pseudothecia of *V. inaequalis* on fallen apple leaves and no asci or ascospores were found in the parasitised ascocarp. *Cladosporium* spp. received no further attention as a biocontrol option against *V. inaequalis*. Heye and Andrews (1983) found that *Athelia bombacina* reduces ascospore production of *V. inaequalis* by 40–100% on overwintered leaf discs. Detailed investigation of the antagonist's mode of action (Young and Andrews, 1990a,b) showed that the pathogen inhibits the pseudothecial development in the infected leaf disc. The antagonistic fungus has also a cellulotic activity on the leaf litter; therefore, the leaves became much softer under the treatment. The fungus also caused nutrient competition and promoted decomposition of leaf litter by earthworms (Heye and Andrews, 1983).

C. globosum was also shown to reduce the number of ascospores by about 30% when sprayed on detached leaves and held in the field to overwinter (Heye, 1982; Heye and Andrews, 1983). However, the orchard application of the fungus did not result in effective control against ascospores of *V. inaequalis*. The reason was that the antibiotics produced by *C. globosum* diffuse to the leaf surface but are degraded very quickly and lose their activity against ascospores of *V. inaequalis*.

More recent studies showed that *Microsphaeropsis* spp. (later *M. ochracea*) were able to reduce the pseudothecium number on leaf litter and ascospore production

Table 3 Summary of some biological control agents (BCAs) against scab, powdery mildew, European canker, brown rot, flyspeck and sooty blotch in preharvest disease management of apple

Target organism and life cycle phase	Biological control agent (BCA)	Exposure system	References
V. inaequalis sexual phase	*Pseudomonas* spp.	Field study, fallen apple leaves; leaf decomposition	Ross and Burchill (1968)
V. inaequalis sexual phase	*Lumbricus terrestris*	Field study, fallen apple leaves; leaf decomposition	Raw (1962); Niklaus and Kennel (1981)
V. inaequalis sexual phase	*Cladosporium* spp.	Field study, fallen apple leaves	Zuck et al. (1982)
V. inaequalis sexual phase	*Chaetonium globosum*	Field study, fallen apple leaves	Heye (1982); Heye and Andrews (1983)
V. inaequalis asexual phase	*C. globosum*	In vitro, agar plate, laboratory study, field study, apple leaves	Andrews et al. (1983); Cullen et al. (1984)
V. inaequalis sexual phase	*Athelia bombacina*	In vitro, laboratory study, field study, fallen apple leaves, leaf decomposition	Heye and Andrews (1983); Young and Andrews (1990a,b); Miedtke and Kennel (1990)
V. inaequalis sexual phase	*Microsphaeropsis* spp. (later *M. ochracea*)	Field study, fallen apple leaves	Carisse et al. (2000); Vincent et al. (2004); Carisse and Rolland (2004)
P. leucotricha sexual phase	Ampelomyces mycoparasites	Apple shoot	e.g. Sztejnberg et al. (1989)
N. galligena sexual and asexual phases	*Bacillus subtilis*	Apple twig	Swinburne (1975); Swinburne et al. (1975); Corke and Hunter (1979)
N. galligena sexual and asexual phases	*Trichoderma viridae*	Fruit brown rot	Byrde and Willetts (1977)
P. fructicola, G. polystigmatus, L. elatus, Z. jamaicensis sexual and asexual phases	*C. globosum* strain NRRL 6296	Field study	Davis et al. (1991)
P. fructicola, G. polystigmatus, L. elatus, Z. jamaicensis sexual and asexual phases	*Trichoderma harzianum* strain T-22	Field study	Kiyomoto (1999)

by 75% in autumn application compared to untreated control (Carisse et al., 2000; Vincent et al., 2004). In a comparative study, *M. ochracea* was more effective against apple scab fungus than *A. bombacina* (Vincent et al., 2004). *Microsphaeropsis* spp. were capable of penetrating into *V. inaequalis* hyphae and parasitising them through their enzymes and antibiosis. This resulted in reduced growth of the hyphae of *V. inaequalis* and finally cell death of the hyphae. In field studies, it was concluded that *M. ochracea* should be applied in August or September, which results in 61–99% reduction of ascospore production (Carisse and Rolland, 2004). Presently, this fungus is the most promising BCA against the sexual stage of apple scab. Recently, commercialisation of this antagonist has started and trials have been conducted with the pre-commercial product in several European countries (W.E. MacHardy, personal communication).

Leaf decomposition by soil organisms is an indirect way of biological control against apple scab, which includes decomposition by *Pseudomonas* spp. (Ross and Burchill, 1968), *A. bombacina* (Heye and Andrews, 1983; Miedtke and Kennel, 1990) and *Lumbricus terrestris* (Raw, 1962; Niklaus and Kennel, 1981). *Lumbricus* spp. was also shown to consume fruiting bodies of different fungi including *V. inaequalis*(Niklaus and Kennel, 1981). Extensive details of leaf decomposition by soil organisms are given by MacHardy (1996).

Pycnidial fungi belonging to the genus Ampelomyces are the most common natural antagonists of *powdery mildew*s worldwide (Sztejnberg et al., 1989). On apple trees, Ampelomyces mycoparasites overwintered as resting hyphae in the dried powdery mildew mycelia covering the shoots and in the parasitised ascomata of *P. leucotricha* on the bark and the scales of the buds. Although commercialised products of Ampelomyces mycoparasites are available against powdery mildew species, there is low practical potential for effective biological control of apple powdery mildew by products prepared from Ampelomyces mycoparasites (Sztejnberg et al., 1989).

Antagonistic microorganisms such as *Bacillus subtilis* strains were investigated as BCAs against *N. galligena*. The bacterium was an effective option against both leaf scar and pruning wound infection (Swinburne 1975; Corke and Hunter, 1979). First, Swinburne (1975) was able to detect that *B. subtilis* has an inhibitory effect on *N. galligena* on branches of apple trees. Later studies in Northern Ireland (Swinburne et al., 1975) showed that two antibiotic-producing strains of *B. subtilis*, sprayed at 10 and 50% leaf fall, provided about 50% higher protection of apple leaf scars against infection of *N. galligena*. Both strains could be recovered from leaf scars during the dormant season and next spring until the end of April, suggesting that the antibiotic strains were able to multiply and grow during winter. However, the protection was not effective in May when the protective layer was shed. Corke and Hunter (1979) also used autumn application of *B. subtilis* to protect pruning wounds in apple trees against infection by *N. galligena*. Their research showed that the number of *N. galligena* conidia, released during the 12 months following treatments of *B. subtilis*, was 96% lower in the *B. subtilis* treatment compared with the untreated control. For the past three decades, no further research has been performed and no commercialised bioproduct has been available against *N. galligena*.

Biological control options of *M. fructigena* have received little attention in the preharvest apple-growing practice. An early study of Byrde and Willetts (1977) showed that *Trichoderma viridae* might have a reducing effect on *M. fructigena* infection of fruit. However, no further studies have investigated biological control efficacy under orchard conditions.

Little progress has been made in biological control against *flyspeck and sooty blotch*. Field sprays of two BCAs showed success against both diseases. A hydrolised, colloidal, cellulose-based formulation of *C. globosum* (NRRL 6296), applied with an oil-based sticker, reduced the number of flyspeck colonies by 63% compared to an untreated control (Davis et al., 1991). The same treatment also controlled sooty blotch as effectively as a standard conventional fungicide (Davis et al., 1991). *T. harzianum* strain T-22 was also tested against both diseases in 7- or 14-day applications from mid-August until harvest (Kiyomoto, 1999), but the fungus did not significantly reduce either flyspeck or sooty blotch incidence compared with the untreated control. None of the above agents were commercialised for control of flyspeck and sooty blotch.

2.3.2 Extracts/Oils of Plants and Composts

Extracts/oils of plants and composts are widely recommended by IFOAM standards for organic fruit growers (Anonymous, 2000). These materials are considered as alternative fungicides against fungal diseases of apple in organic growing and can be sorted into three groups: plant extracts, plant oils and compost extracts (Table 4).

Plant extracts are one of the promising potential sources for environmentally friendly production, as much of the plant kingdom still remains unexplored for possible materials of biological control (Cutler and Cutler, 1999). Most plant extracts contain several active components against fungal diseases from which the most widely studied ones are extracts of *Yucca schidigera*, *Cocos nucifera*, *Inula viscose*, *Hedera helix*, grapefruit seed extract (GSE) and root bark of *Morus alba*.

Yucca extract, made from dried stems of *Y. schidigera,* is reported to have a high content of steroid saponins and to contain polyphenolic compounds (Cheeke, 2001). Heijne et al. (2007) showed that Yucca extract at a higher dose (0.75%) has a similar efficacy against apple scab as standard schedules of elementary sulphur of 0.4% on cluster leaves. However, these tendencies were no longer visible on leaves from extension shoots. Yucca extract is commercially available in Europe such as Norponin BS Liquid (Nor-Natur Aps, Denmark).

Coconut soap, prepared from *C. nucifera*, has been shown to be efficient against sooty blotch and apple scab. Fuchs et al. (2002) evaluated 1% of coconut soap against sooty blotch in a 2-year study in organic apple production. Six applications of the soap, from early July to early September in 10- to 14-day schedules, significantly reduced disease incidence compared to non-treated control; however, the efficacy was not sufficient under high disease pressure. The efficacy of coconut soap treatment was similar to that of four treatments of lime sulphur at 1% dosage. The authors concluded that coconut soap could be effective against sooty blotch if the disease pressure is low. Tamm et al. (2007) evaluated 0.5% of coconut soap

Table 4 Summary of some extracts/oils of plants and composts against apple scab, apple powdery mildew, European canker, brown rot, flyspeck and sooty blotch in apple orchards

Target organism	Extracts/oils of plants and composts	Exposure system	References
Plant extracts			
V. inaequalis	*Hedera helix*	Field study, apple leaf	Bosshard (1992)
P. leucotricha	*H. helix*	Field study, apple shoot	Bosshard (1992)
V. inaequalis	*Cocos nucifera*	Field study, apple leaf and fruit	Tamm et al. (2007); Kunz et al. (2008)
Sooty blotch	*C. nucifera*	Field study, apple leaf and fruit	Fuchs et al. (2002); Tamm et al. (2007)
V. inaequalis	Grapefruit seed extract	Field study, apple leaf	Spitaler et al. (2004); Trapman (2004)
V. inaequalis	Root bark of *Morus alba*	In vitro, laboratory study	Rollinger et al. (2006, 2007)
V. inaequalis	*Inula viscose*	Field study, apple leaf	Tamm et al. (2007)
V. inaequalis	*Yucca schidigera*	Field study, apple leaf	Heijne et al. (2007)
Plant oils			
V. inaequalis	Oil of sunflower, olive, canola, corn, soybean and grapeseed	Field study, apple leaf and fruit	Northover and Schneider (1993, 1996)
P. leucotricha	Oil of sunflower, olive, canola, corn, soybean and grapeseed	Field study, apple shoot	Northover and Schneider (1993, 1996)
Extracts from compost			
V. inaequalis	Compost water extract (CWE)	Laboratory study, fruit scab	Träckner and Kirchner-Bierschenk (1988)
V. inaequalis	Compost water extract (CWE)	Laboratory study, leaf and fruit scab	Gross-Spangenberg (1992)
V. inaequalis	Spent mushroom substrate (SMS)	In vitro, laboratory study	Yohalem et al. (1994); Cronin et al. (1996)
V. inaequalis	Spent mushroom substrate (SMS)	Field study, apple leaf	Yohalem et al. (1996); Earles et al. (1999)

against apple scab and the soap significantly reduced leaf scab incidence compared to untreated control on cultivars ‘Resista’ and ‘Topaz’. The soap was recommended against apple scab in organic apple production though it had lower efficacy than copper compounds (Tamm et al., 2007; Kunz et al., 2008). Coconut soap is available as a commercialised product, for example, in Switzerland and Germany (Biofa Cocana, Biofa AG, Münsingen, Germany).

Strong fungicidal activity of the extract of *I. viscose*, a perennial crop native to the Mediterranean Basin, was reported against plant pathogens of vegetables and grape (Cohen et al., 2002). The authors revealed seven lipophilic compounds of the Inula extract with fungicidal activity. Fungicide treatments with 0.5% of *I. viscose* extract showed significantly lower leaf scab incidence on cultivars 'Resista' and 'Topas' compared to untreated control (Tamm et al., 2007). 0.5% Inula extract was significantly more efficient against apple scab than coconut soap (1%) or potassium bicarbonate (PBC) (0.5%) treatments. The authors evaluated this plant extract as one of the most promising compounds against apple scab in organic production. The commercialised Inula extract is available, for example, in Switzerland (Inulex, Basel, Switzerland).

The plant extract of *H. helix* has been shown to reduce incidence of apple scab and powdery mildew (Bosshard, 1992). Treatments of the extract showed consistently lower apple scab and mildew incidence than untreated control plots (Bosshard, 1992), but no commercialised product of *H. helix* is currently available.

GSE, a plant derivative, decreased infection of apple scab in organic production but had significantly lower efficacy against the fungus than chemical fungicides (Spitaler et al., 2004; Trapman, 2004). A powerful antimicrobial activity of GSE was reported (e.g. Harich, 1999; Von Woedtke et al., 1999); however, considerable amounts of preservatives were detected in all commercial GSEs investigated so far (e.g. Takeoka et al., 2001; Spitaler et al. 2004). GSE is considered to be a potential plant protection material against apple scab.

Rollinger et al. (2006, 2007) showed that methanol extract of Morus root bark revealed distinct *V. inaequalis*-inhibiting qualities. A bioguided fractionation of the extract resulted in metabolites of moracins M (1), O/P (2), kuwanon L (3) and sanggenons D (4), B (5), G (6), O (7), E (8) and C (9). All the Diels–Alder adducts (3–9) showed an antifungal activity against apple scab with IC_{50} values between 10 μM and 123 μM. The in vivo activity of these fractions also confirmed a distinct antifungal activity against *V. inaequalis*. The authors suggested Morus root bark extract as a potential material against apple scab in organic growing.

Previous studies (Martin and Salmon, 1931, 1933; Calpouzos, 1966) demonstrated the role of *plant oils* in the control of plant diseases in general. In the case of apple diseases, Northover and Schneider (1993, 1996) tested the prophylactic and therapeutic activity of three low-linoleic acid oils (sunflower, olive and canola) and three high-linoleic acid oils (corn, soybean and grapeseed) against apple scab and powdery mildew. All six oils were equally effective against *P. leucotricha*, providing 99% control of the disease. The control efficacy against *P. leucotricha* was comparable to that of dinocap treatments. The six oils in ten applications at 6- to 10-day intervals also decreased scab incidence of fruit and leaf by 81 and 66%, respectively. The efficacy of oils against *V. inaequalis* was significantly lower than that of the standard use of captan. Plant oils are recommended mainly against insect pests (e.g. aphids, mites, scales and codling moth) in the organic and the integrated production guidelines (Anonymous, 2000; Cross and Dickler, 1994) though their use during the season can be considered mainly for organic apple production.

The use of water *extracts from compost* has been reported against foliar diseases over the past two decades (e.g. Träckner and Kirchner-Bierschenk, 1988; Träckner, 1992; Weltzien, 1991; Yohalem et al., 1994; Zhang et al., 1998; El-Masry et al., 2002). The presence of protease, chitinase, lipase and β-1,3 glucanase (lysogenic enzymes) in compost water extract (CWE) indicates a possible role in fungal degradation (El-Masry et al., 2002) and can induce systemic acquired resistance in plants (Zhang et al., 1998). Träckner and Kirchner-Bierschenk (1988) were the first to test CWE on apple diseases. They reported a reduction in fruit scab lesions from 2.5 to 1.5 lesion/fruit in treatments of manure-straw-soil extract; however, field application of the extract failed to confirm the scab-reducing effect of the controlled study (Gross-Spangenberg, 1992). Yohalem et al. (1994) tested extracts from more than 40 different composts for biocontrol efficacy and extract prepared from spent mushroom substrate (SMS) showed the largest inhibition of conidial germination of *Spilocaea pomi* in an in vitro assay and a reduction of scab symptoms on apple seedlings. Cronin et al. (1996) demonstrated that a major inhibitory principle of the SMS extract is a low-molecular-weight, heat-stable, non-protein metabolite produced by anaerobic microorganisms in the compost. In a 3-year study, anaerobically fermented SMS was applied at weekly intervals from green tip to petal fall and biweekly thereafter (Yohalem et al., 1996). The spray schedule showed significant reduction of leaf scab incidence and severity compared to water-treated control (Yohalem et al., 1996). The authors also demonstrated that the inhibitory effect of the compost was maintained for 13 weeks independently of storage conditions. CWE, also known as compost tea, was also tested against apple scab in organic orchards, and organic production guidelines recommend it as foliar spray against apple scab (Earles et al., 1999).

In summary, recently several biological control options have become known against fungal diseases of apple, though only few of them are commercially available for the apple-growing practice. Most of these products are suitable only for organic apple growing, as their effectiveness against the key fungal diseases is not able to fulfil the requirements for integrated apple orchards or they are not substantially cost-effective. Developing an effective biological control against polycyclic fungal diseases of apple will be a great future challenge for a preharvest disease management programme.

2.4 Host Resistance

In this section, the role of host resistance is described for each disease by giving examples of suitable cultivars for environmentally friendly production systems (Table 5).

The genetic basis of host resistance to *apple scab* has a long tradition in apple breeding (Williams and Kuc, 1969). Monogenic sources of scab resistance in apple breeding are based on six different major genes (Vf, Va, Vr, Vb, Vbj and Vm), which were recently reviewed in detail by Gessler et al. (2006). Vf from *Malus floribunda* 821 is most used in apple breeding programmes throughout the world (Lespinasse, 1989). Vf and the other genes conferring forms of scab resistance have led to the development scab-resistant cultivars (e.g. 'Prima', 'Priscilla', 'Liberty', 'Jonafree', 'Dayton', 'Novamac', 'Priam') which are available for growers (Sansavini, 1997,

Table 5 Examples of apple cultivars showing different levels of susceptibility to apple scab, apple powdery mildew, European canker, brown rot, flyspeck and sooty blotch

	Cultivar susceptibility		
Fungal disease	Genetically resistant	Low to middle	Middle to high
Apple scab	Roughly 100 scab-resistant apple cultivars are available: e.g. 'Prima', 'Priscilla', 'Liberty', 'Jonafree', 'Dayton', 'Novamac', 'Priam', 'Ariwa', 'Reanda', 'Rebella', 'Remo' 'Rewena', 'Crimson Crisp', 'Harmonie', 'Topaz', 'Brina', 'Ariane', 'Antares', 'Choupette', 'Modi', 'Golden Orange', 'GoldRush', 'Topaz'	e.g. 'Granny Smith', 'Jonathan'	e.g. 'Jonagold', 'Jonica', 'Gala clones, Idared', 'Mutsu', 'Elstar', Golden clones, 'McIntosh', 'Paulared'
Apple powdery mildew	Genetic sources: e.g. 'White Angel', 'David', 'Robusta 5', 'Korea'	e.g. 'Golden Delicious', 'Winesap', 'York Imperial', 'Nittany', 'Lord Lambourne'	e.g. 'Jonathan', 'Baldwin', 'Cortland', 'Idared', 'Jonagold', 'Rome Beauty', 'Monroe', 'Gravensteiner', 'Stayman Winesap', 'Cox's Orange Pippin', 'Granny Smith', 'Ginger Gold', 'Prima', 'New Jonagold', 'Pink Lady', 'Mutsu'
European canker	–*	e.g. 'Golden Delicious', 'Bramley', 'Rome Beauty', 'Jonathan', 'Golden Russett', 'Gloster'	e.g. 'Alkmene', 'Gravenstein', 'Delicious', 'Red Delicious', 'McIntosh', 'Bismark', 'Spartan', 'Newtown', 'Spitzenburg', 'Cox's Orange Pippin', 'Northern Spy', 'Idared', 'Priam', 'Prima', 'Priscilla'

Table 5 (continued)

	Cultivar susceptibility		
Fungal disease	Genetically resistant	Low to middle	Middle to high
Brown rot	–		Cultivars with higher susceptibility to insect damages and growth crack show higher susceptibility to fruit rot. Cultivars 'Jonathan' and 'Beauty of Boskoop' are less susceptible to brown rot.
Flyspeck and sooty blotch	–		Commercial cultivars are not resistant and 'Golden Delicious', 'Granny Smith', 'Cox's Orange Pippin', 'Yellow Newton', 'Buckingham' and 'Jonathan' are highly susceptible to both diseases. Symptoms are more visible on light-skinned cultivars, and the diseases tend to be more severe on those cultivars that mature later in the growing season. Differences in disease severity among cultivars might be related to the permeability of the cuticle.

*Genetic aspects of resistance to the causative agents of sooty blotch, flyspeck and brown rot of apple are not known.

1999). Today roughly 100 scab-resistant apple cultivars are available commercially but only a few are used by growers (Gessler et al., 2006; Holb, 2007a). One of the problems is rooted in the relatively easy breakdown of monogenic resistance. By the end of the 1990s, more and more examples justified that resistance genes have been overcome by *V. inaequalis* and it became obvious that the durability of any form of monogenic resistance is questionable. One of the most promising new strategies was using the combination of different resistance genes in the same genotype (pyramiding) which might provide more durable resistance over time (Lespinasse et al., 1999). The term pyramiding is applied also to resistance to different pathogens in a single plant, producing apple cultivars resistant to scab and mildew and tolerant to canker such as cv. 'Ariwa' (Kellerhals et al., 2000a,b). More recent evaluations showed that pyramiding combined with quantitative resistance in the same genotype would probably decrease significantly the likelihood of resistance breaching by pathogens (Gessler et al., 2006). For instance, the German breeding programme provided several resistant apple cultivars ('Re-cultivars') possessing resistance and/or tolerance against *V. inaequalis*, *P. leucotricha*, *Erwinia amylovora*, *Pseudomonas syringae*, *Panonychus ulmi* and winterfrost (Fischer and Fischer, 1996, 1999). The authors guarantee the possibility that by using these cultivars fungicide spraying can be reduced by 80% or more and suggest them for both organic and integrated apple production. Some of the Re-cultivars, such as cvs. 'Reanda', 'Rebella', 'Remo' and 'Rewena', were suggested as donors for multiple resistance breeding (Fischer and Fischer, 1999). An Italian and Swiss survey showed that currently the best resistant cultivars are the red cvs.: 'Crimson Crisp', 'Harmonie', 'Topaz', 'Brina' and still under assessment, 'Ariane', 'Antares', 'Choupette', 'Modi' and the yellow 'Golden Orange', 'GoldRush' (Gessler et al., 2006). Currently, the most popular scab-resistant cultivar at a European grower scale is 'Topaz' of Czech breeding. It should be noted that severe attacks by other, not commonly occurring, phytopathogenic fungi (such as pathogens of sooty blotch, flyspeck and rust) can be assessed during late summer in orchards where scab-resistant cultivars have not been sprayed with fungicides (Holb, 2008b). Recommendations in Western European countries suggest three to four treatments of scab-resistant cultivars with broad-spectrum fungicides to prevent infection by sooty blotch and flyspeck secondary attacks as well as possible resistance breaching by scab itself (Gessler et al., 2006).

Several studies are available on classifying scab-susceptibility of commercialised apple cultivars (e.g. Aldwinckle, 1974; Norton, 1981; Scheer, 1989; Pedersen et al., 1994; Sandskär and Gustafsson, 2002; Kühn et al., 2003; Dewdney et al., 2003; Quamme et al., 2005). These studies assessed their ratings under unsprayed orchard conditions and suggestions were made mainly for traditional apple production using considerable amounts of fungicide sprays. A recent investigation was aimed to sort 27 cultivars (including scab-resistant, old and popular cultivars) based on their season-long scab reactions under fungicide spray schedules approved for organic or integrated production (Holb, 2007a). The author concluded that popular cultivars (e.g. 'Jonagold', 'Gala', 'Elstar' and Golden clones) were suitable only for integrated apple production and resistant ones (e.g. Re-cultivars) were suitable for organic production.

Despite the large development in scab resistance breeding, established orchards and new planting contain only up to 4% scab-resistant cultivars in European countries. Even many of the organic apple orchards in Europe are planted with popular scab-susceptible apple cultivars, and therefore produce low apple quality. The low acceptance of scab-resistant cultivars is rooted in the poor quality of the first commercialised scab-resistant cultivars. Although many of the later cultivars are appreciably better than their predecessors, these cultivars receive very little marketing promotion now. The future will likely bring better options if molecular identification of major scab resistance genes can be combined with the availability of transgenic R plants. These R genes of apple will allow the option of creating cisgenic apples (Schouten et al., 2006) which may be better accepted by the consumers than transgenic apple transformed with genes not belonging to *Malus*.

In most apple-growing regions, a lower level of *powdery mildew* resistance is more acceptable for a cultivar than that of apple scab resistance. This is due to the fact that low mildew susceptibility of cultivars can already be sufficient to avoid fungicide use. The most known oligogenic resistance sources of mildew are *M. robusta* and *M. zummi* carrying the *Pl1* and *Pl2* resistance genes (Knight and Alston, 1968). *Pl1* and *Pl2* genes have been introgressed into advanced selections and new cultivars (Alston, 1983; Schmidt, 1994; White and Bus, 1999). Other major genes, such as *Pl-w* and *Pl-d*, are in advanced stages of back-cross programmes and genetic markers are being developed (e.g. Evans and James, 2003; James et al., 2005). However, there is a risk of races developing in the pathogen that overcome single-gene resistances, as was experienced with the *Pl-m* gene from 'Mildew Immune Seedling' (Korban and Dayton, 1983; Lespinasse, 1983) and recently the *Pl2* gene (Caffier and Laurens, 2005; Caffier and Parisi, 2007). There was a suggestion that the *Pl-w* from 'White Angel' may have been overcome by a race of the pathogen, too, as all progenies from this cultivar became infected by the end of one season (Lespinasse, 1989). However, absence of the putative races in both cases in the following season suggests that infection may have been the result of high disease pressure. Present resistance-breeding programmes against powdery mildew focus on multiple resistance of genotypes including other fungal diseases of apple (e.g. Laurens, 1999; Fischer and Fischer, 1999; Gessler et al., 2006).

Apple cultivars have been continuously tested for mildew susceptibility in the past century and cvs. 'Jonathan', 'Baldwin', 'Cortland', 'Idared', 'Jonagold', 'Rome Beauty', 'Monroe', 'Gravensteiner', 'Stayman Winesap', 'Cox's Orange Pippin', 'Granny Smith', 'Ginger Gold' and 'Prima' were considered to be moderately-to-highly susceptible. Less susceptible cultivars include 'Delicious', 'Golden Delicious', 'Winesap', 'York Imperial', 'Nittany' and 'Lord Lambourne' (e.g. Aldwinckle 1974; Norton 1981; Scheer 1989; Hickey and Yoder, 1990; Yoder, 2000). Washington et al. (1998) showed that a number of important commercial cultivars are highly susceptible to powdery mildew ('New Jonagold', 23%; 'Pink Lady', 18%); however, there were cultivars with high or moderate levels of resistance to powdery mildew ('Earlidel', no infection observed; 'Red Fuji', 'HiEarly' and 'Redfree', average incidence of mildew between 3 and 6%). Recently Sholberg et al. (2001) developed a technique for better evaluation of apple cultivars for susceptibility to powdery mildew. The authors grafted the selected cultivars to branches of mature 'Jonagold' trees and then evaluated the cultivars in the summer of the same year and in subsequent years after growth on the host tree. The method provided more reliable assessment of powdery mildew resistance than previous assessment methods.

In apple-growing regions with mild winter and humid weather conditions, resistance against *European canker* is also an essential aim of breeding programmes (Van de Weg, 1989). It is well known that cultivars differ in their susceptibility to *N. galligena*, for instance cvs. 'Gravenstein', 'Delicious', 'McIntosh', 'Bismark', 'Spartan', 'Newtown', 'Spitzenburg' and 'Cox's Orange Pippin' have moderate-to-high susceptibility, while others such as 'Golden Delicious', 'Bramley', 'Rome Beauty' and 'Jonathan' are less susceptible to European canker (e.g. Zagaja et al., 1971, Borecki and Czynczyk, 1984; Van de Weg, 1989; Grove, 1990; Van de Weg et al., 1992; Pedersen et al., 1994; Xu et al., 1998). However, Xu et al. (1998) showed high susceptibility of 'Golden Delicious'. Braun (1997) showed that incidence of European canker was greatest on cvs. 'Red Delicious', 'McIntosh', 'Northern Spy' and 'Idared' (>30%) and significantly lower on cvs. 'Golden Russett' and 'Gloster' (<10%). Susceptibility of apple cultivars to European canker shows quite large variations between studies, which may be due to three main reasons: (i) different disease measures used in the studies, (ii) different infection methods and (iii) different types of entry sites.

Most previous studies use canker size as a resistance criterion, whereas canker incidence and the length of incubation period combined with canker size might be better measures (Braun, 1997), as the relationship between canker incidence, the length of incubation period and canker size may depend on cultivar and experimental conditions. Van de Weg (1989) found a significant difference in the incidence of canker between cultivars, whereas in another study, cultivars did not differ in canker incidence but in size, and the incidence was also not affected by initial incubation temperature while in contrast canker size decreased with increasing temperature (Van de Weg et al., 1992).

Infection methods of some studies were based on natural infection (Zagaja et al., 1971; Pedersen et al., 1994) and others on artificial inoculation (Krüger, 1983; Borecki and Czynczyk, 1984; Van de Weg, 1989). In artificial inoculation studies, some used mycelium as inoculum (Borecki and Czynczyk, 1984) and others used a spore suspension (Krüger, 1983; Van de Weg, 1989; Van de Weg et al., 1992); the duration of the wet period (high humidity), the means of achieving the wet period, and the initial incubation temperature also differed. Van de Weg (1989) revealed that the effects of inoculation techniques on canker incidence result in different incidence values.

In addition, resistance to European canker may also vary with the type of entry site. Most previous studies inoculated fresh wounds around leaf scars or tree trunks, and pruning wounds. Pruning wounds were shown to be a better protocol for screening resistance to *Nectria*, which also incorporates healing rate and tree physiological state (Xu et al., 1998). Xu et al. (1998) also concluded that there were significant interactions between cultivars and ages of pruning wounds on the incidence of canker lesions, which implies that cultivars differ in their rates of wound healing, as shown for other woody species (Biggs and Miles, 1998; Doster and Bostock, 1988a,B). The interactions between age-related wound resistance, cultivar and host physiology may have implications for resistance breeding and canker management. Selection for resistance to *N. galligena* is usually based on incidence and size of cankers following the inoculation of fresh wounds (Borecki and Czynczyk, 1984; Van de Weg, 1989; Van de Weg et al., 1992). Xu et al. (1998) suggested that it might be necessary to improve screening by inoculating wounds of various ages on trees at different physiological stages. In the UK, *N. galligena* spores are present in winter (Swinburne, 1975) and readily germinate at low temperatures (Dubin and English, 1975). It may be advisable therefore to restrict winter pruning to canker-free orchards or cultivars with fast-acting defence mechanisms.

The reviews of Byrde and Willetts (1977) and Batra (1991) noted some of the resistant cultivars to *brown rot* such as cvs. 'Jonathan' and 'Beauty of Boskoop'. Susceptibility of apple cultivars to *M. fructigena* is highly dependent on the presence of wounds. Cultivars with higher susceptibility to insect damages and growth crack show higher susceptibility to fruit rot (Xu and Robinson, 2000; Holb and Scherm, 2008).

Some variations among cultivars were found in their susceptibility to *sooty blotch* and *flyspeck* (Gupta, 1989; Williamson and Sutton, 2000). Commercial apple cultivars are not resistant and 'Golden Delicious', 'Granny Smith', 'Cox's Orange Pippin', 'Yellow Newton', 'Buckingham' and 'Jonathan' are highly susceptible to both diseases (Gupta, 1989). In early studies, differences have been related to skin colour and maturity date (e.g. Colby, 1920; Baines and Gardner, 1932). The authors found that symptoms are more visible on light-skinned cultivars, and the diseases tend to be more severe on those cultivars that mature later in the growing season. Belding (1996) noted that the severity of sooty blotch and flyspeck varied among cultivars and he reasoned that since the fungi involved in the sooty blotch complex grow epiphytically on the cuticle, any difference among cultivars might be due to differences in the components of the epicuticular wax. Although differences were found among cultivars in the five principal components of the epicuticular wax, none of the five components supported in vitro the growth of fungi involved in the sooty blotch complex. If dilute apple juice was added to the treatments, the fungi started to grow using nutrients primarily from fruit leachates. Thus, the author concluded any differences in disease severity among cultivars might be related to the permeability of the cuticle to these leachates. Genetic aspects of resistance to the causative agents of sooty blotch, flyspeck and brown rot of apple are not known and no breeding programmes have been initiated against these diseases.

In summary, host resistance, based on breeding programmes for multiple disease resistance, can be evaluated as the greatest potential in effective disease management of environmentally friendly apple production systems. Theoretically, complete host disease resistance would be one of the best approaches in fungal disease management as this would eliminate one of the basic elements of the epidemic triangle. In addition, if complete host disease resistance would succeed for a long-term period, all other chemical or non-chemical approaches could be eliminated from the disease management of apple.

3 Features of Chemical Control for Individual Diseases in Integrated and Organic Apple Production

3.1 General Features and Chemical Control of Apple Scab

3.1.1 Integrated Apple Orchards

Much of the pesticides used in apples are for management of apple scab; therefore, disease components of apple IPM programmes have focused largely on managing apple scab (e.g. Gadoury et al., 1989; Merwin et al., 1994). Chemical control of apple scab has one of the longest and widest histories among plant pathogens and was reviewed in detail by MacHardy (1996). This review, therefore, will emphasise only some of the key elements of apple scab chemical control in integrated apple production (Table 6).

From the end of World War II until the 1970s, growers typically maintained protection throughout a period from green-tip to early fruit set (when fruitlets were approximately 10 mm in diameter) by applying fungicides at approximately weekly intervals (Cooley and Autio, 1997). After this, fungicides were applied at 2- to 3-week intervals during the secondary infection period. A typical fungicide programme before the introduction of IPM involved 15–25 fungicide applications over the growing season in most apple-growing areas (Becker et al., 1982). Generally, growers used fungicides with limited post-infection activity and good protective properties to treat scab. Although the Mills table was available for timing fungicide application after infection, at that time, applying the available fungicides only after a measured infection period had practical limitations.

The first possibility for successful implementation of apple IPM could be achieved by the 1980s when (i) techniques were developed which made it easier for growers to measure Mills infection periods using a modified hygrothermograph (MacHardy and Sondej, 1981), (ii) new findings of apple scab epidemiology were released (e.g. Sutton et al., 1981; Gadoury and MacHardy, 1982, 1986; MacHardy and Gadoury, 1989), and (iii) a new class of fungicides, the ergosterol-biosynthesis inhibitors (EBI), was introduced widely (e.g. Gadoury et al., 1989, 1992; Wilcox et al., 1992; Cooley et al., 1992; Cooley and Autio, 1997). The first use of pathogen monitoring and the Mills infection period table coupled with the post-infection application of EBIs fungicides resulted in a 30–50% reduction in the number of fungicide applications against apple scab. However, the practical use of this system was somewhat complicated as the grower was forced to choose between the optimal timing of post-infection sprays for apple scab and the timing of sprays for other diseases and pests. To solve this contradiction, integration of pesticide application schedules was attempted for disease and pest control in apple orchards (Gadoury

Table 6 Examples of chemical-based fungicides used and/or tested against scab, powdery mildew, European canker, brown rot, flyspeck and sooty blotch in preharvest disease management of apple in integrated orchards

	Fungal disease				
Features	Apple scab	Apple powdery mildew	European canker	Brown rot	Flyspeck and sooty blotch
Common name of fungicide/ Fungicide group	e.g. azoxystrobin, benomyl, bitertanol, captafol, captan, chlorothalonil, copper, cyproconazole, cyprodinil, dichlofluanid, dichlone, difenocnazole, diniconazole, dithianon, dodine, epoxiconazole, fenarimol, fenbuconazole, ferbam, fluazinam, fluquinconazole, flusilazole, folpet, hexaconazole, imibenconazole, kresoxim-methyl, macozeb, maneb, mapanpyrim, methiram, myclobutanil, nuarimol, penconazole, pyrifenox, pyrimethanil, sulphur, tebuconazole, tetraconazole, thiophanate-methyl, thiram, trifloxystrobin, triflumizole, triforine, ziram	e.g. benomyl, bupirimate, Ca-polysulphide, cyproconazole, dichlofluanid, diniconazole, dinocap, epoxiconazole, fenbuconazole, fluqinconazole, flusilazole, hexaconazole, imibenconazole, kresoxim-methyl, mono-potassium phosphate, myclobutanil, nuarimol, oxythioquinox, penconazole, polyoxin B, pyrazophos, pyrifenox, sulphur, tebuconazole, thiophanate-methyl, triadimefon, triadimenol, trifloxystrobin, triflumizole, triforine	e.g. anilinopyrimidines, azoxystrobin, benomyl, bitertanol, bordeaux mixture, captan, carbendazim, copper, dithianon, dodine, fenarimol, fenpropimorph, ferbam, imazalil; kresoxim-methyl, mancozeb, maneb, methiram, myclobutanil, thiophanate-methyl, thiram, triflumizole, ziram	Most fungicides against scab are effective against brown rot on apple, e.g. benomyl, captan, copper, cyprodinil, fluazinam, folpet, iprodion, mancozeb, methiram, thiophanate-methyl, trifloxystrobin	e.g. captan, mancozeb, benomyl, thiophanate-methyl; ziram; kresoxim-methyl, trifloxystrobin, DMI fungicides

Table 6 (continued)

Features	Fungal disease				
	Apple scab	Apple powdery mildew	European canker	Brown rot	Flyspeck and sooty blotch
Key references	e.g. Stanis and Jones (1985); Scheinpflug and Kuck (1987); Hildebrand et al. (1988); Köller (1988); Gadoury et al. (1989, 1992); Wilcox et al. (1992); Cooley et al. (1992); Sholberg and Haag (1993); Merwin et al. (1994); Shirane et al. (1996); Cooley and Autio (1997); Kunz et al. (1997, 1998); Olaya and Köller (1999a,b); Köller and Wilcox (2000, 2001); MacHardy (1996, 2000); Holb and Heijne (2001); Holb et al. (2003a,b); Köller et al. (2004); Fisher and Meuneir (2005); Holb et al. (2005b); de Waard et al. (2006); Grasso et al. (2006); Jobin and Carisse (2007)	e.g. Spotts and Cervantes (1986); Hickey and Yoder (1990); Yoder and Hickey (1995); Sholberg and Haag (1994); Yoder (2000); Reuveni et al.(1998); Reuveni (2000); Reuveni et al. (1998); Berrie and Xu (2003); Lesemann et al. (2006)	e.g. Byrde et al. (1965); Bennett (1971); Swinburne et al. (1975); Swinburne (1975); Berrie (1992); Cooke et al. (1993); Xu and Butt (1996); Lolas and Latorre (1997); Cooke (1999); Latorre et al. (2002)	e.g. Wormald (1954); Byrde and Willetts (1977); Batra (1991); Holb and Scherm (2007, 2008)	e.g. Lewis and Hickey (1958, 1972); Hickey (1960); Brown and Sutton (1986, 1995); Hartman (1995, 1996a,b); Rosenberger et al. (1996a,b, 1997a,b, 1998, 1999); Barden and Marini (1998); Williamson and Sutton (2000); Hernandez et al. (2004); Gleason et al. (1999, 2002); Babadoost (2003); Babadoost et al. (2004); Batzer and Gleason (2005); Cooley and Rosenberger (2005)

et al., 1989; MacHardy, 2000). Three periods were identified for the integration of pesticide applications: (i) prior to pink bud stage, (ii) at petal fall, and (iii) in summer during the secondary infection periods.

Further issues of IPM pesticide application were directed towards reducing fungicide use with the implementation of non-chemical control approaches, which was the so-called advanced apple IPM system (Prokopy, 1993; Prokopy et al., 1994, 1996). In these approaches, chemical control has to be coupled with the use of the potential ascospore dose (PAD) threshold (e.g. MacHardy et al., 1993; Cooley and Autio, 1997), of mechanical sanitation of primary inoculum (e.g. Sutton et al., 2000; Vincent et al., 2004; Holb, 2006, 2007b), and of BCAs against the sexual stage of *V. inaequalis* (Carisse et al., 2000; Vincent et al., 2004).

From the end of World War II until the 1970s, growers typically maintained protection throughout a period from green-tip to early fruit set (when fruitlets were approximately 10 mm in diameter) by applying fungicides at approximately weekly intervals (Cooley and Autio, 1997). After this, fungicides were applied at 2- to 3-week intervals during the secondary infection period. A typical fungicide programme before the introduction of IPM involved 15–25 fungicide applications over the growing season in most apple-growing areas (Becker et al., 1982). Generally, growers used fungicides with limited post-infection activity and good protective properties to treat scab. Although the Mills table was available for timing fungicide application after infection, at that time, applying the available fungicides only after a measured infection period had practical limitations.

Currently, several fungicide groups are registered with preventive or post-infection activities against apple scab all over the world including ftalimides, dithiocarbamates, guanidines, anilino-pyrimidines, benzimidazoles, DMIs and QoIs (Merwin et al., 1994). With this arsenal of fungicides, apple growers follow a combination of pre- and post-infection management strategies against scab using both protectant and curative fungicides. Protectant fungicides are generally used early in the season when there are only few leaves or when infection periods can be forecasted. Curative fungicides are used when a protectant fungicide applied before the infection was washed off by rain, a protectant fungicide was not applied prior to the infection period, or the risk of primary infection was very high (large amount of ascospores, many new unprotected leaves and severe infection period).

DMIs, QoIs and anilinopyrimidines are the principal classes of fungicides used in post-infection management of apple scab in most apple-growing regions. DMI fungicides specifically target C_{14}-demethylation of 24-methylenedihydrolanosterol, disrupting fungal sterol biosynthesis (Scheinpflug and Kuck, 1987; Köller, 1988). DMIs are prone to selecting for development of resistance in microorganims. Resistance mechanisms to DMIs include overexpression of the CYP51A1 gene from the pathogen (e.g. Schnabel and Jones, 2001), as well as efflux mechanisms (e.g. Del Sorbo et al., 1997; Nakaune et al., 1998) and point mutations (e.g. Délye et al., 1997, 1998). DMI resistance in apple scab is well-documented (e.g. Stanis and Jones, 1985; Sholberg and Haag, 1993; Shirane et al., 1996; Kunz et al., 1997; Hildebrand et al., 1988; Köller and Wilcox, 2000, 2001) including practical resistance (e.g. Braun and McRae, 1992; Köller et al., 1997). Resistance to DMIs is quantitatively (e.g. Köller and Scheinpflug, 1987; Smith et al., 1991; Kalamarakis et al., 1991; de Waard, 1993) controlled by more than one gene. Therefore, loss of sensitivity to DMIs by the pathogen is gradual, following a multistep process. The sensitivity of the fungus slowly deviates from the original baseline values and may reach a point at which disease control is affected. However, the critical point of fungicide efficacy loss is hard to identify under field conditions.

As QoIs also have high risk for development of fungicide resistance (e.g. Kunz et al., 1998; Olaya and Köller, 1999a,b; Köller et al., 2004; Fisher and Meunier, 2005; de Waard et al., 2006; Grasso et al., 2006), they are recommended in fungicide alternating programmes as a strategy to slow down fungicide resistance development (e.g. de Waard et al., 2006; Jobin and Carisse, 2007). Strobilurins have been described as being more active against spore germination and host penetration (Gold et al., 1996; Ypema and Gold, 1999). They act as an inhibitor of fungal mitochondrial respiration by binding to the mitochondrial cytochrome bc1 complex subunit and disrupting electron transport (Ypema and Gold, 1999). QoI resistance can be both quantitative and qualitative. In the case of quantitative resistance, a slow decline in disease control can be experienced due to the presence of minor resistance genes that contribute to the avoidance of the intended effects of the fungicide (Grasso et al., 2006). Quantitative shifts can be detected easily with laboratory tests and then can be attributed to the loss of control, which results in a dose-dependent disease control in practice. QoI resistance can also be qualitative, i.e. not showing dose dependence in disease control. In this case, mutational amino acid exchanges can be detected in the cytochrome target site, mostly G143A for *V. inaequalis* (e.g. Olaya and Köller, 1999a,b; Zheng et al., 2000). This mutation is easily detectable with molecular tools but only after resistance has occurred.

Due to the increasing insensitivity of DMIs to apple scab in practice, the use of DMIs is advised to be reduced in several apple-growing areas. Such recommendations may lead to (i) increased use of QoIs and the consequences of possible resistance to QoIs and (ii) increased usage of protectant fungicides which have a higher impact on the environment. This clearly emphasises the essential importance of fungicide antiresistance strategies recommended by the Fungicide Resistance Action Committee (FRAC). Beyond the FRAC recommendation though, the best approach is probably to favour the implementation of integrated tools for apple scab management, such as inoculum reduction by non-chemical approaches, monitoring fungicide resistance and sanitation-improved fungicide timing. Efficacy of these practical management approaches can also be improved by novel disease-warning systems and models (e.g. Seem et al., 1989; Butt et al., 1992; Trapman, 1994; Aalbers et al., 1998; Berrie and Xu, 2003; Holb et al., 2005b). These developments resulted in the creation of more advanced scab management strategies in integrated apple production.

3.1.2 Organic Apple Orchards

One of the key features of chemical disease control in organic apple production is that the effectiveness of approved products is low against the key apple diseases.

Therefore, disease pressure is often high and direct organic disease management often fails especially in humid climatic conditions. Therefore, non-chemical approaches are widely recommended to compensate for the low efficacy of chemical control. According to IFOAM and European standards, only a few chemical products are approved in organic apple production (Anonymous, 2000; EEC, 2000). Most of these inorganic chemical compounds are protectant fungicides providing short-term residual activity. The most widely used compounds are copper, lime sulphur and elemental sulphur in organic apple production; therefore, we focus on the fungicidal features of these compounds. Recently, some other simple inorganic materials, such as SBC and PBC, hydrated lime and kaolin have also received attention and are therefore included in our overview (Table 7).

Copper is one the oldest compounds used in plant protection. Its history started in 1882 when the French botanist Millardet discovered the effectiveness of the mixture of copper sulphate and slaked lime against grape downy mildew. Within a decade, the so-called ‘Bordeaux mixture’ was also used against apple scab (MacHardy, 1996). The use of copper compounds in traditional crop protection sharply decreased after the development of synthetic fungicides but they remained the leading fungicides in organic production. Copper compounds are considered as protective fungicides with good residual activity (e.g. Hamilton, 1931; Holb and Heijne, 2001). However, a recent in vitro study on apple scab demonstrated that some copper salts ($Cu(OH)_2$ and $CuSO_4$) showed 16 and 40 h post-infection activity and killed primary stromata (Montag et al., 2006). $Cu(OH)_2$ was more effective than $CuSO_4$; however, research also showed that application of $Cu(OH)_2$ under dry conditions did not kill primary stromata. For exertion of the post-infection activity of copper salts, leaves must be wet. As this cannot be guaranteed in the field, a post-infection application of $Cu(OH)_2$ cannot be recommended under orchard conditions.

Regarding the mode of action, research showed that copper salts dissolve in water and copper ions (Cu^{2+}) are released into the spray solution, which are the active component of copper fungicides. Copper ions are capable of penetrating into fungal spores and denature proteins with the inhibition of various enzymes in the cell (Heitefuss, 2000). This hypothesis supposes that water solubility of the copper compounds should be important. However, the solubility of copper alone cannot explain the effectiveness of the slightly soluble copper hydroxide and copper oxychloride as well as the insoluble copper oxide. Copper fungitoxicity is believed to be more complex and at least three additional hypotheses are known for explaining the effectiveness of less water-soluble copper compounds. Dissolution of these compounds might be aided by (i) CO_2 and ammonium ions dissolved in rainwater or dew (Pickering, 1912; Reckendorfer, 1936), (ii) exudates from the plant (DeLong et al., 1930) and (iii) secretion of acids or complexing agents by the spore (Barker and Gimingham, 1911; McCallan and Wilcoxon, 1936; Martin et al., 1942). For instance, in a recent study, Montag et al. (2006) demonstrated that *V. inaequalis* spore exudates react with insoluble copper compounds and form highly toxic copper complexes. These copper complexes were more toxic to *V. inaequalis* than dissolved Cu^{2+} ions in the cell. However, copper ions also affect the plant and may

Table 7 Some features of the most widely used inorganic materials against apple scab, apple powdery mildew, European canker, brown rot, flyspeck and sooty blotch in preharvest disease management of apple in organic orchards

	Inorganic materials					
Features	Copper	Lime sulphur	Elemental sulphur	Bicarbonates	Hydrated lime	Kaolin
Fungicide compounds	Copper sulphate Copper oxychloride Copper (I)oxide Copper hydroxide	Calcium polysulphide with calcium thiosulphate	Micronised and non-micronised sulphur	Sodium bicarbonate (SBC) Potassium bicarbonate (PBC)	Calcium hydroxide	Components of stone powder
Target disease in organic apple orchards	Apple scab, brown rot, European canker, flyspeck and sooty blotch	Apple scab, powdery mildew; brown rot, European canker, flyspeck and sooty blotch	Powdery mildew, flyspeck and sooty blotch; apple scab; brown rot	Apple scab	Apple scab	Apple scab, sooty blotch and flyspeck
Fungicide action	Protectant; in vitro 16–40 h post-infection activity against scab	Protectant; in vitro and in vivo 16–72 h post-infection activity against scab	Protectant	Protectant	In vitro and in vivo 16 h post-infection activity against scab	Protectant
Mode of action	Multi-site toxicity, toxic to fungal spores by complex mechanisms	Multi-site toxicity, toxic to fungal spores by complex mechanisms	Multi-site toxicity, toxic to fungal spores by complex mechanisms	–	Kills conidia and germ tubes of *V. inaequalis*	–
General application schedule in organic apple orchards	Early spring spray until early tight cluster, 10–14 days application schedule after the end of June, fallen leaf treatment	Dormant spray, 7–14 days application schedule during the season, fallen leaf treatment	Replacing copper sprays and supplementing elemental sulphur spray, 7–14 days application schedule during the season	Supplementing copper and sulphur sprays, 7–14 days application schedule during the season	Supplementing copper and sulphur sprays, 7–14 days application schedule during the season	Not recommended for season-long spray schedules

Table 7 (continued)

Features	Inorganic materials					
	Copper	Lime sulphur	Elemental sulphur	Bicarbonates	Hydrated lime	Kaolin
Phytotoxicity and side effects	Phytotoxic to leaves and fruit, toxic to some carabid species	Phytotoxic to leaves and fruit, toxic to predatory mites	Phytotoxic above 20°C, toxic to predatory mites	Not phytotoxic to apple fruit at the recommended dose	Not phytotoxic to apple fruit at the recommended dose	Not phytotoxic to apple fruit at the recommended dose
Environmental impact	Toxic to earthworms, heavy metal soil and water pollution	Natural compound with little or no environmental concern	Non-toxic to human and warm-blooded animals	Low mammalian toxicity may increase soil sodium level	Not investigated for apple	Not investigated for apple
Key references in apple	e.g. Hamilton (1931); Byrde and Willetts (1977); Ellis et al. (1994, 1998); Lolas and Latorre (1997); Heitefuss (2000); Holb and Heijne (2001); Holb et al. (2003a,b) (2005a,b); Montag et al. (2006); Jamar and Lateur (2007); Holb (2008a)	e.g. Hamilton (1931); Mills (1944, 1947); Byrde and Willetts (1977); Tweedy (1981); Kelderer et al. 1997 (2000); Heitefuss (2000); Tate et al. (2000); Holb and Heijne (2001); Holb et al. (2003a); Montag et al. (2005)	e.g. Wormald (1954); Hickey (1960); Lewis and Hickey (1972); Byrde and Willetts (1977); Ellis et al. (1998); Yoder (2000); Heitefuss (2000); Holb and Heijne (2001); Holb et al. (2003a,b, 2005a,b); Holb and Scherm, (2007, 2008)	e.g. Schulze and Schönherr (2003); Ilhan et al. (2006); Tamm et al. (2006); Jamar and Lateur (2007); Kunz et al. (2008)	Schulze and Schönherr (2003); Montag et al. (2005)	Thomas et al. (2004); Berkett et al. (2005)

retard plant growth and cause russeting of young plant tissues. Slow drying conditions on the plant surface result in an increase of the availability of copper ions and thus retard plant growth and may cause severe plant injury. Therefore, longer wet weather periods with low temperatures after a spray application can be phytotoxic and may cause fruit russeting during bloom and early fruit development (e.g. Ellis et al., 1994, 1998; Holb and Heijne, 2001).

In most organic apple orchards, scab is controlled by using copper- and sulphur-containing fungicides. In a common fungicide scheme, one to three sprays of copper are applied in the early spring followed by wettable sulphur sprays until harvest during wet periods (Holb and Heijne, 2001). Copper is more effective during the ascospore season than elementary sulphur (e.g. Cooley et al., 1991; Hamilton, 1931; Holb and Heijne, 2001). Copper fungicides were commonly used in organic apple orchards due to their good protective effects, whether applied alone or in various combinations with sulphur.

The largest concern of copper as a heavy metal is that it has serious environmental impacts, especially on soils and waters. In some European countries, copper levels of some agricultural soils exceed the Dutch limit (Anonymous, 1991) of 36 mg kg^{-1} soil following a prolonged use of copper-based products (e.g. van Rhee, 1976; Flores-Veles et al., 1996; Paoletti et al., 1998). Copper pollution in orchards was shown to negatively impact soil ecology and to have detrimental effects on earthworm populations (e.g. Van Rhee, 1976; Paoletti et al., 1998; Holmstrup et al., 1998; Friis et al., 2004). This poor ecotoxicological profile conflicts with the ecological concepts of organic and integrated apple production. Therefore, attempts are being made in several countries to reduce copper application rates in protective spray schedules (e.g. Holb et al., 2003a; Jamar and Lateur, 2007). In addition, strategies are being developed which help to minimise the number of copper spray applications. For instance, Holb (2008b) demonstrated that for apple scab control the use of orchard sanitation by combination of leaf removal and winter pruning could reduce the PAD below 600 ascospores m^{-2} orchard floor on the moderately scab-susceptible cultivar 'Jonathan'. Under such conditions, spray applications could be omitted before early tight cluster in Hungarian organic apple production. This results in omitting two copper sprays at dormant bud and green-tip stages. Omitting these copper sprays would be a benefit for those organic orchards in Europe where the use of copper compounds is restricted or banned.

In the last two decades, several countries restricted copper compounds to an annual use of 2–4 kg ha^{-1} (Anonymous, 1997; EEC, 2000) in organic fruit production. Moreover, in the past few years, some European countries, e.g. the Netherlands and Scandinavian countries, have banned copper-based products (Holb et al., 2003a; Tamm et al., 2004). It seems to be a trend that the use of copper will be forbidden in other European countries in organic growing because of the above environmental reasons. This initiates a more comprehensive research for replacements of copper-based products in European agriculture, including organic fruit production.

Lime-sulphur is also one of the oldest fungicides in use. The common formulation contains a mixture of 29% (wt/vol) calcium polysulphide and a small amount of calcium thiosulphate (McCallan, 1967). Lime sulphur is prepared by boiling hydrated lime ($CaO{\cdot}H_2O$) and elemental sulphur with water. The commonly used fungicide application rate is 2%. At this rate, lime sulphur has a pH of 10.0 and constantly releases small amounts of hydrogen sulphide (H_2S) gas. H_2S gas (Tweedy, 1969, 1981) is able to permeate the fungal membrane (Miller et al., 1953; Tweedy, 1981). It then modifies the respiration complexes of mitochondria when it reaches the cytoplasm (Beffa, 1993). Modifications in the respiratory complexes affect the electron flux in the mitochondrial respiratory chain resulting in multi-site toxicity and broad-spectrum efficacy (Beffa, 1993; Beffa et al., 1987).

As a pesticide, lime sulphur was first described in 1802 in England (Tweedy, 1969). By 1850, the present lime sulphur formula was standardised, and by 1900 it was in common use in California for apple scab, powdery mildew, aphids, mites, brown rot and other pests and diseases (Tweedy, 1969). Most research investigations of lime sulphur were made at the beginning of the twentieth century. Results showed that its efficacy and phytotoxicity were similar to those of copper fungicides (e.g. Goldsworthy, 1928; Hamilton, 1931; Mills, 1947). Moreover, Hamilton (1931) and Mills (1944, 1947) demonstrated that lime sulphur gave sufficient control when applied within 30–72 h after inoculation of *V. inaequalis*. Similarly, the studies of Mills (1944, 1947) have shown that lime sulphur prevented apple scab infection when it was applied within 50 h after the beginning of rain. Some years ago, lime sulphur as a fungicide was newly investigated in relation to season-long disease management in organic apple production. Cooley et al. (1991) and Ellis et al. (1994) claimed that reduced rates of lime sulphur gave better scab control during summer than elemental sulphur applied alone. Kelderer et al. (1997, 2000), in their preliminary studies in South Tyrol, showed that application of lime sulphur shortly after a predicted infection period might have good post-infection activity against apple scab in organic apple production. However, in another study conducted in Austria, satisfactory scab control was not achieved by post-infection treatments with lime sulphur (2–5%) (Steffek, 1999). Trapman and Drechsler-Elias (2000) and Trapman (2001, 2002) in the Netherlands revealed that lime sulphur (1.5–2%) applied at 20–30 h after predicted infection periods gave sufficient scab control under field conditions in organic apple orchards. This result was in agreement with later field trial results of Klopp et al. (2004) in northern Germany. Zemmer et al. (2002) showed that lime sulphur could stop the scab infection until the formation of appressorium. However, when the infection proceeded to the formation of primary stroma, the efficacy of treatments with lime sulphur was insufficient. Further in vitro studies (Montag et al., 2005) showed that lime sulphur (1.5%) applied 16 h after infection killed early infection structures and stopped further development of the apple scab fungus. Lime sulphur reduced the percentage of scab mycelium penetration to below 10% even with treatments of 40 h after infection. In a Dutch study, post-infection treatments with lime sulphur (0.75–2%) applied 35–45 h after predicted infection periods were able to stop primary scab infections (Holb et al., 2003a). The authors used a scab-warning system based on this 35–45 h post-infection activity and saved one to two lime sulphur sprays compared to the preventive treatments of lime sulphur during the primary infection period.

Studies on non-target effects of fungicide sprays claimed that lime sulphur is toxic to plant organs (e.g. Cunningham, 1935; Subhash, 1988; Tate et al., 2000). The mechanism of lime sulphur phytotoxicity is insufficiently understood. The soluble sulphide component of lime sulphur is believed to be responsible for plant injury by reducing carbon dioxide assimilation, which appears to be a fundamental factor underlying the phytotoxic effects (Subhash, 1988). An increased phytotoxic effect was noted when relative humidity and/or leaf wetness increased after spraying (e.g. Cunningham, 1935; Subhash, 1988; Tate et al., 2000). These results suggest that low-volume sprays of lime sulphur can be less phytotoxic than high-volume sprays, because the material applied with the low-volume application dries almost immediately upon contact with the leaf. By contrast, it has been found that the curative activity of lime sulphur might be dependent on wet conditions after spraying (Trapman and Drechsler-Elias, 2000; Trapman, 2001). This is based on the hypothesis that the polysulphide component of lime sulphur is the main active ingredient for its curative activity. The polysulphide component of lime sulphur is able to penetrate into the fungus mycelia only in the water phase (Doran, 1922; Goldsworthy, 1928). If the leaf surface dries, lime sulphur has only a protective effect against the apple scab fungus (e.g. Doran, 1922; Goldsworthy, 1928; Tweedy, 1981; Trapman, 2001). In organic apple orchards, Holb and Heijne (2001) and Holb et al. (2003a) demonstrated experimentally that all lime sulphur treatments had greater curative efficacy against apple scab in wet years but they caused significantly higher leaf phytotoxicity compared to dry years. In addition, the authors showed that lime sulphur has potential for replacing copper fungicides though replacing copper with lime sulphur can result in severe phytotoxicity and reduced yield quality under humid climate conditions.

Elemental sulphur as a fungicide has long been used for plant disease control, including apple fungal diseases, but its efficacy is undoubtedly lower than that of modern, conventional fungicides (e.g. Wormald, 1954; Lewis and Hickey, 1972; Ellis et al., 1998; Holb and Heijne, 2001; Holb and Schnabel, 2005; Schnabel et al., 2007). Elemental sulphur is a contact fungicide with only a weak protective activity (e.g. Ellis et al. 1994, 1998; Lewis and Hickey, 1972). In organic apple orchards, Holb and Heijne (2001) found that 0.5% wettable sulphur applied alone showed acceptable protective activity during the season under low disease pressure. In organic apple scab management, wettable sulphur might be applied successfully on a 5- to 7-day protectant schedule depending on the rate of leaf development during the period of ascospore infections, and it can prevent infections on a 10- to 14-day schedule during summertime if the disease pressure is low (Ellis et al., 1994, 1998; Holb and Heijne, 2001). Under high disease pressure in exceptionally wet years, efficacy of wettable sulphur is unacceptably low, and therefore it is risky to be used against apple scab.

Two types of elemental sulphur-based products are available to commercial growers: non-micronised sulphur and finer-ground micronised sulphur with particle sizes of 4–5 μm. Extremely fine, micronised particles of sulphur were found to act quicker and with greater toxicity against fungal spores compared to larger, non-micronised sulphur particles under controlled conditions (e.g. Wilcoxon and McCallan, 1930; Martin and Salmon, 1932; Tweedy, 1981). Therefore, micronised sulphur products may possess superior fungicidal activity compared to non-micronised sulphur. This effect did not seem to consistently influence field performance of the two forms of wettable sulphur (Holb and Heijne, 2001; Holb and Schnabel, 2005; Schnabel et al., 2007). Although micronised sulphur may act faster and be more toxic than non-micronised sulphur, it acts for a shorter period of time as elemental sulphur particles can quickly penetrate into the fungus cell (Tweedy, 1981; Beffa, 1993). Non-micronised sulphur may be less toxic but it may stay longer on the fruit and leaf surface and can penetrate into the fungus cell for a longer period of time. Moreover, very fine particles of micronised sulphur may be washed off more easily from the plant surface compared to the rough particles of non-micronised sulphur (I.J. Holb, unpublished data). Especially during light precipitations, the rougher sulphur particles may even be re-diluted on the plant surface and penetrate into the fungus cell again. These effects may cancel each other out and make the two forms of sulphur equally effective in the field.

Bicarbonates are one of the control options now attracting attention. They are common food additives allowed under European and North American regulations. Sodium bicarbonate (SBC) and potassium bicarbonate (PBC) have been used against plant pathogens (e.g. Homma et al., 1981; Corral et al., 1988; Horst et al., 1992; Ziv and Zitter, 1992; Palmer et al., 1997; Karabulut et al., 2003) including apple scab (e.g. Schulze and Schönherr, 2003; Ilhan et al., 2006; Tamm et al., 2006; Jamar and Lateur, 2007; Kunz et al., 2008). Recently Ilhan et al. (2006) showed that SBC effectively inhibited spore germination and germ tube elongation of *V. inaequalis* in vitro. In field experiments, 1% SBC treatment reduced the scab incidence on apple leaves to 30% compared with 63% in the water-treated control. The efficacy of 1% SBC was comparable with that of the label dose of tebuconazole on leaves and fruit. The authors showed that treatments of SBC were neither phytotoxic to leaves nor did they adversely affect quality parameters of harvested fruit. Ilhan et al. (2006) concluded that SBC should pose a minimal hazard to humans because of its low mammalian toxicity and it is an inexpensive substance, which would be an advantage to farmers with limited resources. A practical concern with SBC treatments is the presence of sodium, whose addition to agricultural soils is usually avoided; therefore, substitution by the more expensive PBC is recommended (Mlikota Gabler and Smilanick, 2001). Jamar and Lateur (2007) demonstrated that PBC significantly reduced apple scab severity on leaves and fruits compared with water control. The level of scab control was similar to that of wettable sulphur applied alone. The authors indicated that PBC is a contact fungicide with only a weak protective activity. PBC is highly water-soluble and can be washed off from the leaves by a small amount of precipitation. Therefore, frequent spray applications of PCB are recommended against scab in apple orchards (Jamar and Lateur, 2007).

Hydrated lime [$Ca(OH)_2$] has been tried as a replacement for copper fungicides against apple scab. $Ca(OH)_2$ at 5 g l^{-1} was recently shown to quickly kill conidia and germ tubes of *V. inaequalis* (Schulze and Schönherr, 2003). Applications prior to inoculation had no effect as $Ca(OH)_2$ is quickly converted to calcium carbonate ($CaCO_3$) and calcium bicarbonate [$Ca(HCO_3)_2$] by reacting with CO_2 from the air. $CaCO_3$ had no fungicidal effect on apple scab. For this reason, $Ca(OH)_2$ is unsuitable as a protective fungicide and therefore only post-infection activity in combination with a scab-warning system can be expected. Montag et al. (2005) showed that a suspension of hydrated lime (5 g l^{-1}) applied 16 h after infection killed early infection structures and stopped further development. Values of pH 12.4 or higher were necessary (Römpp, 1995) to disrupt the spore membranes and kill germinated conidia of *V. inaequalis* and their primary penetration structures. Montag et al. (2005) observed no phytotoxic reactions of $Ca(OH)_2$, neither with fruit nor with leaves. Their results indicated that a strategy with post-infection treatments of $Ca(OH)_2$ to control apple scab has potential and might become an alternative to lime sulphur. The authors suggest that $Ca(OH)_2$ should be applied with overhead irrigation, which would have several advantages: (i) growers could make applications to an entire orchard in a short time; (ii) if necessary, application of $Ca(OH)_2$ suspensions during rain is possible, as the solid particles act as a buffer and the pH of 12.4 can be maintained even during a light rain or drizzle; (iii) compaction of wet soils by heavy machinery is avoided; and (iv) applications can be automated.

Kaolin is used as a potential insecticide against plum curculio in most organic apple orchards. Berkett et al. (2005) showed that kaolin sprays were able to reduce apple scab too. On cluster leaves and fruit, trees regularly sprayed with kaolin had significantly less incidence of scab than non-sprayed trees. In addition, there was no apparent interference of kaolin with the effectiveness of a standard fungicide programme. The authors concluded that a great advantage of kaolin would be the possible integration of disease and pest management in organic apple production.

3.2 Apple Powdery Mildew

3.2.1 Integrated Apple Orchards

The management of apple powdery mildew is partly based on cultivar resistance in integrated orchards (e.g. Yoder and Hickey, 1983; Hickey and Yoder, 1990). Bower et al. (1995) demonstrated that mildew-resistant cultivars could reduce effectively the need for mildewicides in apple production. Recently, the use of mildew-susceptible cultivars has decreased in apple production; therefore, powdery mildew control is usually coupled successfully with apple scab management as most of the scab fungicides also control powdery mildew sufficiently. Therefore, separate fungicide sprays in apple disease management are not usual against apple powdery mildew. However, in some countries, as DMI are no longer effective against apple scab, they are added to protectant sprays only for powdery mildew control (S. Kunz, personal communication).

Fungicides registered for mildew control are sulphur, oxythioquinox, benzimidazoles, bupirimate, nitrothalisopropy, pyrazophos and EBIs in most countries (e.g. Spotts and Cervantes, 1986; Hickey and Yoder, 1990; Sholberg and Haag, 1994).

The primary mildewicides are DMIs and sulphur in apple orchards (Yoder, 2000). The DMIs have been perceived by many growers as being highly effective, but more expensive than sulphur for mildew control (Yoder, 2000). OoI fungicides were also shown to be highly effective in controlling apple powdery mildew (Reuveni, 2000). Trifloxystrobin (at a concentration of 0.01–0.015%) was superior to most DMIs such as penconazole and myclobutanil (Reuveni, 2000). Highly effective mildewicides, such as most DMIs and OoIs, which strongly suppress the disease, provide acceptable control, even on highly susceptible apple cultivars (Sholberg and Haag, 1994; Reuveni, 2000; Yoder, 2000). However, different levels of DMI and strobilurin resistance in apple powdery mildew isolates were detected (e.g. Reuveni et al., 1998; Reuveni, 2000; Lesemann et al., 2006).

The fungus survives the winter in buds, making it difficult to control during the early spring development of apple trees. First sprays against powdery mildew can only be effective after bud burst when budscales open and overwintered mildew mycelia become available for fungicides. A special action threshold level at 20% leaf infection was suggested in the Mid-Atlantic region of the USA (Yoder and Hickey, 1995; Yoder, 2000). Applications of fungicides should be made from the tight-cluster stage until terminal shoot growth ceases in midsummer. The interval between sprays is generally 7 days during the stages of rapid leaf development before petal fall and 12–14 days during the post-bloom period. Disease assessment and forecasting systems along with sprays of DMIs before mildew becomes severe should be highly effective in minimising losses in commercial orchards (Hickey and Yoder, 1990).

Recent research on control of apple powdery mildew tested spray machines, developed integrated fungicide-fertilizer spray programmes, and improved disease warning. A study by Cross and Berrie (1995) compared axial fan sprayer with the air-assisted tunnel sprayer in integrated apple orchards. The authors concluded that control of powdery mildew was similar when using either sprayer. Approximately 30% of the spray volume applied was collected for recycling with the tunnel sprayer, but the main limitations of the tunnel sprayer were its slow maximum forward speed and the restricted tree size and shape on which it can be used. Other research focused on integrating sprays of mono-potassium phosphate (MPH) fertilizer with systemic fungicides against powdery mildew (Reuveni et al., 1998). Reuveni et al. (1998) showed that the effectiveness of alternating systemic fungicides with a 1% solution of MPH was similar to that of the commercial treatment with the systemic fungicides. In addition, the tank-mix of 1% MPH solution with a half rate of fungicides was as effective or superior to that obtained by the standard fungicide treatment. The authors concluded that the inhibitory effectiveness of MPH fertilizer makes it a potential major component of an IPM programme and the MPH fertilizer can also be useful in mildew resistance management. Recent research also made a great development in forecasting apple powdery mildew. Xu (1999) developed a model to simulate epidemics of powdery mildew on vegetative shoots which generates two types of output: (i) forecasts of disease severity and (ii) indices of the relative favourability of weather conditions on disease development. This model became part of the PC-based disease-warning system, Adem(TM). Field evaluation of Adem(TM) resulted in similar or better mildew control than a routine programme (Berrie and Xu, 2003). In addition, Xu and Madden (2002) argued that the leaf incidence–density relationships for apple powdery mildew may also be incorporated into practical disease management decisions.

3.2.2 Organic Apple Orchards

In organic apple production, mildew resistance of cultivars is a key element of powdery mildew control. The most commonly used fungicide against powdery mildew in organic apple production is elemental sulphur. Of the mildewicides, sulphur was shown to be the least effective but it was demonstrated that increasing the number of sulphur applications from six to eight increased mildew control and yield (Yoder, 2000). Sulphur sprays are also used against apple scab in organic production, and therefore the interval between sulphur sprays is generally 7 days during both the primary and secondary infection periods of powdery mildew. This frequent use of sulphur compounds against powdery mildew fulfils the marketing requirements for organic apple production.

In the last two decades, plant and minerals oils also received attention for control of powdery mildew in order to replace the frequent use of sulphur (e.g. Northover and Schneider, 1993, 1996; Grove and Boal, 1996; Yoder et al., 2002; Fernandez et al., 2006). Sunflower, olive, canola, corn, soybean and grapeseed oils were equally effective in providing over 99% control of *P. leucotricha* when applied to apple foliage 1 day before or 1 day after inoculation (Northover and Schneider, 1993, 1996). The authors also showed that mechanically emulsified canola oil was comparable to dinocap when applied 1, 2, 4 and 7 days after inoculation. Recently, mineral oils were also tested in a three-spray early-season programme targeting apple powdery mildew but the results showed that powdery mildew shoot infestation was suppressed only in 1 year and no differences in fruit damage were found when treatments were compared to untreated control (Fernandez et al., 2006).

3.3 European Canker

Control of European canker is extremely difficult in apple-growing areas where environmental conditions are favourable for the disease such as mild winters and cold summers coupled with high annual precipitation. Removal of infected plant parts is usually not sufficient to control this disease and chemical applications are needed to avoid severe damage (Grove, 1990; Latorre et al., 2002).

Fungicide sprays have already proved effective in preventing canker in early studies (e.g. Byrde et al., 1965; Bennett, 1971). Copper sprays in autumn and/or spring are a general recommendation against the disease, which can be recommended for integrated apple orchards, and the only chemical control option for organic orchards. One to three protective sprays of copper compounds (e.g. Bordeaux mixture, copper oxychloride or copper dioxide) or copper compounds alternated with benzimidazole fungicides (benomyl, thiophanate-methyl or carbendazim) are widely used during leaf fall (Lolas and Latorre, 1997). Benzimidazoles are known to suppress sporulation of the pathogen for prolonged periods (Swinburne et al., 1975) and may thus prevent autumn infection by *N. galligena* without the need for an additional autumn treatment (Cooke, 1999). However, this group of fungicides is now banned for integrated production in several countries. Application timing of fungicides against European canker can be different for apple-growing regions (Swinburne, 1975). In the United States, in the North Pacific Regions, copper sprays are recommended prior to autumn rains and at the onset of leaf fall (Grove, 1990). However, in Northern Ireland Swinburne et al. (1975) showed that spring-summer fungicide programmes caused a greater reduction in canker numbers than autumn fungicides alone. Further investigation in Northern Ireland showed that autumn application of carbendazim gave inadequate control and thiophanate-methyl, bitertanol and fenpropimorph were ineffective (Cooke et al., 1993). According to the above results, spring-summer fungicide sprays were also investigated compared with autumn spray programmes. For instance, Cooke et al. (1993) showed that carbendazim applied as a spring-summer treatment reduced canker development to a similar level to a spring-summer dodine scab programme plus autumn copper oxychloride. Summer carbendazim plus captafol were an outstandingly effective treatment. Berrie (1992) also showed that carbendazim was the only fungicide that reduced numbers of cankers significantly in comparison with captan, dithianon, mancozeb and imazalil. Both Cooke et al. (1993) and Berrie (1992) studies concluded that carbendazim mixed with an effective scab fungicide remains the recommended treatment in an orchard with a serious canker problem. In a more recent study, Cooke (1999) showed also that DMI fungicide programmes during the season including autumn application of copper oxychloride achieved excellent canker control. Curative activities of DMI fungicides were also tested. Xu and Butt (1996) reported that curative fungicide sprays were relatively ineffective in preventing canker development at pruning cuts 48 or 36 h after inoculation with *N. galligena*. The authors showed a dramatic decrease in canker numbers on 1-year-old wood following fungicide treatments, which implied an external source of inoculum rather than the development of cankers from systemic infections. The reason is that the uptake into woody tissue from foliar fungicide sprays is extremely limited and insufficient to produce a fungicidal dose, since their translocation is almost exclusively acropetal (Crowdy, 1977). DMI fungicide sprays are thus unlikely to kill the pathogen within established cankers, to inhibit possible systemic spread via the xylem, and to prevent symptomless systemic infection by *N. galligena* (Cooke, 1999). In addition, not even newer groups of fungicides (such as anilinopyrimidines and the strobilurins) may reduce systemic infection of *N. galligena* with a higher efficacy than other older fungicides.

Efforts were also made to improve canker control by using disease forecasting. As weather conditions are critical, both for inoculum production and infection by *N. galligena*, predictions that use temperature and duration of leaf wetness required for infection have been used for timing fungicide applications on European canker (e.g. Lortie, 1964; Wilson, 1966; Dubin and English, 1974a,b, 1975; Xu and Butt, 1993, 1994). More recently, Latorre et al. (2002) developed a PC-based infection-warning system throughout the analysis of weather parameters and implemented it in the predictive software PatFrut. In the model test, five and six warnings of European canker infection were determined during leaf fall in 2 years, which were associated with rain events and wetness periods. The forecast model showed benefit for canker control. In 1 year, significant differences in disease incidence were obtained reducing disease incidence from 24 to 4.6% when treatments were scheduled according to the model programme (Latorre et al., 2002).

3.4 Monilinia Fruit Rot

Brown rot is rarely a serious problem in integrated apple orchards (Batra, 1991; Holb, 2008a). If fruits are prevented from skin injury then fungicide programmes against apple scab are also effective against brown rot. In the case of specific brown rot problems, fungicides effective against apple scab are also active against brown rot. Sprays applied during bloom reduce blossom blight (in those areas where *M. fructicola* also occurs on apple) and sprays applied 2–3 weeks before harvest help to reduce fruit infection where severe fruit injury is expected (Byrde and Willetts, 1977; Batra, 1991).

In organic apple orchards, brown rot of apple can become a serious disease due to two reasons: first, insect (and especially codling moth) control is insufficient in organic apple orchards, and therefore large numbers of injured fruits are present in the orchards; and second, approved fungicides in organic apple orchards are not effective enough against brown rot. Copper and sulphur compounds were used for brown rot control from the late nineteenth century (Wormald, 1954). Copper fungicides are primarily able to reduce primary inoculum sources produced on mummified fruit (Byrde and Willetts, 1977; Batra, 1991). Sulphur fungicides were widely recommended until the early 1970s, especially against the fruit rot stage of brown rot (Byrde and Willetts, 1977; Batra, 1991). Efficacy of sulphur compounds is based on a reduction of spore germination and spore viability in *Monilinia* spp. (Tweedy and Turner 1966). Over the past 30 years, sulphur has been used against brown rot only in organic apple production, as there is no more effective option against the disease.

3.5 Flyspeck and Sooty Blotch

It can be stated in general that due to the several fungal species associated with the sooty blotch and flyspeck complex, control can be achieved by broad-spectrum fungicides with longer residual activity (Hernandez et al., 2004). However, these fungicides have several harmful effects on the environment; therefore, most of them are banned and no longer used. Research also indicated that fungicide applications from a second cover spray through late August controlled summer diseases more effectively than early-season treatments (Barden and Marini, 1998). The newer site-specific fungicides have narrow-spectrum activities mainly with shorter residual activity, which first, results in a more frequent spray application during summer, and second, not all the fungi associated with sooty blotch and flyspeck complex can be equally controlled. In addition, control of the disease complex with narrow-spectrum fungicides will affect the efficient use of a reduced summer spray programme against scab and probably will limit the omission of the last fungicide sprays against scab in the warm, moist growing areas of the world. All of these will provide a new challenge for scientists and growers in applying chemical control. On the other hand, they provide a clear indication that non-chemical control approaches will play an important role in the successful management of the sooty blotch and flyspeck complex. Recently, a detailed review was published of the current status of sooty blotch and flyspeck control (Williamson and Sutton, 2000); therefore, only the presently available fungicides for integrated and organic apple orchards are discussed here.

3.5.1 Integrated Apple Orchards

Among contact organic fungicides, captan is one of the earliest fungicides which was found to be effective against the sooty blotch fungus, *Gloeodes pomigena,* in in vitro tests and its failure to control sooty blotch in the field was attributed to its short residual activity (Hickey, 1960). Several earlier studies (e.g. Weaver, 1953; Hickey, 1960; Lewis and Hickey, 1958, 1972) led to the development of a spray timing programme similar to the one used today in which the cover sprays are applied every 10–14 days during the summer. Hickey (1977) found that zineb provided up to 60 days residual activity and suggested combinations of captan plus zineb for sooty blotch and flyspeck control for the mid-Atlantic growing region. Brown and Sutton (1986) found that the residual control provided by mancozeb was 20–30 days longer for sooty blotch and 30–50 days longer for flyspeck than that provided by captan. Because of their excellent residual and broad-spectrum activity, these fungicides became widely used from the mid-1960s through to the early 1990s. In the 1980s, the benzimidazole fungicides, benomyl and thiophanate-methyl, were beginning to be inserted into the cover spray programme, often in combination with captan, to improve sooty blotch and flyspeck control. However, most of these fungicides are not allowed to be used in modern integrated orchards anymore. From the 1990s, new fungicides (DMIs and QoIs) with site-specific activity were released and incorporated into the spray programme. This resulted in one or more causative fungi of sooty blotch and flyspeck becoming more important.

Recently, control was also improved via the use of eradicant spray programmes based on forecast models to time fungicide applications. In the first study, Brown and Sutton (1995) monitored hours of leaf wetting (using a DeWit leaf wetness recorder), rainfall and temperature. They noted that the first symptoms of sooty blotch and flyspeck appeared after an average of 273 h of leaf wetting of 4-h duration or greater had accumulated, beginning with the first rain that occurred 10 days after petal fall. They recommended that a threshold of 200–225 h of accumulated wetting should be used to time benzimidazole applications but they suggested that a higher threshold could be used under low-inoculum situations. They indicated also that if the model had been used, the average grower would have saved two sprays each year. The model has subsequently been modified by several researchers. Hartman

(1995, 1996a) and Smigell and Hartman (1998a,b) suggested 175 h of total wetting as a threshold for the insertion of a benzimidazole fungicide in the spray programme in Kentucky. By using the programme, up to four sprays a year have been saved. Hartman (1996b) and Smigell and Hartman (1998a,b) used also their version of the flyspeck and sooty blotch model to time the placement of multilayer fruit bags (used to modify fruit colour for specialty markets) on developing fruit. Gleason et al. (1999), using the Hartman model, compared the accumulation of leaf wetting on-site with an electronic sensor with predicted leaf wetness data. Using the on-site data, they were able to use two sprays less than were used in the protectant programme and one less when using leaf wetness data from the commercial company.

At the same time, a somewhat similar approach was utilised for timing benzimidazole sprays to control flyspeck in New York (Agnello et al., 1999; Rosenberger et al., 1996a,b, 1997a,b, 1998, 1999). The authors reasoned that the last fungicide spray applied to control scab, which typically includes benomyl, thiophanate-methyl, captan or ziram, provided 14–21 days residual activity against flyspeck, depending on the particular fungicide. After the 14- to 21-day period, total hours of leaf wetting are accumulated until 100 h are reached. This 100-h period is referred to as a 'protection gap' and is based on the ability of benzimidazole fungicides to eradicate existing infections during this time period.

More recently, Gleason et al. (2002), Babadoost (2003), and Babadoost et al. (2004) further improved the effectiveness of a disease-warning system and efficacy of reduced-risk fungicides for management of sooty blotch and flyspeck. The authors showed that warning system-timed applications of the second-cover fungicide spray occurred when 175 h of leaf wetness had accumulated; wetness data were derived either from a sensor placed beneath the canopy of apple trees (on-site) or according to remotely sensed estimates. Using sensor measurements as inputs to the warning system saved one to three fungicide sprays per season. The reduced-risk fungicides, kresoxim-methyl and trifloxystrobin provided a control of sooty blotch and flyspeck equal to benomyl or thiophanate-methyl in all trials. Later Batzer and Gleason (2005) also demonstrated that selection of tree canopy sites for leaf wetness duration (LWD) monitoring could profoundly affect the performance of LWD-based disease-warning systems in apple orchards. In addition, Cooley and Rosenberger (2005) revealed that conidia usually do not reach orchards until the cumulative leaf wetting (CLW) from cultivar 'McIntosh' petal fall (PF) reaches 270 h. They concluded that fungicide sprays could be omitted during summer until the time that CLW-PF reaches 300 h. This timing regime should allow growers to omit one to two fungicide sprays during June and July in most years without significantly increasing the risk of flyspeck on fruit at harvest (Cooley and Rosenberger, 2005).

3.5.2 Organic Apple Orchards

Copper, lime sulphur and elemental sulphur can be used against the sooty blotch and flyspeck complex in organic apple orchards. The first report of a fungicide trial for sooty blotch and flyspeck was in 1894, using Bordeaux mixture (Lamson, 1894), and it was reported that Bordeaux mixture could reduce the incidence of disease from 77 to 18%. Bordeaux mixture, applied every 2–4 weeks during the season, was generally recommended during the first decade of the twentieth century to control the diseases (Stevens and Hall, 1910; Ploper and Backman, 1991). By 1910, Bordeaux mixture was being replaced by lime sulphur, which was less phytotoxic (Hickey, 1960). From this time until the late 1940s and early 1950s, Bordeaux mixture and lime sulphur were the principal fungicides used for the control of sooty blotch and flyspeck in commercial apple orchards and in the past 50 years they remained the main fungicides in organic orchards (Trapman et al., 2004). Recently, a disease-warning system is also used against sooty blotch in organic apple orchards based on applications of lime sulphur and copper sprays (Trapman, 2004).

As regards other organically approved fungicide options, coconut soap, prepared from *C. nucifera*, has been shown to be efficient against sooty blotch (Fuchs et al., 2002). Authors showed that effectiveness of six applications of the soap was similar to that of four treatments of lime sulphur at 1% dosage. The authors concluded that coconut soap could be effective against sooty blotch if the disease pressure is low. Kaolin has also been evaluated against sooty blotch and flyspeck in organic apple orchard (Thomas et al., 2004; Berkett et al., 2005). A kaolin-based product, Surround WP (Engelhard Corp., Iselin, New Jersey), was applied at five different rates and frequencies throughout two growing seasons but it was successful at suppressing only flyspeck and only in 1 year.

4 Integration of Multiple Management Tactics Across All Important Fungal Diseases in Integrated and Organic Apple Production

Fungal disease management of apple is based on scab control in both integrated and organic production systems. Management of all other diseases are mainly incorporated into scab management programmes or specific treatments may be attached to scab control schedules such as for the management of European canker, flyspeck and sooty blotch.

4.1 Integrated Apple Orchards

In the past three decades, automatised disease-warning systems have been key elements of fungal disease management in integrated apple orchards. Based on this, the number of chemical applications against fungal diseases has been reduced (Fig. 1/A). As numbers of fungicide sprays are still too high, further reduction is needed especially of sprays against scab. The basic market criterion for integrated apple fruit is that final fruit scab incidence must be below 1% (Holb et al., 2003b). This is a strict criterion; therefore, scab control relies mainly on the effectiveness of chemical fungicides to fulfil the 1% scab threshold level at harvest. Based on annual scab control schedules, advanced IPM against fungal diseases identifies two periods

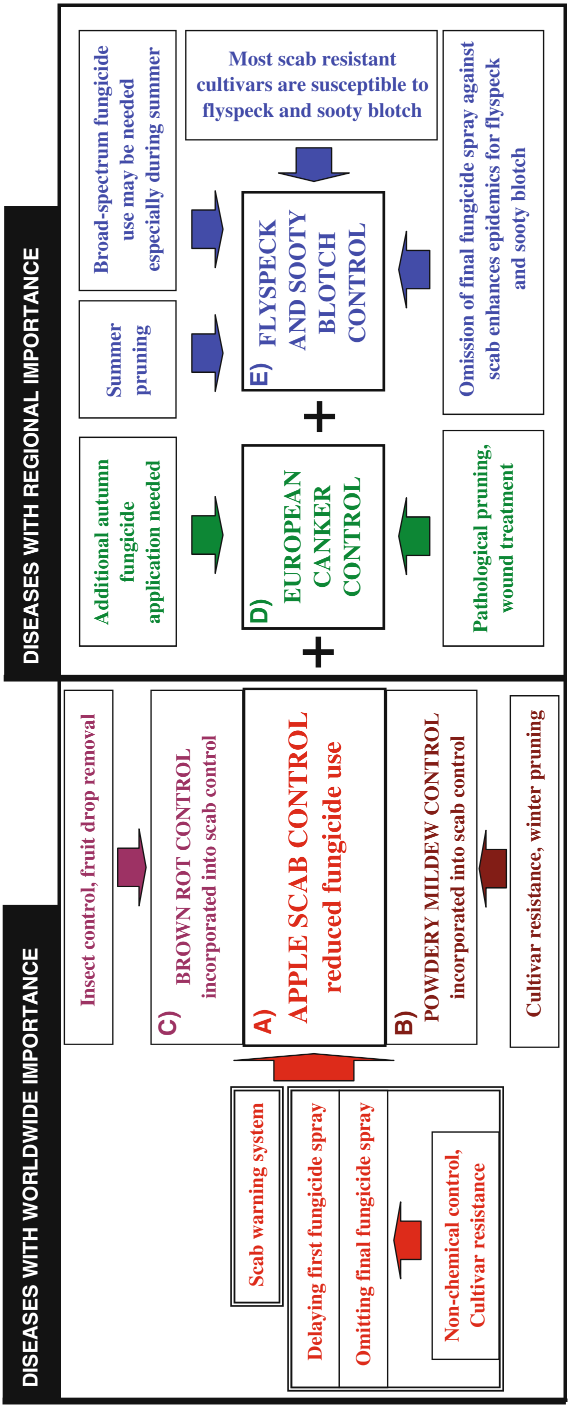

Fig. 1 Summary for integration of management tactics across six important fungal diseases in integrated apple production

for reducing the number of fungicide applications. One is omitting sprays against scab in the beginning of the season by using PAD threshold criteria for delaying first fungicide sprays (MacHardy et al., 1993; Sutton et al., 2000) and the other is omitting fungicide sprays at the end of the season by timing the final spray application against scab (Scheer, 1987; Holb et al., 2003b). Omission of fungicide sprays at the beginning or/and at the end of the season relies on incorporation of non-chemical control options in the disease management schedule, although their biological efficacy and expenses can be highly limiting factors of their use against apple scab (Fig. 1/A). Two options are the most popular in the present apple IPM programmes: one is cultivar resistance and the other is the reduction of primary inoculum by sanitation (Fig. 1/A). Both options presently have limitations. Resistant cultivars are not yet widespread due to low market interest related to their insufficient fruit quality. The reduction of primary sources of scab inoculum is the most effective by leaf removal in the sexual phase and by pruning in the case of the asexual fungal phase. However, the grower attitude is highly dependent on the applicability of these methods, whether it is realistic to omit the first two sprays at the beginning of the season or not (Rosenberger et al., 1996a; Cooley and Autio, 1997).

Control of apple powdery mildew is mainly a question of cultivar susceptibility. The most widely grown cultivars have low-to-moderate mildew susceptibility, which gives a possibility to reduce fungicide use against powdery mildew (Fig. 1/B). This option highly suits to scab control in two aspects: (i) most scab fungicides are highly effective against powdery mildew and (ii) removal of infected shoots during winter pruning is suited to mechanical control of both powdery mildew and scab control (Fig. 1/B). On the other hand, if cultivars are susceptible to powdery mildew (such as 'Jonathan') omitting the first two fungicide sprays against scab until pink bud stage might be impossible as a fungicide spray would be needed against powdery mildew after bud burst. This also indicates that mildew susceptible cultivars cannot be a part of the overall disease management in the future in integrated apple orchards.

Scab fungicides are highly effective against brown rot, though brown rot infection may occur at the end of summer or early autumn when scab fungicide sprays are omitted. However, this late infection is highly correlated with insect injury (Holb and Scherm, 2008); therefore, efficacy of brown rot control is highly dependent on the success of insect control, which is out of the question in a well-managed integrated apple orchard (Fig. 1/C).

Control of European canker during the season is also managed by scab control though it can be more difficult to adjust to scab control than to control of powdery mildew or brown rot (Fig. 1/D). The use of an effective scab control programme based on DMIs and/or non-systemic fungicides such as dithianon should largely prevent canker from becoming established in an orchard as long as the major source of inoculum is external. Supplementing this with two autumn applications of copper oxychloride is worthwhile in wet areas such as Northern Ireland, where it can substantially reduce leaf scar infection (Fig. 1/D). Fungicide spray treatments cannot, however, eradicate existing infections, so if trees are already visibly infected, the programme must be supplemented by cutting out and removing cankers and treating wounds with an effective canker paint (Fig. 1/D).

Control of flyspeck and sooty blotch can be difficult to adjust to scab control in warm and moist growing areas (Fig. 1/E). The major reasons are (i) control of flyspeck and sooty blotch requires broad-spectrum fungicides that might not be used for apple scab for most spray applications; (ii) scab control does not use expensive systemic fungicides at the end of the season due to their cost and fungicide resistance management; (iii) omission of the last fungicide sprays against scab at the end of summer or early autumn might not be applied on flyspeck- and sooty blotch-susceptible cultivars as both diseases build epidemics at the end of summer; and (iv) scab-resistant cultivars receive very few sprays during summer which might allow flyspeck and sooty blotch to cause severe symptoms (Fig. 1/E). Unfortunately, the most favourable scab-resistant cultivars are susceptible to flyspeck and sooty blotch and additional sprays are needed against both diseases during summer. Overall, there is a clear indication that the non-chemical control approach becomes an increasingly essential supplementing option for successful management of the sooty blotch and flyspeck complex. Cooley and Autio (1997) suggested a combination of summer pruning and limited captan use for management of the sooty blotch and flyspeck complex in an advanced IPM programme reducing fungicide use compared to traditional IPM programmes (Fig. 1/E). Although this advanced IPM practice reduced fungicide use, it was also shown that growers were reluctant to incorporate them fully (Cooley and Autio, 1997). Strictly on the basis of expenditures, growers who adopt the advanced IPM programme would save approximately two fungicide applications a year without incurring additional disease damage and would hence save approximately USD 140 ha year^{-1} including application costs (Rosenberger et al., 1996a). Additional costs incurred would include the PAD analysis, estimated at USD 30 ha year^{-1}. Hence, although the advanced IPM methods might save USD 110 ha year^{-1} this would be quickly lost if growers incurred around 1% more than the usual fruit damage (Rosenberger et al., 1996a). As this profit benefit can be lost very easily, growers are reluctant to adopt the advanced IPM programme. This indicates that even if new aspects of the advanced IPM programme are successful, it must be refined to be attractive to apple growers.

4.2 Organic Apple Orchards

In organic apple orchards, there is no efficient chemical control option that could help the low or moderate efficacy of non-chemical disease management. The most effective fungicides are copper and sulphur compounds and their efficacy is below that of the standard synthetic fungicides. Therefore, the risk of disease epidemics is high. To somewhat lower epidemic development, the grower has to use disease-resistant apple cultivars and apply efficient combinations of non-chemical control measures as well. One of the key prerequisites for the successful use of the non-chemical control methods is that the orchard site has to suit to cultivars' optimum, and agronomic measures have to be used to suppress disease development. After this, the frequent use of sulphur and copper fungicides may provide an acceptable disease level and fruit quality for the organic fruit market.

The cultivar planted should possess at least scab and powdery mildew resistance. Cultivar resistance coupled with mixing cultivars within the orchard can be a viable option for reasonable organic apple growing. Most organic apple growers in Europe still use moderately or even highly susceptible cultivars that require large numbers of sulphur and copper sprays, reaching 25–30 sprays per year. This means a weekly fungicide schedule from bud burst until mid-June, then applications at 10–14 days intervals until harvest depending on weather conditions. Due to the restriction of copper fungicides in organic apple production, copper is used in the beginning of the season and the rest of the spray applications contain large amounts of sulphur fungicides, which results in sufficient control of powdery mildew. However, this schedule is not able to control sufficiently other diseases such as apple scab, European canker, flyspeck and sooty blotch. Therefore, cultivar resistance and all previously described non-chemical control options constitute important elements of a successful suppression of apple diseases in organic apple production. All these approaches are very time-consuming and consequently have high labour costs. One of the keys to the success of the non-chemical control options is harmonising management practices during the season based on epidemic features of the pathogen, weather conditions and the efficacy of non-chemical control schemes against the disease. As an example, an option is shown here (Fig. 2) for harmonisation of these elements, which were recently developed to control overwintering conidia of the apple scab fungus in organic apple orchards (Holb et al., 2005b).

In this model, three parameters should be incorporated: (1) Y_{75} as the time for bud closure for cv. 'Jonagold'; (2) previous year autumn scab incidence (40%) as the minimum threshold criterion for overwintering conidia; and (3) minimum values of AUDPC (area under the disease progress curve) and theta (the absolute rate of disease progress) for calculating the present year epidemic intensity until the tree reaches day Y_{75}. The effect of spray application can be modelled based

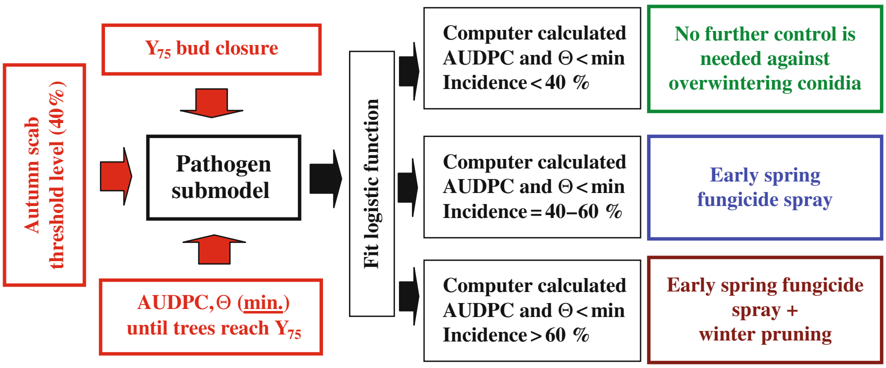

Fig. 2 Possible implications of the most important disease parameters (AUDPC – area under the disease progress curve; theta (Θ) – the absolute rate of disease progress; Y_{75}– the time for bud closure for cv. 'Jonagold'] in apple scab management for the organic production system. Adapted from Holb et al. (2005b)

on the above factors in order to suppress conidial entrapment as much as possible. If the computer-calculated AUDPC and theta are lower than the minimum and until mid-October the orchard has a lower level of scab incidence, less than 40%, then no further control is needed against overwintering conidia. However, if computer-calculated AUDPC and theta are higher than the minimum and autumn scab incidence is between 40 and 60% until mid-October, then an additional copper or lime sulphur spray should be applied at bud burst next spring. Finally, if computer-calculated AUDPCs and theta are higher than the minimum and autumn scab incidence is above 60% until mid-October, then an additional early fungicide spray combined with winter pruning should be applied in order to suppress infections by overwintered conidia.

5 Future Trends

Observing environmentally friendly production in a more general sense, i.e. with regards to the concept of sustainability ('the ability of a system to continue'), most research reveals that integrated production is the future 'sustainable' way to solve crop protection difficulties in arable and tree-fruit production. This is in spite of the fact that, for example, integrated apple production still uses considerable amounts of synthetic pesticides including a large proportion (90%) of fungicides (Penrose, 1995). On the other hand, organic production has received much criticism as to what extent it is sustainable. In fact, researchers believe that there are some basic issues which remain to be solved in order to fully establish the sustainability of organic production. This is due to the fact that the majority of the compounds utilised in crop protection in organic production systems are derived from non-renewable resources and they are not without toxicological hazards to the environment or humans (Edwards and Howells, 2001). Despite these problems, researchers agree that organic farming is more sustainable than conventional production in a bio-physical sense. In summary, both production systems require further development in disease management by improving, for example, warning system, resistance breeding and non-chemical control options.

Current research studies predict that genetic tools are likely to play the most essential role in fungal disease management of apple with the multiple resistance breeding approach if they will be able to produce apples that are not only disease-resistant but also have equally good taste and quality as the susceptible ones. Genetic tools might not be the final approach to solve the fungal disease management as emergence of new pathogen races might break this resistance from time to time. Therefore, other disease resistance mechanisms such as more physiological approaches (e.g. systemic acquired resistance) might be involved in the practice of a successful apple disease management. Either way, the host resistance approach will receive great attention in the future, as basically this seems to be the least costly option for growers and the most environmentally friendly approach for effective disease management.

Often agronomic practices (and also other non-chemical control methods) that are known to reduce disease incidence or severity, even if they are environmentally sound, are not economically feasible. Another problem is that only few of them are absolutes in disease control. For instance, some pathogens are controlled by mulching but others are not or their development is even enhanced by applying mulching. Some diseases can be managed by irrigation, while irrigation makes other diseases worse. In addition, it might be an economic problem if one approach can control only one disease, such as a BCA always has this feature, even if it is effective. In the future, improvement of the present expert systems such as POMME (Travis and Latin, 1991) and their incorporation into precision farming practices for apples might provide the best chance to include agronomic measures and non-chemical control options more efficiently in the current environmentally friendly apple production systems.

As integrated disease management of apple still uses large quantities of chemical-based fungicides, it will undoubtedly benefit from the use of previously discussed non-chemical control methods against fungal diseases. Integration of the novel and more effective non-chemical control methods enables the continuous development of integrated disease management by reducing or replacing fungicides and consequently it will continue in an overall reduction in the use of pesticides. This will result in plant protection schedules gradually approaching the basic concept of the ecologically based production system. The present status of integrated apple production – the combination of chemical, cultural and biological control methods in fungal disease management strategies – is only at the stage of the second level of IPM in the integrated fruit protection. Recently, research programmes have been initiated for the third level of IPM (Prokopy et al., 1994; MacHardy, 2000; Prokopy, 2003), which harmonises scab management strategies with control of other diseases and pests and with other horticultural management practices. The challenge for apple IPM programmes in the twenty-first century is to complete the fourth, final IPM level, which supplements IPM level 3 with cultural, social and political realms.

In organic apple orchards, there are still several efficacy problems in fungal disease management strategies, so further improvements are needed for integrating chemical, cultural and biological control methods in a much more effective way. An urgent task is to develop effective non-chemical control options that are practically feasible and can be incorporated easily into the orchard management practices of organic apple production. These new technological elements must result in acceptable yield and fruit quality parameters. Until these tasks are achieved, an essential change in the current status of organic production cannot be expected.

6 Conclusion

Current fungal disease management in environmentally friendly apple production still relies largely on chemical control. In the past 20 years, continued developments of disease-warning systems and host resistance to fungal pathogens, as well as incorporation of some non-chemical control options into fungal disease management of

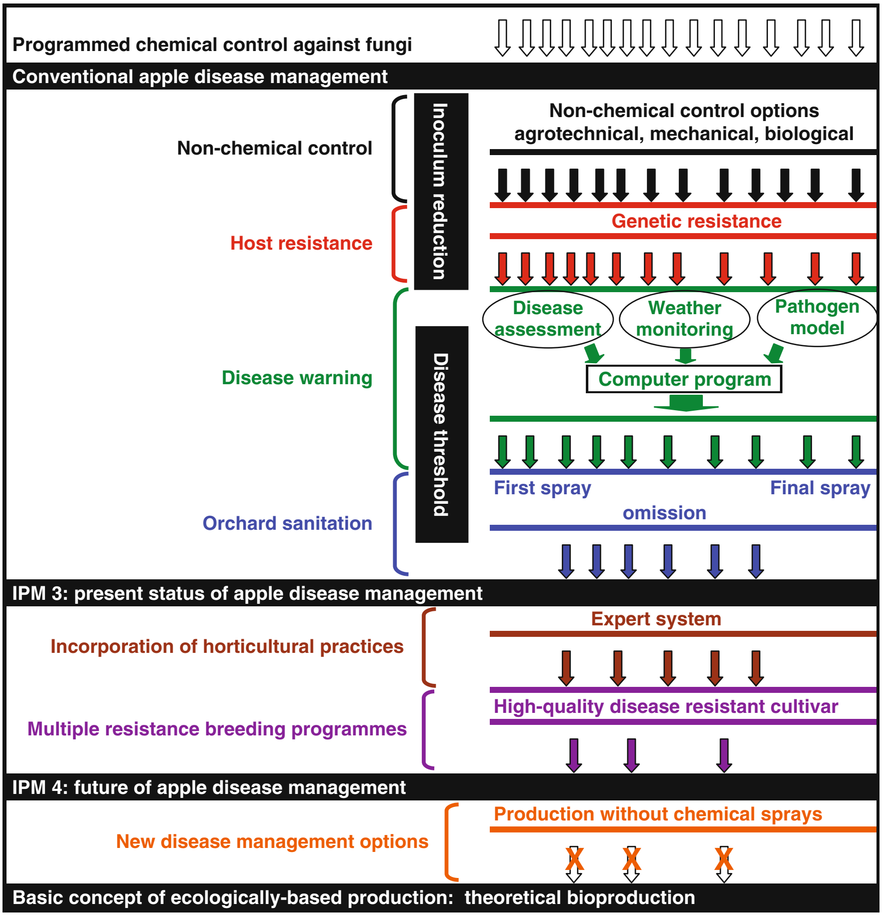

Fig. 3 Basic strategies for reducing chemical-based control against fungal diseases by using several means of non-chemical control approaches in order to reach the basic concept of ecologically based apple production systems. *Arrows* represent fungicide sprays during the season

apple, were able to reduce the number of sprays against fungal diseases considerably (Fig. 3). In addition, the duration of season-long pesticide use was reduced by developing disease threshold levels for omitting fungicide sprays at the beginning or/and at the end of the season (Fig. 3). Despite this, disease management in environmentally friendly apple production still uses large quantities of chemical-based fungicides. Therefore, our future task is to integrate the novel and more effective non-chemical control methods throughout system development. In addition, these methods should be combined with those host resistance components which use genetic tools with multiple resistance breeding approaches. These methods will enable the continuous development of environmentally friendly disease management by reducing or replacing fungicides (Fig. 3). Particularly, the challenge for apple IPM programmes in the twenty-first century is to complete the fourth, final IPM level, which supplements IPM level 3 with cultural, social and political realms. While in organic apple orchards, the most essential task is to develop effective non-chemical control options that are practically feasible and can be incorporated easily into the orchard management practices. Then finally, both environmentally friendly apple production systems may eventually unite into the basic concept of the ecologically based production system (Fig. 3).

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Mitigation of Agricultural Nonpoint-Source Pesticide Pollution in Artificial Wetland Ecosystems – A Review

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Abstract Contamination caused by pesticides in agriculture is a source of poor environmental water quality in some of the European Union countries. Without treatment or targeted mitigation, this pollution is diffused in the environment. Pesticides and their metabolites are of increasing concern because of their potential impacts on the environment, wildlife and human health. Within the context of the European Union (EU) Water Framework Directive and to promote low-pesticide-input farming and best management practices, the EU LIFE project ArtWET assessed the efficiency of ecological bioengineering methods using different artificial wetland (AW) prototypes throughout Europe. We optimized physical and biological processes to mitigate agricultural nonpoint-source pesticide pollution in AW ecosystems. Mitigation solutions were implemented at full-scale demonstration and experimental sites. We tested various bioremediation methods at seven experimental sites. These sites involved (1) experimental prototypes, such as vegetated ditches, a forest microcosm and 12 wetland mesocosms, and (2) demonstration prototypes, such as vegetated ditches, three detention ponds enhanced with technology of constructed wetlands, an outdoor bioreactor and a biomassbed. This set-up provides a variety of hydrologic conditions, with some systems permanently flooded and others temporarily flooded. It also allowed to study processes both in field and controlled conditions. In order to compare the efficiency of the wetlands, mass balances at the inlet and outlet of the AW will be used, taking into account the partitioning of the studied compound in water, sediments, plants and suspended solids. The literature background necessary to harmonize the interdisciplinary work is reviewed here and the theoretical framework regarding pesticide removal mechanisms in AWs is discussed. The development and the implementation of innovative approaches concerning various water quality sampling strategies for pesticide load estimates during flood, specific biological endpoints, innovative bioprocesses applied to herbicide and copper mitigation to enhance the pesticide retention time within the AW, modelling the transport and the fate of pesticides using a 2D mixed

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hybrid finite element model are introduced. These future results will be useful to optimize hydraulic functioning, e.g. pesticide resident time, and biogeochemical conditions, e.g. dissipation, inside the AWs. Hydraulic retention times are generally too low to allow an optimized adsorption on sediment and organic materials accumulated in AWs. Absorption by plants is also not effective (VanBeinum et al., 2006). The control of the hydraulic design and the use of adsorbing materials can be useful to increase the pesticide's residence time and the contact between pesticides and biocatalyzers. Pesticide fluxes can be reduced by 50–80% when hydraulic pathways in AWs are optimized by increasing ten times the retention time, by recirculation of water, and by deceleration of the flow. Thus, using a bioremediation method should lead to an almost complete disappearance of pesticide pollution. To retain and to treat agricultural nonpoint-source pollution at the upstream of the hydrosystems form a part of the management of the environmental activities and thus represents a major stake for a sustainable development.

Keywords Artificial wetland · Pesticides · Agriculture · Stormwater system · Vegetated ditches · Forested plots · Bioremediation · Mitigation · Nonpoint-source pollution · Detention · Retention · Water · Detentions pond · Hydraulic etc

1 Introduction

This chapter aims to present and to review the current knowledge related to the mitigation of agricultural nonpoint-source pesticide pollution: vineyard and crop fields in artificial wetland ecosystems as well as the new prototypes which will be produced in the 3-year EU LIFE project ArtWET which started in October 2006 (LIFE 06 ENV/F/000133, Mitigation of agricultural nonpoint-source pesticide pollution and bioremediation in artificial wetland ecosystems).

To limit natural surface water contamination, several measures can be implemented at different scales. First of all, at the farm scale, prior to or during application, active substance selection and substitution, application rate reduction, application date shifting and proper use and cleaning of pesticide spraying equipment are part of measures that may reduce pesticide transfer to the environment (Reichenberger et al., 2007). As long as pesticides are used, a certain proportion will reach natural systems, i.e. via surface runoff during strong rainfall events (Schulz, 2004). Thus, complementary measures at plot and catchment scale such as conservation tillage on cultivated surfaces and buffer zone implementation on specific areas are needed. Surface waters, including surface runoff and drainage outflows, are accessible contaminated waters contrary to infiltration flows on which implementing treatment measures is hard to perform. Grassed buffer zones could be part of these buffer zones, but they are not included into this study as they are not part of the ArtWET project. Reichenberger et al. (2007) reviewed this mitigation measure for pesticide pollution reduction. It is therefore possible to direct both runoff and drainage flows through mitigation complementary measures, such as artificial wetlands, vegetated ditches or detention ponds, to achieve pesticide pollution reduction by simple landscape management. These different devices form the artificial wetlands (AW).

Statistical calculations conducted using 3,135 references indicated that since 1973 at total of 68% of the publications were devoted to the natural wetlands (NW). As regards natural wetlands, topics initially concerned were fight against the forest fires (Heinselman, 1973), biological conservation (Duelli et al., 1990), natural landscapes and aquatic botany (Wetzel, 1992). Among the communications devoted to artificial wetlands from 1973 to 2007 (i.e. 32%), 39% reported on the fate of the nutrients (nitrogen and phosphorus) in the hydrosystem, 11% dealt with the fate of the heavy metals, 8% were devoted to the study of dairy at the farmer scale and only 2% dealt with the pesticides fate in the environment.

Since the last 7 years (i.e. since 2000), the proportion of the publications concerning pesticides' fate in the artificial wetlands increased (Schulz, 2004) and reached 8% of the publications devoted to the artificial wetlands and natural wetlands. The configurations, the localizations and the uses of these kind of zones are numerous and translated into a wide range of vocabulary: vegetated pond system (Revitt et al., 2004), wet ponds or detention pond (Lundberg et al., 1999), constructed vegetative treatment system (Hares and Ward, 2004), created wetland (Kohler et al., 2004), constructed wetlands mesocosm (Hares and Ward, 2004; Sherrard et al., 2004), surface flow constructed wetland (Tanner et al., 2005), constructed freshwater wetland (Cronk and Mitsch, 1994a), vegetated biofilters (Ellis et al., 1994), dry detention pond and wet bioinfiltration pond (Hares and Ward, 1999) and heterogeneous gravel beds constructed wetland (Maloszewski et al., 2006). These devices are also studied on various scales ranging from the constructed wetland mesocosm (Hares and Ward, 1999) to the regional water management system (Kohler et al., 2004).

Based on the studies of various artificial wetlands located in France, Italy and Germany (continental climate), we want to establish a common relevant methodology to compare all results obtained in various devices and optimize the functioning of physical processes (hydraulic design, soil management, water pathways) and biological processes (plants and bacteria development).

After the definition of the wetlands in the scientific and historical context, we highlight the gaps in the research conducted so far. On the basis of observable, studied and quantified processes in the artificial wetlands of the ArtWET project, we present the effectiveness of artificial wetlands and the pesticides removal mechanism within a physical, chemical, biological and hydrological theoretical framework. We describe the different experimental sites and prototypes conceived from laboratory scale to in situ scale and we highlight the relevant methodologies concerning the evaluation of the effectiveness of the various artificial wetlands described in the ArtWET project.

We aim here to bring together additional and relevant knowledge considering artificial wetlands as a sustainable, low-investment and low-maintenance cost technology that can complement or replace conventional water treatment. Indeed, interest in the engineered use of wetlands significantly increased over the last decades (Tack et al., 2007). Unfortunately, contamination levels in waters at the outlet of these kind of artificial wetlands are most of the time higher than acceptable thresholds, which is the reason why we want to optimize the functioning of these zones as well from the physical point of view (hydrologic dynamic, hydraulic retention time, waterways, pesticide and water sampling, mass balance, channel slope, hydraulic roughness, etc.) as biological (plants selection, bioaugmentation, biostimulation, etc.).

To conclude this introduction, let us note that these kinds of devices can have potentially important implications for long-term agricultural land use in the context of climate change. Indeed, the indirect effects of climate-induced changes in demand for water and other natural and agricultural resources and changes in land use may have a greater effect on fate and transport of pesticides in the environment than direct effects (Bloomfield et al., 2006).

2 State of the Art: Artificial Wetlands as Nonpoint-Source Pollution Mitigation Systems

2.1 Defining a Artificial Wetland in Historical and Scientific Context

Hydrologically, wetlands can be defined as "areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static, flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed 6 m" (Bragg, 2002). Water origin (quantity and quality) and water fluxes inside a wetland are first-order controls of wetland characteristics with prevailing climate and geology acting as boundary conditions.

The wetland is classified as a "free-surface" system, meaning that its water surface is exposed to the atmosphere and contains emergent aquatic vegetation in a relatively shallow bed. It is thus distinguished from "sub-surface" or gravel bed wetlands which are also commonly used for water treatment (Reilly et al., 1999).

According to Vymazal (2005), constructed wetlands (CW) are engineered systems that have been designed and constructed to utilize natural processes involving wetland vegetation, soils and the associated microbial assemblages to assist in treating wastewaters.

Initially wetlands were employed mainly to treat point-source wastewater (Vymazal, 1990; Sooknah, 2000; Kadlec, 2003). The first attempts to use the wetland vegetation to remove various pollutants from water were conducted by K. Seidel in Germany in early 1950s (Vymazal, 2005). The first full-scale free water surface constructed wetland was built in The Netherlands to treat wastewater from a camping site. In 1970s and 1980s, constructed wetlands were nearly exclusively built to treat domestic or municipal sewage. Since 1990s, the constructed wetlands have been studied under different hydrologic regimes (Cronk and Mitsch, 1994b; Fennessy et al., 1994) and used for all kinds of wastewater including landfill leachate runoff (e.g. urban, highway, airport and agricultural), food processing (e.g. winery, cheese and milk production), industrial (e.g. chemicals, paper mill and oil refineries), agriculture farms, mine drainage or sludge dewatering.

The above-mentioned uses were followed later by an increased emphasis on nonpoint-source urban (Shutes et al., 1997; Matamoros et al., 1997A) and agricultural runoff (Higgins et al., 1993; Rodgers et al., 1999). The first reference concerning wetlands for controlling nonpoint-source pollution was published by Mitsch (1992). While the fate and retention of nutrients and sediments in wetlands are understood quite well (Brix, 1994) and even if artificial wetlands are often used for municipal wastewater treatment for removing specific organic pollutants (Haberl et al., 2003; Huang et al., 2004; Matamoros et al., 2007b), the same cannot be claimed for agrochemicals (Baker, 1993; Schulz, 2004), and few studies have assessed the feasibility of using horizontal subsurface flow constructed wetland to remove most of the priority pollutants in the European Water Framework Directive.

Most of the initial studies referred to the potential of wetlands for removal of herbicides and some other organic chemicals (Wolverton and Harrison, 1975; Wolverton and McKown, 1976; Kadlec and Hey, 1994; Moore et al., 2000). Since wetlands have the ability to retain and process transported material, it seems reasonable that artificial wetlands, acting as buffer strips between agricultural areas and receiving surface waters, could mitigate the effect of pesticides in agricultural runoff (Rodgers et al., 1999).

The effectiveness of wetlands for reduction of hydrophobic chemicals (e.g. most insecticides) should be as high as for suspended particles and particle-associated phosphorus (Brix, 1994; Kadlec and Knight, 1996), since these chemicals enter aquatic ecosystems mainly in particle-associated form following surface runoff (Ghadiri and Rose, 1991; Schulz et al., 1998).

Complementing their ecological importance as ecotones between land and water (Mitsch and Gosselink, 1993) and as habitats with great diversity and heterogeneity (Wetzel, 1993), specifically constructed wetlands are used extensively for water quality improvement. The concept of vegetation as a tool for contaminant mitigation (phytoremediation) is not new (Dietz and Schnoor, 2001). Many studies have evaluated the use of wetland plants to mitigate pollutants such as road runoff, metals, dairy wastes and even municipal wastes (Vymazal, 1990; Brix, 1994; Cooper et al., 1995; Kadlec et al., 2000). According to Luckeydoo et al. (2002), the vital role of vegetation in processing water passing through wetlands is accomplished through biomass nutrient storage and sedimentation, and by providing unique microhabitats for beneficial microorganisms. Macrophytes serve as filters by allowing contaminants to flow into plants and stems, which are then sorbed to macrophyte biofilms (Headley et al., 1998; Kadlec and Knight, 1996). In addition to these considerations, complex ecosystems are progressively set up, thanks to sediment accumulation serving as substrate for microorganisms and plants. Bioattenuation is then commonly observed in artificial wetlands. In this work, we define artificial wetland as constructed wetland in forest, agricultural or farm context and also vegetated ditches. Hydraulic detention ponds initially designed to avoid flood in the urban belt (Hong et al., 2006; Kayhanian et al., 2008) are also considered as artificial wetlands.

Indeed pesticides coming from agrosystems (beyond the urban belt) go through these devices and may contaminate some other environmental compartments such as surface waters and sometimes groundwater when the top of groundwater is close to the surface of soils and rivers (Amon et al., 2007; Dahl et al., 2007).

Initially wetlands were employed to treat point-source wastewater. The references concerning artificial wetlands for controlling nonpoint-source pollution are recent and date back to less than 20 years. Attenuation is commonly observed in artificial wetlands. It is thus relevant to consider the remediation of the pesticides in these specific zones.

2.2 Nonpoint-Source Pollution Profile of Pesticides and Pesticide Pathway

Pesticide input into the environment is due to human activities. For agricultural watersheds, the main input routes of entry consist of farmer pesticide applications even if atmospheric deposition via solid particles, rain and snowfall can partly contribute to pesticide input at the farm scale (Dubus et al., 2000). Three major application methods referred to as spraying, incorporation into the soil and fumigation, lead to pesticide losses to the nontarget environment. Indeed, only a portion of the applied product is taken up by plants to meet disease protection or weed elimination objectives. When being applied, pesticide losses into the air typically range between 20% and 30% of the applied active substance during application (mainly because of spray drift) and around 50–60% after application (by volatilization) and can sometimes reach up to 90% (Van den Berg et al., 1999; Aubertot et al., 2005). Once on the soil, pesticide molecules undergo several transfer processes. Groundwater contamination is mainly due to pesticide leaching through infiltration; whereas, surface water pesticide input pathways preferably come from surface runoff or tile drainage water.

As part of this review, surface water is the compartment of concern. At the watershed scale, pesticide losses via surface runoff most frequently represent less than 1% of the applied active substance, rarely exceeding 10% (Carter, 2000; Aubertot et al, 2005). The higher the soil water content, the higher the losses via surface runoff of the active substance and its metabolites. Pesticides from soil water can move up to the surface when the soil infiltration capacity is exceeded. Moreover, because of soil surface erosion, some adsorbed molecules on the soil surface can also be transported to natural receiving surface waters. However, except for highly sorbing compounds, pesticide transport by surface runoff is mainly in dissolved forms (Aubertot et al., 2005).

In artificially drained watersheds, runoff is limited while tile drainage is the major pathway for exporting the drainage area water to natural surface waters (Kladivko et al., 2001). Losses via subsurface drainage are generally less than 0.5% of the applied pesticide but might reach 3% and occasionally greater values (Carter, 2000). However, reviewing a wide range of studies, Kladivko et al. (2001) concluded that tile drainage concentrations and masses are up to one order of magnitude lower than those of surface runoff for artificially drained watersheds. This study also highlighted that even if subsurface drains represent an additional exportation pathway, surface runoff losses were reduced by much more than subsurface drainage losses increased. When crossing the soil from surface to subsurface drains, pesticides can be involved in different mitigation processes of retention or transformation as further described. To sum up, it is clear that pesticide entries via surface runoff or subsurface drainage only represent a small amount of the applied active substance. On the other hand, it is important to note that the resulting concentrations and masses are high enough for receiving surface waters to exhibit biologically relevant effects (Schulz, 2004).

Factors affecting pesticide transport to surface water via subsurface drainage are linked to soil, pesticide and agroclimatic characteristics. Pesticide molecules can be transported either on dissolved or on adsorbed form on suspended solids, the former usually predominating. Soil mineralogy composition accounts for pesticide movement. It is obvious that pesticide retention and degradation characteristics are of importance for assessing their potential to be transferred by subsurface drainage or surface runoff. Similarly to surface runoff generation, soil water content is a key parameter affecting water and solute transport to tile drains.

When comparing dynamics of drainflows and the corresponding pesticide concentrations and loads, it appears that higher concentration occurs during the first significant storm event following pesticide application (Kladivko et al., 2001; Jouzel, 2006).

During a storm event, pesticide concentrations can vary over several orders of magnitudes (Schulz et al., 1998) with peaks occurring generally just before drainflow peaks. A steep concentration decrease after pesticide concentration peak is usually observed while drainflow drops down (Kladivko et al., 2001). The next storm events usually present lower pesticide concentrations and loads. This is mainly due to the fact that the longer the pesticide or metabolite molecules remain in the soil, the more likely immobilization and degradation processes can occur, thus limiting the available quantity for transfer to natural surface water. This supports the fact that the first drainflow event after pesticide application is of most concern for pesticide pollution transfer (Schulz, 2001).

Spray drift is another way of surface water contamination (Padovani et al., 2004, Vischetti et al., 2007, Capri et al., 2005, Ganzelmeier et al., 1995). Spray drift is affected by the climatic condition during the treatment, the device and the formulation used in the farm. The quantity arriving in the water body depends on the distance between treated area and ditch and could be more than 6% of applied rate.

There are various pesticide pathways in the artificial wetlands. Surface water is the major compartment of concern. The pesticide losses present most frequently between 1% and 4% of the applied active substance in surface runoff and the quantity arriving in the water body depends on the distance between the treated area and the artificial wetland. Let us note that concentration at the outlet of the agro-catchment area can reach more than 300 μg/L.

2.3 Typology and Implementation

Several studies concerning more specifically nutrient treatment introduced some elementary rules of constructed wetland implementation (watershed management) (Hammer, 1992; Mitsch, 1992; Rodgers and Dunn, 1992; van der Valk and Jolly, 1992). The main results found in literature deal with design, guidelines and recommendations concerning catchment planning (a unique large wetland and several distributed small wetlands) (Mitsch and Gosselink, 2000). A ratio of about 1% seems to be adequate from a water quality point of view. Nevertheless, this ratio is completely empirical. The appropriate size of a restored wetland will depend on (i) risk assessment between water fluxes superposed with pesticide application period, (ii) the contaminant of greatest local concern that requires the longest residence time for its degradation and (iii) the percent reduction of this contaminant that is required seasonally, annually or interannually. The integration within the catchment planning may follow some general rules: territorial involvement and negotiation might be globalized, runoff and erosion must be reduced, and artificial wetlands must be close to the pollutant source. Nevertheless, we introduce two technical solutions admitted by several authors in highway runoff, agricultural dairy wastewater treatment: linear treatment solution along vegetated ditches or grassed buffer strips. This solution is particularly adapted in case of land pressure. Another solution consists in punctual parallel treatment system intercepting one portion of polluted water volumes and storing into systems parallel to the main ditch, long enough for mitigation processes to take place.

2.4 Artificial Wetland Effectiveness

A review of numerous studies is presented below. In the whole, global efficiency is generally calculated by the ratio of inlet/outlet concentration without describing all the processes involved. This black-box approach does not allow highlighting main processes, but provides evidence of efficiency.

The wetland area should be designed so that it has a very shallow sloping edge and a permanent pool. This configuration provides a variety of hydrologic conditions, with some areas permanently flooded and others temporarily flooded. Those hydrologic conditions provide for the growth and propagation of diverse wetland plants and microbes and promote metabolism of pollutants under aerobic and anaerobic conditions.

Previous studies (Tanner, 1996) presented the advantage of the following aquatic or semi-aquatic plant species in order to provide a large and dense rhizosphere root system favourable to filtration and biological activities: Cattail (*Typha* sp.), Bulrush (scirpes) (*Scirpus* sp.), reed phalaris (*Phalaris* sp.), reeds phragmites (*Phragmites* sp.), glyceria (*Glyceria* sp.) and rushs (*Juncus* sp.). Artificial wetlands require specific plants tolerant to high water level variability from 0 cm to 50 cm, due to hydrological dependence. A range of plants have shown this property, but the common reed (*Phragmites australis*) and the reedmace (*Typha latifolia*) are particularly effective. They have a large biomass both above (leaves) and below (underground rhizome system) the surface of the soil or substrate. The subsurface plant tissues grow horizontally and vertically and create an extensive matrix which binds the soil particles and creates a large surface area for the uptake of nutrients and ions. Hollow vessels in the plant tissue enable air to move from the leaves to the roots and to the surrounding soil. Aerobic microorganisms flourish in a thin zone (rhizosphere) around the roots and anaerobic microorganisms are present in the underlying soil. Natural filtration in the substrate also assists in the removal of many pollutants and pathogenic microorganisms.

The retention capabilities of constructed wetlands will be assessed within the ArtWET project using chemical monitoring supported by ecotoxicological evaluations. Chemical monitoring at the inlet and outlet is used to determine the retention of pesticides within the wetland. These studies are complemented by measurements taken within the wetlands in order to describe and understand processes. There are a number of existing studies which attempted to quantify insecticide retention in wetlands by taking input and output measurements and were done on various current-use insecticides in South Africa. Schulz and Peall (2001) investigated the retention of azinphos-methyl, chlorpyrifos and endosulfan introduced during a single runoff event from fruit orchards into a 0.44 ha wetland covered with *P. australis*. They found retention rates between 77% and 99% for aqueous-phase insecticide concentrations and >90% for aqueous-phase insecticide load between the inlet and outlet of the wetland. Particle-associated insecticide load was retained in the same wetland at almost 100% for all the studied organophosphate insecticides and endosulfan. Other studies performed in the same wetland assessed spray drift-borne contamination of the most commonly used insecticide, azinphos-methyl, and found similar retention rates; however, the retention rate for the pesticide load was only 54.1% (Schulz et al., 2001b, 2003a). In parallel, research was conducted on the fate of pyrethroids such as lambda-cyhalothrin experimentally introduced into slow-flowing vegetated ditches in Mississippi (Moore et al., 2001a: Bennett et al., 2005). They reported a more than 99% reduction of pyrethroid concentrations below target water quality levels within a 50-m stretch due to an 87% sorption to plants. A further study demonstrated retention of approximately 55% and 25% of chlorpyrifos by sediments and plants, respectively, in wetland mesocosms (59–73 m in length) in Oxford, Mississippi as well as a >90% reduction in concentrations and in situ toxicity of chlorpyrifos in the wetlands in South Africa (Moore et al., 2002).

2.4.1 Vegetated Ditches

Among the tens of publication dealing specially with vegetated ditches (Table 1), the main conclusions concerned (i) type of vegetation (cover and density), pesticide adsorption on ditch material (sediment, dead leaves) with isoproturon, diuron and deflufenican (Margoum et al., 2006), (ii) length of the ditch: generally ditch for network drainage hence sections are dimensioned to increase water/macrophytes contact and (iii) low flow velocity: a good efficiency is generally obtained with

Table 1 Copper extracted by macrophytes according to various studies reported in the literature

Species	Source	[Cu] Water (mg/L) Sediment (mg/kg)	[Cu] Aerial biomass (mg/kg)	TF[a]	BCF[b]	References
Phragmites australis	Natural	95**	4.5	0.07	0.05	Denget al. (2004)
Salix acmophylla	Natural	81–1,024**	87–227	–	0.22–1.1	Ozdemir and Sagiroglu (2000)
Eleocharis valleculosa	Natural	5,770**	167	0.11	0.03	Deng et al. (2004)
Juncus effusus	Natural	649**	17	0.35	0.03	Deng et al. (2004)
Phragmites australis	Natural	28.1*	7	–	0.25	Bragato et al. (2006)
		1.23**			5.7	
Phragmites australis	Artificial	10*	167	0.02	16.7	Ait Ali et al. (2002)

[a] Translocation factor ([Cu] aboveground part / [Cu] belowground part).
[b] Bioconcentration factor ([Cu] aboveground part / [Cu] sediment or water).
*The unit in mg/L.
**The unit in mg/kg.

velocity inferior to 0.3 m/s. When velocity is about 1 m/s, pesticide retention is strongly limited.

With a high vegetation density, low flow rate, efficiency could reach a reduction factor of 90% for aqueous-phase insecticides originating from drift (Dabrowski et al., 2005) and of 60% for herbicide. A sufficient stream flow is calculated using measured velocity; otherwise, a stream flow with known channel slope and hydraulic roughness is calculated with the Manning equation (Mitsch and Gosselink, 2000). Streambed roughness and the proportion of flow in contact with the streambed reduce water velocity in agricultural drainage ditches and constructed wetlands. Attributes of vegetated structures include litter and stems from macrophytes that provide dominant drag forces and increase the Manning coefficient (n) by a factor of 10–20 (Kadlec and Knight, 1996). Decreased flow increases retention time and water/macrophyte contact in agricultural drainage systems and removes suspended solids from the water column (Bouldin et al., 2005). Removal of water-soluble as well as particle-bound compounds is accomplished by vegetative communities through water/macrophyte contact and increased deposition of suspended sediment (Bennett et al., 2005; Bouldin et al., 2005).

2.4.2 Forested Plots

Forested plots as buffer zones were most often studied in the case of riparian stream buffers and runoff or nutrient reduction (Willems et al., 1997; Broadmeadow and Nisbet, 2004; Anbumozhi et al., 2005). It is unrealistic to consider planting trees in order to build new, not-yet-existing forested buffer zones like vegetative filter strips. Nevertheless, the landscape presents several areas with forested zones like copses, groves, etc. Improved infiltration rate, root systems and organic matter are the three main advantages of a forested buffer zone (Gril, 2003). Those characteristics are involved in pesticide fate and behaviour and lead to an apparent efficiency above 90% (Lowrance et al., 1997; Vellidis et al., 2002; Gril., 2003). Forested top layers should intercept lateral superficial and subsuperficial flow runoff.

2.4.3 Detention Ponds and Storm Basins

Stormwater wetlands, storm basins or detention ponds are engineered wetlands to temporarily store runoff and are specifically designed for flood control. Typically, stormwater wetlands will not have the full range of ecological functions of natural wetlands. Some studies approach the hydraulic and biological functioning of the stormwater wetlands. Indeed their temporary water storage in shallow pools supports conditions suitable for the growth of wetland plants and bioremediation. But the mitigation studies are mainly focussed on nutrient compounds (Cooper and Knight, 1990; Bouchard et al., 1995) or wastewater (Mallin et al., 2002). As mentioned in the introduction, only few recent studies focus on nonpoint-source pesticide pollution mitigation (Bishop et al., 2000).

It is assumed that stormwater constructed wetland systems can be designed to maximize the conditions of the removal of pollutants from stormwater runoff via several mechanisms: microbial breakdown of pollutants, plant uptake, retention, settling and adsorption. The reduction of pollution particularly depends on the inlet concentrations and is quantified by assessments of input and output without taking into account the biological processes (Hares and Ward, 1999; Lundberg et al., 1999). Fiener et al. (2005) noted a reduction of 46% of the concentration of terbuthylazine. When the detention pond is vegetated, Hares and Ward (2004) postulated that the high reed biomass may be primarily responsible for reducing hydraulic flow thus allowing a greater residence time for sedimentation, filtration and bioaccumulation processes. Bouchard et al. (1995) also noted a seasonal effect: annual removal efficiencies for one system (sedimentation basin, grass filter strip, wetland and detention pond in series) were 85–88% for total phosphorus and 96–97% for total suspended solids and seasonal removals varied considerably, with spring flows leading to a net export of phosphorus and sediment from the system. In order to achieve a good reduction for a variety of pollutants, wet pond design should include maximizing the contact time of inflowing water with rooted vegetation and organic sediments. This can be achieved through a physical pond design that provides a high length-to-width ratio, and planting of native macrophyte species.

2.4.4 Biomassbed

Even if this technique mainly relates to the point-source pollution, it is however interesting to take into account this knowledge to optimize the devices dedicated to nonpoint-source pollution. Point sources of pollution are largely the result of pesticide handling procedures, e.g. tank filling, spillages, faulty equipment, washing and waste disposal and direct contamination. Thus, all farms using pesticides, regardless of quantity, represent a potential pollution risk that can be reduced by good agricultural practices and the installation of suitable handling facilities. One of the tools for the reduction of pesticide point- and nonpoint-source contamination is a biological system where chemicals are bound and biologically degraded (first developed in Sweden in 1993 and afterwards distributed all over Europe), called "biobed" (Torstensson and Castillo, 1997; Torstensson, 2000). In its simplest and original form, the Swedish biobed is a clay-lined hole in the ground filled with a mixture of topsoil, peat and straw in the ratios 25:25 and 50%, respectively. This mixture was used to ensure maximum binding capacity for pesticides, while keeping them bioavailable and creating optimal conditions for their microbial decomposition (Fogg et al., 2004). The Swedish biobed is also adapted to other climatic conditions, especially for the availability of the materials to create the biomix, and called "biomassbed" developed in Mediterranean conditions using residue of grape and citrus cultivation (Vischetti et al., 2004; Fait et al., 2007). During a total study period of 563 days, Spliid et al. (2006) did not find any traces of 10 out of 21 applied pesticides in the percolate of a biobed created as an excavation lined with clay and filled with a mixture of chopped straw, sphagnum and soil with turf on top, and with increased sorption capacity and microbial activity for degradation of the pesticides (detection limits between 0.02 $\mu g\ L^{-1}$and 0.9 $\mu g\ L^{-1}$). Just three pesticides were only detected once and at concentrations below 2 $\mu g\,L^{-1}$. The use of the capacity of species of white rot fungus from a variety of basidiomycete orders to degrade contrasting mono-aromatic pesticides in a biobed was also investigated by Bending et al. (2002). Greatest degradation of all the pesticides was achieved by *Coriolus versicolor*, *Hypholoma fasciculare* and *Stereum hirsutum*. After 42 days, maximum degradation of diuron, atrazine and terbuthylazine was above 86%, but for metalaxyl it was less than 44%. One objective followed in the ArtWET project is to adapt this kind of facilities in particular to improve punctually the treatment of pesticide at the outlet of artificial wetlands and for low flows.

2.4.5 Constructed Wetlands

The use of vegetated wetlands for accelerating pesticide removal from agricultural runoff is gaining acceptance as a best management practice (Rose et al., 2006). Constructed wetlands are promising tools for mitigating pesticide inputs via runoff/erosion and drift into surface waters, but their effectiveness still has to be demonstrated for weakly and moderately sorbing compounds (Reichenberger et al., 2007).

Among 144 references dealing with constructed wetlands, 50% were related to nitrate or phosphorus, 40% to wastewater treatment, 10% to dairy (farm scale), 9% to heavy metals and 10% to pesticides. With respect to constructed wetlands, no other studies with quantitative results were identified than those already cited and discussed by Schulz (2004) and FOCUS (2000). The vast majority of these studies (e.g. Schulz and Peall, 2001) suggest that constructed wetlands are very effective in reducing pesticide inputs into surface waters; however, they may be quite area-consuming: the largest investigated wetland was 134 m long and 36 m wide (Schulz et al., 2001b). However, smaller, less area-demanding wetlands (e.g. 50 m long and 1.5 m wide; Moore et al., 2001b) have been also found to be very effective in removing pesticides from the water passing through the wetland. The land constraints lead to treat the maximum pesticide fluxes within the minimum water fluxes. This challenge could be reached considering technical approaches such as hydrological functioning knowledge of the watershed. Yet, it has to be noted that almost all available studies dealt with strongly sorbing insecticides (e.g. chlorpyrifos) with a strong tendency to adsorb to macrophytes, suspended particles or bed sediment. Some studies (Kadlec and Hey, 1994; Seybold and Mersie, 1999; Moore et al., 2000; Kao et al., 2002; Stearman et al., 2003; Bouldin et al., 2005) investigated the fate and transport of the moderately sorbing herbicide atrazine in constructed wetlands. Moore et al. (2000) found that a travel distance of 100–280 m through the wetland would be necessary to achieve an effective runoff mitigation (more precisely: an atrazine concentration in outflow corresponding to the NOEC for higher aquatic plants). Results obtained in microcosm/mesocosm are generally higher than those obtained in situ: 80% of the experiments in mesocosms/microcosms or in the lab have an experimental efficiency higher than 40% while values below 40% were reported for experimental or in situ constructed wetland.

Based on literature review, the following classification of constructed wetlands could be suggested:

- Silting basins without vegetation in which water elevation ranges from 0 m to 1 m (Braskerud and Haarstad, 2003; Laabs et al., 2007).
- Vegetated basins in which water elevation ranges from 0.2 m to 1 m.
- Combined systems where the first basin is both a silting basin and a hydraulic buffer and the second basin is colonized by a specific vegetation. These systems are more frequently used as they provide better efficiency regarding pesticide dissipation (Braskerud and Haarstad, 2003; Haarstad and Braskerud, 2005; Blankenberg et al, 2006).

A variety of hydrologic conditions in artificial wetlands can occur with temporary and permanent flow. The overall results provided by the bibliography show an attenuation rate ranging between 50% and 99% in the case of aqueous-phase insecticide remediation in an artificial wetland covered with *P. australis*. In the vegetated ditches, the efficiency can reach 90% for aqueous-phase insecticides and 60% for herbicides with a velocity less than 0.3 m/s. As regards forested plot, detention ponds and storms basins, few studies are available but a significative reduction of pesticides is also noted. The additional artificial wetland under consideration in this study is biomassbed. In these devices, the attenuation rate can reach more than 80% for herbicides and 44% for fungicides.

2.5 Main Treatment Objective and Research Needs

Although only a rather low number of publications dealing with pesticides exist in the bibliographical information, several points may be concluded (for nutrients and pesticides):

- Vegetated wetlands are more efficient than nonvegetated ones (Tanner et al., 1995, 1999; Nairn and Mitsch, 1999; Moore et al., 2002; Schulz et al., 2003a,b; Mbuligwe, 2004; Rose et al., 2006; Burchell et al., 2007).
- Retention rate is linked to hydraulic residential time hence to wetland water storage capacity (Rodgers and Dunn, 1992; Tanner et al., 1995; Dierberg et al., 2002).
- Efficiency rate is linked to the inlet load of pollutant (Moore et al., 2000; Moore, 2001b; Schulz and Peall, 2001; Paludan et al., 2002).
- Wetland efficiency is inversely linked to velocity (Tanner et al., 1995; Dierberg et al., 2002; Haarstad and Braskerud, 2005; Nahlik and Mitsch, 2006; Avsar et al., 2007).
- Efficiency depends on the whole system: substrate, vegetation and physico-chemical conditions (Braskerud and Haarstad, 2003; Haarstad and Braskerud, 2005; Blankenberg et al., 2006).
- Efficiency depends on initial concentrations (Blankenberg et al., 2006) and hydraulic retention time is the main factor controlling pesticide degradation.

The question of surface ratio between catchment area and artificial wetland surfaces is crucial, not only from a scientific point of view (for an equal speed or an equal residence time, the larger the artificial wetland is, the higher the performances are), but also regarding the socio-economic aspect of implementation. To reduce the size requires e.g. innovative systems to reach an objective of pesticide fluxes reduction: intercepting a maximum of pesticide flux within a minimum water flow. Our analysis shows that no publications mentioned the intercepted fluxes (to be treated) compared to the total water flow. The other challenge is the knowledge of pesticide degradation/retention processes within all artificial wetlands. These future results will be useful to optimize hydraulic functioning (pesticide resident time) and biogeochemical conditions (dissipation). For instance, in the work of Blankenberg et al. (2007), some pesticides were desorbed in a second year showing a negative mass balance, showing the difference between real and apparent pesticide dissipation. A long-term study is crucial in a sustainable approach to the pesticide problem. Another crucial question is whether the distinction enters retention or degradation (or both) corresponding to a total or apparent mitigation.

3 Theoretical Framework: Pesticide Removal Mechanism in Artificial Wetlands

3.1 Physical and Chemical Pesticide Removal Processes

Significant research effort has been dedicated to understanding the fate and transport of pesticides in the environment, and the relationship between pesticide fate and transport and specific environmental parameters such as organic carbon and pH in soils are generally understood at least qualitatively (Bloomfield et al., 2006).

Artificial wetlands action on pesticides is twofold: either as a sink due to storage, transformation and elimination, or as a source as molecules may be transferred to receiving media like surface and groundwater, or due to plant interception and temporary storage due to sorption into sediment, soil or suspended matter.

It should be noted that the pesticide distribution among the different environmental compartments is quite complex and affected by pesticide chemio-dynamic properties. The soil/water partition coefficient K_{oc}, the pesticide half life DT_{50}, the air/water partition coefficient K_H (Henry's constant), and the octanol/water partition coefficient log K_{ow} are the most important parameters affecting the pesticide environment behaviour. Between two phases, a pesticide can stay in equilibrium (e.g. water–air) or can follow a precise direction (e.g. from water to sediment, soil or plants), according to its properties (Ferrari et al., 2005). These two processes occur with different rates, the former faster and the later slower.

In the environment pesticides are distributed in liquid, solid and gaseous phase; their presence in solid phase (for example in sediment or soil) is due to adsorption phenomena that control the distribution in the other phases, while their most mobile portion is located in liquid and gaseous phases. This portion is available for microbial degradation and for vertical or lateral transfer related to groundwater and surface water contamination. Generally, the solid phase retention minimises the pesticide mobility risk, but makes pesticide disappearance more difficult. Three hydrological factors affect the depuration capacity of the artificial wetlands: the hydro period (Bojcevska and Tonderski, 2007; Prochaska et al., 2007), the residence time of the water into the wetland (Holland et al., 2004; Rousseau et al., 2004; Kjellin et al., 2007), the origin and contents of the feeding water.

Degradation is the transformation with changes in molecular structure and formation of metabolites under the action of chemical, photochemical and biological processes (Tournebize, 2007). The half-life time of pesticides in the environment is determined by their reactivity versus abiotic processes (photolysis, hydrolysis, redox reactions) or biotic processes (biodegradation, conjugation, metabolization). Pesticides either in solution or adsorbed to the solid phase may undergo a chemical degradation by oxidation or photolysis induced or catalyzed by soil components. The abiotic degradation is often incomplete and leads to intermediate substrates for biological reactions.

The term of biodegradation corresponds to the transformation of an organic substance into simple mineral products such as H_2O, NH_3, CO_2, or into simple organic compounds such as CH_4 and other products from microorganism fermentation processes (bacteria, fungi, algae); the biotransformation is a complex process requiring several steps and sometimes generating metabolites more polar, soluble, even more toxic than the parent compound. Wetlands contribute to pesticide degradation on several aspects: bioremediation (bioaugmentation, biostimulation) which consists of reducing mobility of pesticide and transforming it into non-toxic compounds by biological processes (plants and enzymes stimulation) and phytoremediation (rhyzodegradation, phytoaugmentation, phytostabilization) which uses plants and microorganisms to immobilize and extract pesticides (e.g. for plants there are some different ways involved, surface absorption, uptake by roots and distribution, etc.).

All the potential removal processes occurring in natural and constructed wetlands belong to physical, chemical, biological or biochemical mechanisms (Matagi et al., 1998). However, it is difficult to illustrate and separate all the processes, because they are not independent of each other. The extent to which these reactions occur is determined by many wetland parameters: composition of the sediment (clay, minerals, hydrous oxides, organic matter), pH, redox status, type of vegetation. For example, in permanently anoxic conditions in wetlands, decomposition of organic matter is by reduction and organic matter accumulates on the sediment surface; the resulting organic sediment surface is responsible for scavenging pesticides from inlet wastewater.

The pesticide physical removal reactions involve sedimentation, flocculation, absorption, co-precipitation, precipitation; the pesticide movements take place in water, sediments, suspended matters and plants. A pesticide can be transported from one compartment to another, e.g. from water to sediments or biota or suspended materials or vice versa. The most volatile compounds can also dissipate from water to air, while the most lipophilic compounds can be adsorbed more easily on the surface of the sediments, suspended matters, plants and microbial biofilms grown on them.

The pesticide chemical removal reactions involve cation and anion exchange, oxidation–reduction. Chemical removal processes can also include UV irradiation, especially for surface flow systems, where some organic pollutant molecules undergo photolytic decomposition due to exposure to UV wavelengths in daylight.

Ion exchange can occur between the counter ions balancing the surface charge on the sediments, colloids and the ions in the wetland water. Complexation is also a very important phenomenon especially for heavy metal removal; it is a reaction whereby heavy metal ions replace one or more coordinated water molecules in the co-ordination sphere with other nucleophilic groups of ligands (mainly multidentate organic molecules, natural organic matter including humic, tannic and fulvic acids). This process can affect the bioavailability and the toxicity of the involved compounds (Matagi et al., 1998).

3.2 Biological Removal Processes

It should be noted that most studies are not able to distinguish bioattenuation from physical and chemical phenomena since contaminants are almost measured between inlet and outlet, artificial wetlands being considered as a black box. In this section, we aim at showing the respective part of microorganisms and plants in pesticide mitigation, the conditions required for plant and microorganism activity, and to suggest some substantial improvements in biological removal processes. Bioremediation technologies associated or not associated with phytoremediation are presented and discussed in this section in relation to other parameters. In particular, hydraulic retention time of pesticides in artificial wetlands is the main parameter to be considered with the performance of the biological treatment. Contrary to most of plant treatments where flow rates are in a narrow range of values, in case of artificial wetlands, flow rates are often close to zero in a few hours after a storm event while they are very high during storm events with lower hydraulic retention times than the time needed for biological treatment. Thus pesticide storage in artificial wetlands along with close contact between pesticides and plants (and/or microorganisms) must be improved at the same time the biological removal processes are designed.

3.2.1 Indirect and Direct Effects of Macrophytes

Macrophytes play a role in biological removal process through their ability to extract metals and/or organic compounds. Solubilization or complexation with organic acids, root exudates and phytosiderophores may enhance metal extraction by macrophytes. For example, copper (Cu) is widely used in vineyards and sometimes may leave plots at the time of runoff events as soluble forms, as well as adsorbed to soil particles when erosion occurs. Macrophyte performances are shown in Table 1 for this metal. Cu is mainly adsorbed in roots and also accumulated in edible parts at a lower rate (3 up to 50), which has been shown in numerous studies with various metals. Bioconcentration variability recorded for one species, e.g., *P. australis* is almost related to Cu availability. In case of macrophytes, metal extraction is highly subjected to hydroperiod. In well-drained systems, oxides and oxyhydroxides are formed; iron oxyhydroxides can adsorb metals, forming metal/oxide complexes. Conversely in anoxic water-saturated sediments high in organic matter, metal ions can precipitate as insoluble stable sulphide complexes and be retained in the sediment. Metal retention by sulphide complexes is however lower than by iron oxyhydroxides, as shown with *Scirpus californicus* (Sinicrope et al., 1992). In this study, sequential flooding twice a day was the most efficient hydroperiod for removing metals (Cd, Cr, Cu, Ni, Pb, Zn) compared to continuous flooding. Conversely, highest metal retention was observed with twice-daily drainage. pH also plays a crucial role in metal availability for macrophytes. Experiments with *Atriplex canescens* (saltbush biomass) showed that biomass accumulated more Cu, Pb and Zn when pH increased from 2.0 to 5.0 (Sawalha et al., 2007) indicating that carboxyl groups participate in metal binding, most of them in the biomass having pK_a values ranging from 3 to 5. From a technological point of view, Stottmeister et al. (2003) stated that accumulation of heavy metals by plants is usually insignificant when industrial effluent and mine drainage are being treated. Conversely, phytoremediation of dilute solutions containing metals may be relevant. Although a number of terrestrial plants are known to accumulate high amount of metals in their biomass – called hyperaccumulators – intensive research should be undertaken with the aim to select metal-hyperaccumulating macrophytes.

Organic compounds, such as pesticides, may also be absorbed and/or metabolized by macrophytes. Pesticide metabolism in plants was reviewed by Van Eerd et al. (2003). Some available results concern macrophytes. Basically *P. australis* accumulates diuron in leaves (Matamoros et al., 2007a). Conversely, *Schoenoplectus californicus* was shown to accumulate several organochlorine pesticides in rhizomes, roots and stems in same concentrations (Miglioranza et al., 2004). This latter study showed however that pesticide concentration in sediment was two times higher than in the different parts of macrophytes. Pesticide adsorption by macrophytes notably depends on organic compound characteristics. In their review, Stottmeister et al. (2003) reported that molecules with log $K_{ow} < 0.5$ are too much polar avoiding their adsorption onto roots. Conversely log $K_{ow} > 3$ are highly hydrophobic and are only adsorbed at the surface of roots without being absorbed by plant. pKa along with pesticide concentration plays also an important role in pesticide adsorption and in their accumulation by macrophytes.

Macrophytes act most often indirectly on removal processes. Indeed macrophyte peculiarity is aerenchyma (as much as 60% of the total tissue volume) allowing gas molecules, in particular oxygen, to be transported through the plant right down to the deepest roots (reviewed by Stottmeister et al., 2003). Accordingly 1–4 mm-oxygen film thickness (related Eh gradients from –250 mV up to +500 mV) directly on the root surface both protects the roots from toxic components in the anoxic, usually extremely reduced rhizosphere and allows aerobic heterotrophic microorganisms to both grow and to quickly degrade organic compounds such as pesticides. Except during winter, oxygen is continuously released in the vicinity of roots at a rate around 100 up to 200 μmol O_2/h/g of root dry mass according to pH, Eh, temperature and plant (biomass, species, stage of plant development) thus supporting a continuous microbial activity.

Macrophytes also modify hydraulic characteristics such as filtration and physical characteristics, in particular temperature with lower variability from one season to another. As a consequence, macrophytes modify the environmental conditions in artificial wetlands. Permeability coefficient of $>10^{-5}$ m/s is a compromise between efficient circulation of water and sufficient surface for microbial colonization and root development (Stottmeister et al., 2003).

Macrophytes also synthesize rhizodeposits that are sometimes shown to enhance biodegradation (reviewed by Stottmeister et al., 2003). The amount of rhizodeposits has been estimated at 10–40% of the net photosynthetic production of agricultural crops. This percentage appears to be reduced with macrophytes as shown by Richert et al. (2000) with *P. australis*. Until now, the current knowledge of the composition of root exudates of helophytes is very limited along with their positive or negative effect on microbial populations. This knowledge is crucial when phytoremediation-assisted bioaugmentation is chosen as the technique of remediation. Indeed when rhizodeposits can support the growth of inoculated microorganisms, microbial survival may be enhanced. This parameter is thus one of the main limiting parameter able to compromise bioaugmentation. Stottmeister et al. (2003) suggested that in zones of constructed wetlands with a low organic load, root exudates and dead plant material could be involved in the microbial co-metabolic degradation of poorly degradable organic compounds. However, it can be assumed that rhizodeposition is only significant in artificial wetlands if the carbon load in the water is extremely low. Conversely, the amount of carbon released by plant is low in comparison to what is carried by water flow.

3.2.2 Microorganisms as Pillars of Biological Treatments

Although plants play a certain role in biological removal processes, it remains low compared to microorganisms. Atrazine removal by *P. australis* requires 40 days or 7 days, respectively, when microorganisms are removed or not removed from rhizosphere (McKinlay and Kasperek, 1999). Time for atrazine removal also decreased from 40 days to 7 days after successive incubations, suggesting that microorganisms progressively colonized the root system. Extraction of metals by plants is almost improved by rhizospheric microorganisms, thanks to various metabolites such as siderophores, organic acids and biosurfactants that enhance the amount of metals at the plant's disposal and macrophytes are also concerned as shown with *Vallisneria americana* and the community of root-associated heterotrophic bacteria (Kurtz et al., 2003).

3.2.3 Opening the Black Box to Optimize the Treatments

In numerous studies, the decrease in contaminants between inlet and outlet of artificial wetlands is almost related to adsorption due to sediment, organic materials accumulated in artificial wetlands and artificial wetlands materials themselves more than bioremediation and/or phytoremediation as shown by Lee and Scholz (2007) with *P. australis* for Cu and Ni (Table 2). Although adsorption avoids pesticide leakage from artificial wetlands, this storage is not permanent as shown by Braskerud

Table 2 Adsorption and phytoextraction phenomena for Cu and Ni contents removal in artificial wetland (Lee and Scholz, 2007)

	Metal supply (mg/L)	Removal (%)	Adsorption on sediment (%)	Extraction by aerial biomass (%)
Cu	1	96	99.9	0.1
Ni	1	88	99.7	0.3

and Haarstad (2003) with metalaxyl desorption. Indeed, the percentage of retention of the compound evolved from 41% in the first year to –11% (desorption) in the second year for supplies of 140 g and 6.5 g of metalaxyl, respectively.

The use of adsorbing materials can be useful since several studies showed that hydraulic retention time of water and thus pesticides in artificial wetlands is too short at allowing biological catalyzers to be efficient. Hydraulic retention times lower than 1 day were recorded in a storm basin located at Rouffach (France) (C. Grégoire, unpublished data). Several improvements were suggested concerning, e.g., geometrical characteristics of the artificial wetlands by increasing the hydraulic residence time in the artificial wetlands, use of adsorbent materials to increase the pesticides residence time and the contact between pesticides and biocatalyzers.

The choice of macrophyte species depends on pesticides and the part of the plant which is harvested as shown by Bouldin et al. (2006) (Table 3). In addition, the treatment efficiency also depends on the time since plantation was realized more than macrophyte association itself. Interesting example was shown by McKinlay and Kasperek (1999) with atrazine used in their study. Time needed for the disappearance of atrazine 1 year after plantation was 52 days with *Typha latifolia*, *Iris pseudacorus* and *P. australis* association against 32 days with *Schoenoplectus lacustris*. One year later, the delay decreased down to 7 days irrespective of the macrophyte species. One may hypothesize that the microflora settled progressively from the planting time. At the beginning, the macrophyte effect was predominant with varying effects according to the species while 2 years later, microflora was the main parameter explaining both the reduced delay for atrazine degradation and same performance irrespective of the macrophyte species.

Temporal variability of treatments represents one limit of in situ biological treatments. During winter, rhizospheric activity decreases by about 20% and stops at low

Table 3 Effect of plant species and harvested part of plant on pesticide accumulation after a 8-day hydroponic culture (Bouldin et al., 2006)

	μg pesticide/kg aerial biomass (whole plant)	
	J. effusus	*L. peploides*
Atrazine*	4,500 (15,000)	4700 (8000)
Lambda-cyhalothrine*	250 (800)	0 (1,500)

*Concentration below the limit of detection for the solution in which macrophytes have grown.

Table 4 Evolution of plant coverage in a constructed wetland (Rose et al., 2006)

Planting date	November/December 2001	
Plant species	***Persicaria* spp.** + *Ludwigia peploides* + *Myriophyllum papillosum* + *Juncus usitatus* + ***Bolboschoenus medianus*** + ***Typha domingensis***	***Persicaria* spp.** + ***Bolboschoenus medianus*** + ***Typha domingensis***
Covering area	20% (March 2002)	95% (November 2002)

temperatures (Table 4). Three months after the end of this period are thus needed for the recovery of the microbial activity (Brix, 1987; Dubus et al., 2000). Fortunately, risk for pesticide loss is rather low during this period. Also, modification of the plant coverage with time may modify the performances of the treatments (Dubus et al., 2000; Rose et al., 2006). Planting macrophytes known to well-colonize artificial wetlands seems to be the most relevant strategy, thus leading to more regular biological treatment.

Conversely, plant covering avoid high and quick shift in temperature allowing process to be more stable over the course of time.

3.3 Water Management

The water budget of wetlands is strongly site-specific and defined by surface in- and outflows – these can be determined relatively simply, by evapotranspiration (ETP) and by groundwater (GW)–surface-water (SW) interactions. Evapotranspiration from wetlands originates from surface waters, plants (stomata) and soils. Methods used to estimated evapotranspiration from wetlands are, e.g. phytometers/lysimeters (Fermor, 1997), Eddy correlation (Gardner, 1991; Acreman et al., 2003), the Penman–Monteith equation (Allen et al., 1998) or combinations of several methods (Petrone et al., 2004). Artificial influences such as mulching (Price et al., 1998) or changes to vegetation (Petrone et al., 2004) can significantly alter evapotranspiration rates. In general wetlands have the ability to act as groundwater recharge or discharge areas. Groundwater–surface-water interactions are governed by the position of the wetland inside the aquifer system and by hydrogeological characteristics of soil and rock materials (Winter, 1999; Sophocleous, 2002). These interactions influence runoff characteristics (e.g. base flow, response times), biogeochemical processes, habitat patterns or sediment redox (e.g. Hill, 2000; Hayashi and Rosenberry, 2001).

In a catchment-scale perspective, wetlands may reduce or enhance runoff. After long dry periods they may act as buffers reducing flow velocities and runoff volumes due to spreading water over large flat areas. Normally, however, wetlands are characterised by high or saturated soil water contents or even by water tables near or above surface. Then they have low storage volumes and respond quickly to water table rises supporting fast runoff components. Hewlett and Hibbert (1967) were the first to identify the importance of wetlands for catchment-scale hydrology. They described runoff generation by "variable source areas" (VSAs), implying the extent of saturated runoff source areas, i.e. wetlands, varies with catchment's moisture state.

3.4 Sediment Management

In order to increase the hydraulic residence time within the artificial wetlands, it is necessary to decrease the speed of the water flow. The direct consequence then is a deposition of sediments. The process is reinforced by the processes of erosion in the upstream catchment or along the thalweg downslope from the ponds (Fiener et al, 2005). An effective erosion control upslope will reduce the loading of the ponds with runoff and sediments and decrease maintenance costs. The delivered sediments are generally enriched by micro-organic contaminants (Hares and Ward, 2004; Laabs et al., 2007) and their management within the hydraulic devices could be problematic. Currently, their management is considered neither in the artificial wetlands with permanent water, nor in the artificial wetlands with temporary flow as a storm basin or detention pond.

The process of sedimentation is closely related to the hydrological flow patterns of the wetlands; for particles that are light or less dense than water, sedimentation becomes possible only after floc formation. It is not a straightforward physical reaction; other processes like complexation and precipitation have to occur first. Flocculation processes are agglomeration of little particles into larger and heavier aggregates more easily depositing on the bottom. They are enhanced by increased pH, turbulence, concentration of suspended materials, ionic strength and high algal concentration.

In sediments, some pesticides are adsorbed into clay and organic matter by electrostatic attraction and, depending on their characteristics, can be degraded also by biota activities in different periods.

Precipitation is one of the major mechanisms by which pesticides are removed from water and are deposited into sediments. This physical process can occur after other mechanisms aggregate the compounds into particles larger and heavier enough to sink on the bottom.

An assessment carried out by the EU in August 2002 (LIFE99 ENV/NL/000263) mentions that only 7–16% of the contaminated porous matrices are biologically treated. Biological treatments represent the most sustainable solution because they require only little energy compared to the other treatments. The artificial wetlands are complete and complex systems with water, suspended particles and sediments, macrophytes acting as filters by allowing contaminants to flow into plants and stems, and biofilms. The extent of the association of micro-organic contaminants and sediments depend strongly on the nature of the compound and the sediments as reviewed by Warren et al. (2003). To date, the majority of studies have shown that as the extent of sorption increases, degradation rates decrease with only the solution phase fraction of the compounds being available for degradation (Guo et al., 2000). But the negative correlation between K_d values and degradation rates is not universal and in some instances, sorption enhances degradation for the compounds degraded mainly through abiotic pathways or when the compound of interest is toxic to the degrading microbial population. There have been few direct studies of the degradation of pesticides in bed sediments or under simulated bed-sediment conditions.

In the environment pesticides are distributed in liquid, solid and gaseous phase. As regards pesticide distribution among the different environmental compartments, it should be noted that it is quite complex and affected by pesticide chemio-dynamic properties. Physical, chemical and biological removal processes are involved in the pesticides disappearance; that is why different parameters are needed to describe the pesticide environment behaviour. The most important are the soil/water partition coefficient K_{oc}, the pesticide half-life DT_{50}, the air/water partition coefficient $\mathrm{K_H}$ (Henry's constant) and the octanol/water partition coefficient log K_{ow}.

As regards the biological part in the artificial wetland, the direct (with absorption process) and indirect (with microorganism biodegradation) effects of macrophytes lead to an extraction of the metals and organic compounds from the water and accumulated sediments. This interdependence of the processes thus imposes an optimization of the system concerning biology, microbiology, hydrology, hydraulic design and sediment management. This is one of the goals of the EU LIFE project ArtWET.

4 The EU LIFE Project ArtWET

One of the goal in the ArtWET project is to define a common methodology to optimize the performance of the prototypes and to permit their comparison, to identify the relevant processes and the associated parameters, to understand the behaviour and finally to assess the effectiveness, the environmental, economic and social impact and the feasibility of the developed mitigation systems. This methodology must supply a scientific frame for the characterization of the experimental conditions, in situ and in the laboratory, for the choice of the sampling location, the monitored pesticides, the experimental methods, the analysis methods and evaluation criteria, taking into account the parameters varying between the sites.

4.1 An Interdisciplinary Approach in the ArtWET Project

A common methodology is required to be able to compare the results of all the experimental and demonstration objects, in laboratory or in natural conditions, to understand all the relevant physical and biological processes in stake, e.g. adsorption, degradation, and hydraulic movement of water and pesticide, and the associated parameters: hydraulic retention time, pesticide retention time, microbial biomass, plant characteristics (Table 5). The common goal among the different study objects is the necessity to provide reliable and optimized treatments with plants and microorganisms, such as plant and microorganism selection, techniques to be

Table 5 Prototypes involved in the project ArtWET experimental (Exp) and demonstration (Dem) sites associated; F: France, G: Germany; I: Italy; WS: watershed; H: herbicide; F: fungicide; I: insecticides, MVS: maximal volume stored

Type of prototype	Mitigation solution	Location	Dimensions Length (m) Area (m^2)	Max stored volume (m^3)	Discharge (L/s)	System/WS ratio (%)	Percentage of applied pesticides type H (%)	F (%)	I (%)
Dem	Vegetated ditches	Krottenbach, Landau, G	1.7 km length		Mean flow 1,500 Max flow 3,300	–	17	61	22
	Detention pond	Göcklingen Landau, G	4,140 m^2	4650		0.15			
Exp	Vegetated ditches	Landau, G	6 × 65 m length 0.5 m width	195		–			
Dem	Constructed wetland	Loches, F	3 zones: 140 m^2, 487 $m^2$and 363 m^2 1 zone: 1,640 m^2	70 24 3 150	 30	0.22	84	8	8
	Forest plot	Loches, F		980		0.35			
Exp	Forest plot microcosms	Antony, F	8 m length 0.4 m width	1;5		–			
Dem	Storm basin, detention pond	Rouffach, F	350 m^2	1,000	Mean flow 15 Max flow 150	0.17	44	50	6
Exp	Wetland mesocosm	Colmar, F	12 × 7 m^2	12 × 10		–			
Dem	Wetland inside detention pond	Freiburg, G	65 m^2	6.5	Base flow 4 Mean flow 13 Max Flow 7 600	0.004	4	8.5	11
Dem	Outdoor bioreactor	Piacenza, I	4.5 m^2	6.75		–	0	60	40
Dem	Biomassbed	Piacenza, I	0.66 m^2	0.98	0.43	–	60	0	40

used for the microorganisms inoculation, coupling pesticide adsorption on selected materials with bioremediation and phytoremediation, in an optimized hydraulic plan.

4.2 Experimental and Demonstration Sites in the ArtWET Project

4.2.1 General Presentation of the Demonstration Sites

The ArtWET project operates and studies the efficiency of ecological bioengineering methods with the help of different prototypes throughout Europe. Experimental prototypes facilitate experiments under standardized laboratory conditions which can be translated to real-world demonstration prototypes. To guarantee a complete coverage of all possible methods of bioremediation seven new sites were constructed in ArtWET. These sites contain (1) experimental prototypes: vegetated ditches, a forest microcosm and 12 wetland mesocosms, and (2) demonstration prototypes: vegetated ditches, two detention ponds, an outdoor bioreactor and a biomassbed. Table 5 gives the status of the different prototypes which are involved in the ArtWET project. For sites located in Landau, Germany, Loches and Antony, Rouffach and Colmar in France, the demonstration sites are attended with experimental sites. The main goal to this duplication is to facilitate experiments under standardized conditions and translate to real world. The mitigation systems are located in continual and Mediterranean climate: the hottest area is Piacenza with an annual average temperature of 13.5°C, and the driest is Rouffach with an annual average rainfall of 580 mm. The size of artificial wetlands vary from 140 m^2 to 1640 m^2 for the punctual demonstration systems, from 0.66 m^2 to 7 m^2 for the punctual experimental systems, and from 65 m to 1.4 km for the linear systems (experimental and demonstration both). The areas of the upstream watershed are between 42 ha (Rouffach, France) and 270 ha (Landau, Germany). The ratio of artificial wetland system/upstream watershed is always less than 1%. The surface cover of upstream watershed is mostly vineyards (Rouffach, Krottenbach, Göcklingen, Eichstetten, Piacenza) but also has crop (Loches). About half of the watershed area involved is drained (Loches, Eichstetten).

The prototype and experimental sites involved in the project are thus distributed geographically on different climatic conditions. They collect surface waters and present various internal hydraulic designs. This diversity is a source of enriching knowledge. But it is necessary to have common parameters and common methodology in order to be able to compare the performances of these artificial wetlands.

4.2.2 Selection of Common Studied Pesticides

The percentages of applied pesticide type (based on the number of molecules per type and not per applied mass) are mentioned in the Table 6. Fungicides are mainly applied on vineyard and majority of herbicides are spread on crops. The inventory

Table 6 Common pesticides used and/or analyzed at least in two different demonstration sites involved in the ArtWET LIFE project (the concerned sites are marked with X). G: Germany, F: France, I: Italy

Compounds	Landau (G)	Freiburg (G)	Loches (F)	Rouffach (F)	Piacenza (I)
Glufosinate (H)	X			X	
Pyrimethanil (F)	X			X	
Myclobutanil (F)	X	X			
Pyrimethanil (F)	X	X			
Tebufenozide (I)	X	X			
Indoxacarb (I)	X	X			
Mancozebe (F)		X			X
Glyphosate (H)	X	X	X	X	X
Carbendazime (F)		X		X	
Kresoxim-methyl (F)		X		X	
Cymoxanil (F)				X	X
Azoxystrobine (F)	X	X		X	
Dimetomorph (F)	X	X		X	
Cyprodinil (F)	X	X			X
Fludioxonil (F)	X	X			X
Penconazole (F)	X	X	X	X	X

of the compounds used in the different sites allows the identification of common molecules with a wide range of K_{oc} and DT_{50} and some leachable with a GUS > 2.8 (Methalaxyl, Triadimenol, Tebufenozide, Simazine, Cymoxanil, Methoxyfenozide). Even if the compound is sprayed on the agricultural plots, it could not be detected in the concentration of the samples at the outlet of the UW and so in the inlet of the artificial wetlands. Under these conditions, only glyphosate, an herbicide, and penconazole, a fungicide, could be the common studied molecules shared by all the teams.

4.2.3 Experimental Vegetated Ditches Under Natural Conditions

Six experimental ditches were built on the area of the University of Koblenz-Landau. Water is provided by the local waterworks. The tapping point (hydrant) has a maximum flow rate of 800 L/min. The ditches are made of heavy pond foil, the basins and reservoirs of concrete. During the time of pesticide loading the water runs through an activated carbon filter and is disposed into a sewer. At idle time the water is circulating. A mechanism is installed to connect the ditches. Thus it is possible to run experiments with one ditch of 325 m, two ditches of 195 m or six ditches of 65 m. Three ditches are vegetated with emerging plants (*P. australis* and *Typha* sp.), and three ditches with submerged plants with large leaf surface (*Ranunculus fluitans*, *Potamogeton* sp.). The flow rate is controlled by electrical pumps. Water samples may be taken in the inlet basins, the sedimentation basins, the inlet and outlet of the carbon filter and according to experiment requirements also along the watercourse.

4.2.4 Experimental Vegetated Ditches Under Laboratory Conditions

A 6 m canal of glass 0.3 m wide was built in Cemagref in Antony (France). Slope can be set up from 0.01% to 5%. The inlet water is controlled by a precision peristaltic pump. The outlet is monitored by an adapted rain gauge system for discharge and with an automatic water sampler. In parallel, parameters such as electrical conductivity and pH are monitored. Flow in the canal is adopted to simulate the demonstration prototype (30 L/s in the inlet ditch, corresponding to 0.03 L/s/lm; lm: linear meter of widespread ditch). Soil was taken from the forested buffer zone. The topsoil characteristics are clay (<2 μm) 260 g/kg, fine silt (2/20 μm) 271 g/kg, coarse silt (20/50 μm) 225 g/kg, fine sand (50/200 μm) 89 g/kg, coarse sand (200/2,000 μm) 155 g/kg, nitrogen (N) total = 3.28 g/kg, C/N 14.4, carbon (C) organic 47.1 g/kg, and CEC Metson 23.6 cmol/kg (CEC: cation exchange capacity). Soil installation in the canal was made carefully keeping surface roughness and porosity as close to reality as possible. In parallel, a experiment to select the best substrate, e.g. dead leaf rate, clay content, was carried out.

4.2.5 Wetland Mesocosm, Pilot Plant Device

The pilot plant device at Colmar, France, consists of 12 tanks in outdoor conditions made out of high-density polyethylene avoiding any adsorption of organic or mineral pesticides. The tanks can be viewed upon as big buried basins (3.00 m diameter –1.50 m depth). Tank dimensions were chosen to avoid edge effects of plants and rhizosphere. They were filled guided by drainage layer granulometry: at the bottom, 25 cm of 10/14 mm gravels; above, 25 cm of 4/8 mm.

Each tank is connected to a collection basin (1.00 m diameter, 2.55 m depth) allowing to both store the leachate for analysis and to control the water level in the tank and also the hydraulic retention time. For the investigations different hydraulic flows are thus possible: percolation or vertical flow, permanent water level or horizontal flow, percolate collection, storage or recirculation. Following leachate analysis, water is collected in two collection sewers (diameter 200 mm) connected to the municipal sewer system. The tanks are filled with a 40 cm layer of 70% sand (0.25/0.4 mm) mixed with 30% sediment from the storm basin located in Rouffach (France). Sediment is spiked with a mixture of glyphosate, diuron and Cu at the beginning of the experiment and the sediment is regularly watered with sprinkler pipes.

4.2.6 Biomassbed

The installation of the biomassbed at Piacenza, Italy started with the excavation of a hole, which was lined with a plastic tank to avoid any risk of leaching pesticides entering the system. The dimensions of the plastic tank were calculated on the basis of information provided by the farmer: the quantity of water used to wash the equipment was about 800 L, two sets of spraying equipment were used, there were approximately 10 treatments per year, the residual volume in the spray tank after spraying was about 10 L. Evaporation and other probable losses of water were also taken into account. From this information, the maximum volume of the biomassbed was estimated to be 4.5 m^3.

A metal grid was placed inside the plastic tank, 1 m from the bottom, in order to divide the tank into two parts. The lower part of the tank was used for the collection of water, while the upper part held the biomix. Before adding the biomix to the upper part of the tank, a nylon filter, a plastic net and a layer of sand were placed on the metal grid to give better support to the biomix and to prevent the entry of biomix material to the water. The lower part of the tank was fitted with a system to force water circulation through the biomix. The system was connected to a pump and to a timer set to carry out a 15 min cycle every 4 h. An irrigation system was placed above the biomassbed, from which water was discharged to leach through the biomix. The irrigation system kept the biomix uniformly wet and prevented a decrease in degradation rate as a result of low moisture content in the upper layers and decreasing levels of microbiological activity. Some authors suggest that a moisture content of 95–100% is optimal in field biobeds because this is the optimal range for microbial activity. Below 75%, moisture content would be limiting with respect to microbial activity. Finally, a roof was installed above the biomassbed to prevent the entry of rain water.

The biomix used comprised materials available on the farm: 20% topsoil, 40% green compost and 40% chopped vine-branches from winter pruning. The chopped vine-branches were mixed and sieved with a 1 cm mesh, then combined with the green compost and left to compost for 1 month, after which they were mixed with the topsoil. The C/N ratio was 28.7 and the biomix bulk weight was 525 g/L (Vischetti et al., 2004; Fait et al., 2007).

4.3 Relevant Methodologies in ArtWET LIFE Project

The ArtWET project represents an innovative approach to evaluate the artificial wetlands efficiency in real field conditions, even if the complexity of the systems is very high; lots of parameters have to be taken into consideration and it is difficult to define a common methodology to compare the different constructed wetlands present in Europe. What is important in this project is the possibility to study the same reactions occurring in the field under controlled conditions using the prototypes. In order to compare the efficiency of the wetlands, the only identified parameter is the mass balance inlet–outlet of the constructed wetland, taking into account the partition of the studied compound in water, sediments, plants and suspended solids. Finally it is possible to have a percentage of pesticide distribution in the different compartments and their degradation into the wetland.

4.3.1 Relevant Biological Endpoints

With regard to the ecotoxicological effects of pesticides comparing the inlet and outlet situation or various stations within the wetland, there are only very few approaches that have been used so far. Most often organisms were exposed in in situ exposure boxes in the field in order to describe the effects on mortality or sublethal endpoints. The relevant in situ techniques including the endpoints to be used have been extensively reviewed by Schulz (2005).

A toxicity reduction by up to 90% was for example documented by midge (*Chironomus* spp.) exposed in situ at the inlet and outlet of a constructed wetland in South Africa exposed to runoff- or spray drift-related insecticide input (Schulz and Peall, 2001; Schulz et al., 2001a). In another experiment in Oxford, Mississipi, targeted the effects of vegetated (>90% macrophyte coverage) versus nonvegetated (<5% macrophyte coverage) wetland mesocosms on the transport and toxicity of parathion-methyl introduced to simulate a worst-case storm event (Schulz et al., 2003b). Both wetland invertebrate communities and midge (*C. tentans*) exposed in situ were significantly less affected in the vegetated wetlands confirming the importance of macrophytes in toxicity reduction. A parallel study using laboratory testing with amphipod (*Hyalella azteca*) indicated that 44 m of vegetated and 111 m of nonvegetated wetland would reduce the mortality to <5% (Schulz et al., 2003c). The implementation of retention ponds in agricultural watersheds was examined by Scott et al. (1999) as one strategy to reduce the amount and toxicity of runoff-related insecticide pollution discharging into estuaries. However, wetland sizes and retention rates are not further detailed. A positive effect of settling ponds, situated below watercress (*Nasturtium officinale* R. Br.) beds in the UK that were not further described, was documented using mortality and acetylcholinesterase inhibition in scud (*Gammarus pulex*) exposed in situ as endpoints (Crane et al., 1995). Retention rates are not given, as the concentrations of malathion used in the watercress beds were not measured in this study.

4.3.2 Accuracy and Efficiency of Pesticide Sampling

In the objective to evaluate the performance of the artificial wetland systems, the problem most frequently encountered is the quantitative evaluation of the concentrations and flows of pesticides entering and leaving within the systems. However, sampling is as important for the data-gathering as for the analysis and interpretation of the results. Among the different errors and biases, we are interested in those related to sampling. In order to validate a common methodology of acquisition of the samples and to be able to give precision to the results, we carried out preliminary tests on the water-storm basin in Rouffach, France.

Thirty two streaming events were recorded since 2003 until 2006. For each one, the flow is recorded and a water sample is collected every 8 m^3. The number of collected samples varies from 3 to 24 according to the importance of the flow. The real flow of two herbicides (diuron, glyphosate) and one glyphosate metabolite (aminomethylphosphonic acid – AMPA) is calculated by integration, taking account of all the samples available and validated and consists in a linear interpolation of the data of concentration at the moments of measurement of the flow. Three methods of monitoring are tested and evaluated for each event:

M1 – All the samples available are mixed with equal volume, an average sample is thus made up. This strategy consists in calculating the average concentration of an event. We then calculate the flow by multiplying the average concentration by total volume.

M2 – Three sluice box of flow intercepts water in entry by collecting 1 (tank 1), then 1/10 (tank 2) and 1/100 (tank 3) of the past volume in each tank. A sample in each tank is then collected and analyzed. One calculates flow by multiplying each concentration by corresponding volume and then by summing them.

M3 – The selected sample consists of only one manual measurement and is collected at the end of a time *t*, taken starting from the beginning of the streaming, equal to the time of concentration of the watershed upstream.

In all these calculations of flow, we make the following assumption: before the first value of concentration is available, the concentration is considered equal to the first value and after the last value of concentration is also available, the concentration is considered equal to the last value. The evaluation of the performance of these three methods is led by calculating the relative error (average and standard deviation) made by comparing the flow of reference for each event and for each compound. The most powerful method and also the least expensive is the method M1. The average relative error calculated while taking into account all the events is 2.68% for Diuron, 3.89% for Glyposate and 3.94% for AMPA (Table 7).

However, these results must be moderate because the errors can vary during one event from –8.37% to 31.56%, e.g. for the Diuron. A good precision on the annual balance can be provided: the sampling errors are smoothed if the results over the 4 years of observation are taken into account (less than 7%). We can conclude that if this procedure is suitable for long-term survey, the evaluation of each event separately remains problematic.

Table 7 Relative error (%) of pesticide flows for diuron, glyphosate and AMPA between method M1 (mixed synthetic sample for one event) and the reference pesticide flow

	Diuron	Glyphosate	AMPA
Average relative error (%)	2.68	3.89	3.94
Minimal relative error (%)	–8.37	–9.26	–10.42
Maximal relative error (%)	31.56	33.91	38.27

4.3.3 Development and Implementation of an Innovative Process to Herbicide and Copper Mitigation

An innovative bioprocess applied to herbicide and copper mitigation is being developed. With the aim of securizing and optimizing the process, plant-microorganisms-adsorbing materials are closely associated. Well-chosen adsorbing materials should increase the contact time between microorganisms and contaminants. Hydraulic time in Rouffach storm basin is most of the time too short for allowing biological catalyzers to be efficient. At the same time the macrophyte rhizodeposits could stimulate inoculated microorganisms and support their development in the course of the time. Bacterial survival when bioaugmentation is chosen also needs relevant screening schemes.

Culturable non-rhizospheric and rhizospheric bacteria associated with *P. australis* growing in a storm basin located near Rouffach (Haut-Rhin, France) have been characterized. Bacteria were isolated for their resistance to copper, diuron and glyphosate and also for their ability to synthesize siderophores with the aim of increasing copper phytoavailability.

Sediment cores were excavated from the storm basin under different oxic conditions accounted for by two horizons (H1, 0−5 cm; H2, 5−10 cm). H1 was considered as rhizospheric soil (root-adhering soil), whereas in H2 a distinction was made between rhizospheric soil and bulk soil (non-adhering soil). Sediment samples were incubated in a minimal culture medium containing copper (130 mg/L), diuron (20 mg/L) and glyphosate (40 mg/L). K-strategistic and r-strategistic bacteria have been distinguished. Samples were submitted to RISA analysis (ribosomal intergenic spacer analysis) and then to RFLP analysis (restriction fragment length polymorphism) to discard strains that appeared to be similar.

From the sediment samples, 563 strains resistant to the above-mentioned contaminants were obtained. The second step of the bacterial screening consisted in a genetic analysis. Two hundred and nine strains were obtained after RISA and RFLP analysis. Additionally to this genetic characterization, a functional characterization based on herbicide degradation and Cu complexation is still in progress to discard strains.

Four adsorbing materials have been selected to experiment with copper and herbicide adsorption. Two of them are organic, beet pulp and maize cob; the two others are mineral vermiculite and perlite. For copper, the best adsorption rate was obtained for beet pulp and vermiculite with 38% and 37%, respectively. Diuron and glyphosate showed higher adsorption onto maize cob (46%) and beet pulp (25%), respectively.

For mitigation of both copper and organic pollutants, two types of materials will be used.

4.3.4 Constructed Wetland Modelling

Pesticide removal from subsurface flow constructed wetland systems include biological (biological degradation, uptake by plants and aquatic organisms), chemical (sorption, photo-decomposition and degradation) and physical (volatilization and sorption) processes (Chavent and Roberts, 1991). Results obtained by Schulz et al. (2003a) suggest that vegetated wetlands have a strong potential to contribute to aquatic pesticide risk mitigation. According to Rao and Jessup (1982), a model to simulate pesticide dynamics must include at least the following three key processes: water and solute transport, adsorption-desorption and degradation.

Water is a Transfer Vector

The remediation role of the artificial wetland is determined by three hydrological factors:

- The hydroperiod which is defined by the frequency and the duration of saturation with water, i.e. when field capacity is overpassed. It results in a gravitary flow which is driven by the media permeability and the hydraulic head. The velocity of the flow should be preferably slow for best efficiency meaning low hydraulic conductivity and/or gradients.
- The residence duration of the water in the wetland. Tanner et al. (1995) and several other authors (Stearman et al., 2003; Blankenberg et al., 2006; Haarstad and Braskerud, 2005) have shown that pesticide retention increases with residence duration, thus providing better efficiency.
- Origin and contents of the feeding water. The higher the load of agricultural pollutant the more efficient the artificial wetland as expressed in terms of flux (Moore et al., 2000, 2001b; Schulz and Peall, 2001; Stearman et al., 2003).

Surface Hydrologic Model-Based Design

Controlling the average behaviour of water as it flows through artificial wetlands is key to its long-term success. Short-circuiting and dead pools need to be minimized in order to more closely resemble plug-flow conditions. Hydraulic residence times are crucial design elements that assume uniform flow behaviour.

Flow characteristics through the wetlands include

- Velocity: This is controlled by selecting a bed slope that provides a sufficient hydraulic gradient through the wetland to achieve the desired velocity.
- Detention time: The amount of time that it takes a unit of volume to travel from the inlet to the outlet of the wetland is determined by the size, depth and travel path through the wetland.
- Depth of flow: A design depth must be chosen to provide adequate storage and appropriate conditions for the wetland plants chosen.
- Travel path: Providing an appropriate length-to-width ratio will prevent short-circuiting through the system.
- Water balance: The designer must determine the sources and sinks that will occur in the wetland. Groundwater influences are generally minimized by the use of liners. It is important to determine the contribution that precipitation and evapotranspiration will have on wetland hydrology.

The artificial wetlands hydrology will determine many of the controls of the artificial wetlands hydraulics. Hydraulics refers to the physical mechanisms used to convey the water in and through the artificial wetlands. Important components of the hydraulic system include: conveyance system, inlet and outlet mechanism, depth control, isolation devices (for maintenance), collection device (drainage channels).

Several modelling approaches were applied to different aspects of wetlands. Water table variations and flow in saturated and unsaturated zones were modelled using e.g. WETLANDS (Mansell et al., 2000), MODFLOW (Bradley, 2002), HYDRUS-2D (Joris and Feyen, 2003; FEUWAnet Dall'O et al., 2001). Hydrological tracers served as valuable tools for model validation. Simulated groundwater inflows were checked by Hunt et al. (1996) using temperature profiles and isotopic mass balances. In other studies, tracers were used to investigate runoff generation processes (Soulsby et al., 1998; Jarvie et al., 2001), infiltration and solute transport mechanisms (Parsons et al., 2004) or hydraulic parameters (Maloszewski et al., 2006).

For an accurate modelling of pesticide mitigation in artificial wetlands many different processes have to be considered in great spatial and temporal detail. Existing approaches mainly describe the transport through the vadose zone. There are 2D-hydraulic models like Hydrus2D or PRZM3 and conceptual approaches like, e.g. tanks in series (Basagaoglu et al., 2002). Besides general mass transport, pesticide modules include processes like linear-equilibrium sorption or first-order degradation (Helweg et al., 2003). For the investigation of solute transport in surface flow systems mainly watershed models have been used (e.g. SWAT, Holvoet, 2006); two- or three-dimensional numeric hydraulic approaches are less common. However, in principle, finite-element or finite-volume approaches can calculate flow conditions in water bodies including sediment- and also pesticide-transport.

Within the ArtWET project a model approach is in progress which describes two-dimensional surface flow inside artificial wetlands on the basis of the Runge–Kutta–Discontinous–Galerkin method. This method was applied successfully to simulate depth-integrated shallow water flow based on spatial patterns of ground elevation and roughness (Schwanenberg, 2005). In the ArtWET project sediment transport is included by a source/sink term based on the Ackers–White formula (Acker, White, 1973) widely used in many studies (Batalla, 1997; Koskiaho, 2003; Dargahi, 2004). Pesticides are added by linear-equilibrium sorption and first-order degradation. Eventually aspects like decay by sunlight or interaction with vegetation will be considered.

Fate and Transport Using a 2D Mixed Hybrid Finite Element Approximation

In an inventory carried out by Siimes and Kämäri (2003), there were identified 82 solute transport and pesticide models available. In order to find the best available models for herbicide fate simulation for Finnish conditions, a comparative analysis among the models was performed. Besides, a detailed description of the models was provided. The interested reader is referred to review compiled databases as CAMASE (Bergamaschi et Putti, 1999), REM (REM, 2007), or to review papers such as Vink et al. (1997), Vanclooster et al. (2000), FOCUS (2000), Jones and Russel (2001), Dubus et al. (2002) and Garratt et al. (2003).

Vink et al. (1997) studied unsaturated transport of the nematicide aldicarb and the herbicide simazine in a cracked clay soil. They performed a comparative analysis among the models VARLEACH 2.0, LEACHP 3.1, PESTLA 2.3, MACRO 3.1 and SIMULAT 2.4. Their conclusion was that none of these models describe water percolation and pesticide leaching with a complete degree of satisfaction, although, over all the experimentation period (>10 months), the best results on water percolation and pesticide tracer came from PESTLA and SIMULAT. Garratt et al. (2003) compared the capacity of seven pesticide models to predict the propagation of aclonifen and ethoprophos in an environment of arable soil. The tested models were: VARLEACH, LEACHP, PESTLA, MACRO, PRZM, PELMO and PLM. In their study, they observed significant differences in the prediction of the pesticide mobility and persistence. These differences were attributed mainly to the choice of the flow equations, the soil temperature and the degradation kinetics. They suggest that many efforts are certainly still necessary for the parameterization of models that consider flow in the macro-pores.

In order to have a better understanding of the hydrodynamics and the fate of pesticides within the vertical flow sand filter, a two-dimensional numerical model is being developed to simulate solute transport in relationship with the biological treatment in the porous matrix. The hydrodynamic system is simulated by the application of the Richards' equation (1). This formulation physically describes the flow in a variably saturated porous medium.

$$C(h)\,\frac{\partial h}{\partial t} = \nabla\left[K\nabla\left(h+z\right)\right] + W(x,z,t)\,, \tag{1}$$

where $W(x,z,t)$ is the sink/source terms [T^{-1}], x and z (depth) are the spatial coordinates [L], t is time [T], $C(h)$ is the soil moisture capacity [L^{-1}], K is the unsaturated hydraulic conductivity [LT^{-1}], h is the soil water pressure head [L].

The pesticide transport is described by a classical advection–dispersion equation (2) with the presence of sink/source term which takes into account the pesticide degradation.

$$\frac{\partial(\theta C)}{\partial t} + \frac{\partial(\rho S)}{\partial t} - \nabla(\theta D \nabla C) + \nabla\left(\vec{q}\,C\right) = f(C,t)\,, \tag{2}$$

where $\vec{q}$ is volumetric flux [LT^{-1}], ρ is soil bulk density [ML^{-3}], f(C,t) is the sink/source terms [$ML^{-3}T^{-1}$], θ is soil volumetric water content [L^3L^{-3}], C is solution concentration [ML^{-3}], S is absorbed concentration [ML^{-3}], D is the dispersion tensor [L^2T^{-1}], and t is time [T].

The numerical tool used to solve these equations is the mixed hybrid finite element method (MHFEM). This technique is particularly well adapted to the simulation of heterogeneous flow field (Mosé et al., 1994; Younes et al., 1999). It has been applied in previous works concerning mainly to the flow in heterogeneous saturated porous medium. In unsaturated porous medium, the heterogeneity is due to both the heterogeneous sediment distribution and the non-uniform water content in the storm basin. The originality here is to simulate both flow and solute transport, with the application of MHFEM for a variably saturated porous medium.

Mixed Hybrid Finite Element Method – Hydrodynamic Modelling

A two-dimensional (2D) flow domain ω is defined, and it is subdivided into triangular elements K. The Darcy flux $\vec{q} = -K\nabla(h+z)$ is approximated over each element by a vector $\vec{q}_K$ belonging to the lowest order Raviart–Thomas space (Raviart and Thomas, 1977). On each element this vector function has the following properties: $\nabla\vec{q}_K$ is constant over the element K, $\vec{q}_K\vec{n}_{K,E_i}$ is constant over the edge E_i of the triangle, $\forall i = 1,2,3$, where $\vec{n}_{K,E_i}$ is the normal unit vector exterior to the edge E_i. $\vec{q}_K$ is perfectly determined by knowing the flux through the edges (Chavent and Roberts, 1991). Moreover, with the MHFEM, the normal component of $\vec{q}_K$ is continuous from K to the adjacent element K' and $\vec{q}_K$ is calculated with the help of the vector fields basis $\vec{w_i}$, used as basis functions over each element K. These vector fields are defined by $\int_{Ei} \vec{w_j} \cdot \vec{n}_{K,E_i} = \delta_{i,j}, \forall i = 1,2,3$, where δ_{ij} is the Kronecker symbol, so that these functions correspond to a vector $\vec{q_K}$ having a unitary flux through the edge E_i, and null flux through the other edges:

$$\vec{q_K} = \sum_{j=1}^{3} Q_{K,E_j}\vec{w}_j \tag{3}$$

with $Q_{K,Ej}$ the water flux over the edge E_j belonging to the element K.

The estimation of the conductivity can be represented by the relationship $K_K = k_K\left(K_K^A\right)$, where, over each element K, k_K is the unsaturated hydraulic conductivity function [LT^{-1}] given by the modified Mualem–van Genuchten expression (Ippisch et al., 2006), and K_K^A is a dimensionless anisotropy tensor. The transport equation is similarly constructed.

Test Case

A variable saturated flow through layered soil with a perched water table is considered. The soil profile consists of soil 1 from 0 cm to 50 cm and 90 cm to 100 cm and soil 2 from 50 cm to 90 cm. A constant flux boundary condition was applied at the upper boundary, and a zero-flux boundary condition at the lower boundary. The soil hydraulic parameters used are shown in Table 8 in which θr and θs denote the residual and saturated water contents, respectively; Ks is the saturated hydraulic conductivity, α is the inverse of the air-entry value (or bubbling pressure), n is a pore-size distribution index. This case is similar to the example presented by Pan and Wierenga (1995). Initial conditions were considered with moist ($h = -200$ cm) and very dry ($h = -50{,}000$ cm) soil.

Results obtained through the implementation of the numerical approach by the MHFEM to simulate hydrodynamics in very dry to saturated soil presented a good agreement to the results obtained by Pan and Wierenga (Fig. 1).

The objective of this work was to introduce a new formulation to simulate water flow and solute transport in variably saturated porous medium by the application of the MHFEM in a global approach. After a verification stage of the flow and transport equation in porous media, different kinds of pesticide biodegradation kinetics specifically for soil environment (Alexander and Scow, 1989) will be introduced.

Table 8 Hydraulic parameters of the two soils constituting the heterogeneous medium: soil 1 from 0 cm to 50 cm and 90 cm to 100 cm and soil 2 from 50 cm to 90 cm, θr and θs denote the residual and saturated water contents, respectively; *K*s is the saturated hydraulic conductivity, α is the inverse of the air-entry value (or bubbling pressure), *n* is a pore-size distribution index

	θs	θr	α	*n*	*K*s (cm/day)
Soil 1	0.3658	0.0286	0.0280	2.2390	541
Soil 2	0.4686	0.1060	0.0104	1.3954	13.1

Il y a peu de figures dans cet article. Or, les figures sont très efficaces pour communiquer rapidement un point majeur. Ce serait bien de faire une figure/schéma bien pensés par section pour illustrer les points majeurs. En outre les légendes des figures doivent expliquer 1) quel est la tendance à voir (Note the increase...) et 2) sa signification scientifique. La figure doit en effet être compréhensible sans avoir à lire l'article.

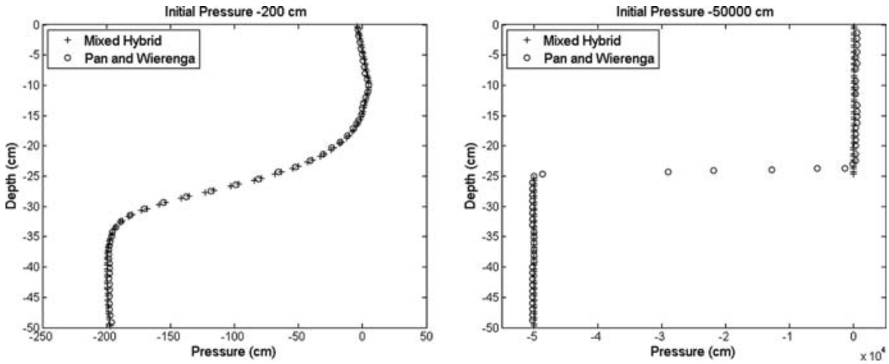

Fig. 1 Pressure head versus depth for cases with moist ($h = -200$ cm) and very dry ($h = -50{,}000$ cm) initial soil conditions

5 Conclusion

Constructed wetlands, named Artificial Wetlands in this article, nowadays have many applications, ranging from the secondary treatment of domestic, agricultural and industrial wastewaters to the tertiary treatment and polishing of wastewaters. Artificial wetlands can also contribute to the self-purification capacity of hydrosystems, specifically agrosystems. The work managed in the ArtWET project aims at integrating solutions for the complete aspect of pesticide loss from agricultural areas into surface waters. Here we present artificial wetlands studied in various designed conditions including permanent and non-permanent flow, drained and non-drained areas. One of the common points is to concentrate surface waters in wetlands whose purifying operation must be optimized. This topical issue is a major stake for sustainable development. It can be reached by considering both hydraulic part and biological part. The control of the hydrologic dynamic must increase the retention time of water and pesticides. This must then allow a first degradation and the adsorption of the active matter into the device. Once the pollution is sequestered into the artificial wetland, biological treatment can be put into action. This treatment lies in an absorption by selected macrophytes and a degradation by microorganisms introduced. This stage consists of a bioaugmentation and biostimulation in order to increase the natural attenuation. Under these conditions, the artificial wetlands must naturally find their place within the landscape and in the chain of devices of treatment of the water resource. The first results show a systematic increase of the rate of degradation between the inlet and the outlet of the artificial wetland ecosystems. The double possibility of acting on the physical and biological parameters must allow to reach an overall degradation rate near to 100%.

This way of pesticide management in agrosystems will become more and more relevant, with the increasing need to develop strategies for sustainable agriculture and to avoid environmental contamination.

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Sustainable Management of Natural Resources for Food Security and Environmental Quality: Case Studies from India – A Review

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Abstract The best management practices of soil conservation, water management (rainwater and groundwater), and soil fertility enhancement were tested and demonstrated in farmers' fields, in red soils (Alfisols), black soils (Vertisols), and alluvial soils (Entisols and Inceptisols) of India, under a range of crops and cropping systems. The project involved a community-based interactive participatory operational research model, with an intrinsic value of large-scale social and extension domain for adoption of sustainable agricultural practices.

In the dry land soils of South India disc ploughing to 30-cm depth during summer and contour bunding, and tied ridges with mulching were conservation-effective measures. Under the bimodal rainfall pattern in the region, intercropping of pigeon pea with pulses and use of compost and green manuring enhanced crop yields. Drip irrigation and arid horticulture were also successful in this region.

On-farm experiments conducted on alluvial soils of Punjab with diverse crop rotations of different water requirements showed that at Nawashahr the average yield of wheat was similar in the rice-wheat system (RWS) (4.0 Mg/ha) and maize-wheat system (MWS) (4.3 Mg/ha). Substituting maize for rice in the monsoon season is preferred because of savings in water. In Faridkot district, the economic returns were higher for the cotton-wheat system (CWS) which optimizes the use of groundwater resource better than the RWS.

Efficient on-farm water management using fertigation technology, agroforestry and fish culture, and intercropping with poplar were useful cyclic interventions in the land use and management strategy for sustainable agriculture in the intensively cultivated alluvial soils of Punjab.

For sustainable management of Vertisols of central India, yield of soybean increased by about 100% with raised bed and furrow system and by 55% in broad bed and furrow system compared with the flat bed system. Adoption of integrated nutrient management (INM) based on soil testing increased the yield of soybean

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(*Glycine max*) and wheat by 71% over farmers' practice at Narsinghpur compared with about 100% in the case of soybean and 187% in the case of wheat at Hoshangabad. Intercropping of soybean with pigeon pea (*Cajanus cajan*) in 4:2 ratio produced higher net return (Rs. 27,620/ha) and benefit:cost ratio (3.3:1) over either of the monocropping system. Aqua-agriculture system was a better alternative to traditional monocropping (Haveli system) in the monsoon season. Aquaculture in the ponded water in the bunded field during monsoon season and growing of wheat or chickpea in the winter season proved successful, and is being adopted in the region.

Keywords Participatory research · Land and water management · Red, black and alluvial soils of India · Sustainable agriculture · Environmental quality

1 Introduction

Of India's total geographical area, around 43% (142 Mha) is under agricultural land use. Monsoon-dependent agriculture is practiced on 58% of the arable land. The impressive and historic increase in foodgrain production since mid-1960s through the Green Revolution is attributed to three factors: spread of high-yielding crop varieties, expansion of irrigation by using surface and underground water resource, and increase in use of fertilizers and other agrochemicals.

The principal challenges on the food front that India faces at the onset of the twenty-first century are:

- Food insecurity: To meet the demand of the increasing population, India's foodgrain production must go up to about 325 million tons (Mt) by 2025. To achieve this target, production has to increase at the rate of 7 Mt yr^{-1} over the next 20 years, without much increase in irrigated cropland.
- Soil degradation: Land area affected by different processes of soil degradation is estimated at 81% of the agricultural land area. This includes water erosion, wind erosion, sand dune encroachment, riverines, ravines and gullies, poor drainage, and excessive salts. Urban sprawl is another factor encroaching upon arable land. Demographic pressure, soil degradation, urbanization, and conversion into non-agricultural land use all contribute to the rapid decline of per capita arable land area.
- Environmental pollution: Excessive and inappropriate use of chemicals and irrigation, removal of crop residues, and simplification of cropping systems have caused pollution of groundwater, eutrophication of surface water, contamination of soil, and deterioration of air quality. In many regions both surface water and groundwater are highly polluted. Agricultural practices such as biomass burning and inefficient use of chemicals and fertilizers emit greenhouse gases (GHGs) that contribute to global warming.

- Social and political stability: There is a strong relationship between food security, employment opportunities, gender/social equity, and social/political stability. Being an agrarian economy, it is important to strengthen agriculture growth which is a vital sector for peace and political stability.

Addressing these complex and interacting biophysical and socio-economic/political factors underpinning food security and environmental quality requires (i) adopting an innovative approach and strategy for sustainable agriculture by assessing soil quality dynamics under changing farming/cropping systems based on intensive off-farm inputs (ii) involving farmers and land managers in the decision-making process, and (iii) developing a mechanism of exchange of information between scientists and farmers/land managers on the one hand and policy makers and industry stakeholders on the other.

2 Project Implementation

The project on "Sustainable Management of Natural Resources for Food Security and Environmental Quality" was initiated by the M.S. Swaminathan Research Foundation (MSSRF) in technical collaboration with The Ohio State University (OSU), Columbus, Ohio, USA, with financial support from Sir Dorabji Tata Trust. The on-farm study was conducted in three major agro-ecoregions and soils of India: the red soils of Pudukottai and Dindigul districts in Tamil Nadu state of southern India, the black soils of Narsinghpur and Hoshangabad districts of Madhya Pradesh (MP) in Central India, and the alluvial soils of Nawashahr and Faridkot districts in Punjab in northwestern India. The methodology adopted consisted of participatory rural appraisals, transfer of the best management practices (BMPs) in soil and water management and crop husbandry to the farmers' fields, training programs for farmers on these practices, and promotion of micro-enterprises for enhancing livelihood opportunities. Organization of Farmers' Days and interaction with the local extension system helped in the dissemination of these results to other farmers in the villages.

The specific objectives of the project were to

1. test, validate, and extend adoption of BMPs to alleviate soil-related constraints through participatory research approach,
2. develop and introduce management practices for efficient utilization of soil, water, and organic resources to enhance and sustain productivity,
3. diversify production system by introducing legumes/pulses, vegetables, livestock, agroforestry, and aquaculture through on-farm demonstration and validation, and
4. restore degraded soils, enhance water recharge, and improve soil organic carbon (SOC) concentration.

The farmer-participatory on-farm research on the validation and adoption of BMPs in three contrasting agro-ecosystems and soils (Fig. 1) is presented below:

Fig. 1 Map of India

3 Alfisols in Semi-Arid Regions of South India

Alfisols of southern India have numerous soil-related constraints to adopting intensive cropping and enhancing crop yields. Effectiveness of highly variable and erratic monsoons is extremely low because of high intensity that generates a large volume of surface runoff, and high temperatures that cause severe losses by evaporation. Consequently, crops suffer from frequent and severe drought stress during critical growth stages. Adverse impacts of drought stress are exacerbated by the severe problem of soil degradation. Alfisols have inherently low soil fertility characterized by low nutrient reserves (e.g., N, P, K, Ca, Mg, Zn, Cu), low soil organic matter (SOM) content, low available water capacity (AWC), and consequently the low microbial biomass C. With predominantly coarse texture and scanty vegetation cover, crops suffer from supra-optimal soil temperatures exceeding 50°C in the top 0–5 cm layer. Adverse impacts of high soil temperatures and recurring drought stress are accentuated by the root-restrictive ground layer at 30–40 cm depth. Thus, alleviating these soil-related constraints by improving soil quality, conserving water in the root zone, moderating soil temperature, improving soil fertility, and enhancing SOM content are essential to increasing agronomic production. Agronomic benefits of improving soil quality can be realized only through improvement of soil quality and diversification of cropping systems.

Based on the participatory rural appraisal approach (Pretty, 1994; Velayutham et al., 2002b) and interaction with the farmers, the on-farm work plan was developed for 5 years (2001–2005). Field demonstrations were established at Ariyamuthupatti Village in Kudumianmalai Panchayat in Pudukottai district of Tamil Nadu. Population of the region is entirely dependent on agriculture. Predominant soils of the district comprise degraded, infertile, red soils (Alfisols – Vayalogam series) in a semi-arid climate. The district is located between 8° 30′ to 10° 40′ N latitude and 78° 24′ to 79° 40′ E longitude in East Central Tamil Nadu, India, and falls in the 8.3 agro-eco sub-region of India (Velayutham et al., 1999; Natarajan et al., 1997). The district has a bimodal rainfall distribution with an average annual rainfall of 685 mm. About 40% of rainfall is received during southwest monsoon season (July–September), 44% during north-east monsoon (October–December), 7% during winter (January–February), and 9% in summer (March–June).

Farmland area of 5.1 ha leased from the community was chosen as the experimental-cum-demonstration farm. Initial soil analysis was done to establish baseline soil properties (Jackson, 1975). The data in Table 1 show a low level of plant-available nutrients of only 146 kg/ha of NPK and low cation exchange capacity (CEC) of 4.6 c mol (+)/kg^{-1} of soil. The SOC concentration (0.26%) was below the critical level of 1.1% for soils of the tropics (Aune and Lal, 1998). The effective rooting depth is shallow, because the soil is underlain by a thick gravelly horizon. The surface soil has low AWC, and is prone to crusting, moderate-to-severe erosion, and acidification. The soils have a high P fixation capacity by iron and aluminum hydroxides.

Table 1 Nutrient status (0–20 cm) of the soil at Ariyamuthupatti village site just before sowing in 2001

Parameter	Value	Remarks
Available nitrogen (kg/ha)	52	Low
Available phosphorus (kg/ha)	14	Medium
Available potassium (kg/ha)	80	Low
Soil organic carbon (%)	0.26	Low
Extractable calcium (cmol kg^{-1} soil)	2.26	Low
Extractable magnesium (cmol kg^{-1} soil)	1.10	Low
Extractable sodium (cmol kg^{-1} soil)	0.34	Low
Cation exchange capacity (cmol kg^{-1} soil)	4.65	Low
Base saturation (%)	82.15	High
Exchangeable sodium (%)	7.3	Low
DTPA extractable zinc (ppm)	0.44	Low
DTPA extractable iron (ppm)	10.03	High
DTPA extractable copper (ppm)	0.62	Low
DTPA extractable manganese (ppm)	9.79	High

3.1 Water Management

At the start of the project, most of the rainfed soils in Ariyamuthupatti village had been uncultivated and left fallow for the past two decades. The farmers were reluctant to undertake the high risks of crop failure involved in cultivating these soils under dry farming conditions. Consequently, the village water pond (locally called tank) was uncared for several years and had been silted up by sedimentation because of uncontrolled and accelerated soil erosion. The pond was overgrown by weeds and shrubs (e.g., *Prosopis* spp.).

Seedbed preparation during summer was done by disc plowing to about 20 cm depth. Bunds were erected across the slope around the individual fields for diverting the excess rainwater into the channels. These channels, about 0.5-m deep with strengthened bunds installed on the contour, were connected to a main waterway. The latter with a gentle gradient, 0.75–1.0 m deep, drained into the pond. The latter was excavated to more than 1 m depth by farmers for storing the excess runoff water carried off from the fields through a network of channels and waterways. Periodic desilting and deepening of the pond was done by the village community throughout the study period. In addition to serving as a water reservoir, the tank also recharged the groundwater.

3.2 Climate and Crop Calendar

Being a rainfed agriculture area, importance was given to millets, pulses, and oilseed crops in crop planning. Improved varieties of pigeon pea (*Cajanus cajan* L.) tested involved those of varying growth duration. These included medium duration or MD varieties (135 days) (APK1, CO5 and VBN1) and long duration or LD varieties (165–180) (VBN2, LRG30, ICPL87119). In general, LD varieties were better suited to this eco-region with a bimodal rainfall pattern than MD varieties. Furthermore, among LD varieties, the highest grain yield of 263 kg ha^{-1} was obtained with the VBN2 variety. Four varieties of black gram (*Vigna mungo* L. Hepper) (VBN2, VBN3, CO6, T9) and three varieties of green gram (*Vigna radiata* L. R. Wilczek) (VBN2, CO6, K851), with growth duration range of 65–70 days, were also tested for their performance. Variety VBN2 of green gram and VBN3 of black gram performed relatively well in these soils and yielded 188 kg ha^{-1} and 180 kg ha^{-1}, respectively. The prolonged dry spell in August coincided with the grain-filling stage and resulted in low yields.

3.3 Intercropping with Pigeon Pea

The best way to increase land equivalent ratio (LER) in rainfed monoculture is through intercropping that fits into the climate regime (Velayutham, 1999). The LD pigeon pea variety (VBN2) was tested for its productivity in an intercropping system (Fig. 2). Both groundnut (*Arachis hypogaea* L.) (TMV 3) and cowpea (*Vigna unguiculata* L.) (VBN1) were grown as intercrops. Pigeon pea was sown at a row-to-row spacing of 1.0 m. In between the rows of pigeon pea, three rows of cowpea and groundnut were sown at a spacing of 30 × 10 cm. It was found that intercropping of pigeon pea is climatically adaptable and economically advantageous for this region. Pigeon pea + groundnut was the best intercropping system in terms of net income (Rs. 4145/ha).

Fig. 2 Red gram + green gram intercropping under mulching moisture conservation

3.4 Double Cropping

Double cropping or sequential cropping was evaluated by disc ploughing to 30-cm depth during the summer. In addition, water harvesting, using diversion channels and waterways, and supplemental irrigation were also used. Enriched press-mud was used to improve soil fertility. Compost was used in conjunction with foliar application of diammonium phosphate. Integrated pest management (IPM) was adopted against the pod borer damage in pigeon pea. The data in Table 2, about the yield of different cropping systems, show that agronomic productivity can be greatly enhanced in ecosystems with a bimodal rainfall pattern by growing two sequential crops under rainfed conditions. Pigeon pea + lablab (*Lablab purpureus* L.) and pigeon pea + groundnut intercropping systems produced the highest grain yields, even under conditions of uneven rainfall distribution. Among the double cropping systems, groundnut followed by cluster bean (*Cyamopsis tetragonoloba* L. Taubert) and radish (*Raphanus sativus* L.) produced the highest monetary returns. In comparison with the monoculture of pigeon pea, the minor millets varagu or Kodo millet (*Paspalum scrobiculatum* L.) and finger millet (*Eleusine coracana* L. Gaertn) also performed well, indicating the potential prospect of including these nutritious minor millets in cropping systems for advancing crop diversification, and food and nutrition security (Velayutham and Palaniappan, 2003).

Table 2 Agronomic yields and economic returns of a range of crops and cropping systems

First crop (July–Sept.)	Second crop (Oct.–Feb.)	Yield of first crop (kg ha^{-1})	Yield of second crop (kg ha^{-1})	Net income (Rs. ha^{-1})
Pigeon pea (LD) (pure crop)	Contd.	263	–	1,315
Pigeon pea (LD) + black gram	Red gram (contd.)	198	107	4,158
Pigeon pea (LD) + cowpea	Red gram (contd.)	109	123	1,780
Pigeon pea (LD) + groundnut	Red gram (contd.)	109	313	4,145
Pigeon pea (LD) + lablab	Red gram (contd.)	198	248	4,712
Pigeon pea (MD)	Red gram (contd.)	140	–	1,120
Black gram	Horse gram	180	63	3,620
Green gram	–	188	–	3478
Cowpea	Bengal gram	27	64	1,457
Groundnut	Cluster bean & Radish	244	257	3,643
Sesame	Horse gram	121	165	1,638
Varagu (Kodo Millet)	Varagu (contd.)	634	–	2,219
Finger millet	–	594	–	2,673

Note: LD – Long duration; MD – Medium duration.

3.5 Summer Ploughing

Summer ploughing to ∼30-cm depth enhanced soil water reserves during the southwest monsoon of 2004, primarily by breaking the crust and improving water infiltration rate. With intense rains received in July 2004, farmers included groundnut in the cropping system. Although there was a continuous dry spell for 52 days, the crop survived due to the moisture conserved through summer ploughing, resulting in an average yield of 1.67 Mg ha^{-1} of pod. In some plots where summer ploughing was not done, the groundnut crop sown in July suffered from severe drought stress at critical stages of flowering, peg formation, and pod filling. The average groundnut yield was 600 kg pods ha^{-1}, or 36% of the yield obtained with summer ploughing. Vittal et al. (1983) reported the beneficial effects of deep tillage on dryland crop production in red soils of India. The importance of summer ploughing is now widely accepted by the farmers of the district, and it forms an important component of the BMPs for dry farming in the region.

3.6 Composting

Farmers, in particular women, participated in the training program for the production of enriched compost using locally available crop residues of cotton (*Gossypium hirsutum* L.), pigeon pea, sugarcane (*Saccharum officinarum* L.), and raw pressmud obtained as a by-product from the nearby sugar refining factory. These materials were wetted and mixed with animal dung. Rock phosphate at 25 kg Mg^{-1} of the material, zinc sulfate at 2.5 kg Mg^{-1}, *Trichoderma viride* at 5.0 kg Mg^{-1}, and phosphobacteria at 200 g Mg^{-1} were added to the residues, mixed, and formed into heaps of 3 × 1.5 × 1 m dimensions. The moisture content of the heap was maintained at about 60% by sprinkling water periodically. The heap was turned over twice at 30 days and 45 days, and well-decomposed compost was ready for field application by 60 days after the start of the composting process.

The enriched compost was mixed with the appropriate inoculum of rhizobium or azospirillum (depending on the crop) and applied at the rate of 2.5 Mg ha^{-1}. Increase in crop yield by compost was 10% in pigeon pea, 58% in groundnut, 500% in onions (*Allium cepa* L.), and 12% in okra (*Abelmoschus esculentus* L. Moench). The strategy of using integrated nutrient management (INM) practices, including P-enriched compost, is important for these depleted and degraded Alfisols of southern India which have high phosphate fixation capacity. Farmers are now practicing the production of enriched compost as a component of BMPs for INM strategies.

3.7 Ridge-Furrow System and Tied Ridging

Ridges, furrows, and tied ridges were formed immediately after the first monsoon rains (Fig. 3). Four techniques of soil conservation and mulching and three of INM

Fig. 3 Tied ridges

were laid out in a strip plot design with cowpea as a test crop in 2002 and 2003 seasons. Total amount of rainfall received during winter 2002 and 2003 crop period was 162 mm and 132 mm with distribution of 7 rainy days in both years. Crop residue mulch was applied at the rate of 2.5 Mg ha^{-1} about 15 days after sowing. The crop yield figures and the derived resource indices are given in Tables 7 and 8.

3.8 Productivity

Productivity was assessed by computing several productivity indices such as production efficiency (PE) and economic efficiency (EE) by the following formula (Singh et al., 2005):

$$\mathrm{PE}\,(\%) = \left\{\left(Y^{\mathrm{IS}} - Y^{\mathrm{TS}}\right)/Y^{\mathrm{TS}}\right\} \times 100, \tag{1}$$

where Y^{IS} is the grain yield in intervention system and management and Y^{TS} is grain yield in traditional system and management.

$$\mathrm{EE}\,(\%) = \left\{\left(\mathrm{NR}^{\mathrm{IS}} - \mathrm{NR}^{\mathrm{TS}}\right)/\mathrm{NR}^{\mathrm{TS}}\right\} \times 100, \tag{2}$$

where, $\mathrm{NR}^{\mathrm{IS}}$ is net returns in intervention system and management and $\mathrm{NR}^{\mathrm{TS}}$ is net returns in traditional system and management. Energy efficiency was computed by evaluating the input and output of energy for each treatment (Dazhong and Pimentel, 1984). Rainfall use efficiency (RUE) was calculated by dividing the yield by total quantity of rainfall obtained during the cropping period. Solar radiation use efficiency (SRUE) for the cropping period was computed as per the procedure of Hayashi (1966) and expressed in g cal^{-1}

$$\text{SRUE} \quad = \quad \frac{\text{Dry matter production } \left(\text{gm}^{-2}\right) \times 4000}{\text{Total incident energy } \times 0.45 \left(\text{Cal m}^{-2}\right)}$$

The data in Table 3 show that tied ridges significantly improved cowpea performance through increasing soil water reserves in the root zone. In addition, mulching with groundnut crop residues reduced soil evaporation and increased crop yield. Among the INM treatments, application of enriched compost produced the highest yield. During the second year, tied ridges replaced the compartmental bunding treatment and ordinary compost application was replaced by application of 50% of inorganic fertilizer and 50% of enriched compost. Significantly more grain yield of cowpea was obtained under ridges and furrows with mulching (716 kg ha^{-1}) and tied ridges with mulching (297 kg ha^{-1}), during 2002 and 2003, respectively, over farmers' practice. Ridges and furrows with mulching had higher PE (25.11%), EE (13.2), RUE (4.4 kg ha^{-1} mm^{-1}), and SRUE (1.61 g cal^{-1}) during 2002 than other treatments. Tied ridges with mulching was superior to farmers' practice and ridges and furrows with mulching in PE (75.25%), EE (7.7), RUE (2.3 kg ha^{-1} mm^{-1}), and SRUE (0.74 g cal^{-1}) during 2003. Among nutrient management practices, application of enriched compost during 2002 and integration of 50% inorganic fertilizers and 50% enriched compost during 2003 produced significantly higher grain yield (731 kg ha^{-1} and 285 kg ha^{-1}, respectively) and at higher resource use efficiencies. Application of enriched compost improved PE (30.46% and 68.07%), EE (12.5 and 6.7), RUE (4.5 kg ha^{-1} mm^{-1} and 2.0 kg ha^{-1} mm^{-1}), and SRUE (1.67 g cal^{-1} and 0.69 g cal^{-1}) during 2002 and 2003, respectively, than inorganic fertilizers application and farmers' practice of no nutrient application. The advantage of tied ridges over farmer's practice on producing higher yield, even during seasons of sub-normal rainfall (2003), is significant (Ramesh and Devasenapathy, 2005, 2007a). Field observations during the cropping seasons showed (Ramesh and Devasenapathy, 2007a, b) that root growth (length, volume, and dry weight), and nodulation characteristics (numbers and dry weight) increased by these practices of soil moisture conservation (e.g., ridges and furrows, and tied ridges with mulching).

Similar results were obtained with pigeon pea crop experiment (Table 4). The highest yield of pigeon pea was obtained with tied ridges and mulching, among soil conservation treatments, and with application of enriched compost among the INM treatments. Soil moisture content (0–30 cm depth) was the highest throughout the growing season under tied ridges with mulching. Improved grain yield (476 kg ha^{-1}), PE (8.9 kg ha cm^{-1}), and EE (15.63) were obtained with tied ridges with mulching. In soil management treatments, application of enriched compost significantly influenced grain yield (471 kg ha^{-1}) and nutrient uptake. The highest net returns of Rs. 5,731/ha from pigeon pea + green gram intercropping was obtained with the BMPs comprising of tied ridges and mulching. In accordance with the increase in agronomic yields, improvements in the post-harvest available soil NPK and SOC concentration were also observed with mulching in ridges and furrows and tied-ridges treatments (Ramesh and Devasenapathy, 2007b). Selvaraju et al. (1999) reported that tied ridging and application of manures in combination with N and P fertilizer improved soil water storage and yield of crops compared to sowing on the flat bed in rainfed Alfisols and related soils of the semi-arid tropics.

Table 3 Effect of in situ rainwater harvesting on sustainable indicators of rainfed cowpea

Moisture conservation	Total biomass (kg ha^{-1})	Grain yield (kg ha^{-1})	Total factor productivity	Production efficiency (%)	Energy productivity (kg MJ^{-1})	Energy efficiency
Winter, 2002						
Farmer's practice	2652.5^{b}	572.2^{c}	0.127	–	0.77	12.60
Ridges & furrows (R&F)	2897.8^{ab}	662.8^{ab}	0.134	15.83	0.78	12.75
Compartmental bunding (CB)	2775.5^{b}	627.8^{b}	0.132	9.71	0.78	12.78
R&F + mulching	3008.8^{a}	715.9^{a}	0.143	25.11	0.81	13.20
CB + mulching	2917.3^{a}	688.9^{b}	0.143	20.39	0.81	13.18
Winter, 2003						
Farmer's practice	956.3^{c}	169.7^{c}	0.066	–	0.37	5.96
Ridges & furrows (R&F)	1117.0^{b}	208.5^{b}	0.072	22.86	0.40	6.53
Tied ridge (TR)	1151.8^{b}	223.2^{b}	0.072	31.52	0.40	6.58
R&F + mulching	1345.2^{a}	279.0^{a}	0.088	64.40	0.46	7.48
TR + mulching	1406.6^{a}	297.4^{a}	0.089	75.25	0.47	7.70

Means within column followed by the same letter are not significantly different (l.s.d at $P = 0.05$).
[a,b,c] Statistical difference among treatment means.

Table 4 Effect of land and nutrient management on pigeon pea yield and efficiency indices (2003)

Treatments	Grain productivity (kg ha^{-1})	Water productivity (kg $ha^{-1}cm^{-1}$)	Energy efficiency
Moisture (M)			
Farmers' practice	292.3	5.5	11.38
Ridges & furrows (R&F)	353.6	6.6	12.23
Tied ridges (TR)	393.7	7.4	13.45
R&F + mulching	454.5	8.5	15.18
TR + mulching	475.4	8.9	15.63
CD (P=0.05)	42.6		
Nutrient (N)			
Farmers' practice	271.2	5.1	14.0
Rec. NPK	391.2	7.4	12.5
50% NPK + 50% enriched compost	442.0	8.3	13.6
Enriched compost	471.2	8.9	14.2
CD (P=0.05)	21.0		

3.9 Low-Cost Gravitational Drip Irrigation System

A ground-level zero-energy drip irrigation system was installed in a farmer's field at Ariyamuthupatti and Kannivadi sites. The system involved installation of a PVC tank of 800 l capacity at an elevation of about 1 m. The tank was connected with a drip irrigation system. Drip line was laid in alternate rows of the crop, with 1 lph dripper spaced at 30 cm. The tank was filled from the nearby borewell once a day and tomatoes were transplanted during the dry season. Yield of fresh tomatoes of 23.2 Mg ha^{-1} was obtained with 73.8% saving in water.

At Kannivadi site, the plots receiving drip irrigation yielded 15.2 Mg green chillies ha^{-1}, (*Capsicum annum* L.) compared with the production of 9.4 Mg ha^{-1} for plots with surface irrigation, an increase of 61%. The green chillies from the drip irrigation plots were larger, longer, and shinier than those from surface-irrigated treatments, and fetched a higher price in the market.

3.10 Arid Horticulture

Adopting rainfed horticulture is an important strategy for providing another income stream for farmers. In cooperation with the state-funded wasteland development project, seedlings of amla (*Phyllanthus emblica* L.), sapota (*Manilkara zapota* L.), and mango (*Mangifera indica* L.) were planted on 35 acres (14 ha) of uncultivated wasteland in the village. The farmers were advised to use pitcher pot irrigation and mulching with coconut husk and crop residues around the saplings for initial establishment and better growth of the seedlings during the summer period. This has been a successful undertaking, and a popular practice with the community.

3.11 Soil Health Card

A soil testing campaign was organized in the project area with the assistance of soil testing laboratory, Kudumianmalai. The services of the mobile soil testing laboratory were also availed. Based on the soil test analysis of individual farms, "soil health cards" were printed and distributed to the farmers. Specific training sessions were organized for the method of soil sample collection, soil test interpretation, and the use of soil health card. Participating farmers appreciated the usefulness of these cards for periodic monitoring of soil health with the adoption of BMPs and recommended cropping systems.

3.12 Social Mobilization and Income-Generating Activities

Self-help groups were formedin order to create other livelihood opportunities and to generate off-season and off-farm income. The women self-help group formed comprised of Mangayi Amman Self Help Group, with 16 founding members, and Akilandeswari Self Help Group, with 12 founding members. Members were invited to the MSSRF centers at Kannivadi and Pondicherry to observe the functioning of the self-help groups in these centers and to interact with them in starting micro-finance-assisted micro-enterprise. The self-help group members mastered the technique of producing *Trichogramma* parasitoid production and supplied the cards to sugarcane cultivators, through a buy-back arrangement with the EID Parry Sugar Factory, located at Aranthangi, 30 km from project site. This arrangement was extremely effective in the biological control of sugarcane internode borer pest. This micro-enterprise and preparation of enriched compost has considerably improved the employment and income-generating capacity of the participating women.

4 Semi-Arid Black Soils (Vertisols) of Central India

Vertisols (black soils) occupy 7% of the arable lands in the semi-arid tropical region, and cover large areas under dry farming in Central and South India. Vertisols are derived from base-rich rocks (basalt) or the related colluvium or alluvium parent materials. These soils have a high clay content, and vary in depth (15–200 cm), clay content (30–70%), and cracking patterns, and are also highly prone to sheet erosion. Surface runoff varies from 10% to 40%, and increases with increase in rainfall. Heavy texture and waterlogging make it difficult for early and rapid seedbed preparation. Important soil groups in the region are Chromusterts and Pellusterts (Murthy et al., 1981; Swindale, 1982; Venkateswarlu, 1987).

In Central India, Vertisols have a large potential of increasing agricultural production provided appropriate technologies for conservation and management of natural resources, particularly soil and rainwater management, are widely implemented. Upland crops grown on these soils in high rainfall areas (>1,000 mm), mainly soybean (*Glycine max*) and maize (*Zea mays*), are prone to temporary waterlogging and anaerobiosis.

In India about 18 Mha of Vertisols (12 Mha in MP) are left fallow during the rainy/monsoon season. Therefore, these soils are cropped only during the post-rainy season on profile-stored soil moisture. The rainy season fallowing, locally called as "Haveli system" of cultivation, leaves the land unutilized and prone to severe erosion and runoff. The low water infiltration rate (3–5 mm h^{-1}) is attributed to high clay content (>40%) containing predominantly expanding-type clay minerals. Through adoption of BMPs, there is a vast scope to improve utilization of soil and water resources in the region for intensification of these inherently fertile soils. A community-based field operational research project was implemented with the specific objectives to (1) promote adoption of BMPs for rainfed and irrigated agriculture in Central India through demonstration of soil and water conservation measures and improved agronomic practices, (2) demonstrate the usefulness of INM practices, and (3) provide training opportunities to researchers, extension workers, and farming communities.

4.1 Ecoregional Characteristics of the Sites

On-farm demonstrations were established on two representative series of Vertisols in Narsinghpur and Hoshangabad districts of MP state (MSSRF, 2006). These two districts come under 10.1 (Malwa Plateau, Vindhyan and Narmada Valley) hot, dry sub-humid eco-sub region (Velayutham et al., 1999). The sub-region is characterized by sub-humid climate with dry summers, mild winters, and ustic soil moisture regime. Temperature ranges from 31°C to 40 in summer and 9–19°C in winter. Annual rainfall ranges from 1,000 mm to 1,500 mm and length of the growing period from 150 days to 180 days. Soils of the demonstration sites were shallow to moderately deep, moderately well drained, slowly permeable, clayey Vertic Ustochrepts and Ustorthents in the gently sloping uplands, and very deep, clayey Chromusterts in the nearly level uplands (Tamgadge et al., 1999).

The water table (WT) was below 10 m at Narsinghpur, and at 5–10 m depth at the Hoshangabad sites. Groundwater was the source of irrigation at the Narsinghpur site, whereas canal irrigation from Tawa Command was used at the Hoshangabad site. The WT's declining at the Narsinghpur site at the rate of 15–20 cm yr^{-1}, whereas the WT in "B" zone of Tawa Command at Hoshangabad is increasing at the rate of 20 cm yr^{-1}. Both areas need judicious use of available water resources for sustaining high agricultural productivity. The characteristics of the soil at the two sites are given in Tables 12 and 13. Total SOC pool to 120 cm depth was 89.6 Mg ha^{-1} for the Narsinghpur site and 74.2 Mg $ha^{-1}$for the Hoshangabad site. The clay content ranged from 42.1% to 46.1% for the Narsinghpur site and 43.5% to 50.0% for the Hoshangabad site. Both soils had a high water holding capacity at 33 K Pa suction or field moisture capacity (30–40% by weight). Total AWC to 120 cm depth (computed on volumetric basis for the specific depths) was 27.5 cm for the Narsinghpur site and 25.8 cm for the Hoshangabad site.

These soils are characterized by typical morphological features called "slicken sides", which are caused by the presence of swell-shrink minerals like smectites. Vertisols are difficult to plough during the rainy season, and need proper land configuration and soil conservation measures for effective irrigation and crop production.

Field demonstrations were established on ten farms in three villages (viz., Dangidhana, Bagpodi, and Murlipodi) in Narsinghpur district, and expanded to 20 farms later. Similarly ten farmers' fields were selected in three villages (viz., Mongwari, Bhairakhedi, Dolariya) in Hoshangabad district, and expanded to 40 farms later during the second and third years.

4.2 Land Forming For Soil and Water Conservation

The raised/sunken bed system (RSBS) of land treatment was established on farmer's fields. The system consists of an array of raised and sunken beds of 8-m width, with elevation difference of 30 cm. The system is created by mechanically moving soil from demarcated 6-m-wide strips, designated as sunken beds. Beds thus created are tied across with small earthen bunds of about 10 cm height at 20 m interval to ensure uniform spread of runoff in sunken beds. The runoff from raised beds is diverted and captured in the adjacent sunken beds. The RSBS (Fig. 8) facilitates drainage for growing upland crops, encourages in situ rainwater conservation, and minimizes soil erosion and nutrient losses.

During the winter season, grain yields of wheat sown in sunken bed and chickpea in raised bed were compared with those from the flat bed system (FBS) of cultivation. During the summer monsoon season, soybean (*Glycine max* L.) was sown in the raised bed and rice in the sunken bed. The results given in Table 14 indicate the beneficial effects of RSBS over the FBS on the yield of wheat and chickpea. The RSBS, once formed, stabilizes over time (cropping cycles) and increases the benefit:cost (B:C) ratio over the FBS.

Two land configuration treatments, namely, ridge-furrow system (RFS) and broad bed and furrow system (BBFS), were compared with the FBS of planting soybean. The ridges were formed 15–20 cm high on 0.5% grade. The BBFS involved erection of 150-cm wide and 15-cm high raised beds on 0.4% grade. The beds were separated by 50 cm wide furrows that drained into grassed waterways. Increase in soybean yield was about 100% with RFS and 55% with BBFS compared with the farmers' practice. Selvaraju et al. (1999) reported that BBF and compartmental bunding land configuration could be adopted on Vertisols for better water conservation and crop productivity.

4.3 Integrated Plant Nutrient Management Practices (INMP)

Eight trials were conducted at the Narsinghpur site to demonstrate the effectiveness of integrated nutrient management practices (INMP) for wheat and chickpea (*Cicer arietinum*). The results given in Table 15 indicate that INMP treatment produced the maximum yield in comparison to the general recommended fertilizer alone. With INMP treatment, an additional income of Rs. 6,978 per ha and Rs. 1,1976 per ha was obtained for wheat and chickpea, respectively, over the farmers' practice. Similar results were obtained with soybean trials established on 15 locations at the Narsinhgpur site. The highest soybean yield (2.58 Mg ha^{-1}) was obtained under INMP + 4 Mg ha^{-1} application of farmyard manure (FYM), which generated an additional income of Rs. 6,847 ha^{-1} compared to the farmers' practice.

4.4 Hoshangabad Site

Ten demonstration trials were conducted at the Hoshangabad site to assess the BMPs for wheat var. DL-788-2 in comparison to farmers' practice. The high yield (4.0 Mg ha^{-1}) was obtained with adoptions of BMPs as compared to 1.4 Mg ha^{-1} obtained under farmers' practice. Similar results were obtained with soybean, with the doubling of yield by the adoption of INMP.

4.5 Intercropping of Soybean

One of the improved agronomic practices in soybean cultivation is intercropping of soybean with pigeon pea. The results of demonstrations conducted in a farmer's field (Table 16) indicate that this system is compatible in 4:2 ratio (four lines of soybean and two lines of pigeon pea) and produced higher net return (Rs. 27,620 ha^{-1}) and B:C ratio (3.3 : 1) over either of the two monocultures.

4.6 Water Management in Rice

One of the improved agronomic practices in rice cultivation is the water management or the frequency of flooding. In general, farmers continuously flood rice fields for the entire growing period. Through irrigation at critical stages of the crop rather than continuous flooding, there is scope for water saving without causing yield reduction. Thus, a demonstration experiment on the effect of irrigation management on rice was established. The data showed that with three irrigations at critical stages, rice yields were (3.8 Mg ha^{-1}) equivalent to that of the continuous flooding (3.5 Mg ha^{-1}). This demonstration has created awareness among farmers in the region about the benefits of water conservation technology for growing rice.

4.7 Aqua-Agriculture

The "Haveli system" of cultivation of vertisols in Central India entails ponding the rainwater in bunded fields during the rainy season (to minimize losses by runoff and erosion) and growing wheat or chickpea in the post-rainy season on stored profile moisture. The available rainwater is not effectively utilized, and the land is left fallow. Raising fish during the rainy season in these fields and growing crops in the post-rainy season can augment farmers' income and enhance employment throughout the year.

A 2-ha field was selected for demonstration and three species of fish (i.e., Mrigal, Rohu, and Katla) were introduced, according to the standard management practices. Wheat and chickpea were sown in the same field after fish harvest in October. The results of this demonstration (Table 17) showed that fish-wheat or chickpea rotation in the field increased the net returns considerably more than fallowing during the rainy season and cropping only during the winter. The data highlights the feasibility and economic advantage of adopting aqua-agriculture in the vast Vertisol region of MP. The demonstration trials of this kind conducted in farmers' fields provided excellent loci for dissemination of this important technology.

4.8 *Optimizing Nutrient and Water Management in Soybean–Wheat System*

Two field experiments to maximize the interactive effect of water and nutrient resources were conducted on soybean-wheat cropping systems under varying fertility and irrigation levels. Three moisture regimes (i.e., irrigation at seeding/transplanting (I_0), moderate moisture regime (I_1) and optimum moisture regime (I_2)) in combination with nine nutrient sources (S_1–S_9) were evaluated. The irrigation schedule for the three treatments for the two crops was as follows:

Irrigation	I_0	I_1	I_2
Soybean (Monsoon)	No irrigation	Irrigation at 60 DAS	Irrigation as per need
Wheat (Winter)	Come-up irrigation + 21 DAS	Irrigation at 21 and 45 DAS	Irrigation at 21, 45, 75, and 90 DAS

DAS = Days after sowing.

The nine nutrient levels were: 100% NPKS optimum dose as per soil test (S_1); 75% of optimal dose + 5 Mg ha^{-1} of FYM (S_2); 50% of optimal dose + 5 Mg FYM ha^{-1} (S_3); S_2 + rhizobium/Azotobacter 1.5 kg ha^{-1} (S_4); S_2 + phosphate solubilizing bacteria (PSB) @ 1.5 kg ha^{-1} (S_5); S_2 + PSB + rhizobium (S_6); S_6 + Zn @ 10 kg zinc sulfate ha^{-1} (S_7); S_6 + Mo as ammonium molybdate @ 0.5 kg ha^{-1} (S_8), and S_6 + Zn + Mo (S_9).

The highest yields of soybean (2.4 Mg ha^{-1}) and wheat (4.4 Mg ha^{-1}) were obtained under I_2 moisture regime. In the case of nutrient sources, grain yield was significantly higher in S_9 than other treatments. The treatment combination I_2 S_9

(optimum moisture level and integrated nutrient sources) was the best for grain yield of both crops.

4.9 The "Seed Village" Model

The availability of good-quality seeds of high-yielding varieties (HYV) of crops is a major constraint among farmers' to realizing the full benefits of HYV technology. The use of harvested grains as seed, in successive seasons, reduces the seed vigor and crop yield. Mobilizing farmers in a village as "seed farmers" to multiply the breeder's seed and ensure seed replacement by all farmers in the village provides the best means of quality seed delivery system at the local level. The project staff assisted this process in the selected villages and the impact of seed replacement and the increase in average yield in the villages are given in Tables 18 and 19, respectively. In 3 years, the production and distribution of HYV of crops increased the average yield of soybean, wheat, and chickpea by 50%, 30–50%, and 50%, respectively. Such an approach is important to achieving self-sufficiency in quality seed production and distribution at the local level.

5 Alluvial Soils of the Indo-Gangetic Plains

The Indo-Gangetic fluvial Plains (IGP) of India extend from 21° 45′ to 31° 0′ N latitudes and 74° 15′ to 91° 30′ E longitudes, and include the states of Punjab, Haryana, Delhi, Uttarpradesh, Bihar, West Bengal, Himachal Pradesh, northern parts of Rajasthan, and Tripura. The IGP cover a total area of 43.7 Mha. Velayutham et al. (2002) have discussed the historical perspective and pedogenesis of the soils of the IGP.

Punjab state, situated in the western part of the IGP, comprises an area of 5 Mha and contributes annually to more than 60% of wheat (*Triticum aestivum*) and 45% of rice (*Oryza sativa*) to the national food reserves. The rice-wheat system (RWS), being highly profitable, has been adopted even in non-traditional areas. In Punjab from 1987 to 2007, the area under rice cultivation increased by more than ten times and under wheat by 2.5 times. The RWS in Punjab is irrigated, and is an intensive input-driven and a soil-exhaustive cropping system. Between 1960/1961 and 2001/2003, irrigated land area increased from 59% to 95%, cropping intensity from 118% to 186%, and food grain production from 3.2 Mt to 24.8 Mt (Statistical Abstracts, Govt. of Punjab).

Irrigation has led to excessive withdrawal of groundwater in most of Punjab. There is a decline in WT in the central districts and a rising trend in the south-western districts of the state. Further, there is a declining trend in soil fertility and a rising trend in salinity and degradation of irrigation water quality leading to the fatigue of the RWS (Sinha et al., 1998). The problems of declining soil fertility are exacerbated by improper management of crop residues. Burning of the rice straw, widely practiced in Punjab, leads to the loss of nutrients, environmental problems of air pollution, and health hazards.

5.1 Ecoregional Characteristics of the Demonstration Sites

The field demonstrations/experiments were conducted in farmers' fields in four locations (Barbha, Sajawalpur, Rakkaran Dhahan, and Baghauraan) in Nawashahr district, and four locations (Kothae Maur, Sadiq, Sandhwan, and Nathewala) in Faridkot district. The Farm Science Centre at Langroya in Nawashahr district and the Regional Agriculture Research Station and the Farm Science Centre at Faridkot provided the technical support. Specific objectives of the project were: (1) demonstration and adoption of techniques for efficient management of soil, water, and crop residues, (2) identification of cropping systems as viable alternatives to RWS which alleviate the pressure on groundwater resources, (3) assessment of agronomic productivity of diversified production systems, and (4) creation of training opportunities for researchers, extension staff, and farming communities.

Nawashahr district is situated in the hot, dry sub-humid climatic zone at 31° 32′ N latitude and 75° 54′ E longitude and forms part of northern flat plain with alluvium-derived soils. It is a part of the 9.1 agro-eco sub-region with growing length of 120–180 days (Velayutham et al., 1999). The mean winter and summer temperatures are 14.2°C and 34.4°C, respectively, with mean annual temperature of 23.5°C. The average annual rainfall is 850 mm. The soils are classified into three sub-groups, namely Typic Haplustalf, Udic Ustochrept, and fluventic Ustochrept (Sidhu et al., 1995). Soils are characterized by an alkaline pH, low EC, and low SOC concentration (Table 5). The RWS is the dominant cropping system in this district, 82% of the area is irrigated by tube wells, and the WT is falling rapidly (Fig. 4).

Faridkot district is situated in the semi-arid climatic zone at 30° 40′ N latitude and 75° 45′ E longitude and forms part of northern alluvial plain with sand dunes. It is a part of the 4.1 agro-eco sub-region, with growing season length of 90–150 days. The mean winter and summer temperatures are 13.9°C and 32.1°C, respectively, with a mean annual temperature of 24°C. The average annual rainfall in the district is 427 mm (Velayutham et al., 1999). The soils of the experimental site are very deep,

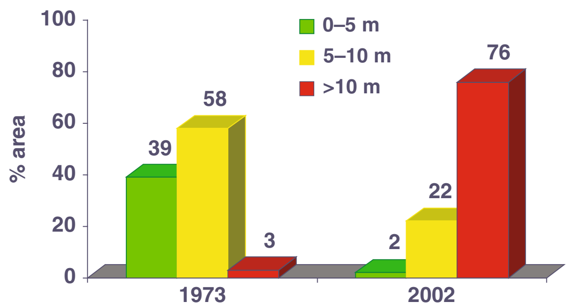

Fig. 4 Percent area under a range of water table depths (m) in Central zone of Punjab

Table 5 General properties of soils at two experimental sites in alluvial soils of Punjab

Soil characteristics	Langroya	Faridkot
Texture	Loam	Sandy loam
pH	8.0 – 8.7	8.5 – 9.4
E.C. (ds m^{-1})	0.2 – 0.3	0.2 – 0.4
Organic carbon (%)	0.4 – 0.6	0.2 – 0.6
Available P (Kg ha^{-1})	6.8 – 13.5	9.2 – 24.8
Available K (Kg ha^{-1})	202 – 397	405 – 562
$CaCO_3$ (%)	0.35 – 1.0	0.7 – 1.2
Extractable calcium ($\mu g\ g^{-1}$)	613 – 754	557 – 681
Extractable magnesium ($\mu g\ g^{-1}$)	351 – 584	97 – 289
Extractable sodium ($\mu g\ g^{-1}$)	18 – 70	41 – 162
DTPA-extractable Zn ($\mu g\ g^{-1}$)	0.4 – 0.8	0.4 – 0.6
DTPA-extractable Fe ($\mu g\ g^{-1}$)	3.1 – 9.8	2.0 – 5.2
DTPA-extractable Mn ($\mu g\ g^{-1}$)	1.7 – 4.1	0.9 – 2.5
DTPA-extractable Cu ($\mu g\ g^{-1}$)	0.5 – 1.4	0.3 – 1.0

well drained, and developed on reworked sand dunes. Theses soils are classified as Ustipsamments, have alkaline pH, and are calcareous. The major source of irrigation is the groundwater (84%). Both RWS and cotton (*Gossypium hirsutum*)-wheat system (CWS) are the dominant crop rotations in the district. Soil characteristics of the two locations, one in each district, are given in Table 5. The chemical properties, organic carbon, available soil nutrients, bulk density, and infiltration rate were determined as per the procedures given in "Methods of Soil Analysis" (SSSA, 2002).

5.2 Alternatives to Rice-Wheat Cropping System

Three cropping systems tested at Langroya site were RWS, maize (*Zea mays* L.)-wheat system (MWS), and maize-field mustard (*Brassica* rapa L.)-rapeseed (*Brassica napus* L.) system (MTRS). The initial soil analysis showed that the SOC concentration ranged from 0.46% to 0.60%, available P from low to medium, and available K from medium to high. Similarly, three cropping systems tested at Faridkot site were RWS, CWS, and cotton-chickpea (*Cicer arietinum*) system (CCS). The SOC concentration at these sites were much lower, (e.g., 0.21%–0.31%), available P from medium to high, and available K was relatively high.

All crops in diverse rotation systems were grown with the standard package of agronomic practices. The yields of crops in the different crop seasons show that the average yield of wheat (second crop) at Langroya was similar for the RWS (4,009 kg ha^{-1}) and the MWS (4,362 kg ha^{-1}). Similar wheat yield in both systems indicates that the lead time available for land preparation for sowing wheat, unless no-till (NT) is practiced, is more following maize than rice cultivation. The average yield of maize was also similar in MWS (4,455 kg ha^{-1}) and MTRS (4,470 kg ha^{-1}).

Comparative economics of diverse cropping systems was assessed for the participating farms. The highest returns were obtained with input of fertilizers followed by that for irrigation. The RWS was the most economic system at Langroya, probably due to the minimum support price offered by the government for procurement of rice and wheat immediately after harvest. However, MWS and MTRS were also profitable cropping systems. Joginder Singh and Hossain (2003) reported the decelerating trend in TFP growth for rice in high-production RWS of North India. In view of the receding WT, rotations substituting maize for rice during the crop cycles in the monsoon season is a viable option that merits attention. At Faridkot, the economic returns from CWS were as good as those for RWS. However, cultivation of cotton is not economical due to heavy dependence on pesticides. In this regard, the use of B_t cotton may be a viable option. The lowest economic returns were obtained from the rice-based system.

A corroborative sample survey was undertaken on selected farms in the project area. The data show that the benefit:cost ratio (BCR) was more favorable for wheat under the present price structure (Table 6). Average return was the highest for irrigation, followed by that for fertilizers.

Other indicators of economic assessment (e.g., GR, VE, ROVE) were higher for the RWS in Nawashahr district and for the CWS in Faridkot district compared with all other cropping systems. In Faridkot district, therefore, the adoption of CWS ensures an economic use of groundwater resource compared to the RWS. Thus, CWS has replaced RWS in most of the districts. The area under cotton in the operational villages (Sadiq and Kothae Maur) increased from 80 ha and 20 ha, respectively, in 2002 to 200 ha and 144 ha in 2004, respectively. Similarly, the area under cotton in the Faridkot district increased from 16,000 ha in 2002 to 30,000 ha in 2004.

Table 6 Comparative economics of alternate cropping systems in sample farms (Rs. ha^{-1})

District	Rotation	GR	VE	ROVE	B:C ratio
Nawashahr	Rice-wheat	62,865	31,365	31,500	2.00
	Maize-wheat	45,005	24,093	20,912	1.87
Faridkot	Rice-wheat	64,590	32,765	31,822	1.97
	Cotton-wheat	63,200	30,858	32,342	2.05

GR – Gross returns; VE – Variable expenses; ROVE – Return over variable expenses; B:C Ratio – Benefit–cost ratio.

5.3 Residue Management in Rice–Wheat Cropping System

With the large-scale adoption of RWS in Punjab, the management of rice straw and seedbed preparation for timely sowing of wheat are principal agronomic challenges that must be addressed. Because of its low nutritional value as a fodder for livestock, rice straw is commonly burnt. Logistically, it is an easier option in the combine-harvested rice fields. However, burning of rice straw has adverse impact on soil quality, especially on SOC concentration and nutrient cycling. Rather than burning it, NT sowing of wheat through the mulch of rice straw can be agronomically viable

Fig. 5 Wheat sown under no tillage

and economically profitable (Fig. 5). Therefore, demonstrations on residue management were established at three locations in Langroya (Barbha, Sajawalpur, KVK farm) and at two locations (Sadiq and Kothae Maur) in the Faridkot sites.

Comparing the data on initial and final soil analyses (Table 7) show management-induced changes in SOC concentration even over a short period. The SOC concentration was affected by residue management after three crops at Nawashahr. It increased from an average initial level of 0.44% to 0.50% when both rice and wheat straw were burnt, 0.51% when rice straw was incorporated but wheat straw was removed, and 0.57% when both rice and wheat straw were incorporated. The increase in SOC concentration due to incorporation of both rice and wheat straw was statistically significant. Although a similar trend was observed at Faridkot location, the differences in SOC concentration were statistically not significant.

The effects of these residue management treatments on the yield of second crop of wheat (third crop in the rotation) are given in Table 8. The wheat yield at Langroya in plots where rice and wheat straw were burnt was somewhat more (5.1 Mg ha^{-1}) than that where the straw was incorporated (4.4–4.6 Mg ha^{-1}). Even with an increase of about 13%, the difference in yield is statistically not significant. However, incorporation of both straws at Faridkot sites was more beneficial than burning. In the latter case, the soils are light-textured (sandy loam) as compared to the loamy soil at the Langroya site. Thus, yield response to residue management seemingly depends on soil texture and other properties (e.g., moisture retention).

Table 7 Soil organic carbon as affected by residue management (winter 2002–2003)

	SOC (%)					
	Nawashahr				Faridkot	
Treatment	Barbha	Sajawalpur	KVK farm	Mean	Sadiq	Kothae Maur
Initial	0.51	0.48	0.33	0.44^{b}	0.33	0.45
Burning of both rice and wheat straw	0.53*	0.47	0.35		0.32	0.45
	0.49**	0.47	0.30		0.28	0.45
	0.60***	0.51	0.40	0.50^{ab}	0.27	0.54
Incorporation of rice straw in wheat wheat straw removed	0.54*	0.54	0.30		–	–
	0.54**	0.54	0.38		–	–
	0.58***	0.51	0.44	0.51^{ab}	–	–
Incorporation of rice and wheat straw + GM	0.52*	0.51	0.39		0.41	0.56
	0.47**	0.51	0.42		0.36	0.55
	0.61***	0.57	0.53	0.57^{a}	0.42	0.57

*After winter 2001–2002, **After monsoon 2002, ***After winter 2002–2003GM – green manure
Means within column followed by the same letter are not significantly different (L.S.D. at *P* 10%).
[a,b,c] Statistical difference among treatment means.

Table 8 Grain yield of wheat (winter 2002–2003) at different sites in residue management experiments

	Grain Yield (Kg/ha)				
	Nawashahr			Faridkot	
Treatment	Barbha	Sajawalpur	KVK farm	Sadiq	Kothae Maur
Burning of both rice and wheat straw	5780	5243	4573	4787	5350
Rice straw incorporation and wheat straw removed	5723	4328	3923	–	–
Incorporation of rice and wheat straw + GM	5165	4348	3790	5017	5512*

*Without green manure

Experiments conducted in farmers' fields in both districts also indicated similar trends in crop yields. The data analyzed to assess comparative economics of residue incorporation in the RWS over 3 years showed that since the GR as Rs ha^{-1} were the same in straw-burnt and straw-incorporated treatments, the improvements in soil quality that the straw incorporation brings about in the long-term may offset the apparent advantage of burning straw giving the higher BCR in the short-term. This is an important management issue brought about by the changing land use scenarios in all agro-ecoregions of the IGP (Velayutham, 1994; Abrol and Gupta, 1998; Velayutham et al., 2000).

5.4 Residue Management and Soil Organic Carbon Sequestration

The positive effects of incorporation of straw in Faridkot sites are also corroborated by the attendant improvements in soil physical properties (Table 9). There was a slight increase in SOC concentration in straw-incorporated plots. With straw incorporation, the SOC pool increased by 0.2 Mg ha^{-1} in 0–15 cm depth for the Nawashahr site. In comparison, increase in SOC pool by straw incorporation was by 1.0 Mg ha^{-1} for the Faridkot site (Table 9). Soil bulk density decreased and there was a perceptible increase in water infiltration rate in soils. These results demonstrate the positive effects of straw incorporation in light-textured soils on improving the soil quality (Lal, 1995).

Swarup et al. (2000) estimated that there was 59% depletion of OSC concentration of cultivated compared with that in undisturbed soils in the IGP during 1960–2000 period. Bhattacharyya et al. (2007) modeled SOC pools and changes in the IGP, India, from 1990 to 2030, using output of the GEFSOC modeling system. The predicted average SOC pool change rate in agro-ecological sub-region 4.1, which includes Faridkot district, by rice-wheat-clover rotation is 0.076 Tg yr^{-1}, 0.025 Tg yr^{-1}, and 0.157 Tg yr^{-1} for 1990, 2000, and 2030, respectively. In contrast, for the RWS the corresponding values are −0.740 Tg, −0.636 Tg, and 0.260 Tg respectively. This trend shows the importance of reviving clover (*Trifolium alexandrinum*) in RWS to enhance SOC sequestration. Lal (2004) described the practices and their potential of SOC sequestration in soils of India.

Table 9 Effect of residue incorporation on soil organic carbon (%), SOC pool (Mg ha^{-1}), bulk density (Mg m^{-3}), and infiltration rate (mm/hr) – 2003–2004

	Nawashahr				Faridkot			
Treatment	SOC	BD	SOC Pool	IR	SOC	BD	SOC Pool	IR
Burning of rice and wheat straw	0.52	1.53	11.9	3.5	0.45	1.65	11.1	6.1
Rice straw incorporated & wheat straw removed	0.53	1.50	11.9	3.5	0.42	1.60	10.1	6.7
Rice & wheat straw + GM incorporated	0.57	1.42	12.1	3.1	0.51	1.58	12.1	7.6

SOC = Soil organic carbon
BD = Bulk density
IR = Infiltration rate
SOC pool (0–15 cm depth)
GM = Green manure.

5.5 More Income Per Drop of Water

In view of the rapidly receding WT, and the need for popularizing efficient on-farm water management, the project staff assisted Mr. S. Kuldeep Singh, a progressive farmer at Kotkapura, in Faridkot district, in installing and demonstrating a drip irrigation system and using fertigation for growing vegetables all through the year

Fig. 6 Drip irrigation in vegetables

in his farm (Fig. 6). The soil of the farm is sandy loam with pH of 8.6, E.C. of 0.2 ds m^{-1}, $CaCO_3$of 1.7%, and SOC concentration of 0.39%. The available P status is low to medium (15.3 kg P ha^{-1}) and that of available K is high (566 kg K ha^{-1}). Irrigation with nutrients (fertigation) was used at the drip discharge rate of 2.0 l hr^{-1} for only a few minutes ¯per day. The yield of tomato (*Lycopersicon esculentum* L.) was 52.5 Mg ha^{-1} and that of bittergourd (*Momordica charantia* L.) was 8.75 Mg ha^{-1}. The quality of produce in both crops was better and fetched higher prices in the market. This farm is now a focal point for dissemination of the fertigation technology to the farming community in the region (MSSRF, 2006).

5.6 Agricultural Diversification

5.6.1 Agroforestry

Only 5.6% of the land area in Punjab is under forests, compared with >84% under cultivation. It is thus logical to popularize agroforestry systems. Poplar (*Populus deltoids*) is among the fast-growing winter trees that can grow well in association with field crops (Fig. 7). Being a deciduous tree, large amount of organic matter and nutrients can be recycled through leaf litter added to the soil.

Two demonstration sites were established to assess the feasibility of intercropping with poplar. Maize as monsoon crop and wheat, mung bean (*Vigna radiata* L.) as winter crops were intercropped with poplar. There was no deleterious shade effect of poplar trees on the yield of either of these crops (Table 10). The leaf samples of poplar trees were collected during the autumn and analyzed for nutrient contents.

Fig. 7 Turmeric and ginger as intercrops with poplar

Fig. 8 Raised bed/sunken bed system. Soybean–rice system

Table 10 Yield of intercrops in poplar (*Populus deltoids*)

	Grain yield (Kg ha^{-1})	
	Baghauran, Langroya	Khara, Faridkot
Crop Rotation	Wheat/Maize	Wheat/Moong
Poplar + wheat/maize	4463/1150	–
Poplar + wheat/moong	–	4625/217

Nitrogen concentration ranged from 1.7% to 1.9%, P from 0.08% to 0.09%, K from 0.26% to 0.28%, S from 0.12% to 0.14% Ca from 2% to 2.5%, and Zn from 4.2 ppm to 4.7 ppm. Thus a large amount of nutrients are recycled. In addition, poplar generates another income stream, from the same unit area under the agroforestry system. It also leads to SOC sequestration in these calcareous sodic soils of the IGP when practiced as a cyclic agroforestry intervention (Gupta and Rao, 1994).

5.6.2 Aquaculture

Towards an attempt at diversification of agriculture in Punjab, a poorly drained agricultural field with impounded water was used for aquaculture. On-farm demonstrations in the farm of S. Guraharan Singh were established on 0.4 ha (1 acre)-size pond at Nathawala Nawan in Faridkot district. The fish feed consisted of FYM 0.48 Mg, poultry manure 0.24 Mg, single superphosphate 15 kg, deoiled rice bran 8.8 kg, deoiled mustard cake (*Brassica juncea*) 8.8 kg, soya meal 6 kg, mineral mixture 240 g, and salt 80 g. Four species of fish were grown in the pond. Fish production (Table 11) proved successful in 3 years, and was an economically viable intervention in regions where water is available in adequate quantities. This is an important diversification to the traditional cropping system.

Table 11 Fish aquaculture on a 0.4 ha pond in Faridkot district
Farmer: S. Gurcharan Singh Village: Nathawala Nawan District: Faridkot

		Average yield (Mg ha^{-1}) of four species		
Species	Weight at maturity	2002	2003	2004
Catla catla (Catla)	1.0 kg			
Labeo rohita (Rohu)	600–800 g			
Cirrhina mrigala (Mirgal)	700 g	2.34	2.75	2.49
Cyrpinus carpio (Common carp)	600–800 g			

5.6.3 Sustainable Management of Vertisols

Vertisols are an important cropland soil in central India. These soils have a high clay content, low organic matter reserves, high water retention capacity, but low water infiltration rate (Tables 12, 13). On-farm experiments have been conducted at two locations in central India, Narsinghpur and Hashangabad in MP. Among soil-related constraints to production, lack of micro-nutrients and low available water capacity are major determinants of crop yield. Growing crops on raised-sunken beds had relatively less beneficial impact on crop yields (Table 14, 15) than the application of INMP. Agronomic productivity and profitability were more with soybean-pigeon pea intercropping than with monoculture (Table 16). Because of low infiltration rate and inundation during the monsoon season, raising fish during the rainy season followed by wheat-gram rotation is an economically viable and sustainable use of these soils (Table 17). Another income stream for the small-land holders of Vertisols is through multiplication of breeder seed (Table 18, 19).

Table 12 Properties of a Vertisol at Narsinghpur site, MP state, India

	Soil depth (cm)				
Soil properties	0–15	15–30	30–60	60–90	90–120
Sand (%)	17.6	17.1	16.1	15.8	15.0
Silt (%)	39.9	40.3	40.7	40.3	38.9
Clay (%)	42.5	42.6	43.2	43.9	46.1
Bulk density (Mg m^{-3})	1.40	1.42	1.48	1.50	1.50
pH (1:2.5)	7.1	7.1	7.4	7.5	7.7
E.C. (1:2.5)(dSm^{-1})	0.40	0.41	0.39	0.38	0.38
Water retention (%)					
33 kPa	32.4	33.0	33.2	33.5	33.5
1500 kPa	17.5	17.6	17.6	17.7	17.7
Organic carbon (%)	0.69	0.54	0.51	0.49	0.42
Carbon pool (Mg ha^{-1})	14.5	11.5	22.6	22.1	18.9
C.E.C. (meq 100 g^{-1} soil)	37.1	38.6	39.3	39.0	41.1
Avail. N (kg ha^{-1})	242	228	–	–	–
Avail. P_2O_5 (kg ha^{-1})	22.4	20.3	–	–	–
Avail. K_2O (kg ha^{-1})	471	465	–	–	–
Zinc (ppm)	0.36	0.37	–	–	–
Cu (ppm)	1.59	1.66	–	–	–
Fe (ppm)	5.4	5.2	–	–	–
Mn (ppm)	3.8	3.7	–	–	–
S (ppm)	4.6	4.5	–	–	–
AWC (cm)	3.1	3.3	6.9	7.1	7.1

Land capability sub-class – IIIes
Irrigability class – 3st
AWC – available water capacity.

Table 13 Properties of a Vertisol at Hoshangabad site, MP state, India

Soil properties	Soil depth (cm) 0–15	15–30	30–60	60–90	90–120
Sand (%)	23.2	22.0	22.0	21.0	21.0
Silt (%)	33.3	33.5	32.3	29.8	29.0
Clay (%)	43.5	44.5	45.7	49.2	50.0
Bulk density (Mg m^{-3})	1.42	1.45	1.48	1.58	1.59
pH (1:2.5)	7.7	7.9	7.8	7.9	8.0
E.C. (1:2.5)(dSm^{-1})	0.2	0.3	0.3	0.3	0.3
Water retention (%)					
33 kPa	32.5	33.1	33.2	34.6	33.7
1500 kPa	18.6	18.7	18.9	20.0	20.1
Organic carbon (%)	0.57	0.49	0.45	0.34	0.32
Carbon pool (Mg ha^{-1})	12.1	10.7	20.0	16.1	15.3
C.E.C. (meq 100 g^{-1} soil)	36.9	36.0	36.2	35.5	35.4
Avail. N (kg ha^{-1})	230	225	–	–	–
Avail. P_2O_5 (kg ha^{-1})	25.4	25.9	–	–	–
Avail. K_2O (kg ha^{-1})	571	565	–	–	–
Zinc (ppm)	0.5	0.4	–	–	–
S (ppm)	3.5	2.8	–	–	–
AWC (cm)	3.0	3.1	6.3	6.9	6.5

Land capability sub-class – IIIs
Irrigability class – 3st
AWC – available water capacity.

Table 14 Effect of raised/sunken bed system on yield of wheat and chickpea

Treatment	Yield (Mg ha^{-1}) Wheat	Chickpea
Raised bed	–	2.0
Sunken bed	3.6	–
Flat bed	3.5	1.9

Table 15 Residual effect of integrated nutrient management (INMP) on yield of wheat and gram at Narsinghpur

Treatments	Wheat: Grain yield (Mg ha^{-1})	Wheat: Percentage increase over the farmers' practice	Chickpea: Grain yield (Mg ha^{-1})	Chickpea: Percentage increase over the farmers' practice
Farmers practice (FP)	1.9	–	1.3	–
INMP	3.1	60.8	2.2	73.0
GRD	2.6	36.6	1.9	45.5

GRD = General recommended dose.

Table 16 Results of soybean-pigeon pea intercropping

Treatment	Yield (Mg ha^{-1}) Soybean	Yield (Mg ha^{-1}) Pigeon pea	Pigeon pea equivalent yield (Mg ha^{-1})	Gross income (Rs. ha^{-1})	Cost of cultivation (Rs. ha^{-1})	Net return (Rs. ha^{-1})	Benefit: cost ratio
Sole soybean (JS-335)	1.5	–	0.8	16,060	7,842	8,218	1.05: 1
Sole pigeon pea (JKM-7)	–	1.3	1.3	25,600	6,471	19,129	2.97: 1
Soybean + pigeon pea (4:2)	1.2	1.2	1.8	36,080	8,460	27,620	3.27: 1

Table 17 Results of fish-wheat/gram rotation

Treatment	Production (Mg ha^{-1})	Cost of cultivation (Rs.)	Gross income (Rs. ha^{-1})	Net return (Rs. ha^{-1})
Fish	1.1	12,576	27,500	14,924
Wheat	2.5	7,092	14,880	7,788
Fish	1.1	12,576	27,500	14,924
Chickpea	1.9	6,616	21,737	15,121
Total revenue (Rs.)		**19,192**	**49,237**	**30,045**

Table 18 Multiplication of breeder seeds in "seed villages"

a. Narsinghpur site

Village	Average of three villages (Dangidhana, Murlipodi, Bagpodi) 2000–2001	2001–2002	2002–2003
Soybean	0.40	7.2	21.5
Wheat	0.40	40.9	55.7
Chickpea	0.35	24.6	28.5

b. Hoshangabad site

Village	Average of three villages (Mongwari, Baihrakhedi, Dolaria) 2000–2001	2001–2002	2002–2003
Soybean	–	1.0	15.0
Wheat	0.4	27.2	30.6
Chickpea	–	0.6	13.2

Table 19 Average yield of crops in "seed villages"

a. Narsinghpur site

Village	Average of three villages (Mg ha^{-1}) (Dangidhana, Murlipodi, Bagpodi)		
	2000–2001	2001–2002	2002–2003
Soybean	1.0	1.8	2.1
Wheat	1.9	2.6	2.6
Chickpea	1.3	1.8	1.8

b. Hoshangabad site

Village	Average of three villages (Mg ha^{-1}) (Mongwari, Baihrakhedi, Dolaria)		
	2000–2001	2001–2002	2002–2003
Soybean	1.0	1.1	1.5
Wheat	1.4	2.7	3.1
Chickpea	1.2	1.4	1.7

6 Conclusions

A community-based participatory operational research project on Alfisols (red soils) of Tamil Nadu in the semi-arid region of India benefited the farming community by adopting rainfed agriculture with adoption of BMPs of dry farming technologies. The on-farm demonstrations revealed the beneficial impacts on soil quality and agronomic production of the BMPs comprising the following components: (1) disc plowing during summer, (2)establishing tied ridges with mulch, (3) application of press-mud compost enriched with rock phosphate, (4) intercropping of pigeon pea as main crop and other pulses and groundnut as intercrop, (5) double cropping with sunnhemp and pulses, (6) diversification of cropping with nutritious minor millets, (7) drip irrigation for high-value vegetable crops, (8) agriculture-centered income-generating activities for self-help groups of farm women, (9) utility of soil testing and use of "soil health card" for monitoring soil productivity and sustainable agriculture, and (10) introduction of arid horticulture.

Soil and rainwater management are the key factors for increasing the production potential of Vertisols in the high rainfall region of Central India. Land treatments (RSBS, ridges and furrows, broad bed and furrows) increased in situ soil moisture conservation, minimized runoff, and increased the yield of principal crops grown in the region (soybean, wheat, chickpea, and rice). INM based on soil testing and application of well-decomposed FYM and biofertilizers increased the yield of soybean and wheat. Intercropping with soybean and pigeon pea in 4:2 ratio was a successful cropping system over the traditional monocropping of soybean. Aqua-agriculture rather than fallowing in the monsoon season is a feasible system in place of traditional monocropping in winter season. The "seed village" model for decentralized production and distribution of quality seeds of HYV of crops among the farming community proved successful in increasing the average yield of crops in the project villages.

Soil fertility and on-farm water management are crucial to sustainability of intensive agricultural production systems of Punjab. On-farm demonstration studies indicated several viable alternatives to the traditional RWS. Substituting rice by maize, cotton, sugarcane were economic and profitable cropping systems. In regions where WT is rising, as in the canal-irrigated area in Faridkot district, replacement of rice with cotton is a viable option. With forest cover of only 5–6% of the total land area in Punjab, agroforestry interventions should form an integral part of long-term land cover and land use management strategy. Incorporating poplar in the rotation with intercrops such as maize, mung bean, wheat, ginger, and turmeric is a suitable agroforestry system.

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Decision Support Systems: Concepts, Progress and Issues – A Review

Shabir Ahmad Mir and S.M.K. Quadri

Abstract Agriculture is a complex process of air, water, weather, soil, plants, animals and micro-organisms, which are uneven in distribution. Because of the uncertainty and risk associated with agricultural production – due to vulnerability in weather, variability in soil, infestation of pests and insects – some most important decisions based on "what if" depends on the existing knowledge base of current and future physical conditions like soil and climate, yield and prices, crop and area. If the past pattern and exact impact of agriculture-associated resources are known, one can predict the likely occurrences of certain crop-related attributes in advance, so that farmers can put together safeguards by tiding over the controllable attributes, which are likely to have serious impact on crop growth and yield. Thus the quality of decision-making can play an important role in complex and uncertain situations.

Empirical evidence reveals that human judgement and decision-making can be far from optimal and could even deteriorate further with added complexity and stress. Therefore, aiding the deficiencies of human judgement and quality decision-making has been a major focus of research throughout the history particularly with the advancement in electronic processing of data and design of Decision Support System (DSS). DSS can help to reduce uncertainty and improve the decision-making process by providing access to data through procedures and analytical reasoning. Computerized decision support for sustainable agriculture is not new. These systems have been designed to address complex tasks involving agronomic, economic, regulatory, climate change and pollution control, enabling us to match the biological requirements of crop to the physical characteristics of land so that the objective specified by the user is obtained. Most agricultural DSSs aim to help stakeholders realize their strategic aim of securing a competitive advantage through timely decision-making.

While addressing the limitations in current decision support technologies, visionaries and researchers throughout times have talked of exploiting our mass

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of information to produce new knowledge automatically, build intelligent DSSs and eliminate human burdens associated with information-seeking and problem-solving activities.

DSSs are widely used and known with agriculture and they have proved to be important tools in the decision-making process from farm to fork. Unfortunately, ease of use, low adoption, failure to show cost benefits, complexity and user inputs, distrust for the output, lack of field testing, lack of integration among heterogeneous components, success measurements, non-involvement of end-user before and after development stages and under-definition of beneficiaries are some issues that need to be addressed. But keeping the amount of input data required as small as possible; keeping the system itself as flexible as possible; providing users with default values; ensured involvement of users from basic development stage; greater user involvement through participatory learning approaches; interactive prototyping as well as keeping DSS development manageable and small in scope can provide avenues for improvement in DSS research and development.

Keywords Decision Support System · Crop modeling · Management Information systems · Agricultural Systems Research · Integrated pollution control · Land use planning

1 Introduction

A problem is considered complex when it entails a web of related problems, covering many disciplines and interacting at various scales, Van Asselt (2000). In order to solve a problem one has to generate alternatives to make choice, supported by estimation, evaluation and/or comparison. The decisions made by the decision-makers are irreversible and have far-reaching consequences for the problem. Decision support systems (DSSs) are valuable in situations in which the amount of available information is prohibitive for the intuition of an unaided human decision-maker, and in which the quality of decision is important. They can aid human cognitive deficiencies by integrating various sources of information, providing intelligent access to relevant knowledge, and aiding the process of structuring decisions. Proper application of decision-making increases productivity, efficiency and effectiveness, and allows one to make optimal choices, e.g. varietal selection of certain crop and their management practices, planning marketing or environmental impact etc. DSS can assist producers in making better decisions by integrating information into a more useable form. It alters production systems, enhances management skills and reduces cost of production by applying modelling, hypothesis and optimization techniques. For making rational decisions, disciplines such as statistics, economics and operational research have developed various methods, which have recently been enhanced by various techniques originating from Information Science, Cognitive Science, Artificial Intelligence and Pattern recognition. These methods have been implemented in the form of computer programs either as stand-alone system or complex computing environments for complex decision making. Such environments are often given the common name of decision support systems (DSSs).

Global agriculture has seen an increasing interest in the potential use of DSSs in recent years. The application of these systems to agriculture seems natural considering widespread use of extension agents in and out of the field. Status of soil, selection and yield characteristics of a variety, likely interaction between the field and variety, disease presence in the area, yield, weather conditions, current market and prices and related information have become important for sustainable agriculture. With the advent of powerful computational power, efficient database management system and on-line analytical processing (OLAP), decision process has improved and reached respectable degree of acceptance (Hammer et al., 2002). The use of rule-based and model-based DSSs as part of everyday monitoring has proven their worth (DTI, 1992). They have allowed rapid assessment of several production systems around the globe for agriculture production, marketing for sustainable development and have facilitated decision-making in marketing at farm and policy levels. These systems have already been widely used and known in different areas of agriculture (Jenkins and Williams, 1998), such as Crop protection, Agronomy, Pomology, Soil Science, Agricultural Engineering, Forestry, Environmental Science, Agro-meteorology, etc. and have the potential to be an important tool in the decision-making process for primary producers and their advisors (Ritchie, 1995).

Use of DSS in agriculture for providing information and recommendation on efficient utilization of fertilizer (K. L. Chai et al., 1994); reducing herbicide use (H. D. Coble, 1994); plant protection (Jorgensen et al., 2006); variety-specific information (Detlefsen and Jensen et al., 2004); management of environment risks (Didier Bazile et al., 2005); Integrated Nutrient Management (Mosseddaq et al., 2005); Forest Management (Riberiro and Borges, 2005); Crop disease control (Ibragimov et al., 2005); Agricultural practices and extension (Rainer M. Sodtke, 2005); Farm mechanization (Suarez de Cepeda et al., 2005); Seasonal Climate Prediction (Hansen, 2002); Integrated Pollution Control (Kseniya, L., 1999); labour requirements and land use planning (Metthews and Buchan, 2003); profit maximization and risk minimization (Meinke et al., 2001) and so on have been designed and implemented successfully mainly for improving economic returns, changing farming practices or minimizing environmental risks.

For land use planning it is increasingly necessary to recognize the complex tradeoffs between the multiple objectives of stakeholders. This is particularly apparent where outcomes of scales above the land management unit are considered important (e.g. water quality, biodiversity and land use planning). Apparently, developed and deployed agricultural DSSs have the capacity to encapsulate scientific, practitioner and stakeholder knowledge and to present the consequences of alternative land use scenarios. They may thus inform the debate on achieving an appropriate balance between economic, social and environmental outcomes (Matthews et al., 1999).

Advancement in hardware and mathematical modelling, artificial intelligence techniques, data warehousing and mining, OLAP, enterprise resource planning (ERP) systems, intelligent agents and World Wide Web (WWW) are adding new capabilities to DSS development. In order to enrich DSS development, database researchers are continuing research on management of data. Management Sciences are coming with mathematical models for use in DSS and are providing evidence on the advantages of modelling in problem solving. Cognitive Science is a step ahead providing descriptive information, which is having a value in DSS design and generation of hypothesis. With these advancements some of the issues raised by various authors before one decade, related to DSS, have no relevance in the present context (Parker and Campion, 1997; Lynch et al., 2000). It is expected that other remaining issues are resolved once validation and appropriateness in relation to intended purpose, introduction of analytical phase to deconstruct professional modes, determination before proceeding to development, actual user's perspective of success, clear definition of beneficiaries, easiness and usability and involvement of end-users in all developmental stages are given due attention and priority (P. G. Cox, 1996; R. L. McCown, 2002a,b). Incorporation of heuristic role to support scientific investigation and promotion of genetic regulation of plant performance in crop modelling, the DSS shall reach to respectable degree of acceptance (G. L. Hammer et al., 2002). Moreover, the four-point criteria proposed by Little (1970) can provide insight to design and develop DSS, which are still relevant in evaluating modern DSS.

This chapter chronicles and explores basic concepts of DSS, articulates a coherent definition of DSS including the features they generally contain, with special mention of their application to sustainable agriculture and future trends. Finally it documents the issues concerning DSS development and adaptations.

2 What Are Decision Support Systems?

The term DSS has been used and defined in various ways depending upon the author's point of view (Power, 2002; Druzdzel and Flynn, 1999). Finlay (1994) defined a DSS as "a computer-based system that aid the processing or decision making". Turban (1995) has defined it specifically as "an interactive, flexible, and adaptable computer based information system, especially developed for supporting the solution of a non-structured management problem for improved decision making". There are also several definitions given by different authors, which fall between these two extremes. Little (1970) defined DSS as "model-based set of procedures for processing data and judgments to assist a manager in his decision-making". Keen and Scott Morton (1978) define DSS as "Computer-based support for management decision making". Moore and Chang (1980) define it as "extensible systems capable of supporting adhoc data analysis and decision modelling, oriented towards future planning and used at irregular, unplanned intervals". Sprague and Clarlson (1982) described DSS as "interactive Computer-based systems that help decision makers to solve unstructured problems using data and models". Keen (1980) is not of the opinion to give DSS a precise definition. For him "there can be no definition of DSS". For Power (1997) the term DSS remains a useful and inclusive term for many types of information systems that support decision making. In order to avoid exclusion of any of the existing types of DSSs, we define them roughly as interactive computer-based systems that aid in making a quality decision. DSSs typically have quantitative output and place emphasis on the end-user for final problem solution and decision-making irrespective of the expert system (ES), which uses qualitative reasoning to solve a problem for decision making. Often ESs are developed around very specific and highly detailed "Domains" and thus tend to be narrow in their range of knowledge (Luconi et al., 1993).

DSSs exist for a wide range of applications including agriculture, water resources, environment, organizational management, health and business. Within these sectors, they are used to improve personal efficiency, expediting problem solving, facilitating interpersonal communication, promoting learning and training and increasing organizational control. For example, in the agricultural field, DSS can be designed for use by agronomists, soil scientists, agricultural engineers, entomologists, weather experts, farmers, students and extension workers. They can be further targeted for a variety of environments or agricultural commodities like temperate or tropical conditions, rainfed or irrigated environments, upland or lowland areas, watershed or field levels, fruits or grains, rice or wheat and others (Bakker-Dhaliwal et al., 2001). Typical information that a decision support application might gather and present would be comparative weather forecasting between one week and next; projected yield of particular crop before harvest; the consequences of different decision alternatives; optimal dosage of fertilizers for a given crop to maximize yield; prediction of pathogen infestation based on the hypothetic climatic conditions; application of water, temperature and fertilizer; and so on. Turban et al. (2005) account ideal set of capabilities of DSS. The key characteristics and capabilities are as follows:

i. Ability to support in semi-structured and unstructured problems, including human judgment and computerized information.
ii. Ability to support managers at all levels.
iii. Ability to support individuals and groups.
iv. Ability to present knowledge on ad hoc basic in customized way.
v. Ability to select any desired subset of stored knowledge for presentation or derivation during problem solving.
vi. Ability to support for interdependent or sequential decisions.
vii. Ability to support intelligence, design, choice and implementation.
viii. Ability to support variety of decision processes and styles.
ix. Ability to support modelling and analysis.
x. Ability to support data access.
xi. Benefits must exceed cost.
xii. Allow modification to suit needs of user and changing environment.
xiii. Support quick decision-making using standalone, integration or web-based fashion DSSs having maximum number of these key characteristics and capabilities can be more useful and adoptable.

2.1 Classification of Decision Support Systems

There is no universally accepted taxonomy of DSSs, as different authors propose different classifications. The classification proposed by different authors, based on different criteria, is summarized in Table 1.

Table 1 Classification of DSS given by different authors

Authors	Classification	Features	Example	Criterion
Bhargava and Power (2001)	Model-driven DSS	Emphasizes on access to and manipulation of statistical, financial, optimization or simulations model	Dicodess (Gachet, 2004); production planning management Decision Support (Scott-Morton, 1971); production scheduling applications (Ferguson and Jones, 1969).	Mode of assistance
	Communication-driven DSS	Emphasizes communications, collaboration, and shared decision-making support	Microsoft's Net Meeting or Groove (Stanhope, 2002); Basic Group Decision Support System (DeSanctis and Gallupe, 1987)	
	Data-driven DSS	Emphasizes on access to and manipulation of time series data.	An Analytical Information management System (Alter, 1980); Data-Driven DSS with OLAP (Codd et al., 1993)	
	Document-driven DSS	Manages, retrieves and manipulates unstructured information in a variety of electronic formats	Search engine (Power, 2002)	
	Knowledge-driven DSS	Specialized problem-solving expertise stores as facts, rules, procedures, etc.	MYCIN, Buchanan and Shortliffe (1984)	
Power (2000)	Enterprise-wide DSS	Linked with large data warehouse, which serves many managers	DSStar (Power, 1998)	Scope

Table 1 (continued)

Authors	Classification	Features	Example	Criterion
	Desktop DSS	Single user, small system that runs on managers Personal Computer	NuDSS, Bell and Chung (2000)	
Haettenschwiler (1999)	Active DSS	It aids the process of decision without bringing out explicit suggestions or solutions	Walmart	User relationship
	Passive DSS	Brings out explicit suggestions	Exsys	
	Cooperative DSS	Modifies, completes or refines the decision suggestions	Co-op (Bui and Jarke, 1986)	
Holsapple and Whinston (1996)	Text-oriented DSS	Works on text as input	POMME, Roach et al. (1985)	Type of Inputs used for decision making
	Database oriented DSS	Has a database in the back end for inputs	DSSAT, Hunt et al. (1994)	
	Spread sheet oriented DSS	Uses spread sheet as inputs, e.g. Excel	SOLVER (in build in Microsoft Excel)	
	Solver Oriented DSS	Mainly designed for solving problems, e.g. Linear Equations	–	
	Rule oriented DSS	Uses inputs in the form of rules based on reasoning	TropRice, Bell and Chung (2000)	
	Component Oriented DSS	Hybrid System that includes two or more five basic structures described by Holsapple and Whinston (1996)	PLANT/ds, Michalski et al. (1982)	
Hackathorn and Keen (1981)	Personal Support	Supports only one user	–	Scope
	Group Support	Supports group of user	–	
	Organizational Support	Supports an organization as a whole	–	

2.2 Components of Decision Support Systems

Like classification, different authors have identified different components in a DSS, and have proposed different architectures. Every DSS does not fit neatly into one category, but is a mix of two or more architectures. The most general architecture of DSS can be divided into four subsystems, namely Database Management Subsystem, Knowledge-based Management Subsystem, User Interface Subsystem and the User (Fig. 1).

Imagine we are making DSS for prediction of a pest infestation for certain crop. The first step of the decision process begins with the creation of databases to create and store historical data of pests (type, characteristics, etc.) and weather (temperature, rainfall, humidity, etc.) in Microsoft Excel or Microsoft Access or Oracle, etc. The Database Management Subsystem stores, manages and provides access to the data using Database Management System (DBMS). DBMS is a set of computer programs that create and manage the database as well as control access to the data stored within it, using database, Data Directory and Query facilities. Knowledge-based Management Subsystem is an inference procedure or control structure that helps solve a broad range of problems. Most of the DSSs use "what if" strategy to solve a problem. In our example, we can use this procedure: "If mean temperature between the periods is greater than 30°C, mean rainfall is greater than 175 mm and relative humidity is 85% then spray such and such pesticide in such concentration". From the user's viewpoint, the User Interface Subsystem is the only part of DSS components with which they have to deal. Typical user interface design consists of choice of input and output devices, screen design, use of colours, data and information presentation format, use of interface styles, reports, etc. In our example an Entomologist can be the one of the end-user. Expert level and input of end-user is important for designing a successful DSS application.

The architectural components proposed by several authors with description of each component are summarized in Table 2.

DSSs are not different from other systems but they require a structured approach. For this purpose a framework was provided by Sprague and Watson (1993) with three main levels: technology levels, people involved and developmental approach.

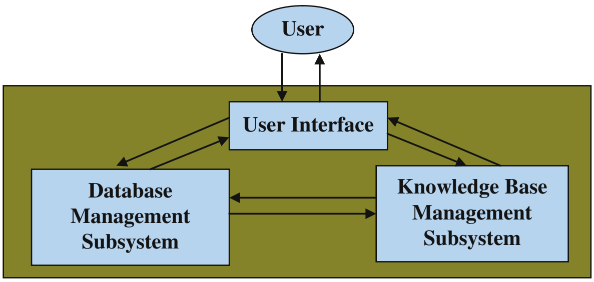

Fig. 1 General architecture of decision support systems

Table 2 Architectural components of DSS given by different authors

Authors	Architectural Component	Description of component
Sprague and Carlson (1982)	i. Database Management System (DBMS) ii. Model Base Management system (MBMS) iii. Dialog Generation and Management System	i. Stores information ii. Integrates models iii. Provides user interfaces to manage system
Haag et al. (2000)	Same as above but describes them in detail	i. Stores information (that can be further subdivided into organization's traditional data repository, from external source such as internet or from experience of individual user) ii. Using various kinds of models it handles representation of events, facts or situations iii. Integrates models and provides user interface
Haettenschwiler (1999)	i. User ii. Decision context iii. Target system iv. Knowledge base	i. Participates in different roles or functions in the data management process ii. Specifically defined decision rules iii. Describes majority of the preferences iv. External data sources, knowledge databases, working databases, data warehouse, metadata bases, models, methods, integrates search engines to responding system
Marakas (1999)	i. Database Management System ii. Memory Management System iii. Knowledge Engine iv. User Interface v. User	i. Stores, manages and provides access to the data ii. Organizes memory efficiently iii. Inference procedure or control structure for utilizing the knowledge iv. Allows user to interact with the system v. One who uses the system
Power (2002)	i. The user interactive ii. The database interactive iii. The model and analytical tool iv. The DSS architect and network	i. Interacts with the user over a command line ii. Interacts with a single or a group of users using a database for heuristics iii. Model designed for analysis iv. Interacts with the other DSS or database server

As far as technology levels are concerned they have suggested three levels of hardware and software for DSS.

Level 1 (Specific DSS): This is the actual application that allows the decision-makers to make decisions in a particular problem area.
Level 2 (DSS Generator): This level allows development of easy specific DSS applications.
Level 3 (DSS Tools): This level includes special languages, functions, libraries and linking modules.

3 Overview of Decision Support Systems

Researchers and technologists have built and investigated DSSs for about 48 years. This concept has evolved from technical studies of organizational decision-making and technical work on interactive computer systems mainly carried at Massachusetts's Institute of Technology (MIT) in 1960s (Keen and Scott Morton, 1978). The first DSS for agriculture cited in the literature is *Televis*, which was developed by Norway in 1957 for plant protection. Another such system named as *Guntz-Divoux* was developed by France in 1963. Use of computerized quantitative models to assist in decision-making and planning was started systematically during this period (Holt and Huber, 1969). First computer-aided DSS for production scheduling was investigated, which was running on IBM 7049 (Ferguson and Jones, 1969). Development of the IBM system 360 and other more powerful mainframe systems made it practical and cost-effective to develop Management Information System (MIS) for large companies (Davis, 1974). These early MIS focused on providing managers with structured periodic reports primarily for accounting and transaction processing. Michael S. Scott Morton's dissertation in 1967 is regarded as major historical turning point wherein building, implementing and testing an interactive and model-oriented DSS was investigated. The concept of DSS is believed to be articulated by Scott Morton in 1964. During 1971 he demonstrated "how computer's and analytical models could help managers to make key planning decisions". DSS became recognized as self-sustained research area in 1970s, when business journals started publishing articles on management decision systems, strategic planning systems and decision support systems (Sprague and Watson, 1979). The term Decision Support System was first used by Gorry and Scott Morton in their review in "Sloan Management" wherein they argued that MIS primarily focused on structured decisions and suggested that information systems supporting semi-structural and unstructured decisions should be termed as "Decision Support System".

DSS gained high reputation during 1980s when its application development started in Universities and organizations, which expanded their scope. During 1982, Ralph Sprague and Eric Carlson's book "Building Effective Decision Support System" further explained the framework of database, model base, dialog generation and software management. It provides a practical and understandable overview of how organization could or should build DSS.

During 1990, Bill Inmon, father of data warehouse, and Ralph Kimball, the doctor of DSS, actively promoted DSS built using relational database technologies, which has exposed many MIS practitioners in a popular computing literature. Beginning in approximately 1995, the WWW and global Internet provided a technology expansion platform for further extending the capabilities and deployment of computerized decision support.

In 2000, Application Service Providers (ASPs) began hosting the application software and technical infrastructure for decision support capabilities. Emergence of enterprise knowledge portals, knowledge management, business intelligence and communication leads DSS to an integrated environment (Bhargava and Power, 2001). Most software developers have regarded WWW as a serious platform for implementing all types of DSSs. Manipulation of quantitative models, accessing and analyzing of large databases and building of group DSSs are major applications of DSS (Ecom, 2002; Arnott and Pervan, 2005). Now a days intellegent decision support systems are adding new capabilities to these systems (Dhar and Stein, 1997).

4 Decision Support Systems in an Agricultural Perspective

Application of DSS in the area of agriculture takes the form of integrated crop management decision support and encompasses fertilizer management, weed management, water management, plant protection, soil erosion, land use planning, drought management, pollution control, etc. These systems are addressing problems related to conservation and improving soil fertility, local water balance, efficient agronomical practices, canopy management, pest and insect management, reducing pre- and post-harvest losses, conservation of forests and global environment change etc. Web-based DSSs are also playing important role in dissemination of technology transfer related to crop management practices, irrigation scheduling, fertilizer application, etc.

4.1 Nutrient Management

Fertilizers and lime are increasingly expensive but are commonly needed to grow high-yielding and good-quality crops. However, unnecessary use is wasteful, reduces farm profits and increases the risk of diffuse nutrient pollution. To maximize profits and avoid waste, farmers need to plan their use of nutrients for each field crop in each year. Organic manures (farmyard manure, sewage sludge, slurries, etc.) contain large quantities of nutrients which can often mean that large reductions are possible in the need for inorganic fertilizers. Nutrient management can play an important role in many of the regulatory and non-regulatory duties of farm-related management, and can protect, restore and enhance the status and diversity of all surface water ecosystems and ensure the progressive reduction of groundwater pollution.

For Nutrient management, different DSSs have been designed to recommend site-specific and need-based parameters that result in an optimized fertilizer management strategy. One example of such system is *CERES*, which simulates the whole soil crop system (Quemada and Cabreva, 1995). Another example is rice fertility DSS, which provides recommendation on efficient utilization of fertilizer for the production of flooded rice in Arkansas (Chai et al., 1994). A DSS for reduction in potential nitrogen (N) losses to the environment has been developed, which saves fertilizer expenditure (Lemberg et al., 1992).

There is an ongoing trend to develop Nutrition Management Decision support tools to make them available to the farmers through WWW. These facilities are enabling farmers to use the service of these tools irrespective of computer ownership, which is being reported as one of the reason for low adaptation of DSS among farmers. *Haifa Nutri-Net* is an example of such system (Achilea et al., 2005). It is a comprehensive crop Nutrition DSS, operated over the web, assisting growers to formulate their crop nutrition programs and irrigation schemes by integrating virtually all relevant cultivation parameters. It is based on comprehensive databases of crop nutrition, irrigation, soil and climate, covering all most every growth environment. *FarmN* is another web based DSS providing INM recommendations (Jorgensen et al., 2005).

Most of the existing DSSs are based on very specific aspects, for example, Nutrient management. One system that addresses all the major manure management systems, such as manure collection, storage, treatment and land application, has been identified (Licklider, 2007). M.M. Gibbons et al. (2005) reported development of DSS for Planning Land Applications of Nutrients for Efficiency and the Environment *(PLANET)*. It provides best management practice tool for farmers and their advisors to adopt in the use of organic manure and fertilizers. Fertilizer recommendations for field are calculated based on the precious cropping fertilizer and organic manure application. To encourage maximum uptake of DSS by the farming community, the logic to generate fertilizer recommendations based on input data was developed and made available to commercial agriculture software developers for integration within their systems, which are being widely used by farmers.

4.2 Insect and Pest Management

Plant protection is definable as the reasoned application of agronomic methods, products as well as chemicals to allow optimal productive factors, while respecting the farm worker, the environment and the consumer.

The concept of computerized DSSs for pest management is not new. DSS models have been developed for diseases that could expand very rapidly or those that should be controlled regularly. Development of weather-related DSS during 1990s resulted in a lower risk of crop damage by diseases and pests and minimal use of other input dosage (Bouma, 2006). Edwards-Jones (1993) reported 67 different DSSs for plant protection. Out of these, 30 deal with insect pest problems, 20 with plant disease and 17 with weeds. Most of the use systems have been developed in North America, particularly United States. Arjoon et al. (1993) reported DSS for pesticide use management. For detection and tackling of air-borne herbicides, a DSS has been reported by Al Khatib et al. (1993). Another model known as *CLIMEX* is used in 20 countries to examine the distribution of insect, plants, pathogens and vertebrates. It was developed by CSIRO Entomology in 1985 and has had numerous applications under practical conditions (White and Walker, 2000).

An insect pest forecasting tool, known as *SOPRA*, has been developed with the objective of optimizing timing of monitoring, management and control measures related to fruit orchards in Switzerland (Samietz et al., 2006). It uses local weather data, and simulates the age structure of the pest populations and crucial events for management activities are provided. Growers in alpine valleys and north of Alps can use it for decision support for the eight major insects and pests of fruit orchards.

In Europe, numerous DSSs exist to predict appropriate pesticide application times for various crops including potato. Of these, the most common are *Negfry*, *Prophy*, *Plant Plus* and *Simphyt*, which mainly focus on reducing fungicide inputs (Dowley and Leonard, 2000). These four late-blight advice systems have the advantage of being in direct competition with each other, resulting to be useful for farmers.

4.3 Agricultural Land Use and Planning

As population and human aspirations increase, land becomes an increasingly scarce resource, calling for land use planning. Land use planning is defined as a systematic assessment of land and water potential, alternatives for land use and the economic and social conditions. It has become essential to mitigate the negative effects of land use and to enhance the efficient use of resource with minimal impact on future generations. Land use planning is becoming complex and multidisciplinary as planners face multiple problems that need to be addressed within a single planning framework. Such problems include non-point-source pollution, water allocation, urbanization, ecosystem deterioration, global warming, poverty and employment, deforestation, desertification, farmland deterioration and low economic growth.

Many different DSS tools for land use-related decision-making have been designed for agricultural planning (Jeffrey et al., 1992), sustainable watershed management (Loi, 2004), forest planning (Riberiro and Borges, 2005), environmental planning (Sargent et al., 1991), site selection (Manos and Gavezos, 1995), species protection plans (Sandstorm, 1996) and conservation preserves planning (Klik et al., 2006). A conceptual framework and a spatial DSS for rural land use planning have been developed for supporting decision making on selected area for different watershed management schemes for conservation planning (Aditnarayana et al., 2000). The system provides suggestions and warnings for land use.

The first linear programming models applied to land use planning were single-objective problems (Campbell et al., 1992; Chuvieco, 1993). However, because of the complexity of agricultural planning, multi-objective models are becoming increasingly more common. Within these models, goal programming is one of the techniques most frequently applied. Giupponi and Rosato (1998) developed a goal programming addressing land use and the cropping system, maximization of gross margin and the minimization of risk. Matthews and Buchan (2003) reported continuous development of the DSS with multiobjective land use planning tools. Linear Programming model and Goal Programming-based DSS for farm regions in Greece have been designed, having development possibilities of agricultural sector in relation with the agricultural processing industries of the region (Barnard and Nix, 1993; Bernardo et al., 1992; Hazell and Norton, 1986; Lee et al., 1995; Manos and Gavezos, 1995). It aims at the development of farm regions through a better utilization of available agricultural resources and agricultural industries.

Most recently trends in modern land use planning increased involvement of stakeholders in the planning process, which causes the need for interactive programming to exchange information between the decision-maker and the system. Interactive multiple objective learner programming has been successfully applied to agricultural development policy analysis (De Wit et al., 1998), land use strategy evaluation (Lu et al., 2004) and land resource utilization (Fischer et al., 1998). DSS for sustainable land use planning to address conservation of land, improving soil quality and fertility, and local water balance with minimization of soil and nutrient translocation into surface water bodies and downstream fields have been designed keeping in view the optimized benefits for farmers as well as for the society (Klik et al., 2006).

4.4 Global Environment Change and Forecasting

Global environment change is happening. Human activities, including those related to the production, supply and consumption of food, are responsible for changing worlds' climate and giving rise to other, globally and locally important environment changes (GECAFS, 2005). These include a change in fresh water supplies, carbon and nitrogen cycling, biodiversity and land cover and soils (Vitousek et al., 1997). There is a concern that meeting society's rising demand for food will further degrade the environment (FOA, 2002) the consequences of these changes must be estimated well in advance to minimize their impact for sustain ability of global agriculture (Steffen et al., 2004) . DSSs for agricultural application of climate forecast can determine where, when and which sections of society are at most risk. They can construct future scenarios from food security point of view and reduce the vulnerability of food systems to Global Environment Change (GEC).

An integrated pollution control DSS has been developed for rapid estimation of the extent and impact of pollution in a given situation (Lvovsky, 1999). Another model based on geographical information system (GIS), remote sensing and precision agriculture has been developed for Minland East Asia (Zuo, 1996). This model has a value in prioritizing and targeting of agriculture research in developed countries (White et al., 1993). Decision Support System for Agro-technology Transfer (DSSAT) has been designed to answer "what if" questions frequently asked by policy-makers and farmers concerned with sustaining an economically sound and environmentally safe agriculture (Hunt et al., 1994). DSS for metrology has been designed by Seghi et al. (2000) which locates and analyzes historical situations of interest. It is used mostly by agronomists to forecast and provide recommendations to the growers. Climate forecasting based DSS are estimating the potential economic values for farm scale management decisions. They are helping in management and adjustment of different crops between specified regions having different climatic conditions (Jones et al., 2000). They are also improving dessimination of grassland technology by predicting impact of climatic risks (Stuth et al., 1993).

4.5 Water and Drought Management

Nearly one billion people worldwide are malnourished. The majority of these people live in developing countries, where increasing water scarcity complicates efforts towards food self-sufficiency. Huge amounts of water are needed to produce more food and eradicate hunger among increasing populations (Falkenmar and Rockstorm, 2005). The current limited approaches to increasing demands of water will not be enough to eradicate hunger, especially in areas with growing populations and amidst dry climates in most developing countries. The central issue is how to manage water for all the different functions for which it is needed. With the advent of agronomic models that show how vegetation is likely to respond to climatic stress, with remote sensing to monitor vegetations conditions from airborne and space borne platforms, and with GIS to display spatial and temporal data in more comprehensible ways, it is now feasible to more accurately assess the impacts of drought (McVicar et al., 1992). Different DSSs have been developed to tackle with the problems related to water and drought management, (Watkins and McKinney, 1995), *TEMPEST* allows to model water flow both saline and fresh and predict the responses to each facet of the landscape to management (Sojda, 1994). *Aussie GRASS*, developed by the Queensland Department of Natural Resources, provides timely estimation of the extent of severity of drought (Hall et al., 1997). A DSS developed in Vietnam formulates the plans for sustainable watershed management, using a combined approach of linear programming, goal programming and GIS for deriving the sustainable watershed management plan (Loi, 2004).

4.6 Other Applications

TropRice, an integrated rice management system being used by researchers, extension workers and some farmers has been used in Asia for irrigated rice areas (Bell and Chung, 2000). It provides some generic and some site-specific information for rice cultivation. It is currently being used in China, India, Indonesia, the Philippines, Thailand and Vietnam and is being translated or localized by national collaborators for local conditions in these countries. Other applications of DSS in agriculture range from conserving soil, local water balance, agronomical practices, canopy management cropping system analysis, and conservation of forest and computer multimedia instruction (Andreas Klik et al., 2006; Lemmon, 1986; Warkentin et al., 1990; Hoogenboom et al., 1994; Caldwell, R. M., 2003; Marrison et al., 1993). Web-based DSSs are assisting in forest management, resource management, and, agricultural emergency response (Venkatachalam and Krishna Mohan, 2000; Jiannong Xin et al., 2005; Riberiro and Borges, 2005). DSS for farm mechanization using GIS based on linear programming provides machinery selection and planning to minimize farmer mechanization has been developed by Suarez de Cepeda et al. (2005). The system includes the natural factors (climate and soil conditions), plot geographic site and the crop and machinery data. The GIS is part of the system to carry out a spatial analysis of the farm results to make machinery grouping.

5 Issues and Concerns

The issues concerning DSS mainly deal with its development and adaptation. Many authors have pinpointed different issues related primarily to DSS development and adaptation. Shim et al. (2002) argues that despite advances in information technology, the promise of technology assisting human's decision-making process has not been fulfilled yet. Parker and Campion (1997) report that use or value of DSS as a vehicle to communicate research results to farmers, agricultural technicians, extension agents, field advisors and researchers is debatable. Literature review of agriculture-related DSS innovations indicates a general realization that although there are many useful, scientifically valid models or knowledge-based tools currently available, a majority of these are underused, due to narrow target audience or environment, insufficient initial attention directed at delivery and poor user interface (Bakker-Dhaliwal et al., 2001). Bell and Chung (2000) have identified multidimensional factors that limit communication and DSS adoption within Asia, including disproportionately large numbers of farmers relative to land ownership; farmer education and beliefs; language and cultural diversity; access to resources and credit; limited communication channels including mass media, radio, television, telephone, cell phone and infrastructure; limited access to extension systems, consultants and universities; underfunded and undertrained extension systems; and limited private sector development. Cox (1996), McCown (2002a), McCown (2002b) and Hayman and Easdown (2002) have also listed various attributes that dissuade DSS users. These include fear of using computers, tedious data entry, complicated setup process, as well as lack of software support, technical interpretation and application or local relevance. Hammer et al. (2002) finds problem of implementation of agricultural DSS in terms of relationship between DSS developer and the potential user. Jorgensen et al. (2006) highlights issues within Online DSS. He argues that information presentation is one of the issues as it does not reach farmers due to the practical usability. He divides the farmers into three groups – systems-oriented, experience-based and advisory-oriented. This varied knowledge orientation necessitated different ways in which DSS need to be developed and implemented. Poor understanding among researchers of how agricultural DSSs are actually used by practitioners is one of the main barriers for adoption of agricultural DSSs (Cox, 1996). Parker (1995) reports 20 agricultural DSSs in UK which are not containing a single name in common use on the farm. Compbell (1999) and Lynch et al. (2000)

have also provided detailed issues corresponding to pre- and post-implantation of DSS. Carberry et al. (2002) identify implementation as main problem with agricultural DSS. The relevance of various issues put forth by authors, mainly by Cox (1996), has been discussed for their applicability in the UK situation by Parker and Campion (1997), based on surveys and advancement in computer technology. They have not agreed on some of the issues due to increase in computer ownership, design of DSS tools supporting far more than exceptional cases and advancement in computer technology. McCown (2002b) advocates that existence of partnership between researcher and practitioner for development of DSS can hopefully give success in their development and implementation. The main reasons provided by different authors have been cross-examined and presented here to avoid confusions and validity in present context.

Usefulness and ease-of-use: Ease-of-use and usefulness are basic features that affect DSS adaptation. User's preferences for simple DSS have been widely documented in the literature (Knight and Mumford, 1994; Armstrong et al., 2003); Cox et al., 2004). For any decision support tool to be useful, it needs to provide information that is relevant to at least one important decision made by the farmers. It suggests that the decision to be addressed by DSS needs to be approached from the farmer's point of view and not the way researchers would approach the problem. Unless the DSS is very simple and quick to use, the majority of farmers are unlikely to use it. Keil et al. (1995) suggested that software that fares low in ease-of-use and usefulness will be rejected. DSSs which are high in ease-of-use and low in usefulness may be embraced by users initially but they have little lasting capabilities. DSSs which are low in ease-of-use but high in usefulness will be used only by competent computer users, as time and effort required to learn outweighs the potential benefits. The ideal DSS aims high in ease-of-use and high in usefulness.

Success measurement: DSS research measuring success has focused on various aspects by making comparison and agglomeration of results. Authors have introduced a taxonomy of the six major dimensions of system success: System quality, information quality, use, user satisfaction, individual impact and organizational impact. Their work differs from other Information System frameworks in its practicality and its recognition that the most important outcomes are those seen from the user's perspective. Many DSS failures after acceptance by the end-users might be attributed to a number of reasons. Some fail to keep pace with the user's needs. Once users interacted and learned from the system, they no longer require to use it for future. The problems concerning underlying model and simplifying technology result in system failure. Example of such failure is the *SIRATAC* expert manager program for cotton users.

Adaptation: Although the adoption of DSS within agriculture is low despite greater use and adaptation. Researchers are aware of the continual high failure rate across all system software (OASIG, 1996). The uptake of DSS is ever more problematic than the uptake of computer system within small business. The OASIG study suggested that part of the reason for the high failure of system was the lack of user involvement from the early stages of development. No universally agreed taxonomy, classification and components of DSS are also narrowing its development. DSS designed for agricultural situations like identification of pests and diseases or irrigation strategies based on ES technology were seen to be useful only in the rare and exceptional circumstances when the user's own experience failed (Parker, 1995; Barrie and Gardener, 1994).

Complexity and user inputs: Most DSSs require input from the users for certain variables (e.g. weather data, sowing date, relative humidity, leaf wetness, etc.). The use of these variables may be ideal for the development of an accurate and productive model but likely to cause problem for the grower or farmer because they are difficult to collect or are inconvenient or too expensive. Eleveld et al. (1992) suggest users often "lack insight into why entries are needed" which in turn results in a loss of confidence in the system. The developers have to give thought to the nature of inputs the user will have to provide.

Underdefinition of users: During the development stage the beneficiaries of the system are not clear, which does not meet the needs of any group. The developers should involve users from the start of the project, primarily conceptual thought with continual feedback (Ludwig et al., 1993). In order to make DSS usable to its potential user, development team should include a researcher who knows something about DSS and a programmer who knows something about the real-world problem being addressed by the DSS with focused end-user group.

Distrust for the output: Producers do not understand the underlying theories of the models, which make them reluctant to consider output as authenticated (Cox, 1996). Sometimes there were problems with the underlying models and opportunity to simplify the technology was not considered.

Failure to show cost-benefits: For design of any software benefits must exceed cost. Literature suggests that most of the decision support tools are not useful to the farmer or consultant. Many of the DSS projects are based on the requests from the industry, others are based on an idea by non-growers that some technology must be useful somewhere. The use of any such tool requires an investment in time on the user's part and initially a certain amount of faith. Therefore, there has to be a fairly obvious cost-saving inherent in its use to take the user from polite interest to purchase and use. It is perhaps not surprising that the results are not always successful (Parker and Campion, 1997).

Moreover, some researchers are of the opinion that since DSSs have not reached to human level by now; therefore, the judgment they suggest is likely narrow and not trustworthy. Some key limitations that lag DSS behind to reach a respected stage are their inability to replicate some innate human knowledge management skills; narrow domain specific; mismatching decision-makers' mode of expression or perception; inability to overcome a faulty decision-maker; knowledge constraints and overdependence dangers (Bell and Chung, 2000).

Some existing DSSs are integrated with other software components such as Crop Simulation, multimedia and GIS. Agent-based approach is lacking to integrate distributed heterogeneous systems to solve integration problem besides problems related to distributed components on different environment. Some DSSs based on GIS approach are raising the problems pertaining to data sharing between GIS and

DSS components and fitting the two components into a common problem-solving framework.

Knowledge sharing and reuse is another topic that has attracted the attention of researchers during the last few years. The researcher sharing knowledge in agriculture can be directed towards identifying common knowledge that can be shared among different DSSs such as identification of agricultural ontology, knowledge related to the same taxonomic categories of crops and knowledge related to common resources like soil, water and climate. The Library of domain-specific tasks like irrigation, fertilization, IPM and other operations from sowing to harvesting needs further research, for reusability and sharing purposes. Intelligent selection of certain media in multimedia data in different forms is a general research issue in DSS. Indexing and searching system is a common problem faced by DSS designers, due to large databases with increasing number of rules together with conflicting sets.

6 Future Trends

Researchers are optimistic about the future of DSS. This optimism continues to produce products and contributions to literature. A host of new tools and technologies are adding new capabilities to DSS. They include hardware and mathematical software development, artificial intelligence techniques, data warehousing and mining, OLAP enterprise resource planning, ERP, intelligent agents and WWW (Berners-Lee, T., 1996).

Data warehousing is a subject-oriented, integrated, time-variant and non-volatile collection of a relational or multidimensional database (MDDB) optimized for decision support, which is separated from operational databases. MDDB organizes data as an *n*-dimensional cube so that users deal with multidimensional data views such as crop, region, yield and area, with speedy query response time. Data mining, also known as knowledge Data Discovery, refers to discovering hidden pattern from data, not known before. It attempts automatic extraction of knowledge from the large databases, either in the data warehouse or elsewhere (spreadsheets, weather observatories, etc.)

Intelligent agent's research is an emerging interdisciplinary research area involving researches from such fields as ESs, DSS, cognitive science, psychology and databases. Intelligent agent's research has contributed to the emergence of a new generation of active and intelligent DSS. This approach will enable us to integrate simulation models, GIS and multimedia with ESs, giving DSS a dominant role to play in modern agriculture. Development of domain-specific tasks will help in knowledge sharing and reuse. Sophisticated user interfaces for different media types are expected to be designed based on the users' expertise and need.

WWW is becoming an infrastructure for the next generation of DSSs and groupware applications. There is also a trend to develop tools and techniques that could facilitate the dissemination of ESs through WWW. High bandwidth, reliable internet connectivity and carefully prepared underlying data will be keys to the future success of web-based decision tools.

ERP, a new generation of information system, is integrating information and information-based processes within and across functional areas in an organization. The extensive databases created by the ERP system provide the platform for decision support, data warehousing, data mining and executive support systems for further development. Global DSSs are emerging as the new frontiers in MIS area. Over the next decade, DSS will focus on teams, work groups, and distributed, decentralized structures (King, 1993). Hammer et al. (2002) foresees future DSS as a “small” tool for aiding farmers’ tactical decisions, a versatile simulator as a consultants’ tool, a versatile simulator as the core of a facilitated “learning laboratory”, and a formal framework that supports regulatory objectives in constraining and documenting farming practice.

DSS integrated with precision agricultural equipments, GIS and site-specific farming are changing the realm of modern agricultural practices. Future developments may include the possibility of implementing a number of DSS models into a GIS, which will support precision agriculture by providing adjusted spraying advice based on plot-specific characteristics (Bouma, 2006).

In future, design and development of DSS is expected to get advantage from promising technologies like data warehousing and mining, agent-based approach, intelligent agents and enterprise resource planning besides advancement in hardware and software technologies. These technologies shall facilitate easier design of more complex DSSs. Agricultural is expected to get maximum benefits out of these as well as new milestones laid by the technologies like modelling, hypothesis, simulations and projections. Continued progress in system modelling combined with projected growth in computer power, near-term improvements in Remote Sensing, GIS, Precision Agriculture, new developments in the data extraction like data warehousing and date mining with new concepts of data exchange over the Internet should all contribute to expanded use of DSS for cropping systems in the future.

7 Conclusion

DSS practice, research and technology continues to evolve though its history covers only a relatively brief span of years. There is no general account of classification and architecture of DSS. But it is possible to reconstruct the history from retrospective accounts considering published and unpublished material and redefine classification and architecture of DSS through in-depth research and literature contribution.

In the agricultural sector, which is in the midst of powerful changes influenced by industrialization and modernization, farm consolidations, reduced or eliminated subsidies, environmental limitations and land use conflicts with overall increased risk, the availability, accessibility and application of contemporary expert agricultural information is of high priority for farmers, technicians and researchers. With these changes and demands, different useful, scientifically valid and user-specific models have been implemented successfully. Many scientific and academic institutions have turned to computerized DSSs as a means of packaging biological, agricultural and technical information to make the information more easily accessible and useful for various beneficiaries in a rapidly transforming and competitive world.

Research issues in agricultural DSS can be categorized under these topics: integration, knowledge sharing and reusability, retrieval of data, automatic data acquisition besides design, development and adaptation. With these challenges work is progressing well. Systems are being developed providing useful access to all human knowledge and expertise for decision-making. Greater user involvement through participatory learning approaches, interactive prototyping, keeping the amount of input data required as small as possible, keeping the system itself as flexible as possible, providing users with default values, involvement of end-users from basic developmental stage as well as keeping DSS development manageable and small in scope can provide avenues for improvement in DSS research and development.

In the last decade researchers have reviewed the use of computer models in land use planning, forecasting, agronomical practices, water resources and emphasized the need for DSSs to make these models more useful. Incorporation of simulation and optimization models with interactive graphical capabilities is encouraging the acceptance of techniques related to literature development in practice. Now we have interactive, user-friendly computer systems which are becoming the rule, rather than the exception. Perhaps the greatest challenge to DSS development is how they are delivered to and used by industry and policy-makers. In order to achieve these goals we must not only learn from experience but be ready to accommodate new technologies and research areas. In addition, adoption to long-term climatic change, integrating process planning, natural hazards mitigation, matching new scientific information with local and indigenous knowledge are being addressed for sustainability of vulnerable areas.

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Olive and Grapevine Biodiversity in Greece and Cyprus – A Review

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Abstract Olive (*Olea europaea*) and grape (*Vitis vinifera*) are among the most important fruit crops. Greece and Cyprus are primeval centers of their early domestication and hence diversification. Despite long selection towards desirable agronomic traits and extensive exchange of selected clones between different regions, both species are still characterized by high levels of genetic and phenotypic variability. Numerous ancient or rare varieties with important agronomic characteristics are still cultivated locally, yet their recent extensive replacement by improved or modern cultivars along with the near extinction of their wild relatives raise concerns about severe genetic erosion. Under the light of the impending climate changes, like global warming and its possible consequences in water availability and expansion of pests/diseases, the need to describe and preserve both cultivated and wild germplasm for future exploitation is imperative now, more than ever. To this end, it is essential to acquire a thorough picture of the existing biodiversity for both species and to understand the molecular mechanisms governing important agronomic traits. More than 170 olive and about 700 grape Greek varieties have been recorded, although the numbers of distinct cultivars may be smaller due to the existence of synonyms. The respective diversity in Cyprus is much lower, though the major Cypriot olive variety 'Ladolia' is actually a highly variable mixture of numerous genetically distinct landraces, and the autochthonous grape cultivars are generally well-adapted to extreme environmental conditions constituting promising plant material for sustainable utilization. Molecular marker techniques have significantly ameliorated the description of local genetic diversity within both species. However, most studies have been restricted to major cultivars and accessions obtained from germplasm collections. Further exploration, description and agronomic evaluation of indigenous germplasm are needed, including minor or underutilized domestic varieties and wild germplasm. Groves or individuals of oleasters and sylvestris grapes should be recorded and preserved in situ or ex situ, i.e., in germplasm collections. Such

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germplasm may constitute invaluable plant material in breeding programs aiming to develop disease-resistant and stress-tolerant clones, thus improving the sustainability of grape and olive growing worldwide. Genetic resources are the foundation of our agricultural future. Sustainable management of olive and grape genetic resources is essential for breeding programs and one of the prerequisites for sustainable agriculture.

Keywords *Olea europaea* · Oleaster · *Vitis vinifera* · Sylvestris grape · Genetic diversity · Molecular markers · Breeding · Sustainability

1 Introduction

> The peoples of the Mediterranean began to emerge from barbarism when they learnt to cultivate the olive and the vine (Thucydides, the Greek historian, fifth century BC).

During some 15,000 years of the agricultural era numerous plants have been domesticated and significant diversification of crop species has evolved. Even though cultivated varieties represent a very small proportion of the total species diversity, they constitute the major part of the human diet and are thus of great importance (Hammond, 1995; Dirzo and Raven, 2003). At the species level, most crops are genetically quite diverse, as evidenced by the existence of many landraces and distinctive varieties. Local cultivars from different geographic regions often show significant variability in their genetic and phenotypic traits. That makes them indispensable for cultivation at the specific environment they have adapted providing sustainability in local communities. Local varieties may provide a vital source of distinctive traits and bioactive molecules extremely valuable in sustainability and quality of food. Genetic diversity is also important for breeders since it offers a broad gene pool for selecting superior alleles that may determine quantity, quality or other attributes relevant to desirable characters. Wild forms of crop plants often serve as an alternative source of allelic genomic regions that control important traits (Wolfe, 1985; Singh, 2001 and references therein). Among others, these genomic loci include genes conferring tolerance against several biotic or abiotic stresses. Intra-species biodiversity of crop plants is thus very important for sustainable agriculture and for the food industry.

Crop biodiversity, despite its high importance, is currently in sharp decline. The Food and Agriculture Organization of the United Nations (FAO) estimated that about 75% of the genetic diversity of domestic agricultural crops has been lost during the last century (Anonymous, 1995). There are several causes for this dramatic depletion of genetic resources, most important of which are the replacement of local varieties by improved or modern cultivars, changing agricultural practices, environmental degradation, legislation/policy and consumer demands. As in most crops, only a few selected cultivars of fruit trees have become predominant in the worldwide agriculture and as a consequence the overall genetic variability has considerably decreased. Reduction of biodiversity, encountered at species or even varietal level, may impose a serious threat to our food security (Thrupp, 2000). Given the dramatic change in the global climate and the spreading of crop diseases, it seems likely that modern varieties may not always be able to resist against striking biotic or abiotic stresses in the near future. Hence, there is an increasing demand to ensure that crop genetic resources will be readily available to meet local and global food demands. A better description and characterization of the heterogeneity within cultivated varieties and wild forms are important factors for planning conservation strategies, better management of available genetic resources and successful breeding programs.

Olive (*Olea europaea* L.) and grapevine (*Vitis vinifera* L.) are two of the most important fruit crop trees in the Mediterranean Basin and also in many other areas with temperate or subtropical climate worldwide. Olive is mainly used as a source of oil or for table consumption, though other end-uses are also important. Recent studies have shown that olives may contain high levels of anticancer agents (e.g., squalene and terpenoids) and valuable antioxidants, such as acteosides, hydroxytyrosol, tyrosol and phenilpropionic acids. The concentration of these bioactive molecules seems to be highly dependent on the specific cultivar, the environmental conditions or the time of harvesting (Owen et al., 2000; Fabiani et al., 2002; Doveri and Baldoni, 2007). Olive oil is a relatively expensive vegetable oil due to its high cultivation and harvesting costs and the limited production. As in any crop, olive breeding and selection schemes target towards bringing together traits of agronomical interest. The breeding process in olive, however, faces many difficulties because the tree has a long juvenile phase and requires time and space to analyze F1 and F2 generations (Hatzopoulos et al., 2002). Moreover, its high heterozygosity greatly diminishes the chances for producing a desired recombination of characters in a single offspring. The utilization of combined morphological and molecular markers for characterizing genetic resources and for direct selection of desirable genotypes is thus essential to accelerate the process. Traits of agronomic importance include shortening the juvenile phase, increasing fruit size and/or oil content and quality, and improving drought or cold tolerance and biotic (pests, diseases) stress resistance. Selection schemes targeting dwarf phenotypes are also important in oleoculture since such trees are ideal for mechanization of harvesting and reduction of the overall costs.

Grapevines are grown in more than 80 countries worldwide for wine production, table grapes or dried fruits (raisins). According to the variety and the cultural conditions, grape berries and wines may contain beneficial organic compounds, e.g., flavonoids that have been linked to a host of health revelations from lowering risk of heart attacks to protection against various cancers. Nowadays, only a small number of *Vitis vinifera* cultivars dominate the global production. More precisely, a few selected cultivars are used in the production of superior wine, though autochthonous or rare cultivars are still of great importance for local communities in the Mediterranean and elsewhere. The primary targets of grape breeding programs are to increase the yield and quality, and to generate cultivars well-adapted to environmental stresses such as soil factors, drought and extreme temperatures, and biotic stresses. At the same time, breeders should take into consideration the requirements for retaining highly desirable characters needed for table, raisin and principally wine production (Riaz et al., 2007). Previous breeding efforts of grapevine have led to the development of novel hybrids derived from crosses between *V. vinifera* and native American species that show pest or abiotic stress resistance. However, they are generally considered to have low fruit quality. Particularly for the wine industry, the utilization of such hybrids has been greatly limited because of the market demands to have traditional varieties with well-documented quality and historical acceptance (Riaz et al., 2007). The knowledge of genetic variation and the amount or pattern of cultivar distribution is central to the development of effective conservation strategies and efficient use of *Vitis* germplasm (Aradhya et al., 2003).

The application of molecular markers for the estimation of genetic diversity along with the knowledge of genetic traits under selection may further facilitate breeding efforts. The development of genomic technologies could provide the necessary tools in order to begin understanding the genetic and molecular basis of fruit ripening and quality, and explain the mechanisms of tolerance/resistance to biotic/abiotic stress in fruit crops (Riaz et al., 2007). In olive, at present, there are only limited data on genome mapping and genome organization (Doveri and Baldoni, 2007). However, efforts have been made on the isolation and characterization of genes involved in important metabolic pathways that influence the quality of drupe and oil, mainly key enzymes in fatty acid biosynthesis and modification pathway, triacylglycerol synthesis and storage (Hatzopoulos et al., 2002 and references therein; Giannoulia et al., 2007; Banilas et al., 2005, 2007). Genomic research in grape is far more ahead, probably because grapes are the most economically important fruit species in the world. It is not surprising that its genome (cv. Pinot Noir) was the first among fruit crops to be sequenced (Jaillon et al., 2007; Velasco et al., 2007). This achievement together with the increasing set of single nucleotide polymorphic (SNP) loci will definitely open a new era in the molecular breeding of grapes. Allelic diversity analysis of genes involved in important metabolic pathways among different cultivars and wild individuals would have direct application for crop improvement (This et al., 2006).

In this article, the magnitude and nature of olive and grapevine biodiversity in Greece and Cyprus are described. These countries are located in the Eastern Mediterranean Basin, where origination and early domestication of both species occurred. Studying genetic diversity in the location where the origination of crops and agricultural development occurred provides insights related to the current structure and geographic distribution of this important facet of biodiversity. The utilization of molecular marker techniques has been invaluable towards the description of genetic diversity within both species and the state-of-the-art for local germplasm in Greece and Cyprus is presented.

2 Domestication and Distribution

Both olive and grapevine have wild progenitors in the West Asia–Eastern Mediterranean region that still exist today. Ethnobotanical data along with recent genetic analyses suggest that the history of olive and grapevine domestication and dispersal share many similarities. These crops have played important roles in the heritage and civilization of the Eastern Mediterranean people from ancient to modern times. Their exact origin, however, and the particular routes of further dispersal are not yet fully explored. In order to shed more light on their origin and to reconstruct their evolution under domestication it is plausible to identify and characterize their wild progenitors (Zohary, 1996). To this end, genetic comparisons between wild-types and domesticated derivatives are required. Historical and biological data should be combined to elucidate geographical areas of crop domestication and diffusion, and to interpret the structure of current genetic biodiversity (Terral et al., 2004). Such information is essential to better manage and utilize the genetic resources in breeding and sustainable fruit production.

2.1 Olive

Olive (*Olea europaea* L.) is a typical woody species found throughout the Mediterranean Basin. Wild forms of olive trees are still natural members of maquis (woodlands) or forests, formed mainly by sclerophyllous evergreen species characteristic of the Mediterranean flora. Olive is believed to be indigenous to the Mediterranean Basin (Green, 2002). The wild variety *O. europaea* var. *oleaster* (Hoffmanns & Link) (or oleaster) is similar to the cultivated form var. europaea (Zohary, 1973). Nevertheless, oleasters (whether original or feral forms) differ from the cultivated clones by the presence of spinescent juvenile shoots, smaller fruits with higher stone/mesocarp ratio and relatively low oil content, and characterized by longer juvenile stage (Lumaret et al., 2004). According to anthracological studies, the exploitation of wild olives is suggested by remains in archaeological sites since Paleolithic times, at about 43,000 years BC (Zohary and Spiegel-Roy, 1975). The exact origins in time and space of olive domestication and diffusion across the Mediterranean are not clear. However, several archaeological, historical, and botanical/biological data agree in that early domestication most likely took place in the Middle East during the Early Bronze Age at about the second half of the fifth millennium BC (Zohary and Spiegel-Roy, 1975; Neef, 1990; Liphschitz et al., 1991; Zohary and Hopf, 1994). The progenitor of domesticated olive is believed to be the indigenous wild oleasters. The domestication process is likely to have involved the selection of genotypes with desirable agronomic traits, like large fruit size and/or high oil content, and their further vegetative propagation either by directly planting of cuttings or through grafting onto wild individuals (Doveri and Baldoni, 2007).

Olive cultivation in Greece may have occurred during the Minoan civilization on the island of Crete at around 3,500 BC. That civilization predates the discovery of Mycenae olive fossils from 1,600 BC. In Greek mythology, Athena (the goddess of wisdom and civilization) offered to Athenians the first domesticated olive tree and the city was named Athens in honor of the Goddess (Rhizopoulou, 2007). Olive was also the symbol of winning and of compromising in ancient Greece (Rhizopoulou, 2004). The cultivation of olive tree in Cyprus seems to have been well established since the Late Bronze Age (Zohary and Spiegel-Roy, 1975). This fact along with the geographical location of the island (Eastern Mediterranean Sea) support the hypothesis that Cyprus belongs to one of the major centers of early domestication of olive and hence variability (Banilas et al. 2003).

Domesticated olive gradually diffused from East to West by the Phoenicians, Etruscans, Greeks and Romans (Terral et al., 2004), largely because of its multiple uses (e.g., oil, fruits, wood and in practical medicine). It is thought that Greek colonizers brought domesticated olive germplasm to Sicily between the ninth and fifth centuries BC. Archaeological and molecular analyses support this possibility suggesting an early introduction of cultivars from the Eastern Mediterranean areas into Italy (Bronzini de Caraffa et al., 2002; Terral et al., 2004; Owen et al., 2005). In accordance to this assumption, the Latin word ‘olea’ has been derived from the Greek ‘aelea’ (in Greek: ελαία), which means olive tree. Following trade routes, Greeks and Phoenicians spread the olive even further to France, Spain, and to Tunisia, on opposite shores of the Mediterranean. It is noteworthy, however, that recent molecular analysis, using both nuclear and cytoplasmic markers, has shown that the Eastern and Western Mediterranean wild populations are strongly differentiated from one another (Besnard et al., 2001a,b; Lumaret et al., 2004). Based on these data and archaeobotanical findings it has been proposed that the selection of olive cultivars occurred in different genetic pools in the East and West Mediterranean (Galili et al., 1989; Terral and Arnold-Simard, 1996; Besnard et al., 2002a,b; Terral et al., 2004). Therefore, it seems most probable that independent domestication events occurred in the Western Mediterranean, further contributing to the richness and heterogeneity of the cultivated germplasm. Nowadays, areas surrounding the Mediterranean Basin account for more than 90% of the world’s olive oil production. However, because of its high quality and healthy constituents, olive oil consumption is steadily increasing all over the world and drives olive cultivation in areas such as Latin America, California, South Africa, China and Australia. More than 1,250 cultivar names have been reported so far world-wide (Bartolini et al., 1998), though only a few cultivars are highly dispersed while the majority is rather localized. For purposes of conservation and sustainable utilization, it is important to determine more accurately the extent of existing genetic diversity in the cultivated and wild germplasm, to decipher true cultivars, their intra-varietal diversity and their characteristics.

2.2 Grapevine

The grapevine, *Vitis vinifera* ssp. *vinifera* (or sativa), along with olive are among the oldest cultivated plants. Grape is thought to have been domesticated in the Near-East, around the northern Zagros, eastern Taurus and Caucasus Mountains (Zohary and Spiegel-Roy, 1975; Zohary and Hopf, 1993; McGovern, 2003) or in the Transcaucasian region (Olmo, 1976) at about 4,000 BC. *V. vinifera* ssp. *silvestris* (or sylvestris) is believed to be the wild form from which the cultivated grape was derived (Levadoux, 1956). What is known by comparisons between the genuine wild vines and cultivars is that, besides alteration of agronomic traits, domestication resulted in dramatic changes in the reproductive biology of grape. Vegetative propagation, either by rooting or grafting, had the practical advantage to maintain desirable characteristics of superior individuals by avoiding wide genetic segregation of numerous traits. The most important change, however, was the shift of wild sylvestris from dioecy to hermaphrodism, due to a single mutation to a pistil suppressing allele. The wild vines are climbing perennial woody plants with coiled tendrils. They are thought to be native to the woodlands south and west of the Caspian Sea, extending along the southern shores of the Black Sea to the Near-East and westwards to northern Greece and southern Balkans (Renfrew, 1996). Wild forms are also found in Portugal, Spain, France, Italy, as well as from the Rhine and Danube Valleys to Tajikistan and the western Himalayas (Zohary and Hopf, 2000; This et al., 2006).

The early exploitation of wild grape fruit as a food source by late Palaeolithic populations (about 11,000 BC) has been well documented from studies at different archaeological sites across Europe (Zohary, 1996). Based on palaeo-ethnobotanical findings it has been suggested that exploitation of wild grapes in Greece occurred shortly before 6,000 BC (Renfrew, 1996). Soon after its primo-domestication, the grapevine was spread to the Middle East and Egypt and then, during the first half of the third millennium BC, to Minor Asia, Greece (Thrace, Aegean Sea, Peloponnese and Crete) and Cyprus (Grassi et al., 2003). During the Neolithic period (4,300–2,800 BC) there is a mass of detailed evidences for the cultivation of grapes in different sites of Greece, the best documented of which comes from Dimitra village in East Macedonia (Logothetis, 1970; Renfrew, 1996). The cultivation of the grapevine in Cyprus dates back to the island's colonization by the Phoenicians in about 3,000 BC.

The distribution of domesticated vine westwards shares many similarities with olive's routes of dispersal. Domesticated grapevines first appeared in Southern Italy at about 2,000–1,500 BC and in Northern Italy, Southern France, Spain and Portugal in the second part of the first millennium (Hopf, 1991; McGovern, 2003). It is likely that domesticated germplasm was brought to southern Italy and France by Greek immigrants. However, as in the case of olive, morphological differentiation between eastern and western germplasm suggests additional domestication events (Negrul, 1938; Levadoux, 1956; Mullins et al., 1992). Consistent to this assumption, recent molecular data from chloroplast DNA microsatellite loci point to the existence of at least two major origins for the cultivated grape, one in the Near-East and another in the Western Mediterranean (Arroyo-García et al., 2006).

About 24,000 cultivar names are currently in use, but it is thought that *V. vinifera* comprises about 5,000 true cultivars (Alleweldt, 1988). The tremendous genetic diversity of the currently existing cultivated germplasm has been primarily originated by domestication of wild vines and then shaped through crosses between cultivars as well as cultivars and wild plants. Grapevines are highly heterozygous and any seedling progeny has a unique combination of parental alleles that often results in a distinct phenotype. The easy-to-propagate vines via cuttings (asexually) contributed to the maintenance of desirable genotypes through the history. However, spontaneous somatic mutations also played a major role in the creation of clonal variation, i.e. vines of the same cultivar that differ phenotypically, which eventually may have led to cultivar differentiation (Doazan and Rives, 1967; This et al., 2006).

In an attempt to trace the origins of modern cultivars, the use of historical records is invaluable and some of those data will be reviewed later in this article. However, it should be mentioned that historical data may not be always reliable and should be critically considered. Molecular biology tools used in agricultural biotechnology, like marker-aided genetic analysis and marker-aided selection, will help to determine more accurately the origin of cultivars and evaluate the extent of existing genetic diversity. In addition, they will help to identify genes or other loci and associate them with traits and functions (Herdt, 2006). This knowledge is fundamental not only for understanding the biology of grape but also for its direct application to improvement or selection strategies. There is a remarkable genetic variation that could be utilized through non-transgenic breeding methods. All we need is the knowledge for selecting superior genotypes more efficiently and rapidly, thus improving decision-making process. Joint historical, morphological and molecular data may contribute to further elucidate grape domestication and routes of diffusion. This information is essential to better interpret the complex structure of current genetic diversity of grapevine.

3 Genetic Diversity in Greece and Cyprus

3.1 Organization of Olive Diversity

Olive genotypes can retain germplasm characteristics over thousands of years due to the remarkable longevity of the olive tree and vegetative propagation of clones. Thus, ancient olive varieties have been preserved through time to our days. Besides, hundreds of novel cultivars have been evolved over the centuries, mainly by strong and long selection towards qualitative and quantitative traits and/or adaptation to various microclimates in different regions (Bartolini et al., 1993). Nowadays, there is a high level of heterogeneity in olive cultivated germplasm in Greece (Fig. 1). Along with the high degree of genetic variation, the existence of synonyms (same clones with different denominations) and homonyms (same or similar denomination for distinct cultivars) has also contributed to a considerable uncertainty about the current status of olive cultivars in many countries including Greece and Cyprus (Connell, 1994; Nikoloudakis et al., 2003; Banilas et al., 2003). Some of this uncertainty could be attributed to environment and genotype interactions, since cultivation conditions may contribute to changes in olive morphology (Booth and Davies, 1995; Vergari et al., 1996). Since lately, cultivar identification has been based exclusively on morphological and agronomic characters. Pomological protocols have been very efficient in cataloguing olive cultivars (Barranco and Rallo, 1984; Barranco et al., 2005) overcoming confusion due to ambiguous denominations and allowing the right establishment of synonyms and homonyms. However, the recognition of olive

Fig. 1 There is a high degree of variability among Greek olive cultivars at both the phenotypic and genetic level. An example of ripening olive fruits from three important indigenous cultivars, 'Amfissis' (**A**), 'Kalamon' (**B**), and 'Koroneiki' (**C**), showing significant variation in drupe size, shape and color

cultivars based solely on phenotypic characters may be problematic, especially at the early stages of tree development (Vergari et al., 1996, Mekuria et al., 1999). The development of biochemical and molecular marker techniques ameliorated accurate cultivar discrimination, identification and germplasm characterization (Trujillo et al., 1995; Belaj et al., 2003). Ouazzani et al. (1993) used isozyme polymorphisms to analyze olive germplasm variability and to differentiate between wild plants and cultivars. More recently, random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) technologies have proved very useful to estimate genetic similarities between or within cultivars (Fabbri et al., 1995; Angiolillo et al., 1999; Mekuria et al., 1999; Gemas et al., 2000; Baldoni et al., 2000; Owen et al., 2005). The isolation of simple sequence repeats (SSRs) or microsatellites in olive (Katsiotis et al., 1998; Cipriani et al., 2002) marked a new era in the genetic diversity studies. The relative advantages of SSR markers over other size-based molecular methods for characterizing olive germplasm have been highlighted elsewhere (Belaj et al., 2003).

Despite the long history and contribution of the Eastern Mediterranean olive germplasm in the overall olive diversity, current knowledge about olive germplasm variability in both Greece and Cyprus is still limited. In Greece more than 170 cultivar names are in existence, although the number of distinct cultivars might be much smaller due to possible existence of synonyms. The denomination of Greek cultivars usually refers to toponyms or place names, e.g., ‘Kalamon’, ‘Koroneiki’, ‘Megaritiki’, ‘Chalkidikis’, the shape of fruit, e.g., ‘Valanolia’, ‘Amigdalolia’ or the end-use, e.g., ‘Ladolia’. The latter has contributed to a great confusion due to extensive homonyms under the denomination ‘Ladolia’, which in Greek means an olive tree that is cultivated for oil production. Table 1 presents some of the most important Greek cultivars that have been previously identified by different biochemical or molecular marker techniques (Hatzopoulos et al., 2002; Nikoloudakis et al., 2003; Owen et al., 2005).

One could hypothesize that a cultivar denomination under a toponym directly reflects the place of origin or principal area of cultivation. However this is not always the case, since recent molecular analysis data suggests extensive exchange of genetic material in the past throughout Greece (Nikoloudakis et al., 2003, Owen et al., 2005). Unfortunately, any ethnological or relevant records for oleoculture in Greece are quite limited. It was only recently, at around 1930, that the first attempts were made to describe Greek olive cultivars based on morphological grounds by Anagnostopoulos followed by Lychnos (1949). Much later, genetic diversity among the most common cultivars was estimated based on allozymic analysis, while recently the most important cultivars were analyzed by RAPD and AFLP molecular markers (Nikoloudakis et al., 2003; Owen et al., 2005). Based on those studies, most of the cultivars do not show clustering in accordance to their principal area of cultivation. This was particularly profound for the Cretan accessions (i.e., ‘Mastoidis’, ‘Throumpolia’, and feral forms) or those originating from the Ionian Islands (i.e., ‘Kalokerida’, ‘Lianolia’, ‘Thiaki’, ‘Tragolia’, and ‘Vasilikada’). These results point to extensive dispersal of elite clones in the past to different geographic areas in Greece. This is in contrast to the confinement of most principal cultivars in other countries, such as in Spain. It seems probable that grafting as the traditional method of propagation in Greece facilitated cultivar diffusion in the past, while in Spain clonal propagation was accomplished mainly through big-sized propagules (Rallo and Barranco, 1983). This suggestion is also supported by the fact that the most important Greek cultivars are nowadays widely cultivated in different provinces of Greece beyond their principal area of ancient cultivation. According to an alternative scenario, which does not exclude the coexistence of the former, the ancestral olive germplasm could be naturally quite variable even within a restricted area, such as an island (Crete or Corfu). Supporting this, recent molecular data suggest that wild olives and feral forms of a given region are genetically more variable than cultivars (Lumaret and Ouazzani, 2001). Nevertheless, in a few cases geographic relatedness

Table 1 List of important Greek olive cultivars

Official denomination (in Greek)	Other common names – synonyms	Principal area of cultivation/current diffusion[a]	Fruit size[b]/ use[c]	Main agronomic traits – characteristics
'Adramytini' (Αδραμυτίνη)	'Fragolia', 'Aivaliotiki'	Lesvos Island (AI)/ northern AI, Cd	M/B	Average fruit and oil yielding. Quality table olives.
'Agouromanakolia' (Αγουρομαν ακολιά)	'Agouromanako'	Argolida (P)/ eastern P	M/O	High yielding. High oil quality. Cold tolerant, late ripening.
'Amfissis' (Αμφίσσης)	'Konservolia', 'Amfissis'	Amfissa (SH)/ wide-spread in Greece, USA, Australia	L/T	High yielding when cultivated on good soil and irrigated. Cold tolerant.
'Kalamon' (Καλαμών)	'Kalamata'	Kalamata (P)/ wide-spread in Greece, USA, Australia	L/T	Average yielding. Early ripening. Susceptible to drought.
'Koroneiki' (Κορωνέικη)	'Psilolia', 'Cretikia', 'Ladolia', 'Lanolia', 'Koroni', 'Vatsiki'	Koroni (P)/P, Cr, II, western SH, AI, Cyprus, Australia, USA	S/O	High yielding. Exceptional oil quality. Tolerant to adverse conditions (drought, arid terrains).
'Kothreiki' (Κοθρέικη)	'Manaki', 'Manakolia', 'Korynthiaki', 'Konservolia'	Fokida (SH)/SH, north-eastern P	M/B	Average to high yielding. High-quality table olives. Tolerant to drought and chilling.
'Lianolia Kerkyras' (Λιανολιά Κερκύρας)	'Souvlolia', 'Merolia', 'Ladolia', 'Corfolia'	Corfu Island (II)/II, western Ep	S/O	High yielding. High oil quality (equivalent to Koroneiki). It may grow in rocky arid terrains. Susceptible to drought.
'Mastoidis' (Μαστοειδής)	'Tsounati', 'Mouratolia', 'Athinolia'	Cr/Cr, southern P	M/O	Medium to low yielding. It may grow up to 1,000 m height. Prefers calcareous soils.
'Megaritiki' (Μεγαρείτικη)	'Ladolia', 'Hondrolia'	Attiki (SH)/ Eastern SH, eastern P	M/B	Medium to high yielding. Average oil quality. Drought tolerant, ideal for reforestation in arid terrains.
'Throumpolia' (Θρουμπολιά)	'Thrubolea'	Cr/Cr, AI, eastern SH	M/B	Average yielding. The edible olives ripen on the tree (also known as 'thrubes'). Susceptible to drought and chilling.
'Valanolia' (Βαλανολιά)	'Mytilinia', 'Valana', 'Kolovi'	Lesvos Island (AI)/ northern AI	M/O	High yielding. Exceptional oil quality. Late ripening.

[a]Abbreviations of provinces are as indicated in Fig. 2.
[b]L = Large; M = Medium; S = Small.
[c]T = Table; O = Oil; B = Both uses.

was observed, such as the close genetic proximity detected between ‘Adramytini’ and ‘Valanolia’, two ancient Greek cultivars that are still being cultivated exclusively in Lesvos Island (Nikoloudakis et al., 2003).

Comparative genetic diversity studies between cultivated and wild germplasm could help to better understand the origin of cultivars. Such studies are also important to evaluate recent concerns about demographic bottlenecks in wild olives, leading to genetic erosion of the genuine oleaster gene pool (Belaj et al., 2007). Unfortunately, the existence of original oleasters in Greece and also in other Eastern Mediterranean countries like Turkey, Syria and Egypt, where olives have been extensively cultivated since ancient years, seems to be rare. It is noteworthy that Lumaret and Ouazzani (2001) surveyed forests from throughout the Mediterranean Basin to identify surviving genuine oleasters but did not find any in the above-mentioned countries, even though such populations are likely to occur in forested areas in those countries (Lumaret et al., 2004). Whether this is the case or not, these data are quite alarming considering that Greek original wild germplasm probably comprises a significant gene pool from which most Greek cultivars have evolved. For this reason we have recently started a thorough survey to find putative genuine oleasters in Greece, in order to further evaluate them by means of molecular marker technologies. Following restrictive criteria, like past and present isolation from cultivated areas and feral morphology, we have recently distinguished some promising sites where oleasters grow in small populations within wild maquis flora, mostly from Sterea Hellas and Crete (Fig. 2). An interesting case is that of an oleaster grove in a gorge of southern Crete (Martsalo gorge in Asterousia Mountain) along with the rare and endemic Cretan date palm (*Phoenix theophrastii*), which is supposed to be remnant of an ancient flora that survived the last Ice Age. Such an olive population is worth to be examined because it seems probable that the distribution of genetic diversity has been reconstructed on the basis of recolonization of the Mediterranean Basin from Ice Age refugia (Breton et al., 2006). Another important population was found close to the above grove, in Mandalo Mountain south of Rethymno (Fig. 3). It is a region that has been included in ‘Natura 2000’, an EU-wide network of nature protection areas established under the 1992 Habitats Directive (92/43/EEC).

In the case of Cyprus, intensive olive cultivation has started since the Late Bronze Age and is still of considerable importance. The main areas of cultivation overlap those of the olden times, while a significant expansion occurred just after the Second World War (Christodoulou, 1999). At about 1970, Cypriots realized the need to increase their productivity by simultaneous reduction of cost and thus started to establish new groves by applying modern production methods. For this reason, foreign cultivars, like ‘Koroneiki’, ‘Kalamon’ and ‘Picual’, were introduced. At the same time, however, the government encouraged cultivation of local genotypes in locations that are considered environmentally fragile, like rocky hills and mountainous regions, in order to prevent devastation and provide sustainability to local farmers.

Today the main variety widely cultivated in Cyprus is an autochthonous ancient cultivar named ‘Local’, which is also referred as ‘Ladolia’. Cypriots prefer to grow

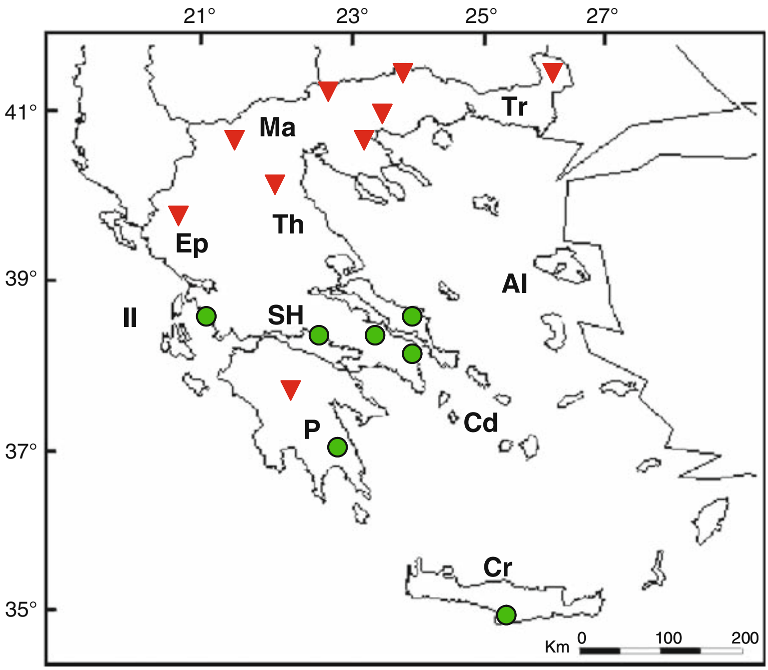

Fig. 2 Map of Greece indicating places where wild oleaster (*circles*) or sylvestris grape (*triangles*) populations have been recently found (see text for details). AI = Aegean Islands, Cd = Cyclades, Cr = Crete, Ep = Epirus, II = Ionian Islands, Ma = Macedonia, P = Peloponnesus, SH = Sterea Hellas, Th = Thessaly, Tr = Thrace

Fig. 3 Genuine wild olives are rare but still grow in small populations in Greece. An example of a grove of oleasters (**A**) and close up of an individual (**B**) from Mandalo Mountain, south of Rethymno in Crete

'Ladolia' than other exotic and potentially higher-yielding cultivars, because it is well adapted to the local environmental conditions, like drought, and appeals to the taste of Cypriots (Gregoriou, 1996, 1999). After thousand years of cultivation and breeding, however, there is doubt whether 'Ladolia' could be still considered as a single variety or actually a highly variable mixture of genetically distinct landraces (Banilas et al., 2003). Since 1987 a collection of 'Ladolia' clones has been established at the Zyghi Agricultural Research Institute of Cyprus (Gregoriou, 1996). Individual plants were selected from many areas of the island for certain desired characteristics with the prime objective of evaluating and preserving the genetic variability of the 'Local' variety, and to create new variants that perform better for the desired fruit and cropping characteristics. Such characteristics were not only targeted towards productivity but also to adaptation to local conditions and resistance to biotic/abiotic stresses. In a recent study, RAPD marker technology was applied to evaluate the genetic diversity and to discriminate among those accessions (Banilas et al., 2003). Results uncovered a relative high genetic diversity among selection clones of 'Ladolia' studied, which was in accordance to the high degree of their morphological and agronomic variation as described by Gregoriou (1996). Based on genetic similarity values certain 'Ladolia' genotypes had clonal relationships, while others sharing low values (<0.4) most likely originated from genetically distant landraces. The occurrence of clonal genetic variation has been also observed by using microsatellite markers in grape (*Vitis* spp.) (Cipriani et al., 2002). This intra-varietal diversification could be attributed to somatic mutations that may occur in such long-lived trees like olives and vines (Cipriani et al., 2002; Cervera et al., 1998). However, the high extent of genetic variation estimated among 'Ladolia' clones could not be addressed only to such spontaneous mutations, unless assuming that they have originated through sexual reproduction. 'Ladolia' is supposed to be a semi-wild olive tree suggesting persisting hybridization between wilds and cultivated clones in such a restricted area as an island (Banilas et al., 2003). Supporting this, it is noteworthy to mention that genuine wild olives still exist in Cyprus (Lumaret et al., 2004). Although the group of 'Ladolia' is characterized by small-sized fruits, some clones like 'Kiti', 'Korakou' and 'Kato Drys' have large-sized fruits and also share desirable characteristics in terms of fruit flesh-to-stone ratio, yield and oil content (Gregoriou, 1999). Based on a previous genetic analysis the above clones were closely related to each other but genetically distant from the small-sized group of accessions (Banilas et al., 2003). Thus, these clones may serve as promising breeding material for the establishment of new cultivars.

To conclude, a high degree of genetic heterogeneity has been recorded among Greek olive varieties, while the major Cypriot 'Ladolia' is actually a highly variable mixture of genetically distinct landraces. Most of the respective studies, however, are incomplete in that they are restricted to accessions from germplasm collections and to major cultivars. Further exploration, description and agronomic evaluation are needed, including more accessions, minor and/or underutilized domestic varieties and wild germplasm.

3.2 Grapevine Diversity

According to the grape cultivar-grouping system of Negrul (1938) Greek grape cultivars belong to *Proles pontica*, a group that is placed between the most ancient cultivars of *Proles orientalis* (Central Asia and Middle East) and the more 'recent' *Proles occidentalis* (western Europe) in terms of evolutionary origin. As opposed to the group of *Proles pontica*, which is characterized by large-sized fruits (table varieties) due to longer selection, *pontica* group comprises mostly wine grapes, while *occidentalis* resembles the wild progenitor (Negrul, 1938; Vivier and Pretorius, 2000; Aradhya et al., 2003). Despite the relatively 'early' establishment of *Proles pontica* cultivars, our knowledge on their origin, domestication and diffusion is far from complete.

Greece has a long history in viticulture and grapevine is nowadays one of the major crops in the area (Fig. 4). Greek ampelographic collections account for about 700 cultivars of which some 300 are still cultivated for wine, raisin production or table grapes (Kotinis, 1985). Few amongst the most important wine-producing cultivars are presented in Table 2. However, as in the case of olive, there are possible synonyms or homonyms, which often make cultivar identification a complicated task. For the discrimination and classification of about 200 Greek cultivars classic ampelography (from the Greek ampelos meaning grapevine and graphy meaning description) was initially applied by the pioneering works of Krimbas (1943),

Fig. 4 Greek ampelographic collections account for about 700 grapevine cultivar names. 'Roditis' (**A**), 'Moschofilero' (**B**), 'Mavrodafni' (**C**), and 'Agiorgitiko' (**D**) are typical domestic cultivars with genetic variation for berry color

Table 2 Major Greek wine-producing cultivars of *Vitis vinifera*

Official denomination (in Greek)	Other common names – synonyms	Principal area of cultivation/current diffusion[a]	Berry color[b]	Main agronomic traits and uses
'Agiorgitiko' (Αγιωργήτικο)	'Mavro Nemeas', 'Mavroudi Nemeas'	Nemea (P) / P, eastern SH	N	High yielding. Red dry wines of exceptional quality.
'Asyrtiko' (Ασύρτικο)	–	Cd / Cd, AI, P, SH, Ma	B	High yielding. Tolerant to drought and biotic stresses. High-quality white dry wines.
'Athiri' (Αθήρι)	'Athiri Lefko', 'Asprathiri'	AI / AI, Cr	B	High yielding. Well-adapted to arid or calcareous soils. White dry wines.
'Debina' (Ντεμπίνα)	'Zitsa'	Ep / Ep, Th	B	High yielding. Susceptible to chilling and biotic stress. High-quality white dry wines.
'Fileri' (Φιλέρι)	'Fileri Mantineias', 'Moschofilero', 'Mavrofilero'	Arkadia (P) / P, II	N	High yielding. Susceptible to pests. High-quality white or rose dry wines.
'Liatiko' (Λιάτικο)	'Liatis', 'Mavroliatis'	Cr / Cr, Cd, II	N	High yielding. Low anthocyanin content. Ideal for sweet wines of high alcohol content.
'Limnio' (Λημνιό)	'Limniotiko', 'Kalabaki'	Limnos island (AI) / northern AI, Ma	N	Average yielding. Low anthocyanin content. Red or rose dry wines of high alcohol content.
'Mandylaria' (Μανδηλαριά)	'Kontoura Mavri'	Cr / Eastern SH, AI, southern P	N	Average yielding. High tannin and anthocyanin content. Resistant to various biotic stresses. Red dry wines.
'Mavrodafni' (Μαυροδάφνη)	'Mavrodafnitsa', 'Mavrodrami'	Patras (P) / Western P, II	N	Medium yielding. Susceptible to various biotic and abiotic stresses. Exceptional sweet wines.
'Moschato Samou' (Μοσχάτο Σάμου)	'Moschato Lefko', 'Moschoudi', 'Moschostafilo'	Samos (AI) / Samos, P	B	High yielding. Exceptional quality sweet wines.
'Roditis Kokkinos' (Ροδίτης κόκκινος)	'Alepou', 'Kokkinostafylo', 'Tourkopoula'	North-western P / P, SH, Th, Ma, Tr	R	Average yielding. When cultivated in hilly areas gives wines of high quality.
'Rompola' (Ρομπόλα)	'Rompola Aspri'	II / II, Ep	B	High yielding. Well-adapted to arid terrains. Susceptible to various biotic stresses. Exceptional quality white dry wines.
'Savvatiano' (Σαββατιανό)	'Kontoura Aspri', 'Stamatiano', 'Perahoritis'	Eastern SH / North-eastern P, SH, Th, Ma	B	High yielding. Dry white wines usually of average quality or retsina wines. Highly tolerant to drought. Well-adapted to arid terrains.
'Xinomavro' (Ξυνόμαυρο)	'Mavro Naoussis'	Naoussa (Ma) / Ma, Th	N	High yielding. Red, rose or white dry wines of high quality.

[a]Abbreviations of provinces are as indicated in Fig. 2.
[b]B = blanc/white; R = rose/pink; N = noir/red, rouge, black, blue, bleu.

Logothetis (1947) and Davidis (1967). Later on, discrimination of about 70 cultivars was achieved by using biochemical methodology (isozyme polymorphic patterns) (Stavrakakis, 1982). However, as it has been repeatedly reported, those approaches are not always sufficiently reliable and consistent due to environmental factors and plant growth stage (Lamboy and Alpha, 1998; Sefc et al., 1998; This et al., 2004). Therefore, since last decade a significant effort has been applied to characterize Greek genetic resources by using molecular marker techniques. In earlier works, some important cultivars grown in Crete (Stavrakakis et al., 1997) or belonging to the group of Muscats (Stavrakakis and Biniari, 1998) were identified by means of RAPD analysis. These studies were the first to show the existence of a relatively high genetic variation among the Greek cultivars by means of molecular marker technology. More recently, Lefort and Roubelakis-Angelakis (2001) by using nuclear microsatellite profiling identified and compared 50 Greek cultivars derived from ampelographic collections, including major wine and table cultivars. This was the first step to characterize Greek cultivars with SSR markers. The authors found considerable overall gene diversity among the genotypes studied, as an excessive heterozygosity was uncovered. Among other important findings, it is noteworthy that in some cases a similar cultivar name does not support any genetic similarity. For instance, 'Roditis Lefkos' (Roditis Blanc) is very distinct from 'Roditis Kokkinos' (Roditis Red), although they were considered to be genetically related (Davidis, 1982). In other cases synonyms were confirmed for certain important cultivars such as 'Moschofilero' and 'Fileri Mantineias' or 'Moschato Mazas' and 'Moschato Kerkyras'.

Microsatellites have been widely employed as powerful and versatile molecular tools for cultivar identification purposes, parentage analysis, evaluation of synonymy/homonymy, genetic diversity studies, genome mapping or genetic linkage maps because they provide high degree of polymorphism and are highly reproducible (Bowers and Meredith, 1997; Sefc et al., 2000; This et al., 2004; Doligez et al., 2002; Adam-Blondon et al., 2004; Riaz et al., 2004; Riaz et al., 2007). The most important achievement towards management of Greek grape germplasm is definitely the release of a multimedia web-backed genetic database (Lefort and Roubelakis-Angelakis, 2000) named 'the Greek Vitis database' (http://gvd.biology.uoc.gr/gvd/index.htm). It is a public database that contains nuclear as well as chloroplast SSR profiles of Greek grapevine cultivars, rootstocks, *Vitis* species and hybrids used as rootstocks. Among others, it includes 1,017 records, corresponding to 1,017 single plants, representing about 670 cultivars of eight ampelographic collections in Greece.

As it was the case in olive cultivars, analysis of genetic data so far suggests no particular correlation between genotypes and modern areas of cultivation, indicating intense cultivar exchange among different regions of Greece or possible introduction of clones from elsewhere in the past (Lefort and Roubelakis-Angelakis, 2001). Unfortunately, there is a lack of sufficient historical records for most cultivars that could possibly provide some evidence for their origin. There are still important questions to be addressed, like whether modern cultivars are directly linked to ancient cultivated germplasm, i.e., whether they were selected locally or introduced. We do have plenty historical records for organized viticulture in different geographic sites, like Thrace, Macedonia, Crete, the Aegean Islands and Cyprus, though there are a few early data for cultivar names. Most of the information we have from archaeological records refers to wine denominations rather than cultivar names. Homer in Iliad and Odyssey provides evidence for the most important centers of ancient viticulture by referring to quality wines from Trace, Ikaria, Lymnos and Lesvos islands. Polydefkis (200 BC) provides some information for viticulture and denominations of cultivars, some of which according to Logothetis (1974) are related to Greek cultivars still cultivated under similar names. For instance the ancient cultivar 'Rodia' could be the modern 'Roditis' or the 'Limnia' might be the present 'Limnio' (Table 2). In the Latin literature, Katon (233–149 BC) refers to varieties cultivated in Italy most of which are likely of Greek origin, like different types of 'Aminaea' (in Greek: Αμιναία). In the first century, there is evidence for introduction of important cultivars into Greece from the Middle East, like the wine-producing 'Ladikino' from Syria that is still cultivated in restricted areas in Crete (Logothetis, 1974). It is quite interesting to note that based on RAPD analysis Ladikino was found to be genetically related to 'Romeiko' and 'Liatiko', two other Cretan cultivars (Stavrakakis et al., 1997). According to the above historical records, molecular analysis suggests influence of Cretan germplasm from the Middle East. It is known that southern coastal Syria, Lebanon and Palestine had well-developed viticulture since late 4th millennium BC, and in Early Bronze Age Byblos (ancient city of Phoenicia, today Lebanon) was an important wine export center (Greene, 1996). Along with the wine, it is likely that there was also trade of vine cuttings for propagation of elite cultivars from Byblos to Thrace and Sicily, a common phenomenon at those times that is often called 'colonization végétale' (Salviat, 1990).

During the following Middle Age (Byzantine period) any relevant scientific information is limited, although viticulture continues to be very important along with intense wine production. Ancient cultivars, like the group of 'Aminaeas', were still cultivated but most cultivar denominations probably had already changed. It is important to note that modern cultivar names started to appear as early as the twelfth century. Interestingly, it was the time that the name 'Athiri', one of the most important Greek modern cultivars (Table 2), first appeared in Crete and the Southern Aegean Islands. The cultivar 'Sultanina' or 'Sultana' (also famous as Thomson seedless) was introduced during the twelfth century in Ionia (Minor Asia) and later into Greece from the Persian province Soultanie in the Caspian Sea. It seems also likely that 'Soultanina' also followed another route of dispersal through northern Africa to Spain, where it was named with the Arabic Kechmich (Logothetis, 1974). In the thirteenth century 'Robola' (Table 2) was already cultivated in Cephalonia and Zakynthos (Ionian Islands) and in the fourteenth century the first records for the famous 'Korinthiaki' cultivar in Peloponnesus appeared. The latter cultivar has been predominantly used for raisin production in the area since then. During the post-Byzantine period and particularly in the first years of the Ottoman occupation the respective information is really scarce. There are data for 'Fileri' (or 'Moschofilero') cultivation in Zakynthos (Ionian Islands) that resembles 'Robola' from Cephalonia in the taste and aroma of the wine produced, and interestingly they share the highest genetic similarity among 50 Greek cultivars studied (Lefort and Roubelakis-Angelakis, 2001). These two cultivars are probably the ones that produce the finest Greek white wines. Their possible common origin is quite interesting because there is a debate among Greek enologists whether 'Moschofilero' is synonym to 'Gewürztraminer' and recently introduced from Germany. Data for other cultivars go back to eighteenth century or later, but their ancient origin may not be excluded. This is because most cultivars have likely changed their names during the ages, and particularly during the last centuries, according to the evolution of the Greek language and the adoption of local toponyms following plant material exchange.

The knowledge of cultivar origin is very important for the modern wine industry, legislation, marketing and enology. A comparable genetic analysis of cultivars with wild sylvestris could also provide some information about their relationship and hence local origin, but very little attention has been paid to wild populations in Greece. Commonly in the Mediterranean areas the wild grapes have been observed in sheltered environments inhabited by species of the genera *Salix*, *Popolus*, *Alnus* and *Ulmus* (Pignatti, 1976). In Greece, Logothetis (1974) identified some wild populations grown in Thrace, Macedonia, Epirus, Thessaly and central Peloponnesus (Fig. 2) climbing mainly on trees of the genera *Olea*, *Platanus*, *Quercus*, *Acer* and *Fagus*. These small populations should be genuine according to classical ampelographic examination and most importantly because they are monosexual, while cultivated vines that escape into the wild remain hermaphrodite. Since genuine wild populations in Greece are considered very rare, it is worthy to examine whether this material is still present and if so, to compare it with modern cultivars or archaeological remains (e.g., seeds). It is generally believed that wild vine in Greece is threatened with extinction and therefore this valuable gene pool should be conserved. A characteristic example is that of a huge and ancient individual close to the village Pagrati in Central Peloponnesus (Fig. 2) called 'vine of Pausanias' after the name of the Greek traveler and geographer of the second century AD. Irrespective its denomination it is definitely very old and according to Logothetis (1974) it is a female individual having all the typical characteristics of the wild sylvestris (Fig. 5).

Grape is also widely cultivated in Cyprus and contributes significantly to the agro-industry of the country. The total area of grapevine cultivation extends to almost 19,000 ha, which is about 13% of the total agricultural land. Vines are cultivated mainly on hilly areas exploiting land on which no other culture could achieve acceptable economic results (Savvides, 2003). It has been proposed that there are about 15 cultivars considered to be autochthonous based on their long cultivation in the Island and historical records (Galet, 1993). To our knowledge the actual number may be somewhat higher, but the possibility some of them being introduced from Greece or elsewhere and the existence of synonyms should be investigated. In the 1960's Cypriot vineyards were dominated by two indigenous wine-producing varieties, namely 'Mavro' and 'Xynisteri', covering about 98% of the total cultivation area. In 1970's Cyprus government imported some of the most important western European cultivars, such as 'Chardonnay', 'Riesling', 'Cabernet Sauvignon',

Fig. 5 Genuine wild grape populations are currently rare in Greece. An ancient sylvestris grape individual, called ‘vine of Pausanias’, still grows in the Central Peloponnesus by the village Pagrati

‘Semillon’ and ‘Grenache Noir’, in the context of a replanting scheme designed by the Ministry of Agriculture and Natural Resources (Roumbas, 1993). This resulted in a reduction of the total area for ‘Mavro’ and ‘Xynisteri’, which nevertheless still remains at relatively high levels (60 and 15%, respectively). A mixture of grapes from these two varieties is used today for the production of the most famous Cypriot wine Commandaria. The name Commandaria was given to this sweet wine by the knights of S. John from Jerusalem (later knights of Malta) in the fourteenth century, though it is believed that its production had started earlier from as yet unknown varieties (Logothetis, 1974).

Since 1990, the cultivation of imported cultivars is gradually increasing. As in other Eastern Mediterranean countries, the spread of introduced cultivars may result in severe genetic erosion and rare local cultivars are likely to disappear. Thus, there is an urgent requirement to conserve and exploit the indigenous Cypriot germplasm. Nevertheless, the genetic diversity of Cypriot germplasm has not been fully evaluated. Except for ‘Mavro’ and ‘Xynisteri’, Cypriot cultivars that are still cultivated, though in restricted areas, include the red wine-producing cultivars ‘Ofthalmo’, ‘Lefkada’ (or ‘Lefkas’), ‘Maratheftiko’, ‘Vlouriko’, ‘Yiannoudi’, and ‘Siderites’, and the white wine cultivars ‘Malaga’, ‘Canella’, ‘Promara’, ‘Morokanella’, ‘Moscato’, ‘Spourtico’, and ‘Verigo’. Some of them are described in Table 3. Of note is that most local cultivars are growing on ancient rootstock, unlike most European cultivars that are grafted on American rootstocks. This is because Cyprus escaped from the phylloxera epidemic in the nineteenth century. In a recent study, 12 Cypriot cultivars were characterized by microsatellite analysis and compared with important Western Mediterranean or Bulgarian cultivars (Hvarleva et al., 2005). The phenogram generated based on genetic distance values revealed a clear-cut between the Cypriot germplasm and the group of the western as

Table 3 Major Cypriot grape cultivars

Official Denomination (in Greek)	Other common names – synonyms	Principal area of cultivation	Berry color[a]	Main agronomic traits – characteristics and use
'Malaga' (Μαλάγα)	–	Pafos, Omodos, Vassa	B	Average yielding. 'Demi-sec' and sweet wines with aroma resembling Muscats.
'Maratheftiko' (Μαραθεύτικο)	'Bambakada'	Hilly regions of Paphos (Panayia, Ambelitis) and in Pitsilia	N	Low yielding. High-quality red wines with elegant tannins and body.
'Mavro' (Μαύρο)	'Local Mavros'	Widely dispersed	N	High yielding. Well-adapted to adverse conditions. Red dry wines usually of average quality. Susceptible to Botrytis infection.
'Ofthalmo' (Οφθαλμό)	'Pophtalmo'	Pafos and Pitsilia area	N	Average yielding. Average-quality red wines.
'Promara' (Προμάρα)	'Bastardiko'	Around Omodos and Kyperountas	B	Average yielding. High-quality white wines with elegant aromas.
'Xynisteri' (Ξυνηστέρι)	'Aspro'	Widely dispersed	B	High yielding. Well-adapted to poor soils and hot-dry climate. White dry wines with elegant aromas.

[a]B = blanc/white; R = rose/pink; N = noir/red, rouge, black, blue, bleu.

well as that of the Bulgarian cultivars. 'Mavro' was found to be genetically similar to 'Morokanella'. 'Xynisteri', the other major cultivar, did not show particular affinity to any genotype in the study but was definitely clustered within the group of Cypriot cultivars. As expected, some synonyms or homonyms between Cypriot and Greek or other exotic cultivars were uncovered after comparison of the above SSR data with those reported by Lefort and Roubelakis-Angelakis (2001). For instance, 'Moscato' is rather synonym to 'Muscat Alexandria', corroborating previous suggestions (Galet, 1993). Also the cultivar 'Lefkada', which is the name of an Ionian island in Western Greece, is a synonym to 'Vertzami' widely cultivated in Lefkada, whereas the Cypriot 'Sideritis' is genetically distant to the Greek 'Sideritis' (homonyms). Results may also imply genetic structure between Cypriot and Greek cultivars, although more genotypes from either site should be compared in order for such differentiation to be justified.

Although significant efforts have been made towards the characterization of Cypriot cultivars, our current knowledge on the local germplasm is far from complete. There are important but rare cultivars that need more attention. For instance, 'Maratheftiko' is the most promising red grape variety of Cyprus for producing high-quality wines (Table 3). It is a rare cultivar tolerant to various stresses, having black-colored and small-sized berries. Interestingly, it is among the very few varieties in the world that is non-hermaphrodic; actually it is female, and for this reason it is planted next to other vines for pollination. Otherwise, it has a natural propensity to severe blossom drop resulting in thinly clustered grape bunches. These features collectively point to the hypothesis that 'Maratheftiko' might be a wild form, rather than a well-established domesticated variety. Genetic comparison with wild sylvestris genotypes would probably help us to address this question and also to elucidate the origin of other domestic cultivars. Unfortunately, it is not known whether genuine wild vines still exist in the island of Cyprus.

The preservation of vine genetic resources is crucial for future generations. As Duchêne and Schneider (2005) noted, since the phylloxera crisis there has not been such a need for the wine industry to review its strategies and techniques in the years ahead. This is due to the changing climate, particularly global warming, having a direct effect on the water availability and pests/diseases that could evolve. Viticulture is exposed to a great potential risk, since high-quality wines are generally associated to optimum climatic conditions. In a recent study, White et al. (2006) estimated that increases in heat accumulation, principally extreme heat days (>35°C) at the growing season, could cause decline up to 81% of the potential premium wine production area in the United States by the late twenty-first century. According to the authors, this will shift wine production to warmer climate varieties and/or lower-quality wines. Alternatively, there will be a need to shift future vineyards to higher terrains or to new geographical areas (Louime et al., 2007). Such accumulating ominous predictions for the future of grape/wine industry strongly suggest revision of grape breeding programs, which have historically focused on cold tolerance and disease resistance (Alleweldt and Possingham, 1988), towards the development of heat-tolerant vine stock (White et al., 2006). It is noteworthy that Cyprus has an intense climate with hot-dry summers and autochthonous vines are watered only through rainfall, which is rare and almost restricted during the winter months. In the light of the above need, indigenous Greek or Cypriot grape germplasm that is generally well-adapted to such extreme conditions may constitute promising plant germplasm material for further exploration and exploitation in breeding programs aiming to develop disease-resistant and stress-tolerant clones.

4 Conclusion

In the present review we attempted to describe and explain olive and grapevine biodiversity in Greece and Cyprus, two important areas of early domestication and dispersal for the two crops. During the last decades, significant efforts have been made to accurately determine the existing genetic diversity mostly based on material obtained from germplasm collections. Molecular marker techniques have significantly ameliorated this aim, and nowadays most important cultivars have been identified and their genetic relationships have been estimated. Tools developed for biodiversity have also allowed clarifications of certain synonyms/homonyms and may help to detect more precisely the origin of cultivars. Both olive and grapevine present extensive diversity in the phenotypic traits and at the genotypic level. Olive genetic diversity in Greece and Cyprus has been studied by dominant molecular markers, like RAPD or AFLP. Further research by using codominant markers like

SSR could provide a more comprehensive picture of their genetic relationships and origins. Using nuclear microsatellite profiling, most important Greek and Cypriot grape varieties have been identified and compared revealing considerable overall gene diversity. However, it is important to explore and conserve the natural range of genetic resources, such as domestic rare or underutilized varieties and wild progenitors that are often well-adapted to specific environments. This is particularly important in the case of viticulture due to the massive introduction of exotic-international cultivars that replace the autochthonous ones, thus contributing to genetic erosion. Wild groves or individuals of oleasters and sylvestris should be recorded and preserved in situ or ex situ (i.e., in germplasm collections). This plant material is invaluable for breeding programs in order to generate clones tolerant to environmental stresses. It is anticipated that the forthcoming developments in genomics, metabolomics and ultimately systems biology will accelerate the development of disease-resistant and stress-tolerant clones and will improve the sustainability of olive and grape growing worldwide.

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Ethyl Carbamate in Foods and Beverages – A Review

J.V. Weber and V.I. Sharypov

Abstract Foods and beverages contain many toxic chemicals that raise health concerns. Ethyl carbamate (EC) or urethane is the ethyl ester of carbamic acid. It occurs at low levels, from ng/L to mg/L, in many fermented foods and beverages. EC is genotoxic and carcinogenic for a number of species such as mice, rats, hamsters and monkeys. It has been classified as a group 2A carcinogen, "probably carcinogenic to humans", by the World Health Organization's International Agency for Research on Cancer (IARC) in 2007. The benchmark dose lower limit of EC is 0.3 mg/kg bw/day and the mean intake of EC from food is approximately 15 ng/kg bw/day. Those levels were calculated for relevant foods including bread, fermented milk products and soy sauce. Alcoholic beverages were not included in this calculation. However, high levels of EC can be found in distilled spirits at concentrations ranging from 0.01 mg/L to 12 mg/L depending on to the origin of spirit. Alcoholic drinks should thus be considered as a source of EC. EC is produced by several chemical mechanisms: first, from urea and various proteins like citrulline produced during the fermentation step, and second from cyanide and hydrocyanic acid, via EC precursors such as cyanate. A large panel of EC formation mechanisms is described from simple ethanolysis of urea in homogeneous liquid phase to photochemical oxidation of cyanide ion or complex heterogeneous gas/solid catalytic reactions. Determination of EC in foods and beverages involves various strategies according to the material, food or beverage, solid or liquid, and according to the concentration, from ng/L to mg/L. Usually adapted extractive techniques and pre-concentration steps are followed by analysis by gas chromatography coupled to mass spectrometry (GC-MS of GC-MS-MS). High-performance liquid chromatography (HPLC) and semi-quantitative spectroscopic methods (infra-red) are also proposed as valuable alternatives to the classical but time-consuming GC-MS. Various preventing methods are developed and used in some cases at industrial scale to lower EC levels in food. Two types of preventing methods are described. First, adapted and optimized

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practices in all steps of the chain of food (or beverages) production lead, in general, to low EC level. Secondly, the abatement of EC precursors can be done by adapted enzymatic, physical-chemical or chemical methods according to the nature of raw materials and conditions of their production processes.

Keywords Ethyl carbamate · Urethane · Foods and beverages · Analysis · Formation · Preventing methods

1 Introduction

Ethyl carbamate (EC), or urethane: $H_2NCOOC_2H_5$ CAS N° 51-79-6, is the ethyl ester of carbamic acid (NH_2COOH). It has been used in several commercial ways like the preparation and modification of amino resins, as cosolvent for pesticides or drugs manufacture and as chemical intermediate in the textile industry to impart wash-and-wear properties (IARC, 1974). In the past, EC has also been used as an antineoplastic agent and for other medical purposes (Paterson et al., 1946), but it saw relatively hard use in the treatment of multiple myeloma (Holland et al., 1966). It was found to be toxic as soon as the 1940s and was discovered as carcinogen in 1943 (Nettleship et al., 1943; Haddow and Sexton, 1946). EC was also used as a hypnotic in man and as an anaesthetic for laboratory animals (University of California 2003). Nowadays EC or other simple carbamates (phenyl, methyl or butyl) have some uses for researches purposes only (Gotor, 1999).

EC is genotoxic and carcinogenic in a number of species, including mice, rats, hamsters and monkeys, which suggests a potential carcinogenic risk to human (Beland et al., 2005; and references cited therein). EC is absorbed rapidly and nearly completely from the gastrointestinal tract and the skin (Cha et al., 2000; and references cited therein; EFSA, 2007). EC metabolism involves three main pathways: hydrolysis, N-hydroxylation or C-hydroxylation and side-chain oxidation. The major pathway is through EC hydrolysis by liver microsomal esterases to ethanol, ammonia and carbon dioxide. In rodents, approximately 5% of EC is excreted unchanged and more than 90% is hydrolyzed. EC is also converted by cytochrome P-450 to form ethyl *N*-hydroxycarbamate (approximately 0.1%), α-hydroxy ethyl carbamate and vinyl carbamate (approximately 0.5%). This last compound, a more potent carcinogenic compound than EC, is converted via an epoxidation reaction to vinyl carbamate epoxide which can covalently bind to DNA, RNA and proteins (Park et al., 1993). Another way is known. P450, contained in the lung, lymph, liver, and skin, catalyzes the metabolism of urethane to *N*-hydroxyurethane. Esterase is also contained within these organs. Consequently, *N*-hydroxyurethane can be metabolized by esterase to hydroxylamine which exerts its carcinogenic effect in multiple organs by generating O_2 and NO to cause oxidation and depurination of DNA (Sakano et al., 2002). Based upon both epidemiological and experimental data, EC has been classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer (IARC) and as a probable human carcinogen by US EPA. In March 2007, the World Health Organization's IARC has re-classified EC as a group 2A carcinogen (probably carcinogenic to humans) from group 2B (possibly carcinogenic) (Baan et al., 2007; De Stefani et al., 2007). Nevertheless, in the recent joint FAO/WHO Food Standards Programme (Codex committee on contaminants in foods) (Joint FAO/WHO Food Standards Programme 2007 – JECFA) it was observed that no specific health effects by EC in humans related to dietary exposure are reported. As estimated by the JECFA, the Benchmark Dose Lower Limit (BMDL 10) of EC is 0.3 mg/kg bw/day and the mean intake of EC from food is approximately 15 ng/kg bw/day. This was based on the relevant foods including bread, fermented milk products and soy sauce. Alcoholic beverages were not included. With the inclusion of alcoholic beverages the estimated intake is 80 ng/kg bw/day. High consumption of stone-fruit brandies could lead to higher intakes of EC. The committee concluded that intake of EC from foods excluding alcoholic beverages would be of low concern. However, the margin of exposure (MOE) from all intakes, food and alcoholic beverages combined, is of concern and therefore mitigation measures to reduce concentrations of EC in some alcoholic beverages should be continued. The committee concluded that the matter of EC was relevant but not of a high priority. Considering that alcoholic beverages represent the higher part of EC intakes, several countries have limitations on their levels (see Table 1). The EC is nowadays under study by the European Food Safety Authority (EFSA) [Question Number: EFSA-Q-2006-076: Ethyl carbamate in foods and beverages, in particular alcoholic beverages (stone-fruit brandies)].

EC is produced at low levels (from several ng/L or ng/kg to mg/L) in fermented foods, baked foods and alcoholic beverages from various precursors like hydrocyanic acid (HCN), urea, citrulline and *N*-carbamyl compounds (including carbamyl phosphate) by reaction with ethanol (Beland et al., 2005). The mechanisms of EC production will be discussed later in this chapter. Some usual values of the EC concentrations in foods and beverages are reported in Table 2. As observed, EC continue to be present at very low levels in several foods and beverages. In the majority of the cases, nevertheless, sensible reductions in EC concentrations over the past years have been achieved using two approaches: firstly by reducing the concentration of the main precursor substances in the food and beverages and secondly by reducing

Table 1 Maximum levels for ethyl carbamate in alcoholic beverages (Ethyl carbamate concentration in μg/L)

Country	Wine	Fortified Wine	Distilled Spirit	Sake	Fruit brandy
Canada	30	100	150	200	400
Czech Republic	30	100	150	200	400
France	nr	nr	150	nr	1,000
Germany	nr	nr	nr	nr	800
USA	15	60	nr	nr	nr
Swiss	nr	nr	nr	nr	1,000

nr: no specific reglementation (at the moment).

Table 2 Domains of EC concentration in some foods and beverages

Food or beverage	Before 2000	After 2004	References
Liquid, mg/L			
Spirit drinks	0.01–6	0.01–6.2	(a,b)
Whisky	0.01–0.02	<0.01	(a,c)
Beer	0.01–0.015	<0.01	(a,d)
Gin	0.011	nd	(a)
Cider	0.08	nd	(a)
Wine	0.07–0.12	0.01–0.025	(a)
Sake	nd	0.08–0.17	(a)
Vinegar	0.03	0.033	(a,g)
Stone-fruit brandy	nd	0.01–22	(g)
Solid, mg/kg			
Cake	nd	<0.01	(a)
Bread	0.01	<0.01	(a,g)
Sauerkraut	0.003–0.035	0.029	(a,g)
Yeast extract	0.003–0.035	0.041	(a)
Cheese	0.08	<0.01	(a,g)
Soybean paste	0.08	<0.01	(a,g)
Sauce and yogurt, mg/kg			
Soy sauce	0.073	0.01–0.084	(a,e,f,g)
Yogurt	0.01	<0.01	(a,g)
Christmas pudding	nd	0.02	(a)
Fermented soy sauce	nd	0.01–0.1	(e)

(a) Hasnip et al. (2007); (b) Lachenmeier et al. (2005b); (c) Riffikin et al. (1989); (d) Ha et al. (2006); (e) Park et al. (2007); (f) Kim et al. (2000); (g) EFSA (2007).
nd: no available data.

the tendency for these precursor substances to react to form cyanate (Hasnip et al., 2007; Food Standards, 2007). We will come back to these points later.

The present review aims to describe the origins and formation mechanisms of EC in natural products, foods and beverages, the methods of EC determination and the preventing actions for their limitation. Some environmental problems connected with processes, waste treatment and consequences of preventing actions will also be discussed. In our knowledge, since 1978 only a few papers in the literature offer review on the subject, but none are bringing together formation mechanisms, determination methods and preventing actions (O'Brien et al., 2006; Sen et al., 1993; Schlatter and Lutz, 1990; Vahl, 1993; Zimmerli and Schlatter, 1991).

2 Ethyl Carbamate in Foods and Beverages: Formation and Mechanisms

2.1 *Naturally Occurring Ethyl Carbamate*

There are many ways of natural EC formation according to the nature of considered foods or beverages, their production processes and the EC precursors available in the raw materials. One of the most common ways of EC production, in acidic medium, is the reaction of urea with ethanol (Delledonne et al., 2001; Wang et al., 2007) given in equation (1):

$$NH_2CONH_2 + C_2H_5OH = C_2H_5OCONH_2 + NH_3 \quad (1)$$

Urea is found in many fermented foods like yogurt, cheese, bread and more generally in baked products, in alcoholic and non-alcoholic beverages (Francis et al., 2002; Larsen, 2006). Urea can be found in raw materials, like milk, in non-negligible concentration (some hundreds of mg/L – Eicher et al., 1999) or formed during food (or beverage) processing. For example, when the yeast metabolizes arginine during the grape fermentation (Arena et al., 1999; Madrera and Suârez, 2009) urea usually amounts to some mg/L (Valero et al., 1999). The EC production from urea and ethanol is assumed in foods and beverages but has a moderate kinetic formation at ambient temperature (Aresta et al., 2001; Matsudo et al., 1993). Nevertheless, it is demonstrated that EC formation from urea increases dramatically (according to the Arrhenius equation) with temperature (in baking, boiling or toasting). For example, a wine-conservation temperature below 24°C is needed to avoid large EC formation (Stevens and Ough, 1993).

Some other mechanisms of EC formation from urea are described. The urea decomposition to isocyanate and cyanate in aqueous-ethanol solutions between 60°C and 100°C was demonstrated (Boulton, 1993; and references cited therein). By reaction with ethanol, isocyanate and cyanate give EC in acidic medium (Equation (2a) – Riffikin et al., 1989; Bruno et al., 2007) (Equation (2a)). Another possible route is the thermal decomposition of urea (in baking, for example) to ammonia and cyanic acid (Equation (2b)) and EC formation occurs by reaction of cyanic acid with ethanol (Schaber et al., 2004).

$$NCO^- + C_2H_5OH + H^+ = C_2H_5OCONH_2 \quad (2a)$$

$$H_2NCONH_2 = NH_4^+NCO^- = NH_3 + HCNO \quad (2b)$$

During fermentation and more especially malolactic fermentation, different EC precursors like citrulline and to a lesser extent carbamyl phosphate are produced by yeast or bacteria from proteins (Aresta et al., 2001). Ethanolysis of these precursors leads to high levels of EC (Ough, 1976a; Matsudo et al., 1993; Butzke and Bisson, 1997; Arena et al., 1999; Mira de Orduña et al., 2000; Uthurry et al., 2006) (see Equation (3)).

$$NH_2COOPO_3H_2 + C_2H_5OH = C_2H_5OCONH_2 + H_3PO_4 \quad (3)$$

Another natural way of formation occurs via cyanide anion. There are more than 2,000 species of plants that produce cyanoglycosides and usually the corresponding hydrolytic enzyme. Consequently cyanoglycosides are able to give a sugar and cyanohydrin; the last compound rapidly decomposes to hydrogen cyanide (Rezaul Haque and Bradbury, 2002; Vetter, 2000). As an example, Cassava the third most important food source in the tropics contains large amounts of cyanogens and may cause cyanide poisoning (Cardoso et al., 2005; Agbor-Egbe and Lape Mbome, 2006). The mechanisms of EC production from cyanide are well documented and in general catalytic pathways are evidenced (Bruno et al., 2007). Furthermore, in the majority of the cases it seems that EC from cyanide (or cyanate) are formed during a heating step of the preparation processes (distillation, baking). The production of EC by catalytic mechanisms from cyanide will be presented and discussed later in this chapter (see Section 2.4). Since HCN is a precursor of EC, a relationship between hydrocyanic (and cyanide) and EC concentrations has been hypothesized in alcoholic beverages. It was demonstrated for 260 samples of different alcoholic beverages having HCN concentrations in the range 1–40 mg/L that only 17.5% of changes in EC concentrations can be explained by changes in HCN concentrations (EFSA, 2007). Nevertheless it was established that the higher the HCN amount the higher the EC concentration. For example, 89.4% of the samples having more than 20 mg/L of cyanide present a EC concentration higher than 0.4 mg/L. Reversely, 63% of the samples having a cyanide concentration lower than 5 mg/L present a EC concentration lower than 0.4 mg/L. In a recent study, Balcerek et al. demonstrated that for distilled fruit spirits the EC concentrations are correlated to HCN concentrations (Balcerek and Szopa, 2006). Furthermore, the authors have shown that the conditions of fermentation (temperature, pH) were crucial for the HCN amounts in the different studied mashes. It could be assumed that EC production from cyanide occurs via the cyanate formation and subsequent reaction with ethanol. Nevertheless, EC formation is a multifactor process depending not only on the EC precursor amounts but also on temperature, light and catalysts.

Photochemical mechanisms are also evidenced in natural EC production. In this case the well-known auto-oxidation of unsaturated compounds by oxygen leading to hydroperoxide and to hydroxyl radical (OH°) occurs leading to EC production via cyanate or isocyanate intermediates by oxidation of cyanide anions or HCN (Muñoz et al., 2000; Aresta et al., 2001; Fox and Stachowiak, 2007; Stodolak et al., 2007). Finally, recently the easy formation of carbamates and carbamic acids by reaction of CO_2 with amines was demonstrated (Dijkstra et al., 2007). This unexplored way is nevertheless a possible route to EC production in foods and beverages where CO_2(from fermentation) and amines (from raw materials) are generally found.

2.2 *Ethyl Carbamate from Additives*

Different foods additives are known as EC precursors. Diethyl carbonate, an additive used for microbial activity control in foods and beverages, and also for many industrial purposes as a solvent or reactive (Tundo and Selva, 2002; Wang et al., 2007), produces EC by reaction with ammonia (Equation (4)) (Ough, 1976b; Aresta et al., 2001):

$$(C_2H_5O)_2\,CO + NH_3 = C_2H_5OCONH_2 + C_2H_5OH \qquad (4)$$

Very interestingly diethyl carbonate is considered as an attractive eco-friendly reactive (Tundo and Selva, 2002). Another example is azodicarbonamide (Hirakawa et al., 2003; Mulder et al., 2007). This compound is used as a blowing agent in the rubber and plastic industries, a flour improver in the bread-making industry (but this use is no longer permitted) (Canas et al., 1997; Dennis et al., 1997), a blowing agent in beers (Joint FAO/WHO Food Standards Programme, 2007) and an additive in closure of glass containers (European Commission, 2003). It is demonstrated that azodicarbonamide leads to aqueous-alcoholic medium to EC (Dennis et al., 1997; Schaefer et al., 2003), but the EC precursor remains unknown (Schaefer et al., 2003). Azodicarbonamide decomposes to gases, primarily nitrogen and carbon monoxide together with some carbon dioxide and ammonia. Normally the residues of non-volatiles are only limited (biurea about 2% of added azodicarbonamide). Under certain conditions however the residual biurea could be as high as 34%. Other non-volatile reaction products from azodicarbonamide can be urazole, cyanuric acid and cyamelide and are all EC precursors. For example, dry decomposition of azodicarbonamide at 190°C leads to 26% (in wt) of cyanuric acid a potent EC precursor (Schaefer et al., 2003).

2.3 Ethyl Carbamate Formation During Food Processing

In general, the higher EC concentrations are observed in baked foods and distilled spirits (see Table 2). The classical Arrhenius rate acceleration of the previously described reactions can be invoked to explain this phenomenon. But it seems that several other mechanisms are responsible for the EC formation: noteworthy is the one from cyanide (and other nitrogenous volatile precursors). It can be assumed that cyanide ion is the most important EC precursor in various fruits and vegetables (Aresta et al., 2001). Its formation by enzymatic or thermal cleavage from cyanogenic glycosides is well documented (Battaglia et al., 1990). The factors influencing EC formation from cyanide are pH, light, ethanol content, temperature and concentration of catalytic metallic species (Riffikin et al., 1989; Aresta et al., 2001). It is demonstrated that cyanate or its isomer isocyanate are the main EC precursors (Equation (2a)). The reaction (2a) is catalyzed by metallic ions like Cu (II) or Fe (III). The non-catalytic formation of cyanate (CNO^-) or isocyanate (NCO^-) from cyanide is until now not clear since oxidation of cyanide to cyanate needs H_2O_2 or hypochlorite ion as reactive and proceeds in basic conditions (Boulton, 1993; Sarla et al., 2004; Pedraza-Avella et al., 2008).

2.4 Production of Ethyl Carbamate in Aqueous and Aqueous-Alcoholic Solutions

The direct conversion of cyanide to cyanate (Equation (5) in aqueous and aqueous-ethanol solutions is possible without catalysis but proceeds slowly in acidic pH (Aresta et al., 2001; Bruno et al., 2007). Consequently, the main ways are the

Table 3 Methods of ethyl carbamate determination in foods and alcoholic beverages

Methods	Details of investigation routines	Analysed products	Limit of quantification (μg/L)	Relative standard deviation (%)
GC -TSD Multidimensional	Liquid–liquid extraction and concentration steps	Liquor (a) Rice wine (a)	1	2 3
GC/MS Multidimensional	Solid-phase extraction and concentration steps prior to determination	Table wine red (b) Table wine white (b) Port wine (b) Sherry-type wine (b) Vermouth (b) Berry wine (b) Sake (b)	1	2
GC/MS	Solid-phase extraction and concentration steps prior to determination	Distilled spirits (c) Fortified vine (c) Table wine (c) Soy sauce (c) Different kinds of fermented liquid and solid foods (c)	 ~10 10	8–10 6–12 8–19 13–28 2–32
	Direct injection method without the need for prior extraction and concentration steps	Stone-fruit brandies (e) Brazilian sugarcane spirits (f)	10 11	13 15
GC/PICI-MS/MS	Extraction and concentration steps prior to determination	Bred (g)	1.2	7
GC/MS/MS	Headspace solid-phase microextraction	Stone-fruit spirits(h)	110	4 (intraday) 8 (interday)
FTIR- PLS	Rapid method without samples pretreatment steps	Stone-fruit spirits (i)	semi quantitative method	
HPLC-FLD	Screening method without the need for prior extraction and concentration steps	Table wine (j) Fortified vine (j) Distilled spirits (j) Cider spirits (k)	0.8 3–4	11 18 14 <5

(a) Ma et al. (1995); (b) Jagerdeo et al. (2002); (c) Canas et al. (1994); (d)Hasnip et al. (2007); (e) Hesford and Schneider (2001); (f) Bruno et al. (2007); (g) Hamlet et al. (2005); (h) Lachenmeier et al. (2006); (i) Lachenmeier (2005); (j) Abreu (2005); (k) Madrera and Suârez (2009).

catalytic mechanisms described in Equations (6a) to (6d) (for Cu(II), commonly found in fermented beverages):

$$CN^- + 2\,OH^- = CNO^- + H_2O \tag{5}$$

$$CN^- + 2\,OH^- + 2\,Cu(II) = CNO^- + 2\,Cu(I) + H_2O \tag{6a}$$

$$4\,CN^- + 2Cu(II) = 2Cu(CN)_2 \tag{6b}$$

$$2\,Cu(CN)_2 = 2\,Cu(CN) + C_2N_2 \tag{6c}$$

$$C_2N_2 + 2\,OH^- = NCO^- + CN^- + H_2O \tag{6d}$$

In solution a complex cyanate-copper (II) is formed and the coordination of the cyanate anions occurs through the nitrogen atom. In such a way, the carbon atom tends to be more positive favouring the nucleophilic attack from ethanol (Aresta et al., 2001) leading to EC formation (Equations (7) and (8)):

$$Cu(CNO)_2 + 2H_2O = Cu(OOCNH_2)_2 \tag{7}$$

$$Cu(OOCNH_2)_2 + 2\,C_2H_5OH = 2C_2H_5OCONH_2 + Cu(OH)_2 \tag{8}$$

It should be observed that the best catalyst for the EC formation from cyanate among the copper (II) salts is copper acetate (Aresta et al., 2001). On the basis of kinetic studies of the EC formation related to cyanate concentration and the evolution of EC concentration in the distillates after distillation some intermediates like carbamic acid or ethyl cyanate were postulated but not evidenced (Boulton, 1993).

2.5 Production of Ethyl Carbamate in Gas Phase

Nowadays the relatively high levels of EC in distilled spirits (0.01 to 12 mg/L according to the origin of spirit) and more generally in alcoholic drinks leads to consider these beverages as the main source of EC intake. The EC precursors and the corresponding formation mechanisms in liquid phase (wines and fruit juices) were described earlier. In distilled spirits several other mechanisms increase the EC production by different gas-phase reactions during the distillation. It could be assumed that only a minority of EC in the liquid phase is vaporized and found in distillates due to its high boiling point, close to 184°C (Lurton et al., 1993). Nevertheless, EC can be transported as aerosol or cloud to the alembic condenser (Bruno et al., 2007; Carley et al., 2003). Furthermore, it is estimated that close to 80% of EC found in spirits is formed during the distillation step and/or within the first 48 h after distillation (Aresta et al., 2001; Riffikin et al., 1989; Bruno et al., 2007; Boulton, 1993; Fox Stachowiak, 2007; Boulton et al., 1993). In the last case the role of EC precursors in gas phase is crucial. HCN and HCNO (or HOCN), present or formed during the distillation in the aqueous-ethanol medium, are the EC precursors. Due to their very low boiling points (<30°C), these acids are vaporized and can produce EC in gas phase or/and by heterogeneous (solid/gas) reactions. More especially in copper-made installation (like alembic) a catalytic way is established (Carley et al., 2003). The authors demonstrated the oxidation of HCN to HCNO (or HOCN) on copper surface at 300 K (Equation 9). The surface oxidation of HCN to HCNO on nickel was also described (Yang and Whitten, 1998).

$$\mathrm{HCN\,gas} + \frac{1}{2}O_2 \quad = \quad \mathrm{HNCO} \ = \ \mathrm{HOCN} \tag{9}$$

Adsorbed or non-adsorbed HCNO and HOCN can react with ethanol or steam in gas phase to produce various intermediates (Equations (10), (11), and (12)) like carbamic acid (Equation (10) or ethyl cyanate (Equation (12), all leading to EC in distillate.

$$\mathrm{HNCO} + H_2O = H_2\mathrm{NCOOH} + \mathrm{EtOH} = H_2\mathrm{NCOOEt} + H_2O \tag{10}$$

$$\mathrm{HNCO} + \mathrm{EtOH} = H_2\mathrm{NCOOEt} \tag{11}$$

$$\mathrm{HNCO} + \mathrm{EtOH} = \mathrm{EtNCO} + H_2O \text{ and } \mathrm{EtNCO} + H_2O = H_2\mathrm{NCOOEt} \tag{12}$$

Other intermediates like lactonitrile and isobutyraldehyde cyanohydrin were identified (MacKenzie et al., 1990). MacKenzie et al. have also studied the naturally occurring solid deposits within the continuous distillation Coffey still by ionic chromatography in combination with X-ray diffraction and secondary ion mass spectrometric techniques. Identified species like CuCN, CuCNO, Cu_2O, Cu_2S, CuS, cluster ions such as $Cu(CN)^{2-}$, $Cu_2(CN)^{3-}$, $Cu_3(CN)^{4-}$ and mixed species $Cu(CN)_2S^-$, $CuSCN^-$, $CuCNO^+$ demonstrate the complex catalytic surface reactions occurring during the distillation.

2.6 Post-distillation and Photochemical Ethyl Carbamate Production

As reported previously, in distilled spirits more than 80% of EC is produced within the 48 h after distillation (Bertrand, 1993; Ough, 1976a). Only a few explanations concerning the involved mechanisms are reported. It is nevertheless established that two parameters are of paramount importance for EC production: the presence of metallic salts (Cu(II) or Fe(III)) and the light (UV, mainly). Furthermore, a synergistic effect between metallic salts concentration and irradiation was observed (Guerain and Leblond, 1993). To sum up, in this case two pathways can explain the EC production: firstly the catalytic ethanolysis of the EC precursors formed in the gas phase during the distillation (see Section 2.5) and secondly photochemical reactions leading to hydroxyl radical production (OH°) by the well-known autooxidation of unsaturated compounds (Fox et al., 2007; Stodolak et al., 2007) allowing the oxidation of cyanide in cyanate (Pedraza-Avella et al., 2008) and finally EC production (see Equation (2a)). Both mechanisms are catalyzed by Cu(II) or other metallic salts, as described earlier. It has to be added that photochemical mechanisms are not restricted to post-distillation reaction but can also be invoked in different cases of natural EC production. It seems also probable that some radical mechanisms occur also during the distillation in the gas phase and produce EC precursors.

3 Determination of Ethyl Carbamate in Foods and Beverages

The EC contents in foods and beverages amount from several ng/L to several hundreds μg/L (see Tables 1 and 2). In general, the low levels of EC concentrations and possible interferences with different molecules contained in analysed substances require specific sensitive methods for its quantitative determination. The most widespread method of EC determination is gas chromatography (GC) with polar columns using flame ionization detector (FID) and/or specific detectors. For quantification purposes the majority of the procedures include propyl carbamate (PC) or butyl carbamate (BC) as internal standards. Although for mass spectrometric detection EC labelled with isotope (deuterium, ^{13}C or ^{15}N) is preferable. Different extractive and concentrative pretreatments are often used (especially for samples with low content of EC) to avoid interferences from co-extracted matrix components and to increase the sensitivity of the methods. To sum up, the method of EC determination has to be adapted to the level of EC and to the studied material.

Simple GC-MS method without prior extraction and concentration steps is adapted for EC determination in stone-fruit brandies having high EC concentrations (in general >200 μg/L – Hesford and Schneider, 2001; international collaborative test). The samples are only adjusted to 40 volume % alcohol by dilution with pure water or by addition of EC-free ethanol. *n*-Propyl carbamate is used as internal standard and the MS detector is employed in selected ion monitoring mode (SIM). Several brandies with EC concentration from 100 to 3000 μg/L were analysed in eight laboratories. The relative reproducibility was found to be 20% of the mean expected concentration for concentrations of EC greater than 200 μg/L. This method was also successfully used for the determination of EC concentration in 34 Brazilian sugarcane spirits from 28 producers (total of 30 different distillation systems – Bruno et al., 2007) and has been now adopted as the official method for the determination of EC in stone-fruit brandies in Switzerland (Office fédéral de la santé publique Suisse – 2003).

Effective separation of alcoholic beverage components from EC can be achieved by GC with two coupled capillary columns having different polarities (multidimensional system). Principles and instrumentation of this method are described in several articles (Schomburg, 1995; Himberg et al., 1989; Krock and Wilkins, 1994). The multidimensional capillary GC method and a thermo-ionic specific detector (TSD) were used to identify EC in various fermented alcoholic beverages (Ma et al., 1995). i-Propyl carbamate (i-PC) was used as internal standard and the samples were pretreated by liquid–liquid extraction technique. Sample solutions were adjusted to pH 10 with 6 M NaOH and saturated with KCl before extraction with methylene chloride. The extracts were dried with anhydrous sodium sulphate and concentrated under reduced pressure in a rotary evaporator. The residue was transferred to a concentrator tube, and 0.5 ml of ethyl acetate was added as a keeper solvent. Under a gentle stream of nitrogen, the volume of the extracts was reduced to 0.5 ml. In the multidimensional gas chromatographic process, EC and i-PC were co-eluted on a polar capillary pre-column, then switched together to a non-polar analytical column by the heart-cutting technique. FID and TSD detectors were used to detect the chromatograms from the pre-column and the analytical column, respectively. For the analysed liquor and wine, the EC concentrations were correspondingly 124.6 μg/L and 15 μg/L and the detection limit was 1 μg/L. GC with FID using two connected capillary columns of different polarities was also applied (Aresta et al., 2001) for EC determination in commercial Brazilian sugarcane spirits (cachaças). The samples were concentrated by extraction from saturated KCl aqueous solution with methylene chloride/ether (4:1) and solvent elimination under vacuum. In most cachaças samples the level of EC concentration was determined in the range 100–1400 μg/L. It is necessary to note that specially prepared synthetic solution of EC (20–120 μg/L), which did not contain the matrix components of cachaças, was successfully analysed without the extraction and concentration steps, using only one polar column.

The application of GC with mass selective detection (GC/MS) or gas chromatography/tandem mass spectrometry (GC/MS/MS) in SIM increases significantly the EC detection. In general, the ions m/z 89, 74 and 62 are used to confirm the identity of EC. The ion m/z 62 characteristic of the carbamates is the most intense ion in the mass spectra of EC, PC and BC. Consequently, the area ratio EC/PC (or EC/BC) is usually used in the quantification. Isotope-labelled (^{13}C or ^{15}N) ethyl carbamate (LEC) as internal standard has the potential to increase the precision of the previously described methods. For example, Canas et al. have developed a GC/MS method for the determination of EC in alcoholic beverages and soy sauce (Canas et al., 1994). The samples were collaboratively studied by 17 laboratories from the United States, Japan, the United Kingdom, the Netherlands and France. Preliminary solid-phase extraction and concentration steps were applied to analyse the samples. Briefly, after addition of internal standard (n-PC) the samples were diluted with H_2O and then transferred to extraction column prepacked with diatomaceous earth. EC and n-PC were eluted with methylene chloride, then concentrated in a rotary evaporator and finally in concentrator tube under gentle stream of nitrogen. Reference values of EC concentration for all the tested samples were obtained by using GC/MS/MS with a triple quadruple mass spectrometer and ^{13}C,^{15}N-labelled EC (LEC) as the internal standard. The determined mean recoveries of EC and n-PC ranged from 89% to 100%. On the basis of the obtained results the authors have recommended to use the method for EC determination in different kinds of alcoholic beverages and foods for EC contents varying from several μg/L to several hundred μg/L. The analytical procedure was adopted by the European Commission (EFSA, 2007). These methods and some of its modifications were used in number of alcoholic beverage investigations (Uthurry et al., 2006, 2004; Woo et al., 2001; Sen et al., 1993, 1992), fermented solids and fermented liquids (beers, wines, fortified wines, spirits, liquors, soy sauces and vinegars) (Hasnip et al., 2007). For liquids analysis solid-phase extraction and concentration procedure followed by GC-MS, according to Canas et al. (1994), was applied with modification of the run time, and LEC was used as the internal standard. For analysis of the solid foods, the samples were homogenized in a blender with distilled water and internal standard solution. The homogenate was mixed with Hydro matrix solid-phase extracting materials and packed into a chromatography column fitted with a glass frit and tap and containing sodium sulfate. The cheeses were homogenized with water and internal standard as above, but the mixture was centrifuged. Non-polar compounds were eluted with hexane. EC (and LEC) was eluted with dichloromethane and the solvent was evaporated, using a rotary evaporator. Lipid material was removed by a Florisil Sep-Pak. The *eluates* were concentrated under a gentle stream of nitrogen and analysed by GC-MS. In all the studied samples, EC could be detected at 1 μg/L level, and its identity could be confirmed at 10 μg/L level by comparing the ratio m/z 62 over m/z 74 areas with those of the calibration standard.

Several types of multidimensional GC-MS methods with preliminary solid-phase extraction procedure are described for EC analysis in wines. For example, Jagerdeo et al. (2002), removed ethanol from samples before the extraction step by using a centrifuge vacuum concentrator. After that, they were brought back to initial volume with water. The samples were applied to a cartridge containing ENV+ (a hyper cross-linked styrene-divinylbenzene copolymer) and allowed to flow through the cartridge under gravity only. After the sample had passed through the cartridge, it was washed with water under gravity, and then cartridges were aspirated under vacuum until most of the water was removed. Second cartridge containing Na_2SO_4 was stacked underneath the ENV+, and the stacked cartridges were eluted sequentially with ethyl acetate. The sample was transferred to GC vials for analysis. For quantization, the ratio of m/z 62 (EC) and 64 (LEC) was monitored as described previously. A limit of EC detection as low as 0.1 μg/L and a 1 μg/L limit of quantization were established.

Sensitive GC/MS/MS method with positive ion chemical ionization (PICI) has been developed for the EC analysis in low-EC-amount products like bread (Hamlet et al., 2005). Ammonia was chose as PICI reactive gas. For identification and quantification, selected reaction monitoring (SRM) was used for the LEC internal standard. The following procedure was used for bread samples analysis. The samples were weighted into centrifuge tubes and spiked with LEC. Deionized water was added and the sample macerated using Ystral 10T generate. Prepared samples were centrifuged until a clear aqueous layer formed. An aliquot of the aqueous extract was mixed thoroughly with Extrelut in a beaker before transferring to chromatography column containing bed of anhydrous sodium sulphate. Acid-washed sand was added and the column was eluted with dichloromethane. The eluate was concentrated by combination of rotary evaporation and nitrogen stream. Application of this method for the analysis of 50 samples of commercial and domestic wheat breads showed that level of EC ranged from 0.6 to 12.2 μg/L. The limit detection and quantification were 0.6 and 1.2 μg/L, respectively. The quantitative performance of the

GC/PICI-MS/MS–SRM compared favourably with GS/MS–SIM method according to Canas et al. (1994). GC/PICI-MS/MS had the advantages of improved sensitivity and unambiguous identification of EC at all concentrations.

The above-stated methods present some drawbacks like their complexity and time-consuming preparation step, which complicates their use in practice especially in the analysis of a large number of samples. Consequently, several simple and rapid methods were described for EC determination in stone-fruit and other distilled spirits characterized by high EC level (>50 μg/L). An automated procedure using headspace solid-phase microextraction (HS-SPME) followed by GC/MS/MS detection was developed for the determination of EC in stone-fruit spirits (Lachenmeier et al., 2006). Solid-phase microextraction method was described in detail by Pawliszyn (1997, 2000). After addition of LEC to analysed samples, the optimized HS-SPME extraction with carbowax/divinylbenzene fibres was done by its salting out with sodium chloride in the presence of pH 7 buffer solution. For quantitative analysis the characteristic fragmentations of *m/z* 74 ▶ 44 and *m/z* 62 ▶ 44 for ethyl carbamate as well as *m/z* 64 ▶ 44 for ethyl carbamate-d5 as the internal standard were monitored in the multiple reaction monitoring (MRM) mode using a triple quadruple instrument. The limits of detection and quantification were 30 μg/L and 110 μg/L. At any concentrations examined the precision never exceeded 4.3% (intraday) and 8.2% (intraday). After optimization of the procedure, the analytical findings of 54 spirit samples were compared to the conventional sample clean-up over diatomaceous earth columns extraction procedure. A good agreement of analysis results between both procedures was found. In comparison to conventional procedures, HS-SPME provided a faster extraction, with concurrently diminished manual clean-up steps and less solvent consumption; therefore, it led to a significant decrease of the total time of analysis (manual sample preparation time of only 5 min instead 2 h for Extrelut extraction).

Besides the classical GC-MS methods, alternative techniques have been developed mainly with the aim of a rapid screening of large number of samples. The rapid method of Fourier transform infrared (FTIR) spectroscopy in combination with partial least squares (PLS) regression using selected wavelength bands was used for the screening analysis of EC in stone-fruit spirits (Lachenmeier, 2005). The PLS procedure was validated using 82 samples as an independent set to test the calibration. A total of 122 stone-fruit spirits were analysed for the determination of EC amounts. No prior preparation of the samples was required. The method was found to lack the accuracy required for a quantitative determination but could be used semi-quantitatively in the context of a screening analysis. If a rejection level of 0.8 mg/L is applied as cut-off, overall correct classification rates of 85–91% for the calibration set and 77–85% for the validation set were achieved. False-negative results can be avoided by lowering the cut-off to 0.6 mg/L. For comparison, the analysis of EC was done using solid-phase extraction and concentration procedures and GC/MS/MS as described previously (Lachenmeier et al., 2005b). The FTIR method is substantially faster (only 2 min per sample) and easy to use as sample preparation is not required. Sample throughput is more than 60 times higher than results obtained by GC/MS/MS. Additionally, some information concerning the EC precursor HCN and the maximum EC concentration which could be formed during storage was available from the FTIR spectra. This semi-quantitative method, however, has the disadvantage of requiring for the analyses a high number of reference samples in order to calibrate the method.

A sensitive screening method for EC determination in alcoholic beverages was also developed by using high-performance liquid chromatography with a fluorescence detector (HPLC-FLD) (Herbert et al., 2002). The method is based on the reaction of EC with 9-xanthydrol in acidic medium to form a fluorescent 1-xanthyluretane detected by FLD. The derivatization reaction was made by adding 500 μL of diluted sample to 100 μL of 9-xanthydrol solution in propanol (0.02 mol/L). After this, 50 μL of 1.5 mol/L HCl was added. The reaction developed during 5 min in the dark. This does not require previous sample extraction or concentration. The presented HPLC-FLD method has a detection limit of 4.2 μg/L and relative precision of 6.3%. The HPLC-FLD technique with some modifications was used to determine EC levels in cider spirits (Madrera and Suârez, 2009). Analytical investigation of 32 cider spirits (seventeen industrial and fifteen experimental) and seven reference spirits shows limits of detection and quantization of 1.6 and 3.6 μg/L, respectively, in the range of EC concentration of 4–67 μg/L in the ciders.

To contribute to a suitable validation of EC determination methods, HPLC-FLD method and GC-MS method including solid-phase extraction and concentration steps (according to Jagerdeo et al., 2002) were compared in an international interlaboratory study (Abreu et al., 2005). Five different samples ranging from 10 to 80% alcohol content (v/v) and representing table wines, fortified wines (red and white), distilled spirits and wine spirits were analysed. Comparison of the standard deviation presented for each sample between the HPLC and GC-MS results revealed that in general the interlaboratory variance was higher in the HPLC than in the GC-MS. Nevertheless, for the lower EC concentrations, as happened for red wine, the standard deviation of HPLC results was not statistically different from that of GC-MS. The study emphasizes the possible routine use of HPLC-FLD as a much simpler alternative analytical method in comparison to the time-consuming GC-MS method for EC concentration determination.

4 Preventing Actions and Related Environmental Issues

Due to the various precursors and the complex mechanisms of EC formation, a general method of its prevention in all foods and beverages is nowadays not conceivable. Therefore, different adapted strategies have been successfully developed and are used in industrial scale. As observed recently by S. Hasnip et al., the level of EC in usual fermented foods has been determined to be, in general, below the detection level with the exception of several alcoholic beverages (Hasnip et al., 2007). In most cases the decrease of EC concentration between 2000 and 2004 is spectacular as illustrated by the example of soy sauce. In 2004, among 11 soy sauce samples tested only one was positive recording 10 μg/kg EC. Earlier survey (1993) revealed a range of contamination up to 70 μg/kg (Hasnip et al., 2007). The solutions used for explaining the decrease of EC levels and some innovative perspectives for the remaining problems will be described below.

In fermented foods a logical solution to decrease EC level is to decrease its precursors (urea, citrulline, arginine, etc.). This is currently accomplished by using selected and well-characterised existing commercial yeast strains with low urea excretion during fermentation (Butzke and Bisson, 1997). Each specific food processing application nevertheless needs a specific optimization step and the use of specific strains in specific conditions (Schehl et al., 2005). Another possibility is to use urease enzyme to limit urea level. However, most of the known ureases are inactivated in acidic medium. To overcome this problem purified acid urease from *Lactobacillus fermentum* are now commercially available and utilized in wines and fermented beverages production (Fidaleo et al., 2006; and references cited therein). For example, the addition of acid urease from crude extract to unrefined Japanese sake (20% (v/v) ethanol) containing 35 mg/L urea reduced its concentration to undetectable levels (Miyagawa et al.,1999). Ough et al. have demonstrated the effect of dead cells of *L. fermentum* on the urea levels in wine (Ough et al., 1991). In this case, due to the lower pH of the medium, high urease concentrations were needed. The same authors have established that citrulline was not removed by the urease and could contribute to further EC production. Although urea removal from sake or California Sherries by acid urease has been industrialized in Japan and United States, the enzyme process has a few drawbacks and notably the processing cost may be uneconomical (Fidaleo et al., 2006). Nevertheless, reduction of the enzyme preparation-specific cost can be imagined by immobilizing acid urease on polyacrylonitrile (PAN) fibres and consequently to operate the process in a continuous manner (Zotta et al., 2007). Recently, the investigation of urease activity of sourdough was described in bread-making processes (Zotta et al., 2007). Another successful way is the preparation of mutant *Saccharomyces cerevisiae*. The urea in *S. cerevisae* is produced by the breakdown of I-arginine by the arginase enzyme encoded by CAR1 gene. Kitamoto et al. (1991, 1993) disrupted the two copies of this gene in sake yeast and the use of the resultant mutant led to non-detectable levels of urea or EC in sake. Some alternative approaches like the introduction of the genes of acid urease from *L. fermentum* in yeast are now under development (Zietsman et al., 2000). The use of urease during wine processing is rather limited mainly due to the cost of enzyme, and only few examples of applications are reported in the literature (Fidaleo et al., 2006). In general for wines, good agricultural techniques and more especially the monitoring of nitrogen fertilization, selection of commercial yeast strains and temperature control during storage and transportation lead to a limited concentration of EC (Butzke and Bisson, 1997). These preventive actions are of importance considering that urea is not, in general, the unique EC precursor.

The distilled spirits are unique in several aspects. Firstly, they have at the moment the highest level of EC (see Table 2) among foods and beverages. Secondly, the precursor of EC is not principally urea nor proteins but mainly HCN. Thirdly, EC in the final product is formed during and mainly after the distillation step. All these points were discussed earlier in this chapter. A last point is the copper alembics used by traditional distillers. Copper is the catalyst of preliminary HCN oxidation into EC precursors in gas phase or in solution (i.e. in distillates). It is well known that distillates obtained in copper alembic have a non-negligible Cu(II) concentration up to more than 10 mg/l in some cases (Cameán et al., 2001). In distilled spirit the ways of EC prevention can be summed up by the elimination of cyanide in the raw material before the distillation step and the elimination of Cu(II) in distillates in order to avoid catalytic EC formation.

As described earlier, high cyanide levels in foods and beverages are not only found in stone fruits but also in different plants like cassava, linseed, beans and peas (Saidu, 2004). A cyanide elimination scenario could be imagined by using microbiological processes. Rhodaneses are widespread enzymes regarded as one of the mechanism evolved for cyanide detoxification (Saidu, 2004; Cipollone et al., 2006). In my knowledge, this potential way of cyanide elimination has not been explored for food and beverage processing. It should be added that the severe health problems connected with high levels of cyanide in foods (at short, medium and long terms) lead to consider the EC levels as secondary (Cardoso et al., 2005). Consequently, the EC preventive actions in processes involving a distillation step could be (1) chemical elimination of cyanides before and during the distillation step, (2) change in the distillation process, and (3) elimination of Cu(II) in the distillates. Finally using good manufacturing practices, low level of EC in distillates (including stone-fruit) can be reached. In this case high-quality raw materials, proper fermentation, short storage and clean copper alembic are well-known conditions that are needed (Laugel and Bindler, 1993).

4.1 Chemical Elimination of Cyanide Before the Distillation

Chemical elimination of cyanide in fermented juices (musts) before the distillation can be performed by the formation of insoluble cyanide salts (silver or copper). Commercially available products mainly Cu(I) salts, like cyanurex® (Schliessmann, 2006), are able to reduce drastically the cyanide concentration up to 90% in the alcoholic medium (Laugel and Bindler, 1993). The results of cyanide elimination on the sensorial properties of the produced spirits are controversial (Laugel and Bindler, 1993; Christoph et al., 1988). But in general no difference was remarked, from an organoleptic point of view, between spirits treated (or not) with cyanurex (Christoph et al., 1988).

4.2 Changes in the Distillation Process

It is demonstrated (see earlier) that during distillation of the spirits the main EC precursors are volatile cyanide, cyanate and isocyanate. Two simple modifications, at least, could be made to the pot still apparatus avoiding the simultaneous presence of cyanide and Cu(II). Firstly, the copper condenser could be changed for one made of stainless steel. Secondly, the gaseous precursors can be fixed on copper pieces before the column (or the head of alembic) in order to avoid EC production (or EC precursors production) and vaporization in the column or in the condenser. In this case a copper surface as larger as possible is needed and lamellar copper system seems to be the most adapted (Guerain and Leblond, 1993)

4.3 Post-distillation Treatment

Due to copper-made alembic, Copper salts can amount in concentrations as high as 10 mg/L in some distilled beverages. These salts are the catalysts of the post-distillation EC production. It seems consequently reasonable to remove Cu(II) anions from the distillates or to remove the EC precursors in order to avoid this problem. Different ways were investigated – firstly, the use of insoluble chelating agents or cationic exchange resins. There are only few studies and patents about removing copper ions from beverages, based on the formation of insoluble chelating agents or the use of chelating resins (Kogyo, 2002; Shuguang and Gibb, 2008). These methods are relatively cumbersome and depend on chemical variables such as pH, contact time with resin, temperature and resin particle size. The removal in the alcoholic distillates, of the EC precursors produced during the distillation from volatile cyanides, was also investigated by using anionic exchange resins in both laboratory scale and pilot scale (Wucherpfenning, 1992; Guerain and Leblond, 1993). The authors noted a strong reduction of cyanides ions (<0.1 mg/L) and surprisingly of Cu(II) ions (<0.1 mg/L) in cherry spirit (45°GL) or plum spirit (65°GL). Copper cations were immobilized as anionic complexes. They report also a strong reduction of EC concentration (< in both cases at 140 μg/L) in the resulting brandies. It should be added that the organoleptical properties of the spirits were deeply modified with the attenuation of stone smell and an unpleasant odour attributed by the authors to the non-adapted conditions of resins regeneration. Recently, Neves et al. (2007) describe an efficient method to remove Cu(II) ions in alcoholic distillates (of sugarcane spirit) without perceptible change in organoleptical properties. The method is based on a treatment with either $CaCO_3$ or $MgCO_3$ which behave as cationic exchanger. The authors report, for freshly distillated samples, a level of Cu(II) ions lower than 0.01 mg/L with both carbonates. A pH increase connected with the neutralization of organic acids is nevertheless logically observed for the treated brandies. It should be added that for sample aged in wood barrel the process is less effective. The formation of Cu(II) complexes with flavour compounds from wood barrels is invoked by the authors to explain the less efficient ion exchange observed. Nevertheless, this interesting method of Cu(II) anions abatement has to be adapted to industrial processes. For example, the stirring of solid carbonate with distillates can change, by oxygenation, the chemical composition of final brandies and consequently their organoleptic characteristics.

4.4 Related Environmental Problems

The treatment of distillery effluents having a non-negligible concentration of copper can be a serious environmental problem (Reaves and Berrow, 1984; Paton et al., 1995; Shuguang and Gibb, 2008). Several solutions are nowadays used in distilling industry for the removal of copper salts in wastewater. These include the cementation reaction between iron and copper, adsorption onto sintered magnesium hydroxide, cation exchangers, electro-chemical deposition and precipitation as hydroxide, carbonate or sulphide. These techniques were effective in reducing copper concentrations by more than 90% (Paton et al., 1995). Recently, the use of biosorbents was described. The potential of spent-grain, a barley by-product from whisky distilling process, was demonstrated for application in the remediation of copper-contaminated wastewater (Shuguang and Gibb, 2008).

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Evaluation of Soil Fertility Using Infrared Spectroscopy – A Review

Changwen Du and Jianmin Zhou

Abstract Soil fertility is conventionally evaluated by soil properties such as C, N, and P contents. Evaluation of soil fertility is now becoming a routine work for soil management and crop production. However, laboratory analysis based determination of soil properties is costly and time-consuming, which is not suitable for precision agriculture. Here, infrared spectroscopy (IR) appears as an alternative and fast technique to measure soil fertility. The IR transmission method is usually used in soil qualitative analysis, while the IR reflectance can be used in soil quantitative analysis, and most of soil-related research is focused on reflectance spectroscopy. Infrared reflectance spectra, including diffuse reflectance spectra and total attenuated reflectance spectra, are involved in soil quantitative analysis. We observe an excellent performance of predicting soil C and N contents using IR spectra. Moreover, in most of cases the predictions of the contents of soil P, K, Ca, Mg, S, and some other microelements are satisfactory. Soil water, soil clays, and soil microbes can also be characterized and evaluated using IR spectroscopy. In recent years, a new method named infrared photoacoustic spectroscopy was applied in soil analysis.

Infrared photoacoustic spectroscopy is indeed more convenient for sample pretreatment and spectra recording, and the recorded soil spectra contain more useful information versus conventional reflectance spectroscopy. Though currently the application of infrared photoacoustic spectroscopy in soil analysis is limited, it appears promising to measure soil fertility. The application of IR in soil fertility is largely dependent on spectra pretreatment and multivariate calibration due to strong interferences in the spectra. Partial least square (PLS) and artificial neural network (ANN) are two widely used mathematical tools in the prediction of soil properties, and more mathematical tools-combined models will benefit the prediction performance. To make full use of soil infrared spectra, soil spectra library construction is needed in future, and a standard procedure should be first

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decided in the construction. Based on soil infrared spectra library soil fertility can be evaluated fast by combining suitable mathematical models, which will play an important role in sustainable agriculture.

Keywords Soil fertility · Sustainable agriculture · Infrared spectroscopy · Reflectance spectra · Photoacoustic spectra · Multivariate calibration · Partial least square · Artificial neural network

1 Introduction

1.1 Definition of Soil Fertility

Soil is a dynamic natural body occurring in the upper few meters of the Earth's surface at the interface between the atmosphere, biosphere, hydrosphere and geosphere, and soil, the foundation for most terrestrial life, has unrivalled complexity. Soil contains minerals, organic matter, countless numbers of organisms, as well varying amounts of air and water which provide life support (Wilding and Lin, 2006). A single gram of soil usually contains tens to thousands of millions of fungi and bacteria, plus thousands of diverse plant and animal species; soil is both an ecosystem in itself, and a critical part of the larger terrestrial ecosystem (Uphoff et al., 2006). From the earliest perceptions of soil as the organic-enriched surface layer to today's pedologic horizonation of profiles, there is a rich history of beliefs and understanding of this vital life-sustaining resource (Richard 2006). This biologically active, structured porous medium, called the *pedosphere*, mediates most of the biogeophysical and chemical interactions among the land, its surface and ground waters, and the atmosphere (Fig. 1) (Wilding and Lin, 2006). Soil is real, it exists, you can touch it, feel it, stand on it, and dig in it, but defining it is far more complex because it can be what you want it to be; thus properties of soils, functions of soils, and classification of soils are important research content in soil science.

It is well known that sandy soils are much easier to prepare but the yields are more difficult to maintain, whereas clay soils are hard to prepare but the yields are much better, in which the difference of this soil property is expressed with soil fertility. Soil fertility is a commonly used concept in soil science, and it is the function of soil properties, including soil nutrients, soil moisture, soil mineral, soil organic matter (Desbiez et al., 2004), and in different areas the limiting factor in soil fertility is different, such as in tropical areas, the important factors are moisture stress, high P fixation, high acidity, etc. (Cardoso and Kuyper, 2006). Therefore, soil fertility is very comprehensive, which cannot be measured directly but can be evaluated by some other soil properties (Bautista-Cruz et al., 2007). Considering sustainable agriculture (both economic and environmental aspects) soil fertility can be defined as the ability of a soil to serve as a suitable substrate on which plants can grow and develop in a sustainable way (Izac, 2003; Adjei-Nsiah et al., 2007). Fertile soils facilitate root development; supply water, air, and nutrients to plants; have little soil erosion; and do not have pest and disease burdens that result in catastrophic impacts

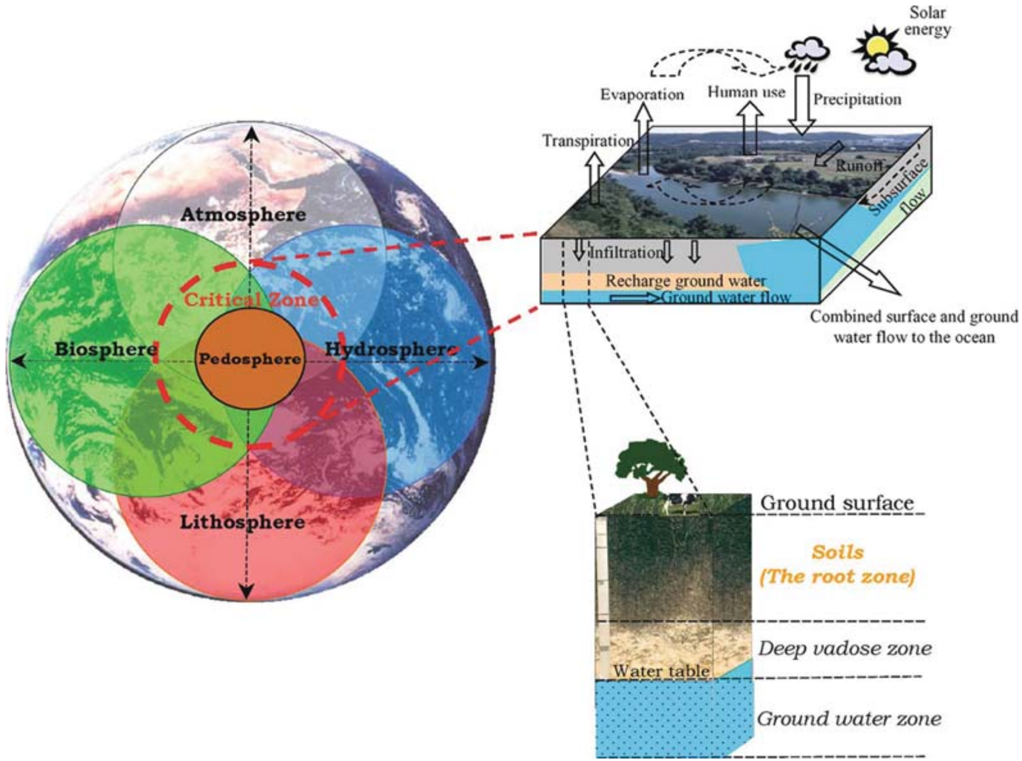

Fig. 1 A schematic of the Earth's Critical Zone and the pedosphere. The pedosphere is the thin skin of soil on the Earth's surface that represents a geomembrane across which water and solutes, as well as energy, gases, solids, and organisms are actively exchanged with the atmosphere, biosphere, hydrosphere, and lithosphere to create a life-sustaining environment. Soil–water interactions create the fundamental interface between the biotic and abiotic, and hence serve as a critical determinant of the state of the Earth system and its critical zone (drawing not to scale)

on the plants. Soil scientists have long grappled with the difficulty of discerning the many variations and gradations in soil chemistry and physical structure that have major implications for soil fertility. Yet this fertility is a function also of gases, liquids, organic matter, and myriad organisms in soils. As wooden bucket principle says that the water capacity is decided by the shortest board rather than the longer one, the soil fertility can be looked on as water capacity in the wooden bucket, which is limited by the poorest soil property. Hence, soil fertility requires a biologically framed understanding of soil system for making their management more productive and sustainable.

1.2 Soil Fertility and Sustainable Agriculture

Maintaining soil fertility is the basis of all forms of sustainable soil use, and maintenance of soil fertility requires preservation of its organic matter, physical properties, and nutrient levels. In most of cases, the leading factor involved in soil fertility is nutrients status (Alfaia et al., 2004). If fertility has fallen below a critical level through long-term agricultural use without replacement of nutrients or as a result of erosion, or if it is naturally very low, the replenishment of soil fertility may be a precondition for productive agriculture. Recent research has provided evidence that soil fertility is declining in many farmed areas, and a significant driver is tillage which dramatically affects the soil organic matter stocks along with associated nutrients (Gobeille et al., 2006). Nutrient budgets are especially threatening for the fertility of soils whose nutrient stocks are already small, such as sandy soils or acid soils with low organic matter contents. Nutrient balance of a site can be achieved both by reducing unproductive nutrient losses from erosion and leaching and by increasing nutrient inputs through nitrogen fixation; they can improve soil structure, water-holding capacity, and crop rooting volume; and they can increase the biological activity in the soil by providing biomass and a suitable microclimate. However, a better understanding of the interactions between crops and soils has also helped to keep expectations at a realistic level and to recognize what agroecosystems can and cannot achieve (Izac, 2003).

Obviously, soil fertility is very important to sustainable agriculture; thus it is necessary to know how to evaluate the fertility of soil, on which basis corresponding measures can be taken to maintain the soil fertility or to delay the declining rate of soil fertility.

1.3 Conventional Evaluation of Soil Fertility

Mineral elements constitute about half of the soil's volume, even though they can appear to be its totality. The mineral portion of soil, which differs from system to system in its chemical composition and its physical characteristics, has long been the focus of most soil science research. These mineral elements exist in different-sized soil particles, classified (from large to small) as sand, silt, or clay.

The mineral composition of soil establishes its physical properties, and it influences and is influenced by the life forms that are present. Besides soil minerals the other main components of soil are as follows (Norman Uphoff et al., 2006):
(1) *Soil water*. Water constitutes about a quarter of soil volume, although the actual amount can vary greatly over time and between soil systems. With too little water, soil systems become desiccated, and with too much, they are saturated. Air in well-aggregated soil can be another quarter of the volume, containing oxygen, hydrogen, nitrogen, and carbon in gaseous forms. The more pore space within the soil, the greater will be its capacity for holding both water and air which benefit plants as well as other flora and fauna in the soil. For any given soil porosity, the amounts of water and air are usually inversely related.
(2) *Soil organic matter*. Organic material usually comprises only a small portion of soil by volume, usually between 1 and 6%, although it can be higher than this. This organic materials category encompasses nonliving organic matter which is derived from the growth, reproduction, death, and decomposition of plants, animals, and microbes and exists in the soil as humus or as other inanimate material, and an immense variety of living flora and fauna, referred to collectively as the soil biota. The organic portion of soils includes both soil organisms and the various biological substances and processes that animate soil systems. The connection between the mineral and organic components of soil systems is intimate, converging at the smallest scale of soil structure and function in what are called clay–humus complexes. At the next higher level of structure, in microaggregates, inert and living materials are practically fused. Although this is well known, the biological dimensions of soil systems are too often regarded more as secondary or intervening variables, rather than as central and determining factors.

Therefore, soil nutrients level and organic matter content are main soil properties used in the evaluation of soil fertility, and the conventional option to determine these soil properties is mainly based on chemical methods, in which sample pretreatment, such as soil extraction and soil digestion, and sample processing, such as colorization, are needed (Fainthfull, 2002). Obviously, the chemical methods provide a useful tool to know the soil characterization, but most of the methods are time-consuming, which makes it unsuitable for fast or in situ evaluation of soil quality as well as the analysis of mass soil samples that is needed in precision agriculture (McCarty and Reeves, 2006; Ortega and Santibanez, 2007).

1.4 The Application Potential of Infrared Spectroscopy in Soil Science

Infrared reflectance spectroscopy has advantages over some of the conventional techniques of soil analysis, e.g., they are rapid, timely, and less expensive, and hence are more efficient when a large number of analyses and samples are required (McCarty and Reeves, 2006; Nanni and Dematte, 2006). Moreover, spectroscopic techniques do not require expensive and time-consuming sample preprocessing or the use of (environmentally harmful) chemical extractants. Infrared spectroscopy may, on instances, be more straightforward than conventional soil analysis and on occasions also more accurate (Viscarra Rossel et al., 2006). For example, McCauley et al. (1993) suggested that infrared spectroscopy may be more accurate than dichromate digestions for analysis of soil organic carbon and Viscarra Rossel et al. (2001) suggested that the precision of the mid-infrared (MIR)–partial least square (PLS) technique for predictions of soil pH and lime requirement is higher than conventional analysis. One other advantage is the potential adaptability of the techniques for in situ field use (Viscarra Rossel and McBratney, 1998). These are particularly important advantages now that there is an increasing global need for larger amounts of good-quality, inexpensive spatial soil data to be used in environmental monitoring and precision agriculture (Mouazen et al., 2007).

2 Techniques of Infrared Spectroscopy

2.1 Absorption of Infrared Spectroscopy

Infrared spectroscopic techniques are highly sensitive to both organic and inorganic phases of the soil, making them useful in the agricultural and environmental sciences. Intense fundamental molecular frequencies related to soil components occur in the MIR between wavelengths 2,500 and 25,000 nm. The visible and infrared portions of the electromagnetic spectrum are highlighted in Fig. 2 (Viscarra Rossel et al., 2006). Infrared spectroscopy is a technique based on the vibrations of atoms of a molecule. An infrared spectrum is commonly obtained by passing infrared radiation through a soil sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule, which makes infrared spectroscopy an alternative method in soil evaluation (Dematte et al., 2004).

2.2 Methods of Infrared Spectroscopy

2.2.1 Infrared Transmission Spectroscopy

Transmission spectroscopy is the oldest and most straightforward infrared method. This technique is based on the absorption of infrared radiation at specific wavelengths as it passes through a sample. It is possible to analyze samples in the liquid, solid, or gaseous forms when using this approach (Stuart, 2004).

Liquid samples can be run neat or by dissolving in a solvent. The sample concentration and path length should be selected to obtain the transmittance in the range of 15–70% in order to get a good infrared (IR) spectrum. This will correspond to about 0.02 mm cell thickness in the case of most neat liquids, and concentration of 10% and cell length of 0.1 mm in the case of most solutions. The solvent selected must be transparent in the region of interest. Neat liquids can be analyzed between

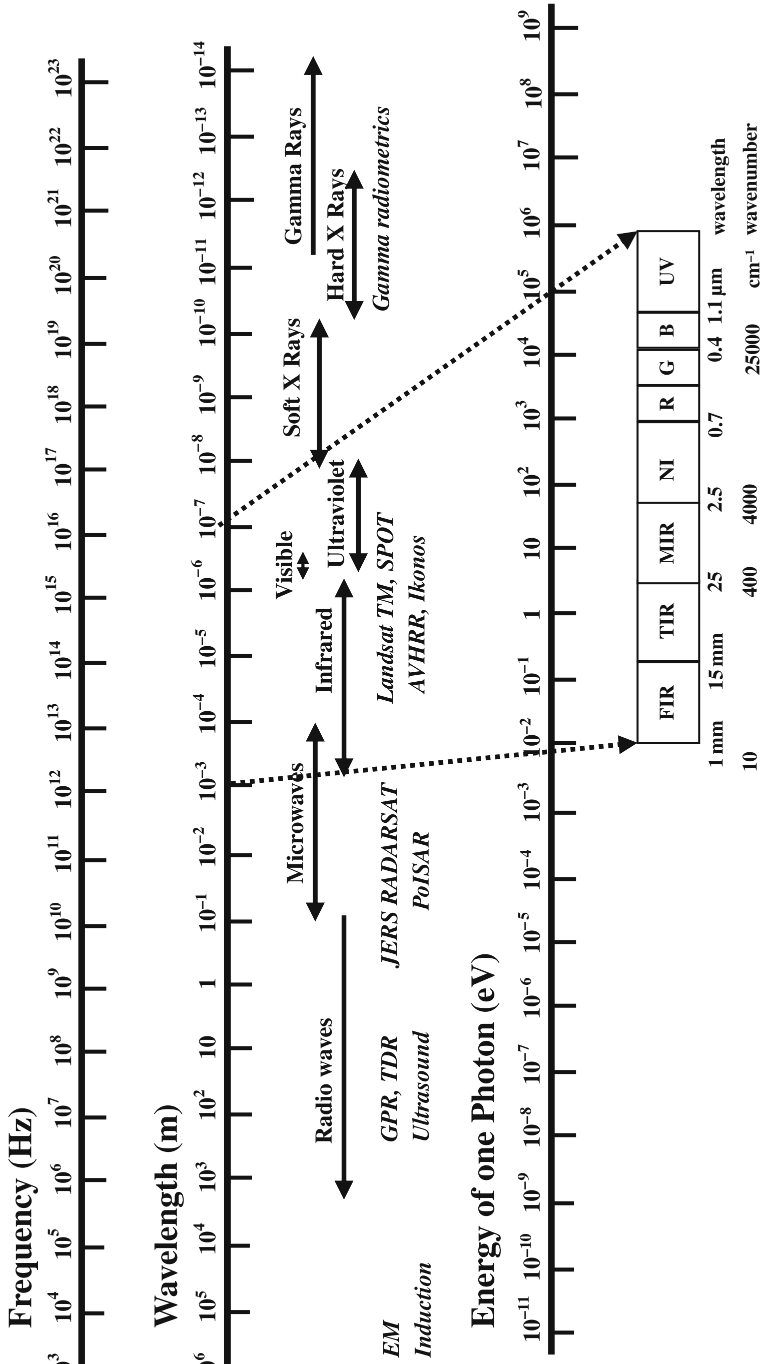

Fig. 2 The electromagnetic spectrum highlighting the visible and infrared portions

salt plates made of NaCl or KBr. Non- or low-volatility liquids can be analyzed by placing a drop of the sample onto specially prepared thin polyethylene (or other) polymer substrates. These supports (called 'IR cards') are cheap and disposable. They absorb IR only in well-known narrow bands, which depends on the material. These absorptions can be accounted for using the clean substrate as a background.

Solid samples, such as soil sample, can be prepared for IR analysis by pellet technique. This technique is based on the fact that a sample in KBr powder can be compressed under pressure with or without vacuum to form transparent disks. In this technique, a solid sample of approximately 2–3 mg is allowed to mix with about 0.2–1 g of KBr (which is transparent to IR). Thoroughly grind the mixture in a mortar, then press the mixture in a pellet die under a pressure of about 6,000–10,000 psi to obtain a transparent disk. Good dispersion of the sample in KBr is critical. It should be pointed out that bands near 3,448 and 1,639 cm^{-1} from moisture often appear in the spectra obtained by this technique. One should avoid moisture by, for example, freeze-drying of the sample as needed. KBr disk can be prepared with a Mini-Press accessory. Pastes and other semi-solids are routinely analyzed with the help of an attenuated total reflectance (ATR) attachment (Linker et al., 2004).

2.2.2 Infrared Diffuse Reflectance Spectroscopy

In external reflectance, the energy that penetrates one or more particles is reflected in all directions and this component is called diffuse reflectance. In the diffuse reflectance (infrared) technique, commonly called DRIFT, the DRIFT cell reflects radiation to the powder and connects the energy reflected back over a large angle. Diffusely scattered light can be collected directly from material in a sampling cup or, alternatively, from material collected by using an abrasive sampling pad. Figure 3 illustrates diffuse reflectance from the surface of a sample.

Kubelka and Munk (1931) developed a theory describing the diffuse reflectance process for powdered samples which relates the sample concentration to scattered radiation intensity, and the Kubelka–Munk equation is as follows:

$$(1 - R^2)/2R = c/k, \tag{1}$$

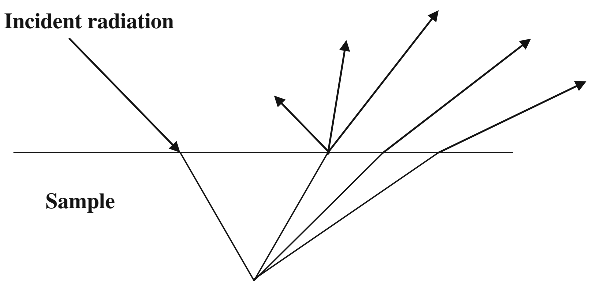

Fig. 3 Illustration of diffuse reflectance

where R is the absolute reflectance of the layer, c is the concentration, and k is the molar absorption coefficient. An alternative relationship between the concentration and the reflected intensity is widely used in infrared diffuse reflectance spectroscopy, namely

$$\log(1/R) = k'c. \tag{2}$$

2.2.3 Infrared ATR Spectroscopy

ATR spectroscopy utilizes the phenomenon of total internal reflection (Fig. 4). A beam of radiation entering a crystal will undergo total internal reflection when the angle of incidence at the interface between the sample and crystal is greater than the critical angle, where the latter is a function of the refractive indices of the two surfaces. The beam penetrates a fraction of wavelength beyond the reflecting surface and when a material that selectively absorbed radiation, such as soil, is in close contact with the reflecting surface, the beam loses energy at the wavelength where the material absorbs. The resultant attenuated radiation is measured and plotted as a function of wavelength by the spectrometer and gives rise to the absorption spectral characteristics of the sample.

The crystal used in ATR cells are made from materials that have low solubility in water and are of a very high refractive index. Such materials include Zinc selenide, germanium, and thallium-iodide. Different designs of ATR cells allow both liquid and solid samples to be examined. It is also possible to set up a flow-through ATR cell by including an inlet and outlet in the apparatus. This allows for continuous flow of soil solutions through the cell and permits spectral changes to be monitored with time.

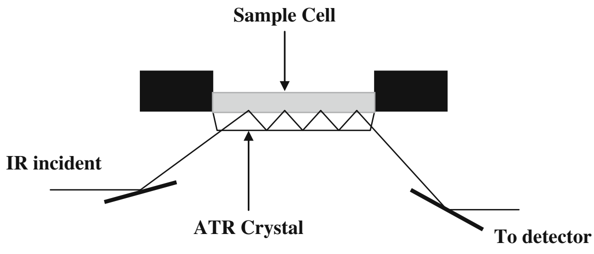

Fig. 4 Schematic of a typical attenuated total reflectance cell

2.2.4 Infrared Photoacoustic Spectroscopy

Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) is based on the absorption of electromagnetic radiation by analyte molecules. Non-radiative relaxation processes (such as collisions with other molecules) lead to local warming of the sample matrix. Pressure fluctuations are then generated by thermal expansion, which can be detected by a very sensitive microphone (Fig. 5) (McClelland

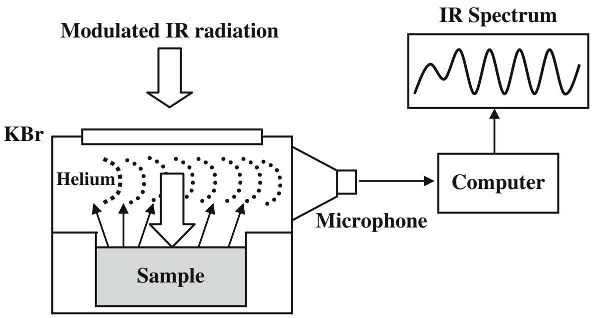

Fig. 5 Schematic of a typical photoacoustic spectroscopy cell

et al., 2001). The resulting spectrum differs from both of equivalent transmission or reflectance spectra since the technique detects non-radiative transitions in the sample. The detected signal in photoacoustic spectroscopy is proportional to the sample concentration, and can be used with highly absorbing samples without any pretreatment. Using multivariate statistic analysis, such as PLS, FTIR-PAS made it a wide application with quantitative purpose in solid substances (Wahls et al., 2000; Bjarnestad and Dahlman, 2002; Irudayaraj et al., 2002; Armenta et al., 2006).

3 Evaluation of Soil Fertility Using Infrared Spectroscopy

3.1 Infrared Spectra Based Soil Qualitative Analysis

3.1.1 Characterization of Soil Components

The soil MIR spectrum (400–4,000 cm^{-1}) can be approximately divided into four regions, and the nature of a group frequency may generally be determined by the region in which it is located. The fundamental vibrations in the 2,500–4,000 cm^{-1} region are generally due to O–H, C–H, and N–H stretching. O–H stretching produces a broad band that occurs in the range of 3,700–3,600 cm^{-1}. By comparison, N–H stretching is usually observed between 3,400 and 3,300 cm^{-1}. The absorption is generally much sharper than O–H stretching and may, therefore, be differentiated. C–H stretching bands occur in the range of 3,000–2,850 cm^{-1}. Triple-bond stretching absorptions fall in the 2,000–2,500 cm^{-1} region because of the high force constants of the bonds. C–C bonds absorb between 2,300 and 2,050 cm^{-1}, while the nitrile group (C–N) occurs between 2,200 and 2,300 cm^{-1}. The principal bands in the 1,500–2,500 cm^{-1} regions are due to C=C and C=O stretching. Carbonyl stretching is one of the easiest absorptions to recognize in an infrared spectrum and occurs in the 1,650–1,8030 cm^{-1} region. C=C stretching is much weaker and occurs at around 1,650 cm^{-1}. It has been assumed so far that each band in an infrared spectrum can be assigned to a particular deformation of the molecular, the movement of a group of atoms, or the bending or stretching of a particular bond. Many vibrations may vary by hundreds of wavenumbers, and these combine most bending and skeletal vibrations, which absorb in the 650–1,500 cm^{-1} region referred to as the fingerprint region.

The absorptions observed in the near-infrared (NIR) regions (4,000–13,000 cm^{-1}) are overtones or combination of fundamental stretching bands which occur in the 1,700–3,000 cm^{-1} region. The bands in the NIR are often overlapped, making them less useful compared to MIR region for qualitative analysis. However, there are important differences between the NIR positions of different functional groups and these differences can often be exploited for quantitative analysis.

Soil, mainly composed of organic matter, clay minerals, and water, is complicated material, and had abundant absorptions in MIR region (Fig. 6), which made it difficult to have a specific identification (Du et al., 2007; 2008a). Kaolin (1:1 clay mineral) and Bentonite (2:1 clay mineral) are two popular clay types commonly encountered in the investigated soils. Main components of the soil can be characterized in the MIR spectrum. Clear absorptions are visible in several spectral regions, and in particular around 2,800–3,700 cm^{-1}, 2,200–2,600 cm^{-1}, 1,800–2,100 cm^{-1}, and 900–1,600 cm^{-1}. The absorptions of bentonite are demonstrated in the regions of 2,800–3,700 cm^{-1} (O–H stretching), 1,500–1,800 cm^{-1} (C=O stretching), and 800–1,200 cm^{-1} (fingerprint region), in which the absorption in the regions of 2,800–3,700 cm^{-1} is a wide band; the absorptions of kaolin are indicated in the regions of 3,500–3,700 cm^{-1}, 1,500–2,000 cm^{-1}, 800–1,200 cm^{-1} (Si–O stretching), in which the absorption in the regions of 800–1,200 cm^{-1} is strong; soil calcium carbonate also shows absorption in the regions of 2,900–3,100 cm^{-1},

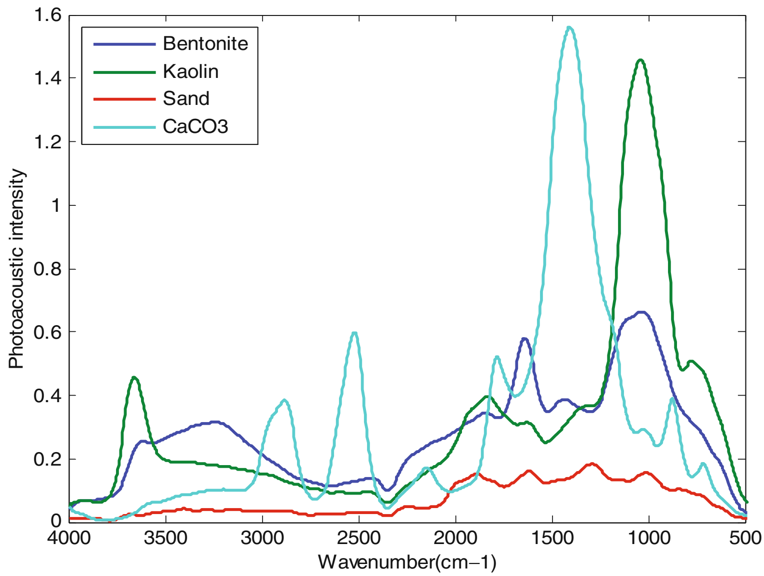

Fig. 6 Mid-infrared photoacoustic spectra of main soil components

2,300–2,600 cm^{-1}, 1,000–1,600 cm^{-1}, 1,600–1,700 cm^{-1}, and 2,100–2,200 cm^{-1}, and the absorption in the regions 1,000–1,600 cm^{-1} is very strong, but is heavily interfered by some other absorptions; the absorption in the regions 2,300–2,600 cm^{-1} is strong enough, and is less interfered, which is useful in quantitative analysis; the absorption in the regions of 2,900–3,100 cm^{-1} might come from the water attached on the calcium carbonate particle surface. Soil water indicates a strong absorption in the region of 1,600–1,700 cm^{-1} and 2,900–3,600 cm^{-1} (Du et al., 2008b), but the interference is very strong in the region of 2,900–3,600 cm^{-1}. In addition, besides the characteristics of soil components' absorptions, soil properties are usually interrelated (Fig. 7) (Cohen et al., 2005), which makes each property directly or indirectly determined by the technique of infrared spectroscopy.

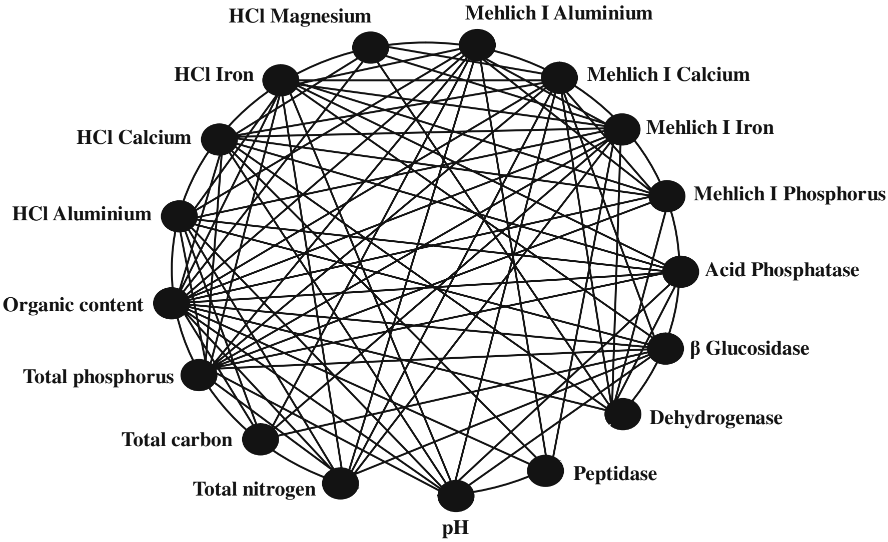

Fig. 7 Graphical depiction of conditional association pattern for observed soil parameters. Nodes represent selected properties; arcs between nodes are significant at $p < 0.001$

3.1.2 Soil Identification

Soil types are due to soil components, and each soil component has a specific characterization of infrared spectrum. Spectral signatures of soils are defined by their reflectance, or absorbance, as a function of wavelength. Under controlled conditions, the signatures are due to electronic transitions of atoms and vibrational stretching and bending of structural groups of atoms that form molecules and crystals. The fundamental vibrations of most soil materials can be found in the MIR region, with overtones and combinations found in the NIR region. Soil minerals such as different clay types have very distinct spectral signatures in the infrared region because of strong absorption of the overtones of SO_4^{2-}, CO_3^{2-}, and OH^-, and

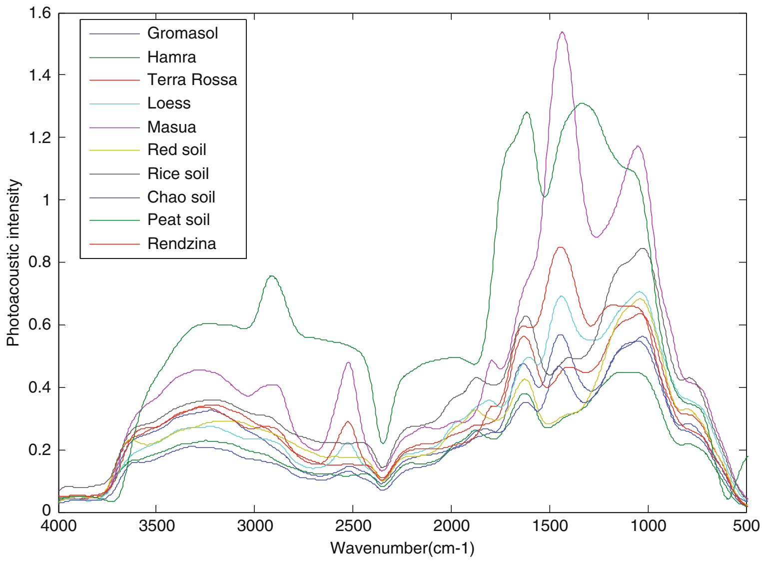

Fig. 8 Mid-infrared photoacoustic spectra of different soils

combinations of fundamental features, for example, H_2O and CO_2 (Fig. 8) (Brown et al., 2006; Du et al., 2007). Therefore, it is possible to have a soil identification based on soil infrared spectrum.

Infrared spectroscopy is a well-established technique for the identification of chemical compounds and/or specific functional groups in compounds, and thus is a useful tool for soil applications (Johnston and Aochi, 1996; Haberhauer and Gerzabek, 2001). In particular, reflectance spectroscopy can be used for nondestructive assessment of physical and biochemical properties of soil and crops (Cozzolino and Moron, 2003, 2006; Chang et al., 2001; Dunn et al., 2002; Shepherd and Walsh, 2002; Shepherd et al., 2003). Although the NIR range (800–2,500 nm) is still the most widely used, MIR spectroscopy is becoming increasingly common due to the specificity of the absorbance bands in that spectral range (Stuart, 1997). In particular, MIR ATR spectroscopy can be used for fast and simple determination of nitrate concentration in water and soil pastes (Shaviv et al., 2003; Linker et al., 2004). Linker et al. (2005, 2006) also showed that MIR ATR spectroscopy could be used to identify major types of agricultural soils based primarily on absorbance bands associated with characteristic soil constituents (e.g., calcium carbonate, clay minerals, and possibly organic constituents), and such identification of soil types led to the significant improvement of ATR-based determination of nitrate in soil pastes (Linker et al., 2006). FTIR-PAS is another spectral technique that can be used for the identification of constituents in complex systems (McClelland et al., 2001). A major advantage of photoacoustic spectroscopy is that it is suitable for highly absorbing solid samples without any special pretreatment. With respect to soil analysis, this is a major advantage compared to transmittance measurements that require time-consuming preparation of KBr pellets, or the ATR configuration that requires a saturated soil paste and suffers from interferences associated with the presence of water (Linker et al., 2004), and very good classification performances were achieved, with correct classification rates of the validation samples typically above 95% (Du et al., 2008a; Linker, 2008).

3.2 Infrared Spectra Based Soil Quantitative Analysis

3.2.1 Soil Nutrients

Soil nutrients, such as, C, N, P, K, S, Ca, and microelements, play the most important role in soil fertility. Usually the nutrients content are determined through laboratory analysis; however, many of the existing methods of soil analysis are resource intensive, and do not lend themselves to the use of large number of samples (Ludwig et al., 2002). It is possible to evaluate the nutrients content using infrared spectroscopy (Chang et al., 2001; Pirie et al., 2005; Verma and Deb, 2007a), and the technique of infrared reflectance spectroscopy could be a faster, cheaper, and more objective way in the evaluation of soil nutrients (Daniel et al., 2003; Brown et al., 2006). Soil properties are usually interrelated, which makes the predictions of most soil properties possible (Fig. 7) (Cohen et al., 2005; Rinnana and Rinnan, 2007). Viscarra Rossel et al. (2006) provided a review of some literature comparing quantitative predictions of soil nutrients using various multivariate techniques and reflectance spectra response in the infrared region of the spectrum. The content of soil C and N are mainly studied because they are more sensitive to infrared effect. The calibration coefficients (R^2) are in the region of 0.80–0.98, and the root mean square errors (RMSE) are very much satisfied in fast evaluation of soil fertility (Reeves et al., 2001; McCarty et al., 2002; Cozzolino and Moron, 2006; Stevens et al., 2008).

Soil C can be spectrally measured with a reasonable accuracy level, depending on the type of instrument and environmental conditions, with RMSE ranging from 1 to 15 g C kg^{-1} (Ludwig et al., 2002; Brown et al., 2005, 2006; Stevens et al., 2006). The calibration coefficient (R^2) is more than 0.8 (Zimmermann and Fuhrer, 2007; Leach et al., 2008; Wetterlind et al., 2008a), and the size of soil particle has a strong influence on the calibration and validation (Barthes et al., 2008). C mineralization is also studied using diffuse reflectance spectra, and it is useful in evaluating C storage potential in soils (Mutuo et al., 2006). Infrared of soil C source materials, i.e., humic acids, fulvic acids, and their interaction products, have provided much information for their characterization and determination (Byler et al., 1987; Francioso et al., 2007), which will benefit the evaluation of soil fertility.

Soil nitrate concentration can be direct measurement using MIR ATR spectroscopy (FTIR-ATR) through the correlation between nitrate concentration and the vibration band around 1,350 cm^{-1} (Borenstein et al., 2006; Verma and Deb, 2007b). Shaviv et al. (2003) showed that MIR spectroscopy using either standard

ATR crystals can be used for direct determination of nitrate concentration in water, soil extracts, or soil pastes. By applying a straightforward chemometric approach, Linker et al. (2004) improved the determination accuracy and overcame some of the interferences associated with direct measurements in soil pastes. However, this correlation between soil nitrate concentration and the infrared absorption band is soil-dependent, due mostly to varying contents of carbonate (Linker et al., 2004, 2005; Jahn et al., 2006). Linker et al. (2005) suggested the use of a two-stage method that can be summarized as follows: (1) determination of the soil type by comparing the so-called 'fingerprint' region of the spectrum 800–1,200 cm^{-1} to a reference spectral library and (2) determination of the nitrate concentration using the model corresponding to this soil type. This soil identification-involved approach led to determination errors significantly lower than those reported earlier (Shaviv et al., 2003; Linker et al., 2004), and determination errors range from 6.2 to 13.0 $mg^{-1}kg^{-1}$, depending on the soil type, with the lowest errors for light sandy soils. These determination errors are appreciably smaller than those obtained using a single model calibrated using all the data (Linker et al., 2006).

For the prediction of the other soil nutrients including P, K, and microelements the calibration results are not stable, which are pending to the variability and capacity of the calibration set (Janik et al., 1998; Ludwig et al., 2002, Cozzolino and Moron, 2003; Brown et al., 2006). It showed that NIR was not a good tool for P and K prediction, with *R*, 0.47 and 0.68, and SEP, 33.70 and 26.54, respectively (He et al., 2007), and future research should be addressed to build calibrations for open populations (Terhoeven-Urselmansa et al., 2008).

Usually infrared reflectance spectroscopy is used in the soil quantitative analysis, but there are certain limits especially in the sample pretreatment. Recently infrared photoacoustic spectroscopy was used in soil quantitative analysis, and a better calibration result for soil C, N, P, K were observed (Du and Zhou, 2007; Du et al., 2009). This technique does not need sample pretreatment, and a fast and in situ monitoring of soil nutrients can be reached, which will be a promising method in the evaluation of soil fertility.

Using the technique of infrared spectroscopy combined with Geographic Information System (GIS) and statistical methods, the N, P, K, and OM spatial variability within the field can be obtained (Odlare et al., 2005; Wetterlind et al., 2008b; Christy, 2008), and their distribution maps can be drawn (He et al., 2005), in which soil nutrient status can be directly indicated. The reference maps for the predicted and measured values of N and OM were almost the same, not being this way for P and K due to the non-successful prediction of these constituents. Phosphorus sensing system could be developed using diffuse reflectance of soil for the soil P testing (Bogrekci and Lee, 2005a; Maleki et al., 2006; Mouazen et al., 2007), and based on this techniques the spectral phosphorus map could be drawn, in which P variability could be well represented (Fig. 9), and would be useful in precision agriculture (Bogrekci and Lee, 2005b). The maps derived from the Infrared spectra data are promising, and the potential for developing a cost-effective strategy to map soil from infrared spectra data at the farm-scale is considerable (Wetterlind et al., 2008b).

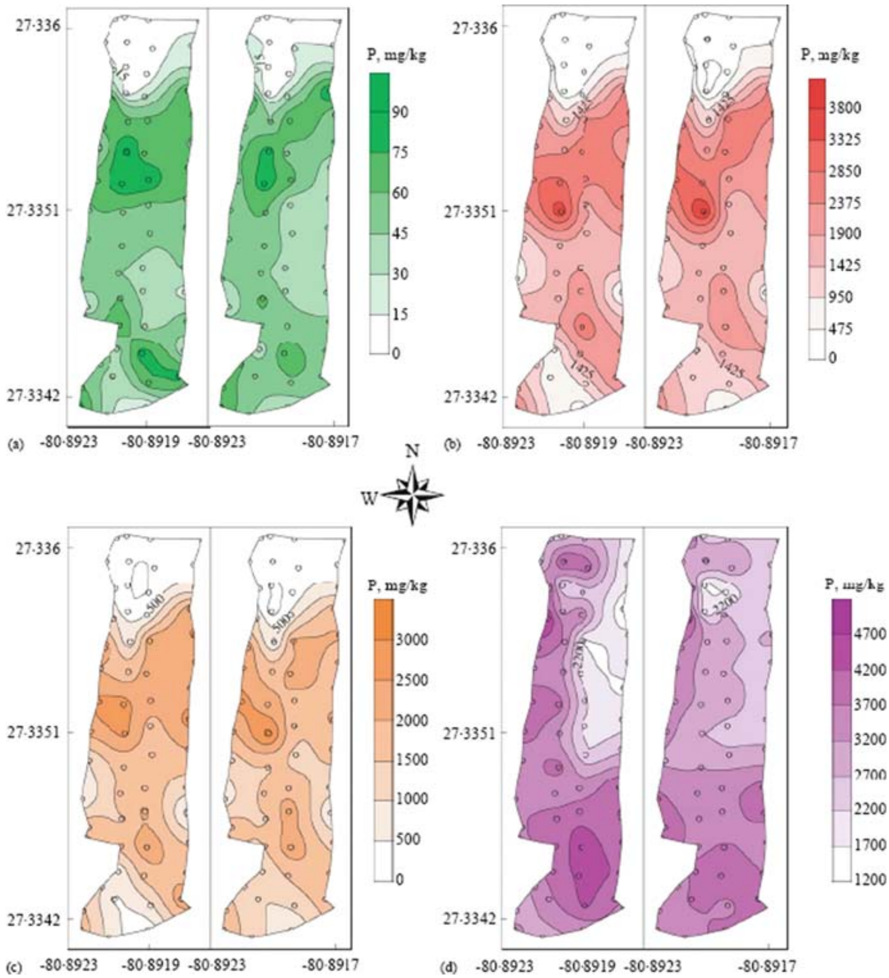

Fig. 9 Actual (*left*) and partial least squares-predicted (*right*) P concentration in Candler Farm, Okeechobee County, Florida: (**a**) water soluble P in soil; (**b**) total P in soil; (**c**) Mehlich-1 P in soil; and (**d**) total P in vegetation. *x* and *y* coordinates are in Lat/Long, decimal degree

3.2.2 Soil Clays

In the IR spectrum, absorptions by water bonds associated with clay content and other bondings associated with clay type provide the opportunity to use IR spectra for quantifying clay information in soil. Using IR spectroscopy research on air-dried ground soil samples has shown predictions of soil clay content with R^2 values ranging from 0.56 to 0.91 and RMSE ranging from 23 to 11 g kg^{-1} (Ben-Dor and Banin, 1990, 1995; Janik et al., 1998; Shepherd and Walsh, 2002; Islam et al., 2003; Sorensen and Dalsgaard, 2005; Brown et al., 2006). An IR spectra data-based calibration model for determination of clay in soil was developed and tested in practice. The model showed ruggedness, linearity, and stable prediction error over the calibrated content range (2–26% clay). The uncertainty of the method was <40% higher than the reproducibility standard deviation of the reference method for clay contents below 26%. As seen in many other cases, the prediction error was dependent on the content range calibrated. When the range was extended from 2 to 74% clay, the estimated prediction error increased to 3.4%. However, an SD (standard deviation)/RMSE ratio of 4.7 demonstrated a high correlation between NIR spectral data and reference data (Sorensen and Dalsgaard, 2005). Visible NIR diffuse reflectance spectra was capable of predicting soil clay content in situ at varying water contents, in which the RMSE was 61 g kg^{-1} (Waiser et al., 2007).

Figure 6 shows the photoacoustic spectra of four major soil components (Du et al., 2007). Bentonite (standing for smectites) and kaolin, which represent the two clay types most commonly encountered in the investigated soils, have spectra that differ notably and share only the strongest band around 1,040 cm^{-1} and have some overlap around the 3,600–3,700 cm^{-1} range. Calcium carbonate is easily identifiable with a series of strong and well-defined bands (900, 1,450–1,550, 2,500–2,550, 2,800–3,000 cm^{-1}), while the overall intensity of the quartz spectrum is much lower and includes only weak bands in the 1,000–2,000 cm^{-1} region. Comparison shows that the spectra of mineral soils agree well with expectations based on soil composition. For instance, the Masua soil, which has a very high calcium carbonate content and relatively high clay content but cannot contain kaolin, has very strong bands around 1,040 cm^{-1} (smectite), 1,430 cm^{-1} and 2,520 cm^{-1} ($CaCO_3$), and two smaller peaks around1,600 and 1,800 cm^{-1} (smectite and $CaCO_3$). The spectra of soils (Fig. 8) with very low calcium carbonate content (Hamra, Terra Rosa, and Red and Paddy soils) are devoid of bands in the 1,430 and 2,520 cm^{-1} region. With regard to clay soils, the differences are more subtle, but the bands in the range of 1,000–1,200 cm^{-1}, around 1,600 cm^{-1}, and the shoulders that can be seen around 780, 1,800, and 3,700 cm^{-1} in the spectra of the Chinese Red and Rice soils point to the presence of kaolin or a mixture of kaolin and smectite in these soils. Furthermore, the Grumosol sample, which is expected to contain only smectite, shows none of the bands that are characteristic to kaolin (780 and 1,800 cm^{-1}). Regardless of type, clay content directly determines the amount of hydroscopic water present in air-dried soil. Figure 8 shows that the Hamra and Chao soils, which have the lowest clay content, have the lowest intensities in the 1,600–1,650 cm^{-1} and 3,000–3,600 cm^{-1} intervals that include the major water bands. This hints to the potential of utilizing PAS spectroscopy for assessing the amount of hygroscopic water or for determining indirectly characteristics such as clay content or specific surface area of a given soil.

3.2.3 Soil Water

Water is essential for plant growth and, as an inevitable consequence of opening their stomata to enable gaseous exchange during photosynthesis, plant transpiration. The soil water content also has a pronounced influence on nutrient uptake from the soil as it affects root growth and the transport of nutrients to the root. Furthermore, soil water influences the availability of oxygen, microbial and faunal activity, leaching of nutrients and agrochemicals into the subsoil, and swelling and shrinking of certain clay soils. Therefore, soil water is one of the most critical soil components for successful plant growth and land management, particularly in arid lands. Measurement of soil water content can be very beneficial for site-specific irrigation, seeding, and land management. The conventional method to determine water content by oven-drying of samples collected from fields is a difficult, costly, and time-consuming procedure. The NIR spectroscopy is a proven technique for the measurement of soil water content (Viscarra Rossel and McBratney, 1998), as it is fast, nondestructive, and cost effective, although a NIR instrument is expensive. Soil water content is considered as one of the most critical factors affecting the accuracy of NIR models developed for the determination of other soil properties, and it can be successfully measured with NIR spectroscopy (Mouazen et al., 2005, 2006).

Most of soil water is absorbed in organic matter or in the mineral surface, and the respective vibration of adsorbed water in MIR region was listed in Table 1 (Richard et al., 2006). Soil water retention is an important property of soil, and has a heavy influence on soil fertility. Soil water retention varies widely with soil composition and texture, but measurements are often time consuming and expensive using traditional laboratory methods since soil water retention is affected by soil density, particle size, mineral and organic composition, and pre-space density and distribution. Soil mineral and soil organic matter are sensitive to IR spectra, and there is a strong absorption band of water around 1,600 cm^{-1} in the IR reflectance spectrum, which makes it possible to analyze water retention using the technique of IR spectroscopy (Janik et al., 2007), and the determination errors range from 0.01 to 0.02 g water/g dry soil (Linker et al., 2006). Soil particle size shows significant influence on determination of soil water, and the soil particles that are less than 45 μm provide a reasonable model for estimating the water content of hydrated asteroids (Milliken and Mustard, 2007).

Table 1 Adsorbed water vibrations in the MIR

Frequency (cm^{-1})	Vibration mode	Water type
3,750–2,900	Stretching OH	OH groups on surface or at specific crystallographic sites and liquid water
3,280	Stretching OH Symmetric, $\nu 1$	Liquid
3,490	Stretching OH Symmetric, $\nu 1$	Liquid
3,300–3,000	Stretching OH; Hexameric and more complex clusters	Adsorbed water
3,430	Stretching OH	Bulk water
3,150	Stretching OH	Water firmly bound to a specific site,
3,050	Stretching OH	perhaps with a cluster structure
3,250	Stretching OH	First spectrum: mix of bulk and adsorbed water components
3,135	Stretching OH	Adsorbed water before vacuum pumping
3,100	Stretching OH	Adsorbed water under vacuum pumping

3.2.4 Soil Microbes

Soil governs plant productivity in terrestrial ecosystems and acts to maintain the equilibrium of biogeochemical cycles through biotransformations (or functions) mediated by living organisms. It has been recognized for many years that microbes are responsible for 80–90% of these functions (Nannipieri et al., 2003). It is thus of great interest, for the sustainability of our environment, to assess if the procedures of restoration of sites degraded by changes of land use may allow the soil to partially or totally recover its microbial functions. Microbial indicators such as changes in total biomass or in the structure of the total microbial community or of a given group of microorganisms have been often used to describe the soil fertility (Schloter et al., 2003).

Out of the many soil functioning monitoring possibilities, a selection of microbial functions and species diversity has been made. General functional aspects are soil respiration, nitrification, nitrogen fixation, and bacterial DNA synthesis. General diversity aspects include ratio of fungi/bacteria, mycorrhiza, suppressiveness to pathogens, and catabolic genes. Functions based on narrow diversity, such as nitrification and nitrogen fixation, are most valuable in relation to monitoring adverse influences. The common approach is to select appropriate microbial functions as relevant indicators of soil functioning. Nevertheless, one of the major limitations is that measurements of microbial functions are often time consuming and require a large number of analysis of soil samples to be representative of a given situation. FTIR is one of the methods that has been successfully used for detecting and identifying microorganisms (Rinnana and Rinnan, 2007), especially in food products (Mariey et al., 2001; Irudayaraj et al., 2002; Al-Qadiri et al., 2006). The possibility of using IR spectroscopy provides many opportunities for understanding both the temporal dynamics and the spatial variability of the recovery of key microbial functions during soil restoration. Some of these studies showed that discrimination was possible not only at the genus level, but also at the species and strain levels (Linker and Tsror, 2008). Calibrations were performed between infrared reflectance spectral data and microbial-based indicators using PLS model, and the microbial functions were precisely predicted (Schimann et al., 2007). Furthermore, identification and speciation of bacterial spores can be made using FTIR-PAS (Thompson et al., 2003), and discrimination was performed at the genus level and at the strain level for five soil-borne fungi. In the discrimination between the five fungi at the genus level, the success rate for the validation samples ranged from 75 to 89%. For discrimination between the two *Colletotrichum* strains, the success rate was 78% (Linker and Tsror, 2008).

3.3 Mathematical Tools in the Treatment of Spectral Data

3.3.1 Data Preprocessing

Preprocessing is a very important part in the analysis of spectroscopic data, and is defined as any mathematical manipulation of the spectral data prior to primary analysis. There are a number of techniques available, such as, normalization, baseline corrections, spectrum smoothing, difference spectrum, and spectral derivatives, in the pretreatment of spectra data, which is helpful to both the qualitative and quantitative interpretation of spectra (Beebe, 1998; Stuart, 2004).

Normalization of a spectral data is accomplished by dividing each absorbance by a constant, which is used to remove systematic variation. Beware that normalization may remove important spectral information, and it is suggested to check the normalization effect combing the primary analysis in calibration.

It is usual to use a baseline joining the points of lowest absorbance on a peak, preferably in reproducibly flat parts of the absorption line. The absorbance difference between the baseline and the top of the band is then used. Spectra were usually preprocessed with a smoothing filter (first-order Savitzky–Golay filter with a 25-point window). The Savitzky–Golay filter method essentially performs a local polynomial regression to determine the smoothed value for each data point. This method is superior to adjacent averaging because it tends to preserve features of the data such as peak height and width, which are usually 'washed out' by adjacent averaging, and the detail of the smoothing filter was given by Savitzky and Golay (1964).

The most straightforward method of analysis for complex spectra is difference spectroscopy. This technique may be carried out by simply subtracting the infrared spectrum of one component of the system from the combined spectrum to leave the spectrum of the other component. If the interaction between the components results in a change in the spectral properties of either one or both of the components, the changes will be observed in the difference spectra. Spectral subtraction may be applied for the data collected for solutions, such as infrared ATR spectrum of soil paste. It is necessary to record spectra of both soil paste and water, and water spectra may then be subtracted from the soil paste spectrum, by which soil spectrum is obtained (Linker et al., 2006). However, water absorptions are very strong under certain circumstances, and in this situation it makes it difficult to investigate the soil spectrum.

Spectra may also be differentiated, and the benefits of derivatives techniques are twofold. Resolution is enhanced in the first derivative since changes in the gradient are examined, and the second derivative gives a negative peak for each band and shoulder in the absorption spectrum (Mark and Workman, 2007). The advantage of derivatization is more readily appreciated for more complex soil spectrum, in which sharp bands are enhanced at the expense of broad ones and this may allow for the selection of a suitable peak.

3.3.2 Model Construction

The Beer–Lambert law is used to do quantitative analysis; however, it cannot be directly used in soil quantitative analysis. The infrared spectra of soil have many overlapping peaks, and isolation of absorptions should be made (Du et al, 2007; Linker et al., 2006). Therefore, multivariate calibration method, such as, principal components regression (PCR), PLS, and artificial neural networks (ANNs), is necessary in soil analysis. Here techniques of PLS and ANN are explained, respectively.

A general form of PLS model is expressed as

$$\begin{aligned} X &= TP^T + E \\ Y &= UQ^T + F' \end{aligned} \tag{3}$$

where X is the variable predictor matrix (absorbance); Y is the variable response matrix (soil properties); T and U are the X-scores and Y-scores matrices; P and Q are the X-loading and Y-loading matrices; E and F are the X-residual and Y-residual matrices. The coordinates of the sample in a coordinate system defined by the principal components (PCs) are called scores. The loading vectors are the bridge between the variable space and the PC space. The loadings provide the information about how much each variable contributes to each PC. In the case here, T contains information about the samples, and P contains information about the wavenumber. The detailed PLS algorithm in the PLS analysis is well described by Blanco et al. (2000) and Wold et al. (2001).

With numerous and correlated X-variables there is a substantial risk for overfitting, i.e., getting a well-fitting model with little or no predictive power. Hence, a strict test of the predictive significance of each PLS component is necessary, and then stopping when components start to be non-significant (Wold et al., 2001). Cross-validation (CV) is a practical and reliable way to test this predictive significance. This has become the standard in PLS analysis. Basically, CV is performed by dividing the data into a number of groups, and then developing a number of parallel models from reduced data with one of the groups deleted. After developing a model, differences between actual and predicted Y-values are calculated for the deleted data. The sum of squares of these differences is computed and collected from all the parallel models to form the predictive residual sum of squares, which estimates the predictive ability of the model. RPD is an important statistical parameter used to evaluate the calibration models. In agricultural application, RPD > 3 was considered acceptable and RPD > 5 excellent (Malley et al., 1999). However, there is no critical level of RPD for the IR analysis in soil science, and acceptable values depend on the intended application of the predicted values. Three categories based on RPD in the ranges >2, 1.4–2.0, and <1.4 were used to indicate decreasing reliability of prediction (Chang et al., 2001). Dunn et al. (2002) and Pirie et al. (2005) reported similar results of suitable limits for RPD: <1.6, poor; 1.6–2.0, acceptable; and >2.0, excellent. In this study the RPD values in optimized PLS models were acceptable, and comprehensively, the PLS models were excellent for soil organic matter, soil available N and P, and they were relatively poor for soil available K. Comparing with research results of Pirie et al. (2005), the predicting ability of soil properties using photoacoustic spectra-based PLS modeling was better enough compared with reflectance spectra in both NIR and MIR region.

Since soil is a complex mixture, and soil nutrient content is related with many soil components, multivariate calibration techniques were used to extract related information in the FTIR-PAS spectra. PLS regression was used to develop a correlation between the PAS spectra and the soil nutrient content in the soil samples. Du et al. (2009) demonstrated results of the leave-one-out CV calibration using different PLS factor numbers varied from 2 to 9. For each soil property, the calibration error kept decreasing to near-zero, and the calibration coefficient kept increasing to near 1. However, the validation error became smaller firstly, then turned larger and larger, which meant that modeling involving too many PLS factors would lead to over-fitting. The PLS factor number could be selected with lowest validation error where the calibration error and calibration coefficient were still good enough. Four PLS factors, which explained 96.98% variance of input vectors and output vectors, were selected for available N and organic matter, and the RPD values were 5.27 and 3.48, respectively; five PLS factors which explained 98.58% variance of input vectors and output vectors, were selected for available P and available K, and the RPD values were 5.51 and 3.85, respectively (Du et al., 2009).

ANN is typically organized in layers where these layers are made up of a number of interconnected nodes which contain an activation function. Input vectors are presented to the network via the input layer which communicates to one or more 'hidden layers' where the actual processing is done via a system of weighted 'connections'. ANN allows one to estimate relationships between one or several input variables called independent variables or descriptors and one or several output variables called dependent variables or responses. Information in an ANN is distributed among multiple cells (nodes) and connections between the cells (weights) (Despagne and Massart, 1998). Most ANN contains some form of 'learning rule' which modifies the weights of the connections according to input patterns that it is presented with. There are many different kinds of learning rules used by neural networks: in Back-Propagation Neural Networks (BP-ANN) 'learning' is a supervised process that occurs with each cycle of 'epoch' (i.e., each time the network is presented with a new input pattern) through a forward activation flow of inputs and the backwards error propagation of weight adjustment (Ramadan et al., 2005). There are many variations of the back-propagation algorithm. The simplest implementation of back-propagation learning updates the network weights and biases in the direction in which performance function decreases most rapidly, the negative of the gradient. One iteration of this algorithm can be written as in Eq. (4):

$$x_{k+1} = x_k - a_k g_k, \tag{4}$$

where x_k is a vector of current weights and biases, g_k is the current gradient, and a_k is the learning rate. In this work, gradient descent with momentum is applied and the performance function was the mean square error, the average squared error between the network outputs and the actual output. For the basic gradient descent algorithm, the weights and biases are moved in the direction of the negative gradient of the performance function. Gradient descent with momentum often provides faster convergence because momentum allows a network to respond not only to the local gradient but also to recent trends in the error surface. Momentum can also help the network to overcome a shallow local minimum in the error surface and settle down at or near the global minimum. Momentum can be added to back-propagation learning by making weight changes equal to the sum of a fraction of the last weight change and the new change suggested by the back-propagation rule. The magnitude of the effect that the last weight change is allowed to have is mediated by a momentum constant which can be any number between 0 and 1. When the momentum constant is 0, the weight change is based solely on the gradient. When the momentum constant is 1, the new weight change is set to equal the last weight change and the gradient is simply ignored. The performance of the network was also tested by reducing the dimension of the input vectors before the training process (Sun et al., 2003).

ANN was implemented to estimate soil organic matter, phosphorus, and potassium content, and satisfactory results were attained (Daniel et al., 2003). An effective procedure for performing this operation is the principal component analysis (PCA), which can reduce input data (Despagne et al., 1998). This technique has three effects: it orthogonalizes the components of the input vectors (so that they are uncorrelated with each other), it orders the resulting orthogonal components (principal components) so that those with the largest variation come first, and it eliminates those components which contribute the least to the variation in the data set. Application of the PC scores instead of the original variables as the net inputs leads to efficient reduction of the net architecture and usually gives better prediction of the Y-variables. PC scores of soil MIR photoacoustic spectra were used as the input of ANN model, and soil properties as well as soil fertility could be successfully predicted (Fig. 10) (Du et al., 2007, 2009).

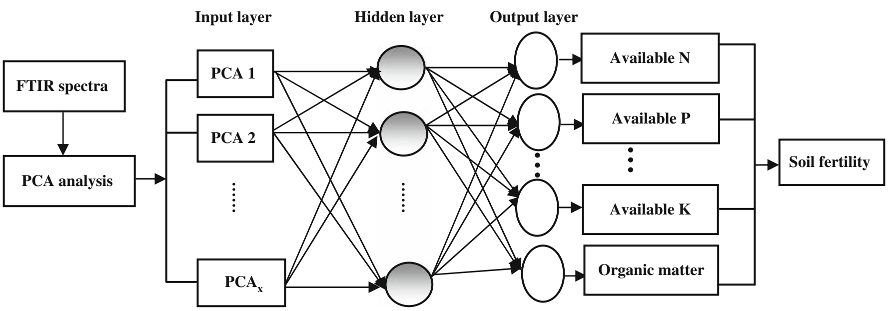

Fig. 10 Schematic diagram of three-layer BP ANN model in the evaluation of soil fertility

3.3.3 Model Verification

Leave-one-out CV was widely used to determine the number of factors to retain in the calibration models. In this instance, 30 bilinear factors were tested. To select the optimal cross-validated calibration model, we computed the RMSE of predictions. Generally, the model with the lowest RMSE is selected.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2}{n}}, \tag{5}$$

where $\hat{y}_i$=values of the predicted variable and y_i=the actual values.

The calibration models were independently validated against the soil data. The procedure employed for the quantification of prediction biases and errors was also that of leave-one-out CV. The validation predictions involved computing each calibration model using n–1 soil samples and predicting the soil property of the sample removed. Explicitly, the procedure entailed removing a soil sample from the prediction set, then computing the calibration model using the n–1 samples and predicting the soil property of the sample removed. This procedure was repeated for all samples and, accordingly, all soil properties. The mean error (ME) was used to quantify bias, the RMSE (their precision may then be easily inferred), and determination coefficients (R^2) (Viscarra Rossel et al., 2006).

A key requirement for empirical modeling is that validation samples be similar to calibration samples; or, put another way, to build a global empirical soil characterization model we would need a calibration library that spanned the range of possibilities for soil composition (Dardenne et al., 2000). It was computed that 5.2×10^9carefully selected calibration samples would be required to span the global soil compositional space, and a far more reasonable calibration size for our tropical soil model, 5.9×10^9 samples. Tropical soils are, on the whole, compositionally much less diverse than less weathered temperate soils and should therefore be more amenable to empirical modeling approaches (Brown et al., 2006). In regions with uniform parent material (e.g., loess deposits), it was expected to construct reliable calibrations with a limited number of samples. Further research is needed to test whether local calibration procedures (Berzaghi et al., 2000) could help to reduce the size of calibration sets for regional or watershed applications. However, parent materials like glacial till with a range of primary and secondary minerals might well require large calibration data sets even in geographically restricted areas.

4 Research Hightlights in Future

4.1 Construction of Soil Infrared Spectra Library

The stable crop production as well as protection and enhancement of the global environment require the development of innovative new methodologies to assess the spatial and temporal variability of soil fertility and soil properties. In particular, spectroscopic techniques like infrared spectroscopy offer the potential to quickly and inexpensively characterize soils relative to standard laboratory techniques. Given the compositional diversity of soils, enough independent samples would be required to construct a complete global, empirical soil library for calibration purposes. However, since we are able to construct useful predictive models, fundamental and partially independent soil-spectral relationships may reduce the required number of calibration samples to a manageable number. At present, with a relatively limited soil spectral library, expanding the soil-spectral library is necessary by scanning previously characterized state and national soil archives (Brown et al., 2006), and it is important to consider the number of samples that are needed to adequately describe the soil variability in the region in which the library is to be used (Viscarra Rossel et al., 2008); this work, necessarily involving multiple labs, will require that we address calibration transfer between spectrometers of the same and different types.

It is proposed that global libraries should be used in conjunction with local calibration samples and with easy-to-measure auxiliary predictors like clay content, organic matter, and pH. Developments in theoretical soil spectroscopy and spectral processing techniques should also improve predictions while reducing calibration requirements. We anticipate that the future development of soil infrared spectroscopy and the expansion of soil spectral libraries will support the assessment of soil fertility at a scale and resolution not previously possible.

4.2 Description of Soil Fertility Using Extracted Information from Soil Photoacoustic Spectra

MIR photoacoustic spectroscopy is a very promising technique for the analysis of soil with higher absorbance, and the characteristics of main soil components can be shown in the FTIR-PAS spectra (Du et al., 2007, 2008a, b). The merits of this technique make it better to construct soil spectral library compared with reflectance spectroscopy. Through calibration with soil fertility related properties and crop production, a soil fertility parameter extracted from soil spectra using mathematical tools can be reached, which will be helpful in the practice of fertilization. However, the application of infrared photoacoustic spectroscopy in soil analysis is very limited, and more soil types and soil samples involved calibrations and verifications should be made to bridge the soil fertility and soil spectra, which needs global collaboration.

5 Conclusion

Soil fertility is a comprehensive soil property, which decides the crop production, and is a very important concept in sustainable agriculture. Soil fertility is usually depicted by some soil properties, such as soil nutrients level, soil organic matter, soil water; therefore, the related soil properties should be first determined, and then a model based on the soil properties is made to give an evaluation of soil fertility. The conventional technique of soil fertility evaluation is based on laboratory analysis, and is costly and time-consuming, which is not suitable for precision agriculture with mass determination. Infrared spectroscopy provides an alternative technique for the evaluation of soil fertility.

Infrared transmission and reflectance spectroscopy are both useful in soil analysis. Infrared transmission spectroscopy is usually used in soil qualitative analysis, such as identification of soil organic matter, and reflectance spectroscopy can be used in soil quantitative analysis. Reflectance spectroscopy, including diffuse spectroscopy and attenuated total spectroscopy, was widely used in soil analysis, but sometimes the prediction error is less satisfied, and sample pretreatment is still required. Infrared photoacoustic spectroscopy is a new technique used in soil analysis, which does not need sample pretreatment, and more useful information can be found in the photoacoustic spectra. The merits of FTIR-PAS technique make it promising in the evaluation of soil fertility in future.

Spectral analysis is very important when infrared spectroscopy is used in the evaluation of soil fertility. Because of the interference of multi-components in the soil, multi-calibration should be involved for extracting the needed information of soil fertility in the soil spectra. PLS and ANN are two important statistical methods to achieve this objective. Enough soil sample number and soil variance are also needed in a good multivariate calibration of soil fertility. Library of soil infrared spectra including at least thousands of soil samples should be constructed, which will be a useful information system in the evaluation of soil fertility. This spectral information system will provide fast and in situ evaluation of soil fertility, which will benefit the sustainable agriculture.

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