CHAPTER 8

THE SOIL AS A RESERVOIR FOR ANTAGONISTS TO PLANT DISEASES

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1. Introduction

The soil is often considered the milieu providing support for plant roots, water and nutrients for plant growth. But it is also considered a hostile environment harbouring plant pathogenic nematodes, bacteria and fungi. The most common attitude is to try to eliminate the plant pathogenic organisms by biocidal treatments such as methyl bromide fumigation, which are dangerous for man and the environment. Beside this pathogen eradication strategy, another approach to control soil-borne plant diseases consists in studying the plant-pathogen interactions at the cellular and molecular level to create new resistant cultivars or to develop new plant protection products based on elicitation of plant defence reactions. This field of research only focuses on plant pathogen interactions, not taking into account the environment in which they take place.

Although a plant disease results from the intimate interaction between a plant and a pathogen, the importance of these direct interactions should not hide the role of environmental factors which influence disease severity. These indirect interactions are particularly important in the case of diseases induced by soil borne pathogens. Indeed, the pathogens are not freely interacting with the plant; they are included in the soil matrix and thus can not escape to the soil environment. Both their inoculum density and infectious capacities are controlled by the soil. Evidence of these interactions is given by the existence of soils that suppress diseases. In suppressive soils disease incidence or severity remains low in spite of the presence of the pathogen, a susceptible host plant and climatic conditions favourable for disease development. These suppressive soils provide examples of biotic and abiotic factors affecting the pathogen, the plant or the interaction between plant and pathogen. In other words, suppressive soils provide examples where biological control, similar to conservation biological control, is active in nature.

Therefore many studies have been devoted to the understanding of soil suppressiveness in order to use suppressive mechanisms in biological control strategies. Since in many cases, antagonistic micro-organisms play a role in soil suppressiveness, the soil has been seen as a reservoir of potential biological control agents. For the two or three last decades the main approach was to identify effective antagonists in soil and try developing them as biological control agents. Most of the biological control agents on the market, even when aerial diseases are targeted, have been isolated from soil. But in order to control soil-borne diseases, one must admit that this strategy has not been as successful as expected. Indeed, even if the soil harbours effective antagonists, soil suppressiveness is due to an association of mechanisms and micro-

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organisms, and a single antagonist is never as efficient as the soil itself. Thus another more ecological approach consists in enhancing natural suppressiveness that exists in every soil. Some cultural practices might modify the microbial balance in a way that soil inoculum potential will be decreased, and/or the soil suppressiveness increased.

In this chapter, we will present the concepts of soil inoculum potential and soil receptivity to diseases, review the mechanisms by which soil suppresses some diseases, give examples of antagonistic micro-organisms selected from the soil microflora and developed as biological control agents, then indicate some alternative approaches such as the use of soil amendments, biodisinfestation and other cultural practices having a beneficial effect on soil quality and soil health

2. Soil receptivity to diseases and soil inoculum potential

Soil suppressive to diseases induced by the most important soil-borne pathogens have been described; they include fungal and bacterial pathogens but also nematodes (Schneider, 1982; Cook and Baker, 1983; Schippers, 1992, Westphal and Becker 2001). These soils control root rot and wilt diseases induced by: *Aphanomyces euteiches*, *Cylindrocladium* sp., several formae speciales of *Fusarium oxysporum, Gaeumannomyces graminis, Pythium spp., Phytophthora spp., Rhizoctonia solani, Ralstonia solanacearum, Streptomyces scabies, Verticillium dahliae, Thielaviopsis basicola (Chalara elegans)*. This large diversity of pathogens controlled by suppressive soils shows that soil suppressivenes is not a rare phenomenon. On the contrary every soil has some potential of disease suppression, leading to the concept of soil receptivity to diseases.

The receptivity of a soil to microbial populations is its capacity to control more or less the activity of the populations present in this soil; in case of plant pathogens, it is the capacity to control the pathogenic activity.

The soil is not a neutral milieu where pathogenic micro-organisms interact freely with the roots of the host plant; on the contrary the soil interferes in several ways with the relationships between and among micro-organisms, pathogens and plants, and it can modify the interactions among micro-organisms themselves. Soil receptivity (or soil suppressiveness) is a continuum going from highly conducive soils in which disease incidence is very high to strongly suppressive soils (Alabouvette *et al*., 1982; Linderman *et al*., 1983).

This concept of soil receptivity was already evoked in the definition of "inoculum potential" proposed by Garett (1956, 1970) as "the energy of growth of a parasite available for infection of a host at the surface of the host organ to be infected". One of the most important words in this definition is "energy" of growth. It clearly states that the presence of the inoculum although necessary is not sufficient to explain the disease. Among the factors that affect the "energy of growth" of the inoculum, Garett (1970) pointed to "the collective effect of environmental conditions", and indicated that "the endogenous nutrients of the inoculum might be augmented by exogenous nutrients from the environment".

Applied to soil-borne pathogens, this concept of inoculum potential led to that of "soil inoculum potential" which was at the origin of both theoretical and practical studies. Baker (1968) gave a definition of inoculum potential as the product of inoculum density by capacity. Louvet (1973) proposed to define inoculum capacity as the product of inate inoculum energy by the effects of the environment on this inoculum. Thus in this definition, the effects of the

environment on the inoculum corresponds to what we have defined above as the soil receptivity to diseases.

Later, the soil inoculum potential was defined by Bouhot (1979) as the pathogenic energy present in a soil. This inoculum potential depends on three main factors: the inoculum density, the pathogenic capacity of this inoculum and the soil factors which influence both the inoculum density and capacity. These factors again correspond to the soil receptivity as defined above.

Thus, whatever the definition all these authors acknowledge that the soil plays a major role in influencing the interactions between a susceptible host plant and its specific pathogens present in soil. It is therefore very important to take into consideration both the inoculum potential of a naturally infested soil and its level of suppressiveness, when elaborating control strategies.

3. Mechanisms of disease suppression

In nature, suppressive soils can be detected by the observation that disease severity in a crop remains low despite the presence of a susceptible host plant, climatic conditions favourable to disease expression and ample opportunity for the pathogen to be present. It is quite easy to experimentally demonstrate that a soil is suppressive to a given disease. The pathogen has to be produced in the laboratory and introduced into the soil at increasing inoculum densities. A susceptible host plant is sown and cultivated under standardized conditions favourable to disease expression. Observations of symptom appearance enable disease progress curves to be drawn with respect to time and inoculum concentrations. Area under the disease progress curve (AUDPC) is the most common method to evaluate disease incidence or disease severity. Appropriate statistical methods (Baker *et al*., 1967; Höper *et al*., 1995, Jeger, 2004) enable these curves to be compared with those obtained from another soil known to be conducive to the disease. All experimental conditions being similar, differences in disease incidence must be attributed to differences in the soil environment, i.e. differences in the level of soil receptivity.

3.1. Nature of soil suppressiveness

Disease suppression does not necessarily imply suppression of the pathogen. In most cases the inoculum is still present but does not provoke the disease. Therefore, Cook and Baker (1983) distinguished: (i) pathogen-suppressive soils, where the pathogen does not survive, from (ii) disease-suppressive soils where inoculum is present but does not induce the disease. Only studies of the mechanisms of suppression enable the distinction between the two types of suppressiveness to be made.

From a theoretical point of view, both the abiotic characteristics of a soil and its biological properties can be responsible for disease suppression. However in most cases, suppressiveness is fundamentally microbial in nature. Disease suppression results from more or less complex interactions between the pathogen, and all or a part of the soil microbiota. Indeed, the suppressive effect disappears upon destruction of organisms by biocidal treatments such as steam or methyl bromide, and can be restored by mixing a small quantity of suppressive soil into the previously disinfested soil (Alabouvette, 1986). Suppressiveness can also be restored in the steamed disinfested soil by re-introduction of a mixture of micro-organisms previously isolated from the suppressive soil (Alabouvette, 1986).

This demonstration of the essential role of the saprophytic microflora does not establish that soil physical and chemical properties do not play any role in the mechanisms of suppressiveness. On the contrary, early studies on Fusarium suppressive soils established correlation between soil type, presence of smectite clays and soil suppressiveness to Fusarium wilt in Central America (Stover, 1962; Stotzky and Martin, 1963). In the case of Swiss soils suppressive to black root rot of tobacco, Stutz et al. (1989) showed that only soils derived from moraine and containing vermiculitic clay minerals were suppressive to *Thielaviopsis basicola*. Abiotic soil characteristics also play a major role in soil suppressiveness to *Aphanomyces euteiches* (Oyarzun *et al*., 1998, Persson *et al*., 1999) and *Rhizoctonia solani* (Steinberg *et al*., 2004).

3.2. Mechanisms of soil suppressiveness

There exist several types of soil suppressiveness and Cook and Baker (1983) proposed three criteria to characterize disease suppressiveness in soils: "the pathogen does not establish; it establishes but fails to produce disease; or it establishes and causes disease at first but then disease severity diminishes with continued growing of the same crop".
The well-known and widespread phenomenon of take-all decline is the best example of soils becoming suppressive with continuous cropping of the susceptible host plant. The disease increases in severity during the first years of wheat cropping, then decreases to an economically acceptable threshold (Hornby, 1998).

Fusarium wilt suppressive soils provide a good example of soils where the pathogen is present in the soil but fails to produce the disease (Scher and Baker, 1982; Alabouvette, 1986). It was established that the dynamics of a marked inoculum of *F. oxysporum* f.sp. *melonis* were similar in a conducive soil and in a suppressive soil from Châteaurenard; thus the difference in disease incidence had to be attributed to a reduced activity of the pathogen in the suppressive soil. Indeed, the percentage of germinating chlamydospores is always extremely low in the suppressive soil. This limited germination of chlamydospores was attributed to the general phenomenon of soil fungistasis (Lockwood, 1977), which is related to competition for nutrients. Addition of increasing concentrations of available carbon, in the form of glucose, resulted in increasing percentages of germinating chlamydospores in both conducive and suppressive soils. (Sneh *et al.,* 1984; Alabouvette, 1986). These results suggest that competition for nutrients, and fungistasis, are much more intense in suppressive than in conducive soils and contribute to reducing the activity of the fungal pathogens. Indeed, glucose amendments that induced chlamydospore germination of the pathogen also induce disease in the suppressive soils. Competition for nutrients, especially competition for energy among heterotrophic micro-organisms, is due to the communities of soil micro-organisms active at any given time and therefore should be linked to the activity of the microbial biomass of the soil.

The microbial biomass, measured by Jenkinson's method (Jenkinson and Powlson, 1976) is always greater in the Châteaurenard suppressive soil than in a conducive control soil. Studies on the kinetics of soil microbial respiration after glucose amendment (Alabouvette 1986; Amir and Alabouvette, 1993) showed further that the soil microflora of the suppressive soil is more responsive to carbon than that of the conducive soil. Consequently, carbon is utilized more quickly and the development of any given organism is stopped more rapidly after glucose amendment in the suppressive than in the conducive soil.

This type of phenomenon corresponds to the "general suppression" described by Cook and Baker (1983) as the inhibition of the pathogen in soil in relation to the total amount of the microbial activity acting as a nutrient sink. A high microbial biomass combined with a very intense competition is responsible for a permanent state of starvation leading to fungistasis inhibiting the growth of the pathogen. This general suppression was already proposed by Gerlach (1968) as an explanation for take-all decline of wheat in polders.

Competition for nutrients other than carbon, especially nitrogen and iron, has been involved in the limitation of germination of fungal propagules in the soil (Cook and Snyder, 1965; Benson and Baker, 1970; Scher and Baker, 1982). Consequently, the population of pathogens faces general competition resulting from the activity of the microbial biomass but also competition exerted by a specific population. For instance, the siderophore-iron competition achieved by fluorescent pseudomonads is responsible for the reduced growth of *Fusarium* spp. in vitro and in suppressive soils (Sneh et al., 1984; Elad and Baker, 1985). Addition of ethylenediaminedi-*o*-hydroxyphenyl-acetic acid (EDDHA), which limits the concentration of iron available for *Fusarium*, results in a lower percentage of diseased plants in a conducive soil. In contrast, addition of Fe-ethylenediaminetetraacetic acid (FeEDTA), which provides iron available for *Fusarium*, results in a higher percentage of diseased plants in the suppressive soils (Lemanceau, 1989). General competition occurs simultaneously for both carbon and iron, in the suppressive soil from Châteaurenard.

Competition for nutrients is not the only mechanism by which antagonistic populations interact with pathogens in soil. Today, antibiosis has been shown to be involved in the inhibition of the pathogen activity in suppressive soils. Indeed, Raaijmaker and Weller (1998) were able to correlate the suppressiveness of soils to take-all with the density of the population of *Pseudomonas fluorescens* producing 2-4 diacetyl phloroglucinol. But it must be underlined that this "specific suppression" always operates on a background of general suppression as stated by Cook and Baker (1983). The high intensity of general competition enhances or increases the significance of specific interactions, either competition or antibiosis, between pathogens and antagonists sharing the same ecological niches in the soil and the rhizosphere. The choice of focusing on specific populations of antagonists is justified by the objective of developing biological control agents.

4. Inoculation and inundation biological control

As stated above, suppressive soils were seen as a source of potential biological control agents. Rather than selecting antagonists at random, selecting them among the micro-organisms isolated from suppressive soils might increase the probability of success.

4.1. Screening of biological control agents

The first step in developing a biological control method is the screening of an effective strain of biological control agent. Two different approaches can be followed.

The first approach, the traditional one, is based on a random screening among many strains owing to a standardised method where the antagonist is confronted with the pathogen, in the soil environment and in the presence of the host plant. Several levels of bioassays are conducted, enabling to progressively decrease the number of strains tested. At the beginning, with the largest number of strains, the bioassay is conducted under artificial conditions,

sometimes in vitro, most often in a sterile substratum such as sand or peat to grow the plant. At the end of the process a very limited number of strains are evaluated for their biological control capacity under conditions similar to that of their application in the targeted crop (Hökeberg *et al*., 1997). This approach does not need any pre-existing knowledge of the modes of action of the antagonists that are most often chosen at random. This approach is space and time consuming, but enables to detect biological control agents fitting with the environment where they will be applied.

On the contrary, the second approach is based on the pre-existing knowledge that a given function, for example antibiotic production, plays a major role in the antagonism expressed by a microbial species against the pathogen. Then, the strategy consists in screening for this function owing to *in vitro* assays. In fact, when the genes coding for this function are known, it is possible to base the screening procedure on the tagging of these genes among a large population of micro-organisms. For example, in the case of the fluorescent *Pseudomonas* spp., most of the genes coding for antibiotic production, such as phenazine or 2-4 diacetylphloroglucinol, are characterised. Therefore it is possible to screen among a large collection of bacteria for the presence of these genes. But, then it is necessary to study the expression of these genes, since the presence of the genomic sequence does not necessary implies the production of the given metabolite in the environment where the biological control agent will be used.

Scientists in favour of the first approach argue that to be effective a biological control agent must not only possess the required modes of action but be also well adapted to the environment where they have to express theses functions (rhizosphere, spermosphere). And until now, only a few teams have been involved in the study of the genes coding for the "ecological fitness" of the biological control agents. Therefore there is a risk of selecting potentially very active antagonists that will not be able to survive or to express their beneficial properties in the soil environment. Scientist in favour of the second approach argue that knowing the most important functions will enable the manipulation of the biological control agents in order to add several modes of action in a single strain or to deregulate the production of an important metabolite in order to have it produced in greater quantity or at the right time.

4.2. Modes of action of biological control agents

Antagonistic effects responsible for disease suppression results either from microbial interactions directed against the pathogen, mainly during its saprophytic phase, or from an indirect action through induced resistance of the host-plant.

Microbial antagonism implies direct interactions between two micro-organisms sharing the same ecological niche. Three main types of direct interactions may be characterised: parasitism, competition for nutrients and antibiosis.

Parasitism of a plant pathogen by other micro-organisms including viruses is a well-distributed phenomenon. The parasitic activity of strains of *Trichoderma* spp. towards pathogens such as *Rhizoctonia solani* has been extensively studied (Chet and Baker, 1981) and other mycoparasites such as *Coniothyrium minitans* and *Sporidesmium sclerotivorum* are efficient in controlling diseases caused by *Sclerotinia* spp. and other sclerotia forming fungi (Adams and Fravel, 1993; Whipps and Lewis, 1980).

Competition for nutrients is a general phenomenon regulating population dynamics of micro-organisms sharing the same ecological niche and having the same physiological

requirements. Competition for carbon in soil is considered as responsible for the well-know phenomenon of fungistasis (Lockwood, 1977) describing the inhibition of fungal spore germination in soil. Energy deprivation in soil is also partly responsible for "general suppression of a pathogen which is directly related to the total amount of microbial activity at a time critical to the pathogen " (Cook and Baker, 1983). This general suppression has been demonstrated to play a role in the determinism of the suppressiveness of soils to fusarium wilts, where it controls competition for carbon between pathogenic and non-pathogenic *Fusarium oxysporum* (Alabouvette *et al*., 1986). Some strains of nonpathogenic *F. oxyxporum* are more competitive than other and should be selected for biological control (Couteaudier and Alabouvette, 1990). Competition for minor elements also frequently occurs in soil, and for example competition for iron is one of the modes of action by which fluorescent pseudomonads limit the growth of pathogenic fungi and reduce disease incidence or severity (Schippers *et al*., 1987; Bakker *et al*., 1991; Lemanceau and Alabouvette, 1993).

Antibiosis is the antagonism resulting from the production by one micro-organism of secondary metabolites toxic for other micro-organisms. Antibiosis is a very common phenomenon responsible for the biological control activity of many biological control agents such as *Bacillus* spp., *Streptomyces* spp., *Trichoderma* spp or fluorescent *Pseudomonas* spp. A large diversity of antibiotics, bacteriocines, enzymes and volatile compounds have been described and their role in suppression of several plant pathogens has been documented (Loper and Lindow, 1993; Thomashow and Weller, 1996). A given strain of a biological control agent may produce several types of antifungal compounds effective against certain species of fungal pathogens. For example, the strain CHAO of *Pseudomonas fluorescens* is producing siderophores, phenazines, 2.4-diacetylphloroglucinol and cyanide, a different combination of these metabolites being responsible of the antagonism expressed against *Gaeumannomyces graminis* var. *tritici* and *Chalara elegans* (Défago and Haas, 1990). It is important to emphasise that a single antifungal metabolite generally does not account for all the antagonistic activity of a biological control agent.

Induced systemic resistance classically occurs when an inducing agent pathogenic or not is applied prior to challenge inoculation with a pathogen, resulting in reduced disease in comparison to the non-induced control. More and more studies are devoted to induced resistance of the host plant after application of biological control agents. Kuc (1987) reported the first evidence of systemic protection of cucumber against *Colletotricum orbiculare* after pre-inoculation of the cotyledons of the plant with the same pathogen. It is also well established that the pre-inoculation of a host-plant with an incompatible *forma specialis* of *F. oxysporum* results in reduced disease severity when the plant is inoculated with the compatible pathogen (Biles and Martyn, 1989). The fluorescent pseudomonads selected for their plant growth promoting capacity or for their biological control activity have been shown to induce systemic resistance in the plant (Kloepper *et al*., 1996; Van Loon *et al*., 1998). Since induced systemic resistance is a general phenomenon that can protect the plant against several pathogens and can be induced by many biological control agents it retains more attention today than any other modes of action of biological control agents. However, it must be said that induced systemic resistance is not exclusive from other modes of actions and might, most often, only exert a complementary effect to microbial antagonism.

More generally, consistency of biological control needs the association of several modes of action, acting simultaneously or successively. As stated above, it is proposed to associate several modes of action in a single antagonistic strain, by genetic manipulation and the first

improved strains of *Pseudomonas fluorescens* producing phenazine and phloroglucinol have been evaluated for their improved biological control activity (Thomashow and Weller, 1996). Another approach consists in associating several strains of biological control agents in the same product. It has been well established that association of certain strains of *Pseudomonas fluorescens* with nonpathogenic *Fusarium oxysporum* always improves the control of fusarium diseases. Obviously these associations have to be based on the knowledge of the compatibility of the strains and of the modes of action in order to create a synergetic effect (Alabouvette *et al*., 2001, Olivain *et al*., 2003).

4.3. Production, formulation and application of biological control agents

Production and formulation of the biological control agents, the two last steps before application probably constitute one bottleneck for the development of biological control strategies. Indeed, too often these steps are not carefully considered by the academic research laboratories, which consider that these technological problems have to be solved by the industry. However, producing and formulating an efficient biomass at a low cost need a scientific approach based on the knowledge of the physiology of the micro-organisms. The aim of the fermentation is to produce and harvest a viable biomass that will have to express its beneficial properties after some time of storage and application to the crop. Moreover, this biomass must be pure, without contaminants. To achieve this goal, it is absolutely needed to study the physiology of the micro-organism to determine the fermentation parameters that will enable to obtain an effective biomass at an affordable cost. Several review papers have addressed these questions of how to produce and formulate an active biomass (Lewis *et al*., 1991; Lumsden *et al*., 1995). In most examples the micro-organisms are grown in liquid fermentation and the propagules after harvest are either mixed with a solid substratum, such as clay talc or peat, or embedded in alginate pellets (Fravel *et al*., 1985; Lumsden and Lewis, 1989). The final product must be easy to handle, to store and then to apply.

An alternative to liquid fermentation is the solid state fermentation, where the biological control agent is directly produced on solid material that provides nutrients and a substratum that can help to solve the formulation problem. The inoculum being stored in the substratum on which it has grown usually presents a better survival (Olivain *et al*., 2003). Moreover solid state fermentation enables to utilise different types of agricultural waste products that are cheap and can be found on the local market especially in developing countries.

The final step in developing a biological control method is to choose a method of application that will enable to deliver the biological control agent, at the right time and at the right place, where it has to be active. Depending on the target pathogen, the antagonist will be delivered with the seed or in the potting mixture to let it colonise the young roots of the plant. Obviously seed coating is the best approach to introduce a biological control agent in the rhizosphere of open field crops and this is the technique used to apply *Pseudomonas chlororaphis* to wheat seeds in Northern Europe (Hökeberg *et al*., 1997).

In any case, it is always necessary to study the compatibility of the biological product with the chemical pesticides used in the same crop. Indeed, the biological control agent is most often targeting a single type of pathogens; therefore it has to be integrated into the pest management programme. Much more research is needed to determine the exact use of biological strategies in disease and pest management.

4.4. Registration of biological control agents

As other plant protection products, biological control agents are subjected to the European directive 91-414 CCE, which lists all the plant protection products allowed to be on the market. It means that a full dossier giving all information related to the characterization of the micro-organisms, its biology, its toxicity for man and the environment will be reviewed at the European level to decide whether or not release of this micro-organism will pose an unacceptable risk for the applicator, the consumer or the environment. Obviously, the risks resulting from the application of a living organism are not the same as the risks posed by chemical molecules. Thus the directive 91-414 has been adapted to the specific case of micro-organisms in the directive 2001-36. It is not useful here to describe in details all the requirements necessary to characterize the dangers and evaluate the risks, but it must be stressed that this procedure is both expensive and time consuming. It represents a bottleneck for development of biological plant protection products. Experts are just afraid that the biological control agents could proliferate in the environment and threaten the ecological balance. But as presented above, all these micro-organisms have been isolated from the natural environment, mostly from soil, where they will be applied and where they will be submitted to various types of constraints (competition, antibiosis, UV radiation etc) limiting their growth and preventing their proliferation. Indeed, there is no example today of an uncontrolled proliferation of a biological control agent.

To promote biological control, the procedure of registration should be less expensive. Indeed, most of the time, these biological control agents are targeting niche markets which will never pay back the actual cost of registration. Obviously, one can not claim that being natural biological control agents present no danger. Thus, since registration is compulsory, one should find a more realistic approach to identify the dangers and estimate the risks.

4.5. Inoculation biological control versus inundation biological control

When applying a micro-organism isolated from a suppressive soil to a conducive soil, the expectation is to succeed in the establishment of the biological control agent in the soil and consequently transform the conducive soil into a suppressive soil. This corresponds to inoculation biological control, which, according to the definition given by Eilenberg et al., (2001), means that the biological control agent will multiply and control the pest for an extended period of time. Unfortunately, the introduction of a given antagonist in a soil is not sufficient to make the conducive soil suppressive even if it can control the disease efficiently for one season. Indeed, as underlined in paragraph 3 the mechanisms of soil suppressiveness are always complex and involved several populations of micro-organisms. Therefore, introduction of a single biological control agent to soil refers to inundation rather than to inoculation biological control.

5. Conservation biological control

Another ecological approach towards biological control of soil-borne plant pathogens consists in the management of the biotic and abiotic properties of a soil to reach a quality promoting beneficial microbial and physico-chemical interactions and thus limiting the pathogenic activity below a tolerable level of expression. Adaptation of cultural practices has been proposed in order to decrease the soil inoculum potential or increase the level of soil suppressiveness to diseases. Indeed, disease suppressive soils were developed through crop rotation (Cook *et al*., 2002), intercropping (Schneider *et al*., 2003), residue destruction (Baird *et al*., 2003), organic amendments (Tilston *et al*., 2002), tillage management practices (Sturtz *et al.,* 1997, Pankhurst *et al*., 2002) and combination of those regimes (Hagn *et al*., 2003; Peters *et al*., 2003; Garbeva *et al*., 2004). In the second part of this chapter we will review some of the practices which are developed or already in use to control diseases in a sustainable way.

5.1. Pathogen eradication versus microbial management

Forty years ago, in intensive vegetable cultivation in greenhouses, the use of heat-treatment by soil steaming was a common practice. Most of the pathogens are highly susceptible to heat, the lethal temperatures for pathogenic fungi being reached between 55 and 65°C for 15 to 30 minutes (Bollen, 1969). With the oil crisis, the cost of soil steaming became too expensive and the growers moved to application of chemical biocides which are dangerous for man and the environment. These molecules being biocide they kill not only the pathogens but also most of the beneficial soil micro-organisms, leading to an unbalanced equilibrium in soil. The use of the most dangerous product, methyl bromide will be banned at the end of year 2004. But most of the chemical products still in use produce ephemeral results including un-controlled side effects on both existing and forthcoming microbial communities leading to the infernal circle of applying repeatedly the same treatments to the soil. Fortunately less drastic techniques of pathogen eradication have been proposed, they have in common that they do not kill every micro-organisms but modify the microbial balance in a positive direction for pest control and plant growth.

5.1.1. Solarisation

Solarisation or solar heating is a method that uses the solar energy to enhance the soil temperature and reach levels at which many plant pathogens will be killed or sufficiently weakened to obtain significant control of the diseases. Solarisation does not destroy all the soil micro-organisms, but modify the microbial balance in favour of the beneficial micro-organisms. Indeed, many papers report situations where the efficacy of soil solarisation is not only due to a decrease of the pathogenic populations but also to an increase of the density and activity of populations of micro-organisms such as *Bacillus* spp., *Pseudomonas* sp. and *Thalaromyces flavus* antagonistic to the pathogens. Several review papers are available that describe both the technology of solar heating and the mechanisms involved in the control of pests, pathogens and weeds by soil-solarisation (DeVay *et al.*, 1991; Katan and DeVay, 1991; DeVay, 1995; Katan, 1996).

Solarisation is a hydrothermal process; its effectiveness is not only related to the temperature but also to the soil moisture. Indeed, temperature maxima are obtained when the

soil water content is about 70% of the field capacity in the upper layers and the soil should be moist to a depth of 60 cm. Various kinds of plastic films have been used. Polyethylene film, as thin as possible (25 to 50 μm) is recommended because it is transparent to most solar radiations and less transparent than some other plastic to terrestrial radiation. The duration of solarisation is an important factor determining the effectiveness of the treatment. The longer the mulching period, the greater the depth of effective activity, the higher the pathogen killing rates. Usually, in Mediterranean areas, four weeks are required to achieve control of the diseases. As stated above, disease control results both from the reduction of inoculum density and from increased activity of some antagonistic micro-organisms. Depending on the target pathogen one or the other mechanism is predominant.

An important characteristic of soil solarisation is its very large spectrum of activity. This method controls fungi, nematodes, bacteria, weeds, arthropod pests and some unidentified agents. Indeed, solarisation often results in increased yield when applied to monoculture soils, where specific pathogens have not been identified. In this case, solarisation probably controls the weak pathogens or deleterious micro-organisms responsible for "soil sickness". All the pathogens do not present the same susceptibility to solar heating, if most of the fungi are well controlled some failures have been reported. Another interesting property of solarisation is its long-term effect. Disease control and yield increase have been reported two and sometimes three years after solarisation. This long time effect is probably due to both the reduction of the inoculum density and some induced level of suppressiveness of the soil (Katan *et al*., 1983). Obviously, solarisation is effective in warm and sunny areas in the world and particularly under the Mediterranean climate. However some interesting data have been reported from cooler regions of the world where solarisation may be applied under plastic frames or in greenhouses.

5.1.2. Biofumigation or biodisinfection

Better adapted to cooler regions of the world, biological soil disinfection is based on plastic mulching of the soil after incorporation of fresh organic matter (Blok *et al*., 2000). The mechanisms involved by this newly developed technique are not totally understood.

Two main mechanisms contribute to the efficacy of the biodisinfection: the fermentation of organic matters in soil under plastic results in the production of toxic metabolites and in anaerobic conditions which both contribute to the inactivation or destruction of the pathogenic fungi.

Many species of *Brassicaceae* (*Cruciferae*) produce glucosinolates, a class of organic molecules, which may represent a viable source of allelochemic control for various soil-borne plant pathogens (Kirkegaard and Sarwar, 1998). Toxicity is not attributed to glucosinolates but to products such as isothiocyanates, organic cyanides or ionic thiocyanates resulting from their enzymatic degradations achieved by a group of similar-acting enzymes called myrosinase. Myrosinase and glucosinolates are separated from each other in intact plant tissues. When the *Brassicaceae* (cabbage, mustard, horseradish), grown as intermediate crop are buried into the soil as green manure, the disruption of cellular tissues allows mixing of glucosinolates and myrosinase resulting in the rapid release of glucosinolates degradation products. The hydrolysis products have a broad biocidal activity towards nematodes, insects and fungi as well as putative phytotoxic effects. They act either as selective fungicides or as fungistatic compounds limiting therefore the development and activity of fungal populations, some of them being putative pathogenic agents for the forthcoming crop (Sarwar *et al*., 1998). For that purpose new

cultivars of *Brassicaceae* have been selected for their high content in glucosilonates, they are now available on the market

Other plant families are able to release other types of toxic compounds. This is the case of *Alliacae*. Degradation of garlic, onion, and leek tissues is releasing sulfur volatiles such as thiosulfinates and zwiebelanes which are converted into disulfides having biocidal activities against fungi, nematodes and arthropods (Arnault *et al*., 2004). Based on the type of mechanisms involved, two definitions have been proposed by Lamers *et al.* (2004). Biofumigation corresponds to the use of specific plant species containing identified toxic molecules, biodisinfection refers to the use of high quantities of organic matter which after soil tarping results in anaerobic conditions mainly responsible for the destruction of the pathogens.

5.2. Crop rotation versus mono-cropping

In general, continuous cropping with a susceptible host causes the build up of populations of specific plant pathogens resulting in increasing soil-borne disease occurrence or severity. On the contrary, rotation including non-host plants or plant less susceptible to the pathogenic agents will limit the build-up of the population of the pathogens and in some cases will even lead to a decrease of the inoculum density. Indeed, some non host-plants are able to trigger the germination of the conservation structures (sclerotia, chlamydospores, oospores). But in the absence of a susceptible host, some pathogens are not able to survive saprophytically in soil. Therefore the cropping of such a non-host plant will result in a decrease of the inoculum potential of the soil. Moreover, crops in a rotation scheme may also stimulate some microbial populations resulting in the development of a suppressive effect towards the pathogens. For example Mazzola (1999) showed that growing wheat in orchard soil prior to planting apple reduced infection by elements of a fungal complex including: *Cylindrocarpon destructans*, *Phytophthora* spp., *Pythium* spp. and *Rhizoctonia solani*. This beneficial effect was correlated with the increased population of specific antagonistic populations of fluorescent pseudomonads making the soil suppressive towards *Rhizoctonia solani*.

On the contrary the case of take-all decline of wheat illustrates the benefit obtained through long term monocropping. Indeed, monoculture will first favour disease which in return will favour the antagonists of the pathogens. Therefore, the take-all disease of wheat caused by *Gaeumannomyces graminis* var *tritici* can be naturally controlled by monocropping of the cereal providing that monoculture lasted for more than 4 years (Dulout *et al*., 1997). This feature was related to the development of populations of fluorescent pseudomonads within the root and straw fragments remaining post harvest which make the soil suppressive to the disease. These bacterial populations produce antibiotic compounds (phenazine, 2,4-diacetylphloroglucinol) which are deleterious to the pathogen (Thomashow and Weller 1988; Raaijmaker and Weller 1998). However, several consecutive crops of wheat enduring take-all are necessary to ensure an effective threshold of disease suppression and the best yields following take-all decline are rarely equal to those achieved with crop rotation. Therefore, although wheat monoculture does induce take all decline, short crop rotation based systems are preferred (Cook, 2003).

5.3. Residues management

As in the case of take-all decline of wheat, plant residues left on or near the soil surface may contribute to an increase of soil suppressiveness to disease through the promotion of the general microbial activity which is involved in the mechanisms of disease suppression. The incidence and severity of Fusarium wilt of cotton increased when levels of plant residue in the soil were increased by the incorporation of whole cotton plants into the soil (Wang *et al*., 1999). Indeed, the debris not only promote the microbial activity but also help to preserve the pathogens, preventing a decrease of the inoculum density. This is the case for *Macrophomina phaseolina* causing charcoal rot in soybean (Baird *et al*., 2003), *Fusarium* sp. causing root and crown rot on maize (Cotten and Munkwold 1998), *Rhizoctonia solani* causing crown and root rot on sugar beet (Guillemaut 2003). Some practices used by growers to kill living plants at crop termination (foliar application of herbicide and mechanical destruction of the vines) could be counterproductive with respect to disease management. Indeed, such putatively preventive strategies might enhance the fungal reproduction and increase the soil inoculum as it was shown in the case of the root-infecting fungus *Monosporascus cannonballus* causing vine decline of melons. In such cases, destruction of infected roots prior to pathogen reproduction would be a method of preventing inoculum build-up in soil (Stanghellini *et al*., 2004).

Therefore, attention should be paid to residue management by burial through tillage practices or promotion of rapid decomposition (Toresani *et al*., 1998). When residues are buried, the pathogens are displaced from their niche to deeper layers in the soil thus their ability to survive is severely decreased. Repeated incorporations of crop residues can affect a change in the activity of the residue-borne microorganisms that in turn influence the decomposition of crop residues. Carbon released from this decomposition contributes to a more general increasing soil microbial activity and so increases the likelihood of competition effects in the soil, resulting in enhancement of general suppression. Developing disease suppressive soils by introducing organic amendments and crop residue management takes time, but the benefits accumulate across successive years improving soil health and structure (Bailey and Lazarovits, 2003).

5.4. Soil tillage

It is difficult to assess the role of tillage on disease suppression as its evaluation is often combined with the effects of other agricultural practices such as organic amendments and green manure burial, residue management or crop rotations (Bailey and Lazarovits, 2003, Peters *et al*., 2003). Therefore tillage appears as giving conflicting effects on disease suppression. Conventional tillage results in considerable disturbance of the soil but removes residue from the surface. Tillage also disrupts hyphae altering for instance the ability to survive of *R. solani*, (Roget *et al*., 1996, Bailey and Lazarovits, 2003). On the contrary, reduced tillage can favour pathogens by protecting the pathogen's refuge in the residue from microbial degradation, lowering soil temperature, increasing soil moisture, and leaving soil undisturbed (Bockus and Shroyer 1998). Reduced tillage systems change the availability of nutrients in the soil increasing microbial biomass, microbial activity and subsequent competition effects. Total soil nitrogen, organic matter and denitrification processes are increased but mineralization and nitrification processes are reduced. Soil inoculum potential and disease incidence might be differently altered according to the pathogens considered. Indeed, the impact of tillage

practices depends on specific pathogen-soil-crop-environment interactions, environment being sometimes, the most important factor limiting the severity of disease regardless of tillage or crop rotation practices (Bailey *et al*., 2000).

5.5. Organic amendments

In the sixties and seventies, organic amendments have been proposed to control soilborne diseases (Lumsden et al., 1983). Although their effects were not studied in relation to induction of suppressiveness in soil, many papers reported a beneficial effect of organic amendment on the reduction of disease incidence or severity. In one case, the beneficial effect was clearly linked to induction of suppressiveness in the soil. Indeed, the Ashburner system to control *Phytophthora* root rot of avocado in Australia is based on the incorporation of large amounts of organic matter to reproduce the environment of naturally suppressive soils that exist in the rain forest (Baker, 1978). Since that time, addition of organic amendments to control soil borne pathogens has been extensively studied. Hoitink (1980) has developed a growth medium based on composted bark to grow rhododendron and azaleas. This substrate is suppressive towards root rots induced by several species of *Pythium* and *Phytophthora*. After the peak heating that creates a biological vacuum, the compost can be colonized by a great diversity of micro-organisms some being antagonist of the pathogens. The level of disease control obtained depends on many factors such as the chemical properties of the parent material, the composting process and obviously the type of micro-organisms present. This is probably why such contrasted data have been published regarding the efficacy of disease control obtained by organic amendments of soil. Under the frame of a European project (Compost Management in Horticulture QLRT-2000-01442: http://www.agro.nl/appliedresearch/compost) 18 composts from different origins were evaluated for their capacity to suppress 7 different diseases. It appeared that there is no general rule, some compost controlled some diseases but not others, and the only exception is Fusarium wilt which is controlled by almost all the composts

To enhance the suppressive potential of composts and thus to improve the efficacy of disease control it has been proposed to inoculate these composts after peak heating with specific strains of biological control agents. Although promising, this strategy has not yet been successfully applied. Indeed, as every soil, every compost possesses a certain level of suppressiveness towards introduced micro-organisms. Thus it is not easy to establish some biological control agents in composts even after peak heating.

Despite these difficulties, compost amendment has been successfully used to increase soil suppressiveness to diseases including nematode diseases (Erhart *et al*., 1999; Lumsden *et al*., 1983; Oryazum *et al*., 1998; Serra-Wittling *et al*., 1996; Steinberg *et al*., 2004; Windmer *et al*., 2002) as well as disease suppression in farm truck and horticultural crops (Tilston *et al*., 2002, Cotxarrera *et al*., 2002; Hoïtink and Boehm, 1999). The mechanisms involved in these examples of successful disease suppression are diverse and not clearly understood. In a recent study (Perez *et al*., 2005) the effects of three composts added to two different soils were carefully addressed. Assessing the density and the activity as well as the physiological and genetic structure of the soil microflora revealed that the phytosanitary state of the soil might be governed by the repercussions of the organic amendments at the functional level but no general rule could be stated. The impact of organic matter on the soil biota differed with the nature of the compost and the soil types. The structures of the bacterial and fungal communities were perturbed in different ways according to the soil-compost mixtures. More generally, looking

through all the already published data, there has been no definitive work linking narrowly biological control in soil to applications of organic amendments. This is probably due to the large diversity in the chemical composition of the composts, manures and other organic matters that does not fit in a suppressive way with the large biodiversity and ecological requirements of the pathogens.

Composts can also act as a non-host plant: an interesting example is provided by the incorporation of composted onion wastes into the soil to control Allium white rot due to *Sclerotium cepivorum*. This fungus is an obligatory parasite which can survive as dormant sclerotia in the soil for many years but can only germinate in the presence of the host plants. The stimulus for germination is the exudation of alk(en)yl cysteine sulphoxides by the roots of Allium species. Properly composted, onion wastes contained some sulphoxides (di-n-propyl disulphide) which trigger the dormant sclerotia to germinate in absence of the root while these germinated sclerotia are unable to survive without the living host, what contributes to the decrease in the primary inoculum faced by the next onion crop (Coventry *et al*., 2002).

6. Conclusion

In this chapter, we presented two approaches towards biological control of soil-borne plant pathogens. The first one consists in the selection of an antagonistic micro-organism which will be developed as a plant protection product; the other consists in a modification of the soil management practices to increase the level of soil suppressiveness to diseases. These two approaches are not novel; both have already been proposed during the first congress on "ecology of soil-borne plant pathogens, prelude to biological control" hold in Berkeley in 1965. Most of the ideas presented above were already discussed, and one may wonder why so little progress has been made during these last 40 years.

The first reason is linked to the high complexity of the soil ecosystem. The few examples presented in this chapter show how complex are the interactions between soil abiotic characteristics, soil microbiota and soil suppressiveness to diseases and pathosystems. It is therefore clear that one single population is unlikely to be responsible for the whole functioning of the soil. On the contrary, all the microbial populations including bacteria, fungi, protozoa and microfauna are involved in this functioning but with the constraints of the environment. One must admit that we still are at the descriptive stage, and that we have difficulties addressing the question of soil health following a holistic approach. At least we made progress understanding that disease incidence or disease severity does not rely only on the inoculum density. When reviewing some of the papers published in the seventies and dealing with organic amendments to control soil borne diseases, one must admit that scientists were always trying to explain disease control by a direct effect of the soil environment on the inoculum density. Today we know that the interactions are much more complex and that the effects of a given organic matter depend on the soil environment to which it will be added. The objective is no necessarily to induce a *decrease* of the inoculum density but to *increase* soil suppressiveness to diseases. Many agricultural practices may result in an increased level of soil suppressiveness but in order to advise farmers one need to better understand the effects of management practices on the diverse components of soil health and to determine when and what kind of management is necessary to increase soil suppressiveness to diseases.

The fast development of molecular and physiological tools is enabling the characterization of the structure of the microbiota as a whole. Until today, we were obliged to focus on a very

limited number of microbial populations, either pathogens or antagonists, but were unable to detect changes affecting the soil microbiota without having, a priori, a specific hypothesis. Thus the development of new techniques enabling the evaluation of biodiversity in soil microbiota is totally changing our views of the microbial balance (Mazzola, 2004). If, as expected, these new methods can be run automatically, they will make possible the characterization of many samples and thus enable comparison of the microbial communities in different soils under different cropping systems or in a same soil submitted to different practices. These techniques will be useful for correlating changes affecting the level of soil suppressiveness with shifts affecting microbial communities. We will be able, for example, to detect and characterize shifts in the microbial communities following application of any treatment to the soil (e.g. fertiliser, pesticide, biological control agents, and organic matter) and to correlate these changes with variations in the level of soil suppressiveness to a set of diseases. Moreover, the molecular techniques should enable by consulting a gene data bank, to determine which populations are affected by the treatments and then to study their role or their function, in the ecosystem. It will be possible to determine if these populations are really involved in mechanisms controlling soil health or if they are only indicators (markers) of soil health. But, it is obvious that several, or probably many, indicators will be needed to characterize soil health. Therefore mathematical modelling will be necessary to organize all these data and to follow the dynamics of the measured parameters either biotic or abiotic. The resulting and evolving models will allow us to propose management techniques useful for farmers and for the preservation of the environment. Solutions proposed to farmers will be more complex to achieve that the traditional chemical control applied as insurance. Thus it will demand the active participation of farmers with the support of the consumers which should understand what the benefits will be for the society.

The second approach which was favoured during the 20 last years consists in developing plant protection products based on micro-organisms. The discovery of soil naturally suppressive to diseases, which should have promoted research on ecology of soil micro-organism, paradoxically stimulated the development of bio-control agents isolated from these suppressive soils. As already stated, the soil is a reservoir of beneficial micro-organisms, not only for plant but also for human disease control, and it seemed easier to solve all the questions related to the development and application of a biological control agent than to understand all the conditions that make a soil suppressive. But it was a mistake, because to be successful inoculation biological control requires a full understanding of the ecology of the biological control agent. In fact, at that time people were thinking at developing a biological control agent as a chemical pesticide, with the same requirements for formulation, shelf life, and efficacy. This way of thinking partly explains the failure of this strategy since only a very few of the antagonists studied in the laboratories are actually on the market. Being living organisms, biological control agents have special requirements that both the producer and the applicator must take into account. Moreover, most of the antagonistic micro-organisms have narrow host specificity; they are able to control a single disease, when the farmer has to deal with several soil-borne pathogens. Consequently, biological control has to be integrated in a strategy of disease management. Several approaches have been proposed such as the use of an association of several antagonists or the application of antagonists after solarisation or mixed with organic amendments.

The difficulties encountered to apply biological control agents invite us to think in a different manner and as presented above consider biological control application as part of the agricultural practices which have to be chosen to promote soil suppressiveness to diseases.

Developing disease suppressive soils by introducing organic amendments and or biological control agents, crop residue management, crop rotations and adapted tillage practices will probably not provide immediate return compared to the use of fumigants or pesticides, but the benefits accumulate across successive years and improve soil health and structure. Farmers should not rely exclusively on a single management practice but a combination of practices should be integrated to develop a consistent long term strategy for disease management that is suited to their production system and location.

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