CHAPTER 19

POST-HARVEST *BOTRYTIS* INFECTION: ETIOLOGY, DEVELOPMENT AND MANAGEMENT

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Abstract. *Botrytis* is regarded as the most important post-harvest fungal pathogen that causes significant losses in fresh fruits, vegetables and ornamentals. Its ability to attack a wide range of crops in a variety of modes of infection and its ability to develop under conditions prevailing during storage, shipment and marketing make its control a challenge. Harvested crops are particularly vulnerable to *Botrytis* infection because unlike vegetative tissue harvested commodities are senescing rather than developing. Control of *Botrytis* on harvested crops has relied mainly on pre-harvest chemical fungicides for reducing inoculum density and incipient infections before harvest. Control programmes were developed specifically for each crop and largely depend on epidemiological and etiological information. The future of many of these chemicals, however, is now doubtful and their use has come under scrutiny. This is due to severe restrictions and regulations imposed especially on post-harvest chemical treatments for the majority of freshly harvested fruits and vegetables. To develop better and more efficient methods for controlling post-harvest *Botrytis* rot it is essential to understand the relationship between infection of various plant parts in the field and incidence of grey mould in storage. This relationship has still not been fully elucidated in tomato, kiwifruit, strawberry, grapes and roses. These crops are discussed in this chapter as examples for different research strategies to tackle the problem. It is concluded that control methods based on holistic strategies which incorporate modelling and prediction systems, early detection techniques, biological and physical methods, and cultural practices, should be tailored to meet the demands of each crop.

1. Introduction

Much of post-harvest losses of fresh fruits and vegetables are due to fungal and bacterial infections. These losses are serious because the value of fresh produce significantly increases while passing from the producer to the consumer's table (Eckert and Sommer, 1967). These losses are estimated at 50% or more in developing countries due to the lack of adequate handling and refrigeration facilities; while losses may be lower in developed countries. Because of the lack of precise statistics, however, it is hard to determine the extent of real losses that may vary depending on the commodity and the producing country. Post-harvest decay can be traced to infections that occur either between flowering and fruit maturity or during harvesting and subsequent handling, storage, marketing and even after

Y. Elad et al. (eds.), Botrytis: Biology, Pathology and Control, 349-367.

purchase by the consumer. Intensive efforts have been made to minimize these losses through better understanding of the biology and etiology of post-harvest diseases as well as developing adequate control strategies.

A number of fungi from several genera cause post-harvest decay on agricultural produce. Among these are representatives of *Botrytis*, *Penicillium*, *Mucor*, *Alternaria*, *Colletotrichum, Diplodia, Rhizopus, Fusarium* and *Aspergillus*. However, *B. cinerea* is regarded as the most important since it has a wide host range (Sommer, 1985). It is an important post-harvest pathogen because environmental conditions prevailing during shipment and storage facilities are favourable to its development and infection. The losses inflicted by *B. cinerea* after harvest to fruits and vegetable crops as well as ornamentals are considerable if the disease is not properly managed. Susceptibility of vegetable, fruit and ornamental crops to grey mould infection and development increases after harvest as plant tissues senesce. Factors involved in acceleration of senescence, such as ethylene, in certain crops greatly enhance susceptibility to grey mould (Chapter 10). Delaying senescence by post-harvest treatments e.g. controlled atmosphere (CA), modified atmosphere (MA), cold storage, ethylene inhibitors and gibberellin can considerably reduce susceptibility to decay (Sommer, 1985). It was demonstrated that *B. cinerea* isolated from strawberry and kiwifruit actively produce large quantities of ethylene when grown in culture medium supplemented with methionine (Altaf et al., 1997; Chapter 10). This feature could be essential for post-harvest pathogenesis of *B. cinerea.*

This chapter will provide a general overview of post-harvest rot caused by *Botrytis* in selected crops. It will touch on the great body of research done on this subject and illustrate all aspects of the *Botrytis* problem in major crop model systems. Etiology of the infection, key current strategies and alternative approaches of control will be described.

2. Etiology of post-harvest botrytis rots

While most fungal post-harvest pathogens have a restricted host range, damage caused by *B. cinerea* is widespread on many crops causing grey mould on various plant parts, including roots (e.g. flower bulbs, sweet potato), shoots (e.g. lentils, kale), leaves (basil, lettuce), flowers (e.g. rose petals) and fruits (Jarvis, 1980; Maude, 1980). The largest damage inflicted by *Botrytis* is recorded for senescing fruit tissue after harvest since it is the most vulnerable to infection and development. Vegetables and small fruit crops that are susceptible to grey mould after harvest include artichoke, apple, asparagus, bean, beet, blackberry, black-eyed pea, blueberry, broccoli, brussels sprouts, cabbage, carrot, cauliflower, celery, chives, cucumber, currant, eggplant, endive, gooseberry, grape, kale, kiwi, kohlrabi, leek, lentil, lemons, lettuce, okra, onion, parsnip, pea, peanut, pepper, pears, peaches, plums, pumpkin, quince, raspberries, red chicory, rhubarb, rutabaga, shallot, squash, strawberry, sunflower, sweet cherry, sweet potato, tomato and turnip. *Botrytis* is a major problem for fruits and vegetables in cold storage and subsequent shipment, because the fungus is able to grow effectively at temperatures just above freezing.

The mode and time of infection vary depending on the plant species and the plant part affected. Symptoms of *Botrytis* diseases vary greatly depending on the host and plant part attacked. Symptoms include a grey to brown discoloration, water soaking, and fuzzy whitish grey to tan mould (mycelium and conidia) growing on the surface of affected tissue and restricted lesions. The following are possible modes of infection of *Botrytis* that develop after harvest:

Infection through blossoms: The pathogen can infect fruits via the flower remaining in the very early stages of development and develop only after harvest when the fruit reaches full ripeness. This mode of infection is important in strawberry and is supposed to be the main cause of decay in mature fruit after harvest. Blossom blights often precede and lead to fruit and stem end rots. Ageing flower petals of beans, carrot, celery, eggplant, onion, pepper, squash, cucumber and tomato are particularly susceptible to colonization by *Botrytis* species, and under cool, humid conditions abundant mycelium and conidia are produced. The fungus often grows from the fading petals into the rest of the inflorescence and develops on the fruit causing blossom-end rot. From there it can spread and destroy part or all of the fruit (Chapter 14).

Infection through surface injuries and cracks: Conidia can infect the fruit directly through growth cracks, cut stem scars, insect wounds or lesions made by other pathogens. Infected fruits develop water-soaked, yellowish green or greyish brown irregular lesions which can be somewhat soft and spongy in texture.

Infection of bulbs and roots: Lesions can develop on any part of the root or bulb surface, but they are more likely to form at the crown, at wounds. Lesions usually appear soft, watery and tan colour, later becoming somewhat spongy, dark brown, and light-weight. Affected tissue eventually may dry to form a greyish leathery decay. Pockets of mycelium may develop between decayed bulb scales on the surface of root lesions. Black sclerotia can also be formed on and in decayed tissue, except at low temperatures in darkness, when only a fine white mould develops. Grey mould causes considerable damage on stored carrot, parsnip, mangel, sweet potato, beet, endive, chicory, turnip and rutabaga, but usually affects only topped roots. The bulb rot phase is common on onion and garlic, but also occurs on other bulb crops (Chapter 15).

Infection through harvest cuts and trimming: Grey mould rot caused by *B. cinerea* commonly occurs after harvest on the cut surface on leafy vegetables and fresh herbs (e.g. celery, basil, shiso). The cut surface is a perfect infection site for *Botrytis* because of the presence of nutrients leaking from the damaged tissue. Once infection is established decay can proceed to entire leaf tissue. The picking wound also is known to be good site for *Botrytis* infection. In kiwifruit, picking wounds are regarded as the main site of infection pathway for the majority of infections by *B. cinerea* (Michailides and Elmer, 2000).

Insect mediated-infection: In most cases, diseases have a pre-harvest component. The activity of various types of invertebrates can play a critical role in contaminating the produce with inoculum. The most substantial evidence in this regard has been shown in relation to infection of kiwifruit through flowers with *B. cinerea* facilitated by thrips (*Thrips obscuratus)* and honey bees (Michailides and Elmer, 2000). High *Botrytis* incidence was also reported on kiwifruit with garden

snail (*Helix aspersa*) damage; slime secreted by the snail stimulated the germination of the conidia. Honey bees and other types of insects visiting flowers potentially have the capability to disseminate *Botrytis* in strawberry (Michailides and Elmer, 2000). For details see Chapter 14.

3. *Botrytis* on major crops

3.1. Table grapes

More than 2 million tonnes of table grapes are shipped from the southern to the northern hemisphere, and to a smaller extent, by other routes. Surplus yields that accumulate in the peak of production are stored for local consumption for timely distribution. The table grape season can be extended substantially by the correct composition of early and late varieties and by diversifying the local geographical production areas. Shipping and storage of table grapes requires development of commercial technologies which are focused on preserving the fresh appearance of the grapes without compromising fruit taste. The major challenge for this technology is post-harvest fruit decay caused by *B. cinerea*.

Multiple pathways of infection can lead to grape decay during storage (Coertze and Holz, 1999, 2002; Chapters 2 and 14). It is evident that *Botrytis* can infect grapes during fruit set and bloom (McClellan and Hewitt, 1973), resulting in latent infection and grape decay in the vineyard. The actual contribution of this route to decay in storage may be low since removal of external inoculum by disinfecting the surface can prevent much of the damage (Lichter et al., 2002). During ripening the susceptibility of the berries increases partly due to a decrease in the content of phytoalexins such as resveratrol (Jeandet et al., 1991). Bird, insect and mechanical damage, sun scald and rain cracking serve as vectors and pathways for infection (Broome et al., 1995; Coertze and Holz, 2002). Rain events or failure in insect control can result in grey mould epidemics in the vineyard, making post-harvest storage a challenge. However, in subtropical climates, *Botrytis* infection in the vineyard is infrequent and development of grey mould in storage remains the primary problem. Microclimate plays an important role in the epidemics of *Botrytis* in the vineyard or after storage (see Michailides and Elmer, 2000). The first symptom during storage is ‘slip-skin’ (separation of the skin from the flesh upon touch). This is usually accompanied by red-brown colouration easily scored on white varieties. Internal disintegration of the berries is typically followed by appearance of aerial mycelium and sporulation which serves as an inoculum source for subsequent infection. The decaying berry itself infects neighbouring berries forming ‘nests’ that can eventually spread over the entire bunch. Infection can also develop from the pedicel-berry interface. Less typical decay symptoms can be observed as lesions with clear boundaries and limited area.

The berries of modern table grape varieties are large with thin skin. Early season horticultural practices have a substantial effect on bunch and berry structure and quality, and hence its storage potential. For example, ‘berry thinning’ performed manually or by chemical induction of flower abscission with gibberellin prevents excessive fruit set (Coombe, 1973). Failure in thinning may result in ‘corn’-like

appearance of the bunch with tightly compressed berries, creating a convenient microclimate for decay beyond the reach of botryticides (Marois et al., 1986; Vail and Marois, 1991). Earlier application of gibberellin results in bunch 'stretching' which loosens the berries. Gibberellin-mediated manipulation of berry size (Coombe, 1973) is another treatment with many implications for the post-harvest quality of the berry, but it may decrease fruit resistance to decay both at harvest and during storage.

Table grapes can withstand extreme cold storage and the recommended storage temperature is -0.5°C. This low temperature is of high value for prevention of fungal decay, but it is only sufficient to slow down development of *B. cinerea*. Therefore, a complementary technology of fumigation with sulphur dioxide (SO_2) was developed. SO_2 damages fungal membranes, inhibits various enzymes and if applied correctly is a very effective tool (Harvey and Uota, 1978). SO_2 is also a strong antioxidant and prevents the necrosis of the rachis. This technology is currently the only commercial practice for storage of table grapes and it is applied by different means. Gaseous application is common in California and is implemented by exposing the grapes to a large dose before storage, followed by weekly fumigations according to a formula accounting for the exposure time, volume of the room and its capacity (Combrink and Ginsburg, 1972; Smilanick et al., 1990). Most other grape producers use 'generator pads' containing sodium metabisulphite that release SO_2 in a controlled manner upon reaction with water vapours. The pad is placed over the grapes and the gas is contained in ventilated or non-ventilated liners, or without liners, wrapping the pallet with stretch-polyethylene, as practiced in Chile, South Africa and Israel, respectively. Special care is necessary to avoid high concentrations of SO_2, expressed as bleaching, typically around the pedicel-end or around microscopic holes or cracks in the cuticle. The damage may also be expressed as an undesired taste and rare hypersensitivity in the human population. Because of the deleterious side-effects of SO_2 and the world-wide scale of the *B. cinerea* problem, several lines of alternative technologies have been suggested over the years.

Cultivar selection and horticultural practices are extremely important for integrated pest management (IPM) and have the potential to increase the quality and integrity of grapes. To date, this approach has not developed into a strategy which is sufficient for current commercial demands. Pre-harvest application of fungicides can protect grapes from post-harvest decay, but that is not always the case (Kock et al., 1991). Fungicide application during flowering, fruit set and before veraison were ineffective in reducing infection by *B. cinerea* during storage (Kock et al., 1991). However efficient fungicides may be, their acceptance by consumers is low and the potential for resistance is increased.

The practices at harvest**,** such as handling and removal of damaged and decaying berries, are very important for the quality of the bunches after storage. Forced-air cooling may be significant for prevention of fungal development in the initial stage of storage (Jooste, 1987), but that is not a rule (Witbooi et al., 2000). Because forced-air cooling provides benefits to the appearance of the bunch, its added potential to reduce *B. cinerea* infection is likely to be considered as part of IPM. Current technology largely ignores the microbial contamination of fruits which

are packaged with soil particles, carposphere microflora and insect debris. Dipping bunches after harvest in ethanol addresses these problems without compromising fruit quality. Interestingly, this treatment is very effective in preventing *B. cinerea* from developing decay (Lichter et al., 2002), partly due to the high sensitivity of the conidia to ethanol (Lichter et al., 2003). It is estimated that this technology can be used for short-term storage, but ethanol is unsuitable for latent and internal infection. Another treatment which can be used after harvest and before storage is vapour heat applied in the range of 50-55°C for 12 to 32 min (Lydakis and Aked, 2003). Fumigation of grapes with volatiles, such as hexanal, prevents decay after 7 days storage at 15°C (Archbold et al., 1997). UV-C (220-280 nm) is known to induce resveratrol and pre-treatment of berries with low UV-C doses followed by artificial inoculation with *B. cinerea* reduced post-harvest grey mould of table grapes (Nigro et al., 1998). Dipping bunches after harvest in resveratrol was found to inhibit development of *B. cinerea* (Urena et al., 2003). It is noteworthy that resveratrol intoxicates *B. cinerea* following laccase processing (Schouten et al., 2002).

Biocontrol is a very appealing option in IPM programmes for grapes (Chapter 13) and several reports demonstrate its efficacy against bunch rot in the vineyards and for prevention of grey mould after harvest. Among the organisms reported to be effective are *Trichoderma harzianum* (Elad, 1994), *Aureobasidium pullulans* (Lima et al., 1997), *Pythium periplocum* (Paul, 1999), *Metschnikowia fructicola* (Karabulut et al., 2003), various yeasts (Wilson et al., 1991; Zahavi et al., 2000) and *Bacillus subtilis* (Pusey, 1989). Currently, no biocontrol agent seems to be efficient enough to cope on its own merit with *B. cinerea* in the arena of table grapes and cold storage. The balance in future may, however, be shifted in favour of biocontrol with the support from IPM programmes.

Several alternative technologies have been examined for continuous protection during storage. CA of grapes with 5-10% CO_2 failed to prevent development of *B. cinerea* (Uota, 1957; Laszlo, 1985). Increasing CO_2 levels to 15% or higher suppressed *B. cinerea* (Berry et al., 1997; Crisosto et al., 2002; Retamales et al., 2003). However, a lower concentration of CO_2 was recommended for cv. Red Globe to avoid damage to the taste and appearance (Crisosto et al., 2002). Apparently, there is a narrow threshold between efficacy and damage expressed as browning of berries or rachis and undesired after-taste. Carbon monoxide at 10% was also reported to offer good decay control without loss of quality (Yahia et al., 1983). MA depends on CO_2 released from the bunch by respiration and its accumulation to the desired concentration in the liner. Probably due to its limited efficacy on its own merit, it was reported mainly in conjunction with other molecules, such as acetic acid, chlorine and volatiles (Moyls et al., 1996; Archbold et al., 1997; Zoffoli et al., 1999; Westercamp and Blargues, 2002). Fumigation with acetic acid at intervals during cold storage was also reported (Sholberg et al., 1996). Application of ozone to grapes elevated the level of resveratrol and had a significant effect on *Rhizopus stolonifer* rather than *B. cinerea* (Sarig et al., 1996). Continuous exposure to 0.3 µl/L ozone inhibited 'nesting' of *B. cinerea,* but did not reduce incidence of decay of grapes (Palou et al., 2002). Ozone applied in solution for detached berries significantly controlled grey mould, although its efficacy was

variable and dependent on grape condition (Gabler and Smilanick, 2001). This study also identified ammonium bicarbonate and ethanol as the most interesting compounds for storage of detached berries. None of the above techniques has so far reached commercial application and this situation is unlikely to change unless consumer demands reverse regulation.

3.2. Tomato

Unlike table grapes, failure to control *B. cinerea* before harvest makes it difficult to control the fungus during storage. This is partly because the optimal temperature for *B. cinerea* development on tomatoes is 15°C (Takeda and Nakamura, 1990) and close to the optimal temperature for storage of tomatoes (12°C). Another matter is the lack of an efficient method (such as SO_2 fumigation of grapes) to control the pathogen during storage. Therefore, much of the effort is dedicated to pre-harvest prevention, with a focus on IPM programmes that take into account climate and horticultural practices (Shtienberg and Elad, 1997; Chapter 18). Latent infections of tomato during flowering give rise to stem-end rot (Lavy Meir et al., 1988; Jarvis, 1994). During fruit development, wet climate in field-grown tomatoes and a humid microclimate in greenhouses play a crucial role in disease development. Hence, in a dry climate or well-ventilated indoor growth, *B. cinerea* is not a major post-harvest problem. The extent of post-harvest decay of tomato fruits is positively correlated with the amount of *B. cinerea* present at harvest (Chastagner et al., 1977). During storage, injuries such as those incurred by the calyx of adjacent fruit may be a focus for infection (Thomas et al., 1977). However, the variation in incidence and extent of infection is high between cultivars, growers, and specific growth conditions.

Fungicides have played an important role in maintaining the fruit free of decay (e.g. Chastagner et al., 1977), but fungicide resistance appeared frequently. For example, in one study more than 90% of tomato samples contained benomyl-resistant strains (Staunton et al., 1975). Such specific resistance is attributed to point mutations in the tubulin gene (Yarden and Katan, 1993; Chapter 12). New fungicides with a dual mode of action offer better protection for tomatoes (Siviero et al., 2003), but environmental concerns may limit their use. Many types of chemicals have been suggested for control of *B. cinerea* after harvest. For example, acetic acid (Sholberg and Gaunce, 1995), the anionic surfactant Nacconol (Hoy and Ogawa, 1984), the phytoalexin glucohexatose (Ning et al., 2003) and a peptide with antifungal activity (Lopez-Garcia et al., 2000). The efficacy of biocontrol agents was demonstrated with *Candida oleophila* (Saligkarias et al., 2002), *Bacillus amyloliquefaciens* (Mari et al., 1996) and *Aureobasidium pullulans* (Schena et al., 1999). The interactions between microorganisms on the fruit surface may lead to negative effects, as was observed for tissue co-inoculated with *Salmonella typhimurium* and *B. cinerea* (Wells and Butterfield, 1999). Physical treatments such as hot water dips, hot air and brief hot water rinsing and brushing (Barkai Golan et al., 1993; Lurie et al., 1998; Fallik et al., 2002) reduce the inoculum on the surface of the fruit and also decrease chilling injury, increase expression of heat shock proteins and activities of enzymes, such as peroxidase, which may limit decay development (Lurie et al., 1997). Application of UV-C light retarded ripening and decay caused

by *B. cinerea* (Liu et al., 1993) and decay was also delayed by short-term anoxia treatments (Fallik et al., 2003).

There are many reports on the contribution of MA and CA for protection of tomato from fungal diseases (Dennis et al., 1979; Marangoni and Stanley, 1991). However, this approach has never reached the practical phase, probably because the benefits did not justify the commercial adoption of the new technology. Tomato is considered a climacteric fruit that produces and requires ethylene for development and ripening. CA supplemented with reduced level of ethylene resulted in increased susceptibility to *B. cinerea*; 1-3 µl/L of ethylene was required for optimal decay control (Geeson et al., 1986). These results correlate with the negative effect of the inhibitor of ethylene action, 1-methylcyclopropene (1-MCP), on resistance of tomato to *B. cinerea* (Diaz et al., 2002). On the other hand, ethylene induced elongation of germ-tubes of *B. cinerea* after incubation with tomato fruit and a tomato mutant with reduced responsiveness to ethylene produced less decay than the control cultivar (Barkai Golan et al., 1989; Chapter 10).

Tomato is a good model for studying molecular aspects of post-harvest *B. cinerea* problems (Botella, 2000). Functional analysis of these interactions can be carried out by testing *Botrytis* gene knock-out mutants for virulence on harvested tomato fruit, or by testing tomato mutants for susceptibility to fruit decay. Several *Botrytis* genes such as cutinase A and the polygalacturonase *Bcpg1* were evaluated for pathogenicity on tomato fruits and the Bcpg1 mutant had reduced pathogenicity (Van Kan et al., 1997; Ten Have et al., 1998). Other *Botrytis* gene disruptants displayed reduced virulence on tomato leaves and fruits (Zheng et al., 2000; Gronover et al., 2001). Tomato fruits over-expressing pear PGIP were more tolerant to *B. cinerea* (Powell et al., 2000). Suppression of a ripening-related expansin gene which increased consistency of tomato paste did not influence fruit tolerance to *B. cinerea* (Brummell et al., 2002). In general, genes affecting cell wall metabolism have a potential to affect the interaction with *Botrytis* (Brummell and Harpster, 2001), but this issue was not addressed systematically. See also Chapters 4, 7 and 20.

3.3. Kiwifruit

Botrytis causes three types of post-harvest rots on kiwifruit (*Actinidia deliciosa*) that develop mainly during cold storage at 0°C. The major symptom is stem-end rot in which the fungus penetrates the picking wound at, or soon after, harvest. The second type is spread of the infection from fruit to fruit by contact. When fruits become over-mature *Botrytis* conidia can directly infect through cracks, wounds or through any other damaged skin area (Brook, 1992). *Botrytis*-infected fruit can also enhance premature softening of the remaining fruits in a tray or bin as a result of the enhanced ethylene production by the infected fruit. Stem-end rot begins at the picking wound of otherwise undamaged kiwifruit and progresses down the fruit; resulting in the classic 'tide mark' on the outside of the fruit. The diseased pericarp is glassy and water-soaked. Secondary rotting results in the complete disintegration of the fruit. Symptoms are not usually manifest until between 4-12 weeks of storage at 0ºC, or sooner if the fruit is kept at higher temperatures. Costly repacking in the

offshore market is often necessary. In California, research showed that most grey mould developing in kiwifruit in storage was due to latent infections of *B. cinerea* in the fruit sepals and receptacles (Michailides and Elmer, 2000). The greater the level of sepal and receptacle infection prior to harvest, the greater the incidence of grey mould in cold storage. In New Zealand, however, studies demonstrated that the main site of infection is through the picking wounds. At the middle of the growing season *Botrytis* is found on senescent petals attached to fruit. By harvest, the green leaves with necrosis (dead patches) and dead leaves are the primary source of inoculum. Research has shown that the hairy kiwifruit acts as a conidia trap and thousands of *Botrytis* conidia have been found on the fruit surface. At the time of harvest, conidia on the skin surface contaminate the picking scar which leads to stem end rot in fruit under cold storage conditions. In California, fruit damaged by the brown garden snail (*Helix aspersa*) increased grey mould incidence in storage compared to undamaged fruit (Michailides and Morgan, 1996). Knowledge of the *Botrytis* life cycle after harvest is less clear, but it can be expected that infection occurs through wounds. Infection of kiwifruit at pre-harvest stages is described in Chapter 14.

Dicarboximide sprays at harvest were the common means of chemical control of *Botrytis* in kiwifruit orchards. In California, Sommer et al. (1984) showed that two sprays with vinclozolin at bloom and two more pre-harvest sprays were the most effective treatments for controlling grey mould in storage. However, *Botrytis* has developed resistance to the dicarboximides, such as vinclozolin and iprodione. Some strains of *Botrytis* are now also resistant to the benzimidazoles (benomyl). A new generation of low risk chemicals (e.g. fenhexamid; Chapter 12) for the control of grey mould have been also evaluated. The development of the BOTMON predicting system, in which the level of *B. cinerea* colonizing the sepal or receptacle is determined, has greatly improved decisions by growers regarding the timing and number of fungicide applications in the field (Michailides and Morgan, 1996). Fumigation of kiwifruit with SO_2 was also reported to reduce grey mould without fruit injury (Cheah et al., 1992).

Alternative control methods were suggested for management of the disease. An open canopy provides plenty of air movement in the plantation and subsequently creates unfavourable conditions for conidial germination. Good orchard hygiene to remove sources of *Botrytis* conidia such as dead leaves, petals etc. can reduce the risk of *Botrytis* infection of the fruit. Careful post-harvest handling of the fruit (picking, grading and packing) reduces the risk for skin injury and minimizes the prospects for infection sites. Picking bags contaminated with conidia can become a major source of inoculum; research has shown that nylon mesh bags may reduce *Botrytis* build-up compared to enclosed bags.

Incubating or 'curing' the fruit at ambient temperatures for at least 2 days before packing and cold storage significantly decreases *Botrytis* storage rots. Fruit should be checked during cold storage for presence of rots. The optimum time for checking is after 10-12 weeks of storage, by which time almost all the primary infections will have produced visible symptoms. Early condition checking (e.g. after 6 weeks storage) will identify any major problems, but fail to detect c. 80% of infections (Manning et al., 1995).

Biocontrol of *Botrytis* on kiwifruit tissues was first reported by Menzies et al., (1989) but the results were variable. In Italy, post-harvest applications of a yeast to freshly harvested fruit significantly increased the proportion of healthy kiwifruit after artificial inoculation with *Botrytis* and cool storage for 15 days (Testoni et al., 1993). Cook et al. (1999) reported that application of yeasts (e.g. *Candida sake, C. pulcherrima, Trichosporon pullulans*), or inducing host resistance by incubating kiwifruit at 10°C with high humidity were each effective in significantly reducing incidence of post-harvest stem-end rot in cool stored kiwifruit. However, when these two means were combined, the timing of the yeast application determined whether their effect was additive. There were also attempts to use *Bacillus subtilis, Pseudomonas syringae, Acremonium breve* and *Cryptococcus laurentii* as pre- and post-harvest treatments, but their efficacy was variable (Michailides and Elmer, 2000).

3.4. Roses

Botrytis blight is a widespread disease of greenhouse-grown roses (Coyier, 1985). Susceptibility to *B. cinerea* is regarded as an important factor of vase-life. Infection of petals reduces the ornamental value and can be the reason for flower collapse. Symptoms of infection are visible within 24 h at 18-25°C and > 90% relative humidity (RH) (Salinas et al., 1989) initially as restricted lesions that later become necrotic (Elad, 1988). The infection will eventually spread, leading to the necrosis of whole petals (Pie and Brouwer, 1993). The infection can spread to receptacle and then cause abscission of all the petals. The problem is aggravated by latent infections that are symptomless at harvest, but become apparent during storage and transport (Elad, 1988). Various rose cultivars show differing susceptibility to *B. cinerea* (Hammer and Evensen, 1994) and different methods have been developed for determining the relative susceptibility of the cultivars (Hazendonk et al., 1995). Cut flowers senesce after harvest during transit and marketing. Ethylene produced during senescence predisposes the flowers to infection and development of *Botrytis*. Infected flowers produce elevated amounts of ethylene that lead to premature senescence of flowers (Elad, 1988; Chapter 10).

Sanitation, cultural practices and chemical control using fungicides have been the main recommendations for reducing infection in rose production. The dicarboximide fungicides are recommended for the control of *Botrytis* in roses. However, their efficacy on greenhouse roses is limited (Elad, 1988). Flowers which may be closed at time of treatment develop and are harvested before the next spray, meanwhile later infections may have occurred. In addition, the pathogen has developed resistance to these fungicides in many areas. Cultural methods, such as reduction of RH and avoidance of free water on the rose canopy by forced aeration and heating, are currently the most effective control measures (Hammer and Evensen, 1994).

Pre-harvest sprays, fertilization or pulsing buds by fertigation with calcium compounds reduced disease severity and extended vase life of the cut flowers (Volpin and Elad, 1991; Bar-Tal et al., 2001). Microbial antagonists of *B. cinerea* are reported to reduce grey mould on rose crops. Redmond et al. (1987) identified

isolates of yeasts and bacteria that reduce the number of lesions produced by the pathogen on rose petals, and Elad et al. (1993) found that *Trichoderma harzianum* reduced symptoms on rose flowers harvested soon after treatment. Hammer and Marois (1989) used two antagonists to reduce the symptoms caused by *B. cinerea* in roses stored at 2.5°C, but control was ineffective when flowers were removed from storage and kept at room temperature.

Meir et al. (1998) have demonstrated that pulsing of cut roses with 200 μM methyl jasmonate (MJ) provides systemic protection against *Botrytis* rot by inducing resistance mechanisms in cut roses of six cultivars without impairing flower quality. Also, a direct antifungal effect of 100-400 μM MJ on conidial germination and germ tube elongation of *B. cinerea* was obtained *in vitro*, with complete inhibition at 400 μM MJ (Meir et al., 1998). These results suggest that a combined treatment of spraying and feeding with MJ may be efficient for control botrytis blight in cut roses. Indeed, application of this treatment on cvs Frisco and Red Charm resulted in excellent control. Based on these results, a practical application of MJ consisting of simultaneous pulsing and spraying was developed for use by growers. The treatment included MJ pulsing for 4 h at 20°C and MJ spraying, followed by continuation of the pulsing for additional 20 h at 6°C.

3.5. Strawberry

Botrytis fruit rot is one of the most important diseases of strawberry worldwide. This disease causes severe pre-harvest and post-harvest losses, primarily due to infection that is initiated in the field during flowering and remains quiescent until fruit ripens. Although the disease can be important pre-harvest, it is most important when developing during shipment or storage. After harvest, this disease is the major limiting factor in cold-chain marketing of strawberries.

On ripe strawberry fruits, lesions are typically found on the proximal end of the berry and are frequently associated with infected stamens, or with dead petals that stick to the fruit or become trapped under the calyx. Lesions begin as small, firm, tan spots that quickly enlarge and become covered with white fungal mycelium and grey to brown conidia. Epidemics of botrytis fruit rot in perennial strawberry production systems are initiated primarily by conidia produced in dead strawberry leaves in the field (Braun and Sutton, 1987). Conidia are either splash- or air-dispersed to infect floral parts, including stamens and petals (Bristow et al., 1986). Mycelium of *B. cinerea* then invades the adjacent receptacle as it ripens and causes fruit rot. The pathogen sporulates on diseased flowers and fruits, which become important sources of secondary inoculum in annual production systems where there are multiple flowering and harvest cycles over several months.

Effective management of fruit rot developing after harvest involves regular pre-harvest applications of botryticides during peak flowering periods. Blacharski et al. (2001) reported that weekly applications of captan and thiram were effective in reducing pre- or post-harvest incidence of grey mould. Fenhexamid (750 g a.i./ha), applied as a programme of treatments from early flowering, demonstrated good control of *B. cinerea* in the UK (Duben et al., 2002). Stensvand (1998)

has also evaluated the use of new chemicals as well as biocontrol agents for the control of grey mould of strawberry.

The removal of all diseased and unmarketable fruit from within the plant canopy is critical for effective management of botrytis fruit rot, as this fruit is an important source of inoculum that directly infects nearby flowers and fruit. The removal of senescent foliage also reduces inoculum, but provides only limited control. Use of drip- instead of overhead-irrigation reduces free moisture on plants and prevents splash-dispersal. Growing strawberries under clear plastic tunnels also reduces leaf wetness duration and botrytis fruit rot incidence (Xiao et al., 1999). Reducing plant density by use of wider plant spacing reduces the incidence of grey mould (Legard et al., 2000) but may reduce yield. The combination of cultural and chemical methods is essential for an effective control programme. Although no cultivar is fully resistant, there are significant differences in susceptibility among cultivars used commercially (Legard et al., 2000). Public concern about potential chemical pesticide residues in fruits is common. These problems high-light the need for development of alternative control methods which are effective and safe for the producer and the consumer (Wilson et al., 1998; Droby et al., 2000; Janisiewicz and Korsten, 2002).

Biocontrol of rot-causing pathogens of strawberry has been attempted in several laboratories (Peng et al., 1992; Lima et al., 1997; Helbig, 2001; Boff et al., 2002; Guetski et al., 2002). The main approaches involved either interference with pathogen infection at flowering and during fruit development, or the suppression of inoculum production (Peng and Sutton, 1991). Field application of biocontrol agents to strawberry at the flowering stage, by spraying (Tronsmo and Dennis, 1977; Peng and Sutton, 1991), or carried by bees has proved to be effective in suppressing *B. cinerea* on stamens and petals and reducing fruit rot (Peng et al., 1992; Bilu et al., 2004). *Aureobasidium pullulans* and *Candida oleophila* were more effective against *B. cinerea* storage rots on strawberries when applied in the field at bloom compared with their application immediately after harvest (Lima et al., 1997); both antagonists were shown to colonize the flower to such an extent that their activity prevented colonization of senescent floral parts (stamens) by *B. cinerea*. More recent work (Karabulut et al., 2004) showed that a pre-harvest application of the yeast *Metschnikowia fructicola* effectively reduced the development of post-harvest strawberry grey mould. The yeast significantly suppressed post-harvest incidence of fruit rot better than fenhexamid. Systemic acquired resistance (SAR) was used to suppress *B. cinerea* on strawberry fruit. Acibenzolar (S-methyl benzo[1,2,3]thiadiazole-7-carbothioate) is a chemical activator of SAR. When applied to strawberry plants at 0.25-2.0 mg/ml, acibenzolar delayed by about 2 days the development of grey mould on harvested strawberry fruit held at 5^{o}C. This delay was equivalent to a 15-20% increase in storage life of the fruit (Terry and Joyce, 2000).

MA packaging using low density polyethylene (LDPE) is commercially used in Spain and other countries as a means to reduce botrytis rot and improve the overall quality of strawberries (Artes et al., 2001). The elevated CO_2 and reduced O_2 level inside the package seem to greatly reduce grey mould development on the fruit.

4. Conclusions and future prospects

At present, control of post-harvest decay caused by *B. cinerea* in various fruit and vegetable crops relies mainly on the pre-harvest use of chemical fungicides (Chapter 12). Control programmes were developed specifically for each crop and largely depend on epidemiological and etiological information of *Botrytis* attack (Chapter 18). The future of chemical fungicide control, however, is questioned and their use has come under scrutiny. This is because of 1) failure to effectively control grey mould in several harvested fruit and vegetable commodities due to resistance problems, 2) consumers' concerns over the possible effects of toxic residues on human health and the environment, and 3) restrictions imposed by authorities on the use of agro-chemicals. These issues that were the driving force for the development of reduced-chemical post-harvest disease control measures have now become an economic imperative, not just an option. The use of alternative non-chemical control methods as stand-alone treatments, however, does not provide the efficacy and consistency required under commercial post-harvest situations. A more realistic scenario to combat decay of harvested commodities would be the use of an integrated control approach combining biological and physical control strategies, with or without limited quantities of fungicides pre-harvest, and with efficient management and handling practices.

The availability of efficient post-harvest chemical control treatments for certain crops, such as the SO_2 technology for grapes, in some respects delayed development of alternative control means. Conversely, the lack of such dedicated control technologies in other harvested commodities has promoted the development of IPM programmes. Kiwifruit represent a crop in which horticultural and epidemiological knowledge has significantly contributed to generating control strategies for the control of botrytis rots. Strawberries are a model for a highly perishable crop in which harvesting over a relatively long season places a serious challenge as far as disease control in general and botrytis rots in particular. Roses are highly susceptible to *B. cinerea* and have relatively short shelf life, but because they are inedible, the use of chemical control strategies theoretically can be continued, subject to environmental regulation.

It is an enormous challenge to meet the demands for improved quality and safety, and reduction of post-harvest losses due to *Botrytis* infections in an affordable and environmentally compatible manner. After decades of research on *Botrytis* several questions related to the relationship between infection level occurring in the field and development of post-harvest decay remain unanswered. To develop more efficient methods for controlling post-harvest grey mould it is essential to elucidate the relationship between infection of various plant parts in the field and incidence of grey mould in storage. Development of control strategies based on a holistic approach in which modelling and prediction systems, early detection techniques, biological and physical methods and cultural practices should

be encouraged specifically to meet requirements of each crop. For example, management of grey mould on kiwifruit in New Zealand using non-chemical methods has been a success story. Adoption of summer pruning to create more open canopy, pre-harvest prediction and post-harvest curing has effectively reduced *Botrytis* losses and led to the perception by the industry that grey mould is no longer a problem. This success story may be repeated in other crop systems following the allocation of appropriate research efforts.

It is reasonable to assume that IPM approaches are unlikely to offer completely satisfactory answers for *Botrytis* control in certain commodities. However, to complement available solutions we need to systematically explore the potential of genetically manipulated plants which are more tolerant to grey mould (Chapter 20).

5. Acknowledgment

We wish to thank Prof. Ruth Ben-Arie for critical reading of the text.

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