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Integrated management of postharvest gray mold on fruit crops

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Highlights

- Gray mold one of the most important postharvest disease of fresh fruit
- Disease management requires the use of a series of preharvest and postharvest actions
- In conventional agriculture, gray mold management relies mainly on fungicide use
- Interest in developing and commercializing alternative treatments is increasing
- Losses can be minimized by the integration of preharvest and postharvest approaches

Abstract

Gray mold, incited by *Botrytis cinerea*, causes major postharvest losses in a wide range of crops. Some infections that occur in the field remain quiescent during the growing season and develop after harvest. The pathogen is also capable of infecting plant tissues through surface injuries inflicted during harvesting and subsequent handling; these develop during storage, even at 0 °C, and spread among products by aerial mycelial growth and conidia. The postharvest decay by this pathogen is controlled by a combination of preharvest and postharvest practices. To minimize postharvest gray mold, control programs rely mainly on applications of fungicides. However, mounting concerns of consumers and regulatory authorities about risks associated with chemical residues in food have led to imposition of strict regulations, the banning of use of certain chemical groups, and preferences by wholesaler, retailers and consumers to avoid chemically treated produce. These developments have driven the search for alternative management strategies that are effective and not reliant on conventional fungicide applications. In this review, conventional and alternative control strategies are discussed including their advantages and disadvantages. They include the use of conventional fungicides, biocontrol agents, physical treatments, natural antimicrobials, and disinfecting agents. Based on examples to control gray mold on specific crops, it is concluded that an integrated management program where adoption of a holistic approach is the key for meeting the challenge of minimizing postharvest losses caused by *B. cinerea*. To optimize the efficacy of treatments, it is essential to understand their mechanism of action as much as possible. Information about direct and indirect effects of each approach on the pathogen is also presented.

Keywords: biological control, *Botrytis cinerea*, cold storage, natural antimicrobials, postharvest decay.

1. Introduction

In a report by the United Nations Food and Agricultural Organization, it was estimated that one-third of the food produced worldwide for human consumption is lost after harvest (Gastavsson et al., 2011). Losses inflicted throughout the supply chain due to pathogen-induced diseases are the major component of food wastage. Pathogen attack may take place during harvesting and subsequent handling, storage, marketing, and after consumer purchase. Among these pathogens, *Botrytis cinerea*, the cause of gray mold, is considered one of the most important postharvest decays of fresh fruit and vegetables (Droby and Lichter, 2004; Elad et al., 2015). According to a recent review, *B. cinerea* ranked second into the world Top 10 fungal plant pathogens list based on scientific and economic importance (Dean et al., 2012). *B. cinerea* is an important postharvest pathogen because of the conducive conditions prevailing throughout the postharvest handling chain, including injuries, high humidity, senescing plant tissue and high sugar content. Major postharvest losses due to *B. cinerea* occur in a long list of fresh fruits: apple, blackberry, blueberry, currant, grape, kaki, kiwi, pear, pomegranate, quince, raspberries, strawberry, grapes and many others (Droby and Lichter, 2004; Romanazzi and Feliziani, 2014) (Fig. 1). In other fruits (*e.g.* apricot, lemon, orange, peach, plum, sweet cherry), although it is not the main pathogen, it is still capable of causing considerable postharvest losses.

Harvested agricultural commodities are highly vulnerable to pathogen attack since they undergo accelerated senescence processes, and in many fruit ethylene plays a major role in enhancing susceptibility to gray mold as well as to other postharvest diseases (Lougheed et al., 1978). Manipulation of fruit ripening processes using various postharvest technologies (*e.g.*, inhibition of ethylene production or action, modified and controlled atmospheres, plant hormones) can greatly affect infection and development of postharvest gray mold (Crisosto et al., 2002).

B. cinerea can survive in the field under a wide range of conditions as a saprophyte, where it colonizes flower residues, fruit juice drops, dead leaves, or other non-living plant tissue. This type of survival is well known in strawberry where the pathogen overwinters on dead leaves and starts its pathogenic phase at flowering, where it can remain latent on the stamens and below the sepals, and later infect the fruit close to or soon after harvest (Powelson, 1960). For this reason, the origin of most infections in strawberry fruit is located close to the sepals, which are often located under flower residues (Fig. 2). In many cases, it is possible to find gray mold developing on packed produce in the market, with the pathogen infection occurring on infected petals. In grapes, colonization of flower residues by *B. cinerea* is considered to be an important mode of infection. The pathogen can remain into the cluster and start additional infections of the berries when environmental conditions are favorable to the development of the disease (Pearson and Goheen, 1988). In this case, treatment at pre-bunch closure is recommended in table grapes to avoid infections soon before and after harvest. This is due to the current lack of systemic active ingredients that target *B. cinerea*. These infections occur because the inoculum of *B. cinerea* surviving on flower residues is capable of initiating infections on tissue lesions due to biotic (grape moth, powdery mildew infections, fruit fly) or abiotic damage (striking among berries, hail, wind).

After harvest, *B. cinerea* is capable of infecting fruits and vegetables through the damaged tissue in the stem end, which is rich in nutrient exudates. Stem end infections can develop and spread to the entire fruit. This mode of infection is mostly known in kiwifruit as the majority of fruits are infected through picking wounds (Michailides and Elmer, 2000). In pome fruit, gray mold infections can originate from wounds, stem punctures, or the stem or calyx end of the fruit (Sutton et al., 2014). Although *B. cinerea* is a common saprophyte on decaying organic matter on the orchard floor, gray mold is seldom seen in the field on pome fruit, while it becomes visible during storage. Indeed, conidia of *B. cinerea* are carried into the storage on bins and containers, transported with other organic matters, air-dispersed or commonly water-dispersed in flumes in packinghouses (Sutton et al., 2014). In addition, there is substantial evidence indicating an important role of insects in mediating contamination of harvested agricultural commodities with *B. cinerea* inoculum. In this relation, *Thrips obscuratus* and honeybees were shown to facilitate deposition of conidia into fruit injuries and surface cracks (Michailides and Elmer, 2000).

Efforts to minimize gray mold infections and the subsequent development of decay have focused on a better understanding of its biology and etiology on harvested commodities and using this information to develop pre- and postharvest control strategies for the pathogen. Among these approaches, the use of biocontrol agents (BCA) or natural compounds, when applied shortly before or soon after harvest, was found to be relatively successful (Calvo-Garrido et al., 2014). Overall, control of the infections on the fruit during storage is considered easier compared to those inflicted in the field, and several appropriate disease management strategies have been suggested in this regard (Ippolito and Nigro, 2000; Feliziani and Romanazzi, 2013; Teles et al., 2014).

This article provides a general overview of strategies and approaches for management of postharvest rots caused by *B. cinerea*.

2. Postharvest control of gray mold in conventional and organic agriculture

In conventional agriculture, we cannot avoid the use of synthetic fungicides, and there is a long list of registered active ingredients on different crops for gray mold control for both pre- and postharvest use (Romanazzi and Feliziani, 2014). However, growers are currently stimulated to adopt alternative approaches as stand-alone treatments or in conjunction with conventional fungicides. This development is taking place due to several reasons, including requirements from supermarket chains for commodities with low number of residual pesticides (e.g. a maximum of four to five active ingredients) used during production and subsequent postharvest handling. In addition, in some cases, due to the limited number of active ingredients on the fruit, the overall level of residues should not exceed 70-80% of the total allowed maximum residue limits (MRLs). For example, if we have four residual active ingredients, each should be present on average at the level of 20% of the allowed MRL. Unfortunately, these commercial policies do not take into consideration that the presence of fungicide residues in the fruit below certain thresholds will allow the pathogen to develop after harvest, resulting in significant losses throughout the handling chain. Furthermore, the presence of sub-lethal concentrations of fungicides in the fruit could increase the occurrence of mutations for fungicide resistance in fungal population, as at low doses of fungicides, the frequency of mutations is usually higher, due to the larger size of the sensitive pathogen population (van den Bosch et al., 2011).

In recent years, there have been registrations of several low-risk fungicides classified as a minimal risk to human and environmental health, for the control of gray mold with pre-harvest application intervals (e.g. fenhexamid) as brief as one to a few days prior to harvest (e.g. strawberry, table grapes). At the same time, more environmentally persistent older active ingredients that are considered less safe, such the benzimidazoles, are no longer available in the European market. Others are likely to be banned soon or withdrawn from sale (mostly dicarboximides) in other countries because of a high frequency of resistant isolates and a lack of interest among companies to continue their marketing due to a loss of profitability. In addition to chemicals used in conventional agriculture, there is increasing interest in using alternatives to conventional fungicides for the control of postharvest decay. This is based on the use of registered biocontrol agents alone to eliminate or reduce fungicide residues in the fruit or, in conjunction with conventional decay control for the purpose of managing fungicide resistance problems.

Recently, there has been an increase in the number of products available and registered that promote plant defense; these contain living organisms (biocontrol agents) or chemical plant stimulators such as glutathione, oligosaccharides, laminarin, and chitosan, which are known to inhibit postharvest decay. Most usually they have dual inhibitory effects on the disease due to direct inhibition of pathogens and induction of defense mechanisms in the host tissues. As an example, *Metschnikowia pulcherrima* depleted iron in apple wounds resulting in decreased infection by *B. cinerea* (Saravanakumar et al., 2008). Treatment with chitosan, benzothiadiazole, and a mixture of calcium and organic acids reduced pathogen growth and increased the expression of enzymes linked to defense mechanisms in strawberry tissues (Landi et al., 2014). Regulation EU 2014/563 included chitosan chloride as the first member on a basic substance list of plant protection products (as planned with Regulation EU 2009/1107), so it can be used in plant disease management since July 1, 2014.

3. Management of gray mold on stored products

Once harvested, most fruits need to be cooled as quickly as possible to remove field heat, to decrease respiration and water loss so as to retain harvest quality. This practice is particularly important when air temperature at harvest is relatively high, and can lead to enhanced loss of water resulting in drying that starts from stems or pedicels and enhanced senescence processes. Loss of even relatively small amounts of water from table grapes has a large negative impact on their quality (Crisosto et al., 2001). In addition, the temperature during cold storage needs to be optimal and constant, especially for long distance shipment, because any interruption of the cold chain can allow the development of a pathogen from quiescent infections. This favors rapid disease development particularly under the high humidity conditions within packages (Fig. 3). Thermometers with wireless remote access are commercially available and their use is increasing to monitor the temperature of fruit during the transport.

Usually fresh fruit are stored at temperatures between 0 to 10 °C, depending on the commodity, for a few days (small berries), up to two months (for some table grape cultivars as ‘Crimson Seedless’), or even many months (for kiwifruit, apples or pears). Reduction of the temperature in a period as rapidly as possible is indispensable for perishable fruits and vegetables. For example, highly perishable wild strawberry (*Fragaria vesca*) fruits are harvested in the field directly into containers and placed in a cold proof box with an ice pad on the bottom (Fig. 4). Under these conditions, the fruits can have a shelf life of three to four days. In Italy, some packinghouses pay a higher price to growers when strawberry fruits are harvested in the early morning. It was estimated that the harvest of these fruits for every hour after 10 AM resulted in one day shorter shelf life (G. Savini, personal communication). Table grapes are usually packed directly in the field (Fig. 4) to minimize handling that removes their waxy bloom and causes detachment of berries from the clusters, then they are pre-cooled within a few hours using forced air ventilated rooms to reduce the temperature to about 0-1 °C. High humidity that occurs within table grape packages minimizes water loss but it can cause condensation to occur if the cold chain is broken and the cold fruit are placed in a warm environment. High humidity and free water conditions facilitate conidial germination and penetration through cracks or microlesions that can occur during harvest and subsequent handling. These conditions are ideal for infection because fruit tissues after harvest and during cold storage are less reactive due to weakening of defense mechanisms. Once decay has developed, it can progress rapidly by contact and aerial mycelial growth to nearby healthy fruits. This type of infection is known as nesting, because of clustering of infected fruit close to a source of mycelial inoculum. Low temperatures during storage slow but do not stop the growth of *B. cinerea* since it is able to grow at a wide range of temperatures, from 0.5 °C to 32 °C (Coertze and Holz, 1999).

The use of conventional synthetic fungicides for controlling pathogens on most commodities is prohibited after harvest in most EU countries. In grapes and some other fruits, however, the use of sulfur dioxide during storage is permitted since it is considered as processing aid and not as a fungicide. When it was recognized that hypersensitive reactions occurred in people sensitive to sulfites in food, sulfur dioxide was classified was classified as a pesticide and MRL 10 mg kg^{-1} of sulfite residues in table grapes was established by the U.S. Environmental Protection Agency (Anonymous, 1989). In California, many organic growers use ozone fumigation of grapes after harvest (Feliziani et al., 2014), and this technology has also been used to some extent among packinghouses working with conventionally grown grapes. An interesting side of ozone treatment resides in its oxidant activity that can reduce fungicide residues on the berries (Karaca et al., 2012; Mlikota Gabler et al., 2010). Sulfur dioxide can damage the fruit by causing surface cracks (Zoffoli et al., 2008) and bleaching color from red cultivars (Luvisi et al., 1992). In addition, the treatment is non-selective in eliminating the vast majority of epiphytic microflora left on the fruit without natural protection allowing gray mold to develop more readily compared to non-fumigated fruit. To achieve good levels of control, usually sulfur dioxide is applied in storage room of grapes weekly, following a first treatment during cooling prior to cold storage and/or grapes are packed with pads releasing sulfur dioxide (Luvisi et al., 1992; Leesch et al., 2014). Due to the problematic use of sulfur dioxide, there are several reports about alternative methods, including application of ethanol after harvest (Karabulut et al., 2003), ethanol in conjunction with chitosan or calcium chloride (Romanazzi et al., 2007; Chervin et al., 2009), organic salts (Nigro et al., 2006), controlled atmosphere (Crisosto et al., 2002), or ozone (Palou et al., 2002; Feliziani et al., 2014). However, few of these methods are used at a commercial scale (Romanazzi et al., 2012). Recently, Teles et al. (2014) reported that 40% CO_2 for 48 h pre-storage treatment followed by controlled atmosphere during subsequent storage markedly reduced gray mold incidence. High CO_2 pre-storage alone limited disease incidence both in naturally and artificially infected grapes, but it was more effective when combined with CA in cold storage. In another study, the use of ozone gas followed by sulfur dioxide was examined (Feliziani et al., 2014). The combination of a single initial sulfur dioxide fumigation, followed by continuous low level of ozone during cold storage, was effective. Also ozone gas was effective in cold storage between biweekly sulfur dioxide fumigations. Both approaches controlled postharvest gray mold of table grapes and matched the effectiveness of the commercial practice of initial and weekly sulfur dioxide fumigations. They are of value since they reduced the amount of sulfur dioxide currently applied by half or more.

4. Potential of alternative strategies for controlling postharvest gray mold

Synthetic conventional fungicide treatment has been the primary strategy for managing postharvest diseases. However, there are many risks associated with these chemicals, including the development of fungicide resistance (Fillinger et al., 2008), mounting health concerns of consumers and health authorities leading to the demand to reduce human and environmental exposure to chemicals, and increased restrictions imposed by regulatory agencies on specific agro-chemicals and/or their allowable residues, especially after harvest. Furthermore, some of these chemicals are expensive. These issues have caused a significant research effort during the past twenty-five years to develop effective and useful alternative technologies to the synthetic fungicides to preserve quality and prolong the storage and shelf life of fruit. Innovations in this area can be grouped in four categories of treatments: i) microbial biocontrol agents (BCAs); ii) natural antimicrobials; iii) disinfecting agents; and iv) physical means. Among these, considerable work focused on the use of various microbial antagonists (yeasts and bacteria) that occur naturally on fruit surfaces and disrupt the ability of postharvest pathogens to establish infections in wounded fruits. Gray mold is one of the main targets of these antagonists.

Preharvest application of alternative strategies

A number of antagonistic microorganisms were suggested for use in the field before harvest to protect the crop from postharvest gray mold infections (Sharma et al., 2009; Feliziani and Romanazzi, 2013; Liu et al., 2013; Mari et al., 2014) (Tab. 1).

In a study aimed to characterize the effect of cropping system on epiphytic microbial community on grapes, Schmid et al. (2011) showed that in organically grown grapevines, the number of antagonistic species, such as *Aureobasidium pullulans*, was enhanced. *A. pullulans* was reported as the active ingredient in different biocontrol products to control *B. cinerea* (Boniprotect and Botector; bio-ferm, Tulln, Austria). Recently, major companies involved in crop protection (including Syngenta, Bayer, and BASF) have been investing in the field of biocontrol, natural compounds, and resistance inducers, because of consumer demand for fruit free of pesticide residues along with increased restrictions imposed by legislation. They realize that the market of organic agriculture is growing and it is time to develop products for it. In conventional agriculture, the introduction of biological control of postharvest diseases is not extensive since their effectiveness is often relatively low and not always consistent when compared to the chemical control. In the field, yeasts and bacteria are exposed to a wide array of stressful environmental conditions and their viability and effectiveness are challenged by high temperature, freeze/spray drying (desiccation), and oxidative stress. Combination of yeast and bacteria with other antimicrobial compounds could be an effective method for improving biocontrol performance. Combinations of salts, such as bicarbonates (Droby et al., 2003; Qin et al., 2015), and natural compounds, such as chitosan (Meng et al., 2010), have reported to improve the performance of biocontrol agents.

The use of organic and inorganic salts before harvest has been increasingly popular in several organic crops (Nigro et al., 2006; Feliziani et al., 2013a; Khamis and Sergio, 2014). The application of calcium chloride is widely used in southern Italy (Nigro et al., 2006) and it can be considered as one of the few examples of success of preharvest treatment alternatives to conventional fungicides to control postharvest decay on table grapes (Romanazzi et al., 2012). However, these salts can alter the rate of maturity and leave a visible residue on the berry, that harms their marketability. A delay in ripening was caused by preharvest calcium chloride applications to ‘Italia’ grapes (Nigro et al., 2006). Conversely, application of potassium salts enhanced maturity of ‘Thompson Seedless’ grapes (Feliziani et al., 2013b; Obenland et al., 2015).

Postharvest application of alternative strategies

The research on BCAs for postharvest use resulted in several commercial products able to control *B. cinerea* (Droby et al., 2009; Nunes, 2012; Feliziani and Romanazzi, 2013; Liu et al., 2013; Mari et al., 2014). These products (e.g. Shemer, Candifruit, Boniprotect, Yield Plus, Nexy, Pantovital, Biosave) have reached the market and their use has been promising (Feliziani and Romanazzi, 2013; Mari et al., 2014). However, because of the expense of registration and limited market for them as plant protection products, the number of registered BCAs is low as compared to the huge mass of research work that has been conducted in this field. This occurred because it is often particularly difficult to move from the discovery phase of an effective antagonist to its introduction as an approved and profitable commercial product. Some products were commercially available for limited time, because they were not successful, or because they were developed and sold by small companies that lacked a large market presence. However, the largest obstacle to their widespread use is the development of product that performs effectively and reliably under a wide array of conditions, and that integrates easily to a range of commercial processing systems. The reasons for the variability in performance may be due to the presence of pre-established infections, high levels of inoculum, poor storage of the biocontrol product prior to application, or improper application. Considerable efforts, however, have been made to integrate the use of postharvest biocontrol products into a production systems approach. The incorporation of various additives is a method that has been used to increase the applicability, effectiveness, and reliability of postharvest BCAs. Despite these limitations, some of the major producers of conventional fungicides have acquired specialized companies that develop BCAs. Currently research on the discovery and characterization of old and new BCAs able to control fruit gray mold is very active (Fiori et al., 2008; Saravanakumar et al., 2009; Oro et al., 2014).

A large variety of volatile compounds, plant extracts, and animal-derived materials with antifungal activity have been reported. Plant volatiles such as acetaldehyde, benzaldehyde, benzyl alcohol, ethanol, methyl salicylate, ethyl benzoate, ethyl formate, hexanal, (E)-2-hexenal, lipoxygenases, jasmonates, allicin, glucosinolates and isothiocyanates have been shown to inhibit *B. cinerea* infection on various commodities when tested under laboratory and small scale conditions (Tripathi and Dubey, 2004). Although proven effective at the level of laboratory and small-scale practical experiments, their efficacy needs confirmation under large scale and commercial conditions, and safety issues need to be addressed. The use of essential oils is getting interest for the control of postharvest decay (Sivakumar and Bautista-Baños, 2014). These compounds were reported to control gray mold of table grapes (Abdollahi et al., 2010, 2012), and were applied alone or together with other treatments (Sivakumar and Bautista-Baños, 2014). In the case of essential oils, issues such as formulation, method of application, phytotoxicity, and organoleptic quality should be taken in consideration. Treatments with emulsions of 1% essential oil from oregano, savory and thyme showed significant efficacy in reducing diameters of lesions caused by *B. cinerea* in 4 cultivars of apple; while the same essential oil emulsions tested at 10% were phytotoxic for all the apple cultivars evaluated (Lopez-Reyes et al., 2010). Among animal-derived compounds, treatment with chitosan was effective in the control of preharvest gray mold in wine grapes (Elmer and Reglinski, 2006), and in the management of postharvest gray mold on different fruits (Romanazzi et al., 2015).

Disinfecting agents (ethanol, acetic acid, electrolyzed oxidizing water) have been used for fruit surface sterilization, mainly when the process of washing is included in postharvest fruit packaging. Acetic acid was successfully used as fumigant to control postharvest decay of table grapes (Sholberg et al., 1996), as well as ethanol (Mlikota Gabler et al., 2005). The application of electrolyzed oxidizing water is effective in disinfection of water used in packinghouses operations and has shown to decrease conidia contamination of different pathogens, including *B. cinerea* (Guentzel et al., 2010). However, these alternatives have been tested only in the laboratory or in a small scale tests and further research is necessary to assess their potential issues such as phytotoxicity and/or their possible integration into current commercial practices (Romanazzi et al., 2012).

The use of physical means (UV-C irradiation, ozone, CA/MA, hypobaric or hyperbaric treatments) has been demonstrated to be effective in controlling gray mold on table grapes (Romanazzi et al., 2012). These control means have the advantage in that they avoid direct contact with the fruit (Sanzani et al., 2009), although often their effect is maintained last only as long as they are applied. Among physical means, heat treatment could reduce the application dosage of fungicides. When pear fruit were immersed for 3 min in water at the temperature of 50 °C mixed with the fungicide fludioxonil, a reduced concentration of the active ingredient was required to achieve a control of gray mold comparable to the control obtained with the full dosage of the unheated fungicide (Schirra et al., 2008).

A strategy to further improve the effectiveness of alternative control methods is the integration of different approaches. However, once a treatment is considered effective, it is necessary to carefully verify its potential introduction at a commercial scale in the packinghouse, transport and market chain (Romanazzi et al., 2012). To have effectiveness comparable to the conventional synthetic fungicides the combination of two or more alternative approaches may be needed to accomplish commercially acceptable control of postharvest decay. Several combinations were applied in the case of gray mold. For example, application of hydroxypropyl methylcellulose and beeswax edible coatings reduced gray mold of stored tomatoes (Fagundes et al. 2014) and the application of garlic extract and clove oil decreased infections of *B. cinerea* on apples (Daniel et al., 2015). However, effectiveness in the lab needs to be confirmed in large-scale tests and the existence of possible negative effects needs to be evaluated. Some studies concerning the effectiveness of alternative strategies present only disease severity data. However, an alternative that only reduces disease severity but does not reduce disease incidence is not commercially acceptable because the consumers and industry need is to have fruit lot with a very low level of decay incidence. For example, a maximum 0.5% infected berries is the threshold in the inspection standards for table grapes in California; if exceeded, the grapes cannot be shipped (1999, USDA Agricultural Marketing Service).

5. Concluding remarks and future challenges

Postharvest decay caused by gray mold has great economic importance and in some cases can lead to complete loss of the product. Reducing these losses to a level that is acceptable still poses a great challenge for producers, packers, and marketing at the wholesale and retail levels. In this regard, gray mold remains a challenge to control in certain highly perishable crops, such as small berries.

Extensive research has been done and will continue in the future to find effective management technologies and innovative approaches for the control of gray mold on fresh fruit and vegetables after harvest. Most of the efforts, however, have been devoted to the development of management programs at the preharvest level. Although applications of conventional fungicides constitute the most common practice for controlling gray mold in the field/orchard or in the packinghouse, their use after harvest on fruits is not allowed in many countries. Their continued use as preharvest treatments has come under increased scrutiny and their future as a control strategy is somewhat questionable. This is because of problems associated with (1) failure to effectively control pre and postharvest gray mold due to development of fungicide resistance; (2) consumers desire to reduce human and environmental exposure to chemicals; and (3) increased restrictions imposed by marketing chains and governmental regulatory agencies on the use and food residues of agro-chemicals in fresh agricultural commodities. These have been the driving forces for the development of postharvest disease control measures that do not rely on conventional fungicides. Currently, the use of alternative methods as stand-alone treatments for the control of postharvest gray mold, however, does not provide the efficacy and consistency required for commercial situations.

B. cinerea uses several modes of infection to attack fruit and vegetables before and after harvest. To increase control of these infections, it is important to influence the process of infection at different levels: the pathogen, the microenvironment, and the host. For example, application of a BCA or any other alternative method at a time that prevents establishment of the pathogen in the host tissue, given that the attachment of pathogen propagules to the host surfaces and the early stages of germination are critical to successful infection. The microenvironment (e.g. surface wounds) can also be altered to directly or indirectly affect the pathogen. The pH and nutritional composition of the infection site can be manipulated by the addition of salts, organic acids, or surfactants/adjuvants. In certain crops, surface injuries can be cured to resist infection by various thermal treatments, and subsequently the chances for infection are lowered. Susceptibility of the commodity (host) may also be reduced by changing its physiology using various treatments to either retard senescence or induce natural resistance.

It is anticipated that the continuing withdrawal of key synthetic postharvest fungicides from the market, due to exclusion by regulatory agencies or the high-cost of registration, will lead to an absence of effective conventional chemical tools for reducing postharvest losses due to gray mold. Hence, the use of alternative control methods is expected to gain popularity in the coming years and become more widely accepted as a component of an integrated strategy to manage postharvest diseases. Along with this approach, effective alternative control strategies would rely on elements such as: (i) classical microbial antagonists; (ii) natural plant resistance; (iii) natural antimicrobials which are the product of a biological process; and (iv) combinations among the above cited methods such as thermal curing treatments, plant growth regulators, ethylene inhibitors, MA, CA, and heat treatments. Also, it is very important to reduce the inoculum load and conditions conducive to establishment of infections through well-established cultural and management practices.

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Tab. 1 – List of some commercial formulations based on BCA available on the market for the control of gray mold.

Trade name	**Microrganism**	**Company**	**County**
Shemer	*Metschnikowia fructicola*	Bayer/Koppert Biological Systems	Germany/Netherlands
Candifruit	*Candida sake*	IRTA (former Sipcam-Inagra)	Spain
Pantovital	*Pantoea agglomerans*	IRTA	Spain
Boni Protect /Botector	*Aureobasidium pullulans*	Bio-Ferm/Manica	EU (preharvest) Austria
Nexy	*Candida oleophila*	Lesaffre	France
Serenade	*Bacillus subtilis*	Bayer (former BASF)	Germany
Bio-Save	*Pseudomonas syringae*	Jet Harvest Solutions	USA
Yield Plus	*Cryptococcus albidus*	Lallemand	South Africa
Amylo-X	*Bacillus amyloliquefaciens*	Biogard CBC	Italy

Figure captions

Fig. 1 – Gray mold development on some fruits. From left to right, in the first row: quince strawberry, kiwi, raspberry. Second row: baby kiwi, table grapes, pomegranate, blueberry. Third row: persimmon, peach (infection on the left), orange, sweet cherry.

Fig. 2 – On the left, infection in strawberry starting from sepal area, where it is possible to see a petal residue. On the right, strawberry box in a store with gray mold infection, with necrotized (bottom) and healthy (top) petals. In the middle, an infection from *Penicillium* spp.

Fig. 3 – Black continuous line indicates the ideal dynamic of temperatures during cold storage of fruit. Blue dotted line indicates accidental increase in temperatures that should be avoided, as any interruption of the cold chain can allow the development of an infection from quiescent pathogen.

Fig. 4 – Harvest of table grapes in Southern Italy (top left). Bunches are packed directly in wood boxes (top right). Cold proof containers used to harvest wild strawberries with ice pad on the bottom (bottom left) and cardboard onto which strawberry boxes are placed in (bottom right).

Fig. 1

Fig. 2

Fig. 3

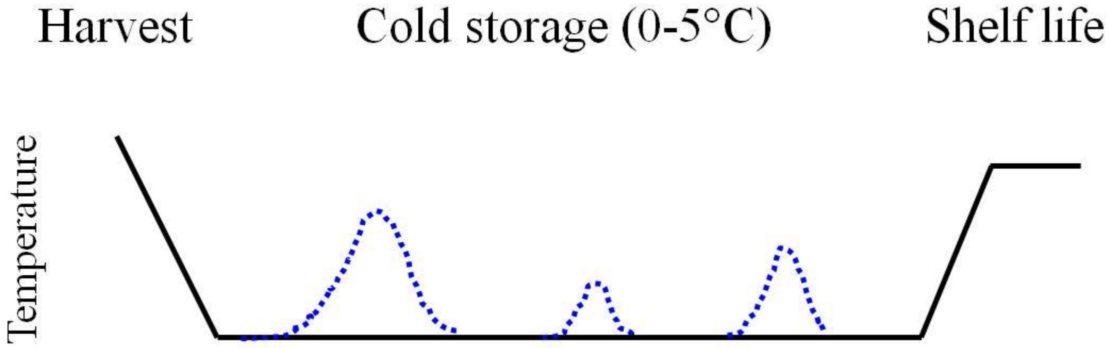

Fig. 4