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(Article begins on next page)

1 **Integrated management of postharvest gray mold on fruit crops**

2
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15
16 **Highlights**

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

22 **Abstract**

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

44

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

47

48 **1. Introduction**

49 In a report by the United Nations Food and Agricultural Organization, it was estimated that
50 one-third of the food produced worldwide for human consumption is lost after harvest
51 (Gastavsson et al., 2011). Losses inflicted throughout the supply chain due to pathogen-
52 induced diseases are the major component of food wastage. Pathogen attack may take place
53 during harvesting and subsequent handling, storage, marketing, and after consumer purchase.
54 Among these pathogens, *Botrytis cinerea*, the cause of gray mold, is considered one of the
55 most important postharvest decays of fresh fruit and vegetables (Droby and Lichter, 2004;
56 Elad et al., 2015). According to a recent review, *B. cinerea* ranked second into the world Top
57 10 fungal plant pathogens list based on scientific and economic importance (Dean et al.,
58 2012). *B. cinerea* is an important postharvest pathogen because of the conducive conditions
59 prevailing throughout the postharvest handling chain, including injuries, high humidity,
60 senescing plant tissue and high sugar content. Major postharvest losses due to *B. cinerea*
61 occur in a long list of fresh fruits: apple, blackberry, blueberry, currant, grape, kaki, kiwi,
62 pear, pomegranate, quince, raspberries, strawberry, grapes and many others (Droby and
63 Lichter, 2004; Romanazzi and Feliziani, 2014) (Fig. 1). In other fruits (*e.g.* apricot, lemon,
64 orange, peach, plum, sweet cherry), although it is not the main pathogen, it is still capable of
65 causing considerable postharvest losses.

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

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

90 After harvest, *B. cinerea* is capable of infecting fruits and vegetables through the
91 damaged tissue in the stem end, which is rich in nutrient exudates. Stem end infections can
92 develop and spread to the entire fruit. This mode of infection is mostly known in kiwifruit as
93 the majority of fruits are infected through picking wounds (Michailides and Elmer, 2000). In
94 pome fruit, gray mold infections can originate from wounds, stem punctures, or the stem or
95 calyx end of the fruit (Sutton et al., 2014). Although *B. cinerea* is a common saprophyte on
96 decaying organic matter on the orchard floor, gray mold is seldom seen in the field on pome
97 fruit, while it becomes visible during storage. Indeed, conidia of *B. cinerea* are carried into the
98 storage on bins and containers, transported with other organic matters, air-dispersed or

99 commonly water-dispersed in flumes in packinghouses (Sutton et al., 2014). In addition, there
100 is substantial evidence indicating an important role of insects in mediating contamination of
101 harvested agricultural commodities with *B. cinerea* inoculum. In this relation, *Thrips*
102 *obscuratus* and honeybees were shown to facilitate deposition of conidia into fruit injuries
103 and surface cracks (Michailides and Elmer, 2000).

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

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

115

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

117 In conventional agriculture, we cannot avoid the use of synthetic fungicides, and there is a
118 long list of registered active ingredients on different crops for gray mold control for both pre-
119 and postharvest use (Romanazzi and Feliziani, 2014). However, growers are currently
120 stimulated to adopt alternative approaches as stand-alone treatments or in conjunction with
121 conventional fungicides. This development is taking place due to several reasons, including
122 requirements from supermarket chains for commodities with low number of residual
123 pesticides (e.g. a maximum of four to five active ingredients) used during production and
124 subsequent postharvest handling. In addition, in some cases, due to the limited number of

125 active ingredients on the fruit, the overall level of residues should not exceed 70-80% of the
126 total allowed maximum residue limits (MRLs). For example, if we have four residual active
127 ingredients, each should be present on average at the level of 20% of the allowed MRL.
128 Unfortunately, these commercial policies do not take into consideration that the presence of
129 fungicide residues in the fruit below certain thresholds will allow the pathogen to develop
130 after harvest, resulting in significant losses throughout the handling chain. Furthermore, the
131 presence of sub-lethal concentrations of fungicides in the fruit could increase the occurrence
132 of mutations for fungicide resistance in fungal population, as at low doses of fungicides, the
133 frequency of mutations is usually higher, due to the larger size of the sensitive pathogen
134 population (van den Bosch et al., 2011).

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

148 Recently, there has been an increase in the number of products available and registered
149 that promote plant defense; these contain living organisms (biocontrol agents) or chemical
150 plant stimulators such as glutathione, oligosaccharides, laminarin, and chitosan, which are

151 known to inhibit postharvest decay. Most usually they have dual inhibitory effects on the
152 disease due to direct inhibition of pathogens and induction of defense mechanisms in the host
153 tissues. As an example, *Metschnikowia pulcherrima* depleted iron in apple wounds resulting
154 in decreased infection by *B. cinerea* (Saravanakumar et al., 2008). Treatment with chitosan,
155 benzothiadiazole, and a mixture of calcium and organic acids reduced pathogen growth and
156 increased the expression of enzymes linked to defense mechanisms in strawberry tissues
157 (Landi et al., 2014). Regulation EU 2014/563 included chitosan chloride as the first member
158 on a basic substance list of plant protection products (as planned with Regulation EU
159 2009/1107), so it can be used in plant disease management since July 1, 2014.

160

161 **3. Management of gray mold on stored products**

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

174 Usually fresh fruit are stored at temperatures between 0 to 10 °C, depending on the
175 commodity, for a few days (small berries), up to two months (for some table grape cultivars
176 as 'Crimson Seedless'), or even many months (for kiwifruit, apples or pears). Reduction of

177 the temperature in a period as rapidly as possible is indispensable for perishable fruits and
178 vegetables. For example, highly perishable wild strawberry (*Fragaria vesca*) fruits are
179 harvested in the field directly into containers and placed in a cold proof box with an ice pad
180 on the bottom (Fig. 4). Under these conditions, the fruits can have a shelf life of three to four
181 days. In Italy, some packinghouses pay a higher price to growers when strawberry fruits are
182 harvested in the early morning. It was estimated that the harvest of these fruits for every hour
183 after 10 AM resulted in one day shorter shelf life (G. Savini, personal communication). Table
184 grapes are usually packed directly in the field (Fig. 4) to minimize handling that removes their
185 waxy bloom and causes detachment of berries from the clusters, then they are pre-cooled
186 within a few hours using forced air ventilated rooms to reduce the temperature to about 0-1
187 °C. High humidity that occurs within table grape packages minimizes water loss but it can
188 cause condensation to occur if the cold chain is broken and the cold fruit are placed in a warm
189 environment. High humidity and free water conditions facilitate conidial germination and
190 penetration through cracks or microlesions that can occur during harvest and subsequent
191 handling. These conditions are ideal for infection because fruit tissues after harvest and during
192 cold storage are less reactive due to weakening of defense mechanisms. Once decay has
193 developed, it can progress rapidly by contact and aerial mycelial growth to nearby healthy
194 fruits. This type of infection is known as nesting, because of clustering of infected fruit close
195 to a source of mycelial inoculum. Low temperatures during storage slow but do not stop the
196 growth of *B. cinerea* since it is able to grow at a wide range of temperatures, from 0.5 °C to
197 32 °C (Coertze and Holz, 1999).

198 The use of conventional synthetic fungicides for controlling pathogens on most
199 commodities is prohibited after harvest in most EU countries. In grapes and some other fruits,
200 however, the use of sulfur dioxide during storage is permitted since it is considered as
201 processing aid and not as a fungicide. When it was recognized that hypersensitive reactions
202 occurred in people sensitive to sulfites in food, sulfur dioxide was classified as

203 a pesticide and MRL 10 mg kg⁻¹ of sulfite residues in table grapes was established by the U.S.
204 Environmental Protection Agency (Anonymous, 1989). In California, many organic growers
205 use ozone fumigation of grapes after harvest (Feliziani et al., 2014), and this technology has
206 also been used to some extent among packinghouses working with conventionally grown
207 grapes. An interesting side of ozone treatment resides in its oxidant activity that can reduce
208 fungicide residues on the berries (Karaca et al., 2012; Mlikota Gabler et al., 2010). Sulfur
209 dioxide can damage the fruit by causing surface cracks (Zoffoli et al., 2008) and bleaching
210 color from red cultivars (Luvisi et al., 1992). In addition, the treatment is non-selective in
211 eliminating the vast majority of epiphytic microflora left on the fruit without natural
212 protection allowing gray mold to develop more readily compared to non-fumigated fruit. To
213 achieve good levels of control, usually sulfur dioxide is applied in storage room of grapes
214 weekly, following a first treatment during cooling prior to cold storage and/or grapes are
215 packed with pads releasing sulfur dioxide (Luvisi et al., 1992; Leesch et al., 2014). Due to the
216 problematic use of sulfur dioxide, there are several reports about alternative methods,
217 including application of ethanol after harvest (Karabulut et al., 2003), ethanol in conjunction
218 with chitosan or calcium chloride (Romanazzi et al., 2007; Chervin et al., 2009), organic salts
219 (Nigro et al., 2006), controlled atmosphere (Crisosto et al., 2002), or ozone (Palou et al.,
220 2002; Feliziani et al., 2014). However, few of these methods are used at a commercial scale
221 (Romanazzi et al., 2012). Recently, Teles et al. (2014) reported that 40% CO₂ for 48 h pre-
222 storage treatment followed by controlled atmosphere during subsequent storage markedly
223 reduced gray mold incidence. High CO₂ pre-storage alone limited disease incidence both in
224 naturally and artificially infected grapes, but it was more effective when combined with CA in
225 cold storage. In another study, the use of ozone gas followed by sulfur dioxide was examined
226 (Feliziani et al., 2014). The combination of a single initial sulfur dioxide fumigation, followed
227 by continuous low level of ozone during cold storage, was effective. Also ozone gas was
228 effective in cold storage between biweekly sulfur dioxide fumigations. Both approaches

229 controlled postharvest gray mold of table grapes and matched the effectiveness of the
230 commercial practice of initial and weekly sulfur dioxide fumigations. They are of value since
231 they reduced the amount of sulfur dioxide currently applied by half or more.

232

233 **4. Potential of alternative strategies for controlling postharvest gray mold**

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

249

250 ***Preharvest application of alternative strategies***

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

254 In a study aimed to characterize the effect of cropping system on epiphytic microbial
255 community on grapes, Schmid et al. (2011) showed that in organically grown grapevines, the
256 number of antagonistic species, such as *Aureobasidium pullulans*, was enhanced. *A. pullulans*
257 was reported as the active ingredient in different biocontrol products to control *B. cinerea*
258 (Boniprotect and Botector; bio-ferm, Tulln, Austria). Recently, major companies involved in
259 crop protection (including Syngenta, Bayer, and BASF) have been investing in the field of
260 biocontrol, natural compounds, and resistance inducers, because of consumer demand for fruit
261 free of pesticide residues along with increased restrictions imposed by legislation. They
262 realize that the market of organic agriculture is growing and it is time to develop products for
263 it. In conventional agriculture, the introduction of biological control of postharvest diseases is
264 not extensive since their effectiveness is often relatively low and not always consistent when
265 compared to the chemical control. In the field, yeasts and bacteria are exposed to a wide array
266 of stressful environmental conditions and their viability and effectiveness are challenged by
267 high temperature, freeze/spray drying (desiccation), and oxidative stress. Combination of
268 yeast and bacteria with other antimicrobial compounds could be an effective method for
269 improving biocontrol performance. Combinations of salts, such as bicarbonates (Droby et al.,
270 2003; Qin et al., 2015), and natural compounds, such as chitosan (Meng et al., 2010), have
271 reported to improve the performance of biocontrol agents.

272 The use of organic and inorganic salts before harvest has been increasingly popular in
273 several organic crops (Nigro et al., 2006; Feliziani et al., 2013a; Khamis and Sergio, 2014).
274 The application of calcium chloride is widely used in southern Italy (Nigro et al., 2006) and it
275 can be considered as one of the few examples of success of preharvest treatment alternatives
276 to conventional fungicides to control postharvest decay on table grapes (Romanazzi et al.,
277 2012). However, these salts can alter the rate of maturity and leave a visible residue on the
278 berry, that harms their marketability. A delay in ripening was caused by preharvest calcium
279 chloride applications to 'Italia' grapes (Nigro et al., 2006). Conversely, application of

280 potassium salts enhanced maturity of ‘Thompson Seedless’ grapes (Feliziani et al., 2013b;
281 Obenland et al., 2015).

282

283 ***Postharvest application of alternative strategies***

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

305 discovery and characterization of old and new BCAs able to control fruit gray mold is very
306 active (Fiori et al., 2008; Saravanakumar et al., 2009; Oro et al., 2014).

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

327 Disinfecting agents (ethanol, acetic acid, electrolyzed oxidizing water) have been used for
328 fruit surface sterilization, mainly when the process of washing is included in postharvest fruit
329 packaging. Acetic acid was successfully used as fumigant to control postharvest decay of
330 table grapes (Sholberg et al., 1996), as well as ethanol (Mlikota Gabler et al., 2005). The

331 application of electrolyzed oxidizing water is effective in disinfection of water used in
332 packinghouses operations and has shown to decrease conidia contamination of different
333 pathogens, including *B. cinerea* (Guentzel et al., 2010). However, these alternatives have been
334 tested only in the laboratory or in a small scale tests and further research is necessary to assess
335 their potential issues such as phytotoxicity and/or their possible integration into current
336 commercial practices (Romanazzi et al., 2012).

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

346 A strategy to further improve the effectiveness of alternative control methods is the
347 integration of different approaches. However, once a treatment is considered effective, it is
348 necessary to carefully verify its potential introduction at a commercial scale in the
349 packinghouse, transport and market chain (Romanazzi et al., 2012). To have effectiveness
350 comparable to the conventional synthetic fungicides the combination of two or more
351 alternative approaches may be needed to accomplish commercially acceptable control of
352 postharvest decay. Several combinations were applied in the case of gray mold. For example,
353 application of hydroxypropyl methylcellulose and beeswax edible coatings reduced gray mold
354 of stored tomatoes (Fagundes et al. 2014) and the application of garlic extract and clove oil
355 decreased infections of *B. cinerea* on apples (Daniel et al., 2015). However, effectiveness in
356 the lab needs to be confirmed in large-scale tests and the existence of possible negative effects

357 needs to be evaluated. Some studies concerning the effectiveness of alternative strategies
358 present only disease severity data. However, an alternative that only reduces disease severity
359 but does not reduce disease incidence is not commercially acceptable because the consumers
360 and industry need is to have fruit lot with a very low level of decay incidence. For example, a
361 maximum 0.5% infected berries is the threshold in the inspection standards for table grapes in
362 California; if exceeded, the grapes cannot be shipped (1999, USDA Agricultural Marketing
363 Service).

364

365 **5. Concluding remarks and future challenges**

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

371 Extensive research has been done and will continue in the future to find effective
372 management technologies and innovative approaches for the control of gray mold on fresh
373 fruit and vegetables after harvest. Most of the efforts, however, have been devoted to the
374 development of management programs at the preharvest level. Although applications of
375 conventional fungicides constitute the most common practice for controlling gray mold in the
376 field/orchard or in the packinghouse, their use after harvest on fruits is not allowed in many
377 countries. Their continued use as preharvest treatments has come under increased scrutiny and
378 their future as a control strategy is somewhat questionable. This is because of problems
379 associated with (1) failure to effectively control pre and postharvest gray mold due to
380 development of fungicide resistance; (2) consumers desire to reduce human and
381 environmental exposure to chemicals; and (3) increased restrictions imposed by marketing
382 chains and governmental regulatory agencies on the use and food residues of agro-chemicals

383 in fresh agricultural commodities. These have been the driving forces for the development of
384 postharvest disease control measures that do not rely on conventional fungicides. Currently,
385 the use of alternative methods as stand-alone treatments for the control of postharvest gray
386 mold, however, does not provide the efficacy and consistency required for commercial
387 situations.

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

401 It is anticipated that the continuing withdrawal of key synthetic postharvest fungicides
402 from the market, due to exclusion by regulatory agencies or the high-cost of registration, will
403 lead to an absence of effective conventional chemical tools for reducing postharvest losses
404 due to gray mold. Hence, the use of alternative control methods is expected to gain popularity
405 in the coming years and become more widely accepted as a component of an integrated
406 strategy to manage postharvest diseases. Along with this approach, effective alternative
407 control strategies would rely on elements such as: (i) classical microbial antagonists; (ii)
408 natural plant resistance; (iii) natural antimicrobials which are the product of a biological

409 process; and (iv) combinations among the above cited methods such as thermal curing
410 treatments, plant growth regulators, ethylene inhibitors, MA, CA, and heat treatments. Also, it
411 is very important to reduce the inoculum load and conditions conducive to establishment of
412 infections through well-established cultural and management practices.

413

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418

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- 600

601

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

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

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614 **Figure captions**

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

619

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

623

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

628

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

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635 **Fig. 1**

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643 **Fig. 2**

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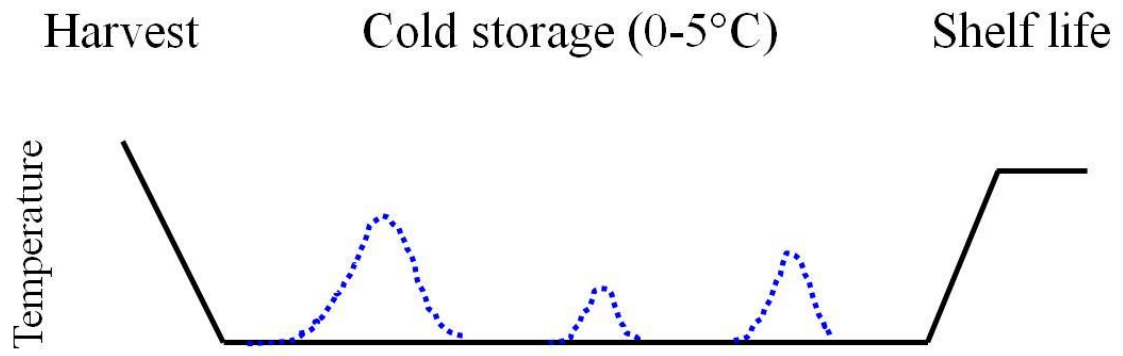
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651 **Fig. 3**

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658 **Fig. 4**

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