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Integrated management of postharvest gray mold on fruit crops

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

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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

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 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% CO_2 for 48 h pre-

222   storage treatment followed by controlled atmosphere during subsequent storage markedly

223   reduced gray mold incidence. High CO_2 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

418

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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	*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

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614   **Figure captions**

615

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

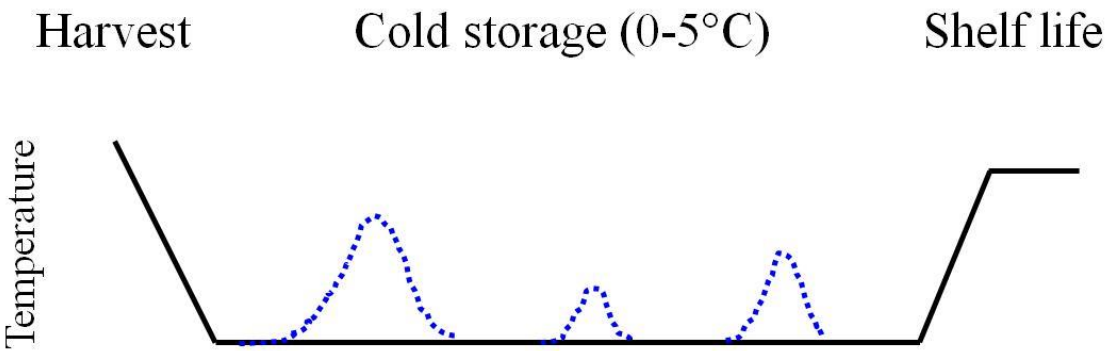631   on the bottom (bottom left) and cardboard onto which strawberry boxes are placed in (bottom

632   right).

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Fig. 1

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643 **Fig. 2**

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Fig. 3

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Harvest   Cold storage (0-5°C)   Shelf life

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Fig. 4

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