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Review

Brown rot strikes Prunus fruit: an ancient fight almost always lost

Leandro Oliveira Lino, Igor Pacheco, Vincent Mercier, Franco Faoro, Isabelle Bonard, Daniele Bassi, and Benedicte Quilot

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1 **Brown rot strikes *Prunus* fruit: an ancient fight almost always**
2 **lost**

3 **Leandro Oliveira Lino^{1,2,§}, Igor Pacheco^{3,5§,*}, Vincent Mercier⁴, Franco**
4 **Faoro⁵, Daniele Bassi⁵, Isabelle Bornard⁶, Bénédicte Quilot-Turion².**

5 ¹ CAPES Foundation, Ministry of Education of Brazil, Brasília - DF 70040-020,
6 Brazil.

7 ² INRA, UR1052 Génétique et Amélioration des Fruits et Légumes, Avignon,
8 F-84000, France

9 ³ INTA, Universidad de Chile. Av. El Líbano 5524, Macul, Santiago, Chile.

10 ⁴ Unité Expérimentale de Recherches Intégrées, INRA, Domaine Gotheron, F-
11 26320 Saint Marcel-lès-Valence France

12 ⁵ Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di
13 Milano, Via Celoria 2, 20133 Milano, Italy

14 ⁶ INRA, Unité de Pathologie Végétale, UR0407, Domaine St. Maurice, BP 94,
15 F-84140 Montfavet, France

16 [§] equally contributing authors

17 * Corresponding Author: Igor Pacheco. Email: igor.pacheco@inta.uchile.cl

18

19 **Abstract:** Brown rot (BR) caused by *Monilinia* spp., has been an economic
20 problem for the stone fruit market due to the dramatic losses, with the biggest
21 losses mainly during the postharvest period. There is much literature about
22 basic aspects of *Monilinia* spp. infection, which indicates that environment
23 significantly influences its occurrence in the orchard. However, progress is
24 needed to sustainably limit this disease: the pathogen is able to develop
25 resistance to pesticides and most of BR resistance research programs in plant
26 models perish. Solving this problem becomes important due to the need to
27 decrease chemical treatments and reduce residues on fruit. Thus, research
28 has recently increased, exploring a wide range of disease control strategies
29 (e.g. genetic, chemical, physical). Summarizing this information is difficult, as
30 studies evaluate different *Monilinia* and *Prunus* model species, with diverse
31 strategies and protocols. Thus, the purpose of this review is to present the
32 diversity and distribution of agents causing BR, focusing on the biochemical
33 mechanisms of *Monilinia* spp. infection both of the fungi and of the fruit, and
34 report on the resistance sources in *Prunus* germplasm. In this review, we
35 comprehensively compile the information currently available to better
36 understand mechanisms related to BR resistance.

37

38 **Keywords:** *Monilinia* spp., brown rot, *Prunus*, QTL

39

40

41 1 Introduction

42 The genus *Prunus* has hundreds of species with some economically
43 important members, including the cultivated almond, peach, plum, cherry and
44 apricot. The five most important countries for the production of these fruits are
45 China (10.7MTon), the United States (2.9MTon), Italy (1.9MTon), Spain
46 (1.4MTon) and Greece (0.8MTon)¹.

47 Different cropping practices are employed for the production of this variety
48 of fruit, according to their different environmental and nutritional requirements.
49 In addition, the broad range of pests has to be controlled to reach a high
50 quality final product. This latter point is a crucial issue in current fruit cropping,
51 since the demand of fresh fruit with reduced residual quantities and the
52 regulation of fungicide use has become stricter in EU countries, after the
53 release of the European Directive 2009/128/EC which indicates the use of
54 Integrated Pest Management (IPM) as mandatory^{2,3}. In the United States, the
55 government has strongly promoted IPM in order to reduce chemical pesticide
56 input with the creation of Regional IPM Centres, resulting in progressive
57 decreases in pesticide use and toxicity for humans^{4,5}. Reduced pesticide
58 applications have been advised in China⁶, Brazil and other countries⁷.

59 Among the plethora of pathogenic agents attacking *Prunus* crops (and
60 other Rosaceae), brown rot (BR) is the economically most important
61 disease of stone fruits⁸. *Monilinia* spp. are able to infect various plant organs,
62 causing blossom blight, twig blight, and BR in immature and mature fruits, the
63 latter being the most sensitive host phenological phase. The relatively long
64 period of incidence, extending from bloom to post-harvest, the multiplicity of

65 climatic and cropping factors favouring disease spread, the occurrence of
66 diverse fungicide resistances in some BR agents and the poor availability of
67 host resistance, result in severe, unavoidable and sometimes unpredictable
68 losses in the fruit market ⁷. According to Martini and Mari ⁹, the worldwide
69 yearly value of *Monilinia* losses is 1,7 thousand million Euro; in the United
70 States, yearly losses are estimated to be 170 million USD for peach, cherry
71 and plum production¹⁰; and in Australia yearly losses are estimated at 1
72 million AUD for peach and apricot crops ¹¹. Under laboratory conditions, BR
73 can result in losses of over 60% of peaches and nectarines after 5 days of
74 infection at room temperature.

75 To avoid these damages, *Monilinia* spp. diseases are controlled by
76 chemical methods. Fungicide applications are necessary to diminish BR
77 damage in humid seasons, but lead to sustainability challenges in pome and
78 stone fruit cropping, as there are many fungicide-resistant strains (*Monilinia*
79 *fructicola*, see below). An important research field has been dedicated to the
80 epidemiology of BR, as well as aspects related with traditional chemical
81 control and emerging alternative control strategies (e.g. tree management)
82 ^{12,13}, compatible with IPM and organic agriculture (biologic agents, post
83 harvest bio-chemical-physical agents). These topics are thoroughly reviewed
84 and discussed in recent publications ^{14,9}. Significant efforts are being invested
85 in order to characterize and enhance fruit resistance to BR for the generation
86 of new varieties with reduced requirements of application of exogenous
87 methods for BR control. These have been included as important objectives of
88 international collaborative initiatives for new cultivar development around the

89 world, such as Fruit Breedomics European project and ROSBREED American
90 initiative.

91 In the present review, we will focus on stone fruit characteristics
92 conferring resistance to BR. For this aim, we compiled information from peer-
93 reviewed articles, congressional acts, and unpublished data obtained over
94 years working on this topic. After a brief description of the taxonomy,
95 morphology and geographic distribution of *Monilinia* species, we will focus on
96 fruit features representing points for the start of infection. We will examine the
97 steps of infection development and discuss the main biochemical and
98 molecular host factors for BR resistance in fruit. To finish, we will describe the
99 breeding programs aimed at enhancing BR resistance in stone fruit,
100 generating knowledge for the genetic dissection of fruit BR resistance.

101 **2 *Monilinia* spp. fungi cause brown rot**

102 2.1 Taxonomy

103 The agents causing BR are polytrophic fungi belonging to the Phylum
104 Ascomycota, Class Leotiomycetes, Order Helioteliales, Family
105 Sclerotiniaceae, Genus *Monilinia*. They attack members of the Rosaceae and
106 Ericaceae families^{15,16}. The generic name *Monilinia* includes those members
107 of *Sclerotinia* that produce moniloid conidia and pseudosclerotia.

108 Of the 35 species of the genus *Monilinia* Honey, three are the main
109 species that are pathogenic to pome and stone fruits: *Monilinia fructicola* (G.
110 Winter) Honey; *Monilinia laxa* (Aderhold & Ruhland) Honey and *Monilinia*
111 *fructigena* (Aderhold & Ruhland) Honey¹⁷. At least two species have been
112 described to be important pathogens of Ericaceae: *Monilinia vaccinium-*

113 *corymbosi* causing mummy berry of blueberry ¹⁸ and *Monilinia oxycocci*
114 causing cottonball of cranberry ¹⁹. According to phylogenetic analyses based
115 in rRNA sequences of *Monilinia* and *Sclerotinia* species, the separation of the
116 genus in two sections is consistent: *Junctoriae*, attacking Rosaceae hosts,
117 and *Disjunctoriae* attacking Ericaceae hosts ^{15,20,21}, moreover, partial
118 congruence found in the branching topologies of hosts and pathogen
119 phylogenies, lead to suggest the hypothesis of co-speciation between them ¹⁵.
120 In this review, we will focus on *Monilinia* spp. and BR in stone fruits.

121 The disease cycle of *Monilinia* species is represented in Figure 1.
122 Primary inoculum sources in the spring are overwintering BR fruit mummies
123 either on the tree, which produce asexual fruiting structure (sporodochia) and
124 spore (conidia) or on the orchard floor, which produce sexual fruiting
125 structures (apothecia) and spores (ascospores). The spores are dispersed by
126 wind and rain to susceptible host tissues, and germinate under favourable
127 wetness and temperature conditions. In general blossom blight reduces the
128 crop load in fruit crops, but it can destroy the crop at flowering in susceptible
129 almond cultivars. The infections of blossoms typically remain attached and the
130 infection spreads into the peduncle and down into the twig. The infection
131 continues with the formation of a twig canker that often develops a gumdrop
132 as a host response. Conidia form on infected tissue and serve as secondary
133 inoculum for infection of immature and mature fruit ²². Infections on immature
134 fruit, after the endocarp lignification, may give place to conidia, providing
135 additional inoculum.

136 2.2 Differentiation of *Monilinia* species

137 By observation with naked eye, it is possible to identify the differences
138 between the three agents of monilioses in fruit in orchard conditions ²³. *M.*
139 *fructigena* has colour ranging from white to light beige, large (1.5 mm on
140 average) conidiospores tufts, and disposition in concentric circles in the fruit.
141 *M. fructicola* has brown-coloured, medium size (1 mm on average)
142 conidiospores tufts and 10% black spots. *M. laxa* can be distinguished by
143 greenish-grey conidiospores tufts less than 0,5 mm on average that cover the
144 whole infected surface. However the differentiation in fruit between *M. laxa*
145 and *M. fructicola* may sometimes be difficult and the use of molecular
146 techniques is required (Figure 2).

147 Studies to identify the *Monilinia* species reported that, in culture medium
148 with potato dextrose and agar (PDA) at 22°C, *M. laxa* is characterized by
149 concentric rings of mycelium with lobbed margins, while in *M. fructigena* it is
150 possible to observe fragmented radial colonies. Differences in colony growth
151 rates between the three species were observed (20 – 25°C). The highest
152 growth rate on PDA was found for *M. fructicola*, followed by *M. fructigena* and
153 *M. laxa* respectively. However, *M. laxa* showed the biggest lesion growth rate
154 on peach fruit ⁸. In culture medium it is possible to analyse characters as
155 conidial size and germ tube morphology. These methods have been used
156 since 1920 and their simplicity makes them useful still ²². Differences in
157 conidia size among the species are reported. On average the conidia size of
158 *M. laxa* is smaller compared to *M. fructigena*, 13x9 µm and 22x12 µm,
159 respectively. *M. fructigena* produces one or two germ tubes per conidium, and

160 *M. laxa* and *M. fructicola* isolates consistently produce only one germ tube per
161 conidium⁸.

162 Several molecular biology techniques (mostly based in the Polymerase
163 Chain Reaction, PCR) have been used to develop reliable and sensitive
164 methods to identify and detect *Monilinia* species. Fulton and Brown²⁴,
165 proposed the study of the small sub unit of ribosomal DNA (rDNA) to
166 differentiate *Monilinia* isolates from the three major species. Many PCR
167 protocols for *Monilinia* spp. identification, based on the comparison of internal
168 transcribed spacers, sequence between the 18S small and the 28S rDNA
169 subunits of *Monilinia* genes, have been proposed^{22,25,26}. Ma et al²⁷ and Hu
170 et al⁸ reported a detection and identification method of *Monilinia* fungi based
171 on species-specific microsatellites^{8,27}. Identification methods based on
172 amplified fragment length polymorphism (AFLP) are also reported^{28,29}. In
173 addition, molecular techniques have been developed for species identification
174 on quiescent fruit infections of stone fruit³⁰, and for the early detection of
175 infections in cherry fruit³¹. In Banks et al³², monoclonal antibodies are
176 reported to be useful for identification and detection of *Monilinia* spp. in pome
177 and stone fruit³². Some of these approaches have set the basis for several
178 studies about morphological and molecular diversity of *Monilinia* spp.,
179 describing the geographical distribution and host range of the three main
180 species of *Monilinia* that caused BR of stone and pome fruits,^{33,34,35}.

181 2.3 Host range and distribution of *Monilinia* spp.

182 *M. fructigena* is an economically important BR-agent that has been
183 associated with European BR of pome fruits^{15,36}. However, its occurrence in

184 stone fruits has also been well documented in Europe ^{37,38}, Brazil ³⁹ and
185 China ⁶.

186 *M. laxa* has been historically associated with European blossom blight
187 and BR of stone ^{36,38} and pome fruit ^{40,41}. However in the last two decades it
188 has been also reported in different regions of the world, including Brazil ^{39,42},
189 United States ^{43,44,45}, China ⁶ and Iran ⁴⁶.

190 *M. fructicola* (G.Wint) is the most widely distributed species, occurring in
191 Asia, North and South America, New Zealand and Australia ^{7,47}. In Europe, it
192 was a quarantine pathogen until early 2014, when it was removed from the
193 European quarantine pest list due to its current spread in the following
194 countries: France ⁴⁸, Hungary ³⁴, Switzerland ^{49,50} Germany ⁵¹, Czech
195 Republic ⁵², Slovenia ⁵³, Italy ^{54,55} Austria (subsequently eradicated) ⁵⁶,
196 Poland ⁵⁷, Slovakia ⁵⁸, Serbia and Spain ³⁵.

197 The low genetic diversity found in Spanish and French populations of *M.*
198 *fructicola*, compared with American or New Zealand diversity, indicates few
199 and recent introduction events of the pathogen to Europe ⁵⁹. In addition to its
200 wide distribution, *M. fructicola* has been reported to infect other hosts such as
201 Cornelian cherry ⁶⁰ and others that do not belong to Rosaceae family, for
202 example grapes ⁶¹ and dragon fruit ⁶².

203 These three species share high levels of DNA similarities. *M. fructicola*
204 and *M. fructigena* exhibited 97,5% sequences identity while *M. laxa* and *M.*
205 *fructigena* displayed more than 99,1% for the *Cyt b* gene ⁶³. In this way, we
206 may expect that part of the knowledge acquired from one species may be
207 extrapolated to the other members of *Monilinia* genus.

208 A fourth species, *M. polystroma* (also called 'Asiatic Brown Rot') is
209 native of Japan, where it had been formerly confounded with *M. fructigena*. It
210 was described as a new species after finding significant biological and
211 morphological characteristics with respect to European isolates of *M.*
212 *fructigena*⁶⁴. Molecular differences between European and Japanese isolates
213 of *M. fructigena* were previously demonstrated, on the basis of the ITS region
214 of ribosomal DNA⁶⁵. *M. polystroma* has been reported to occur in pome and
215 stone fruit orchards from China⁶⁶, Poland⁵⁷ and Hungary⁶⁷.

216 Two other less-distributed *Monilinia* species are described. *M.*
217 *mumecola* was reported to infect *Prunus mume* in Japan⁶⁸, and be the causal
218 agent of the BR of papaya in Hubei, China in 2009^{8,69}. Finally, *M.*
219 *yunnanensis* has been recently designated as a new species causing BR in
220 Chinese peach orchards and, based on the DNA sequence similarity analyses
221 of marker genes, was found to be very close to *M. fructigena*⁸; this species is
222 also able to infect fruits of *Crataegus pinnatifida*⁷⁰.

223 In summary, it is no longer relevant to affirm that the different BR-agents
224 are distributed in specific regions. Indeed, all of the three main *Monilinia*
225 species are present in almost all stone and pome fruit-producing countries⁷¹,
226 likely due to open trade around the world. The worldwide distribution of *M.*
227 *laxa* is very well illustrated in Rungjindamai et al¹⁴. In the same way, the fact
228 that *Monilinia* species have the ability to colonize fruit of virtually any *Prunus*
229 or *Malus* hosts, suggests a relative wide host range of these agents. Few
230 studies of host specificity in *Monilinia* spp. have been reported to date, among
231 which the proteomic analysis conducted by Bregar et al⁷², showed a host-

232 specific expression of some proteins between apple and apricot *M. laxa*
233 isolates.

234 **3 Penetration sites in relation to fruit growth**

235 As stated before, in this review we only discuss aspects of fruit infection.
236 Different biologic mechanisms may be involved in pathogenesis of fruit and
237 flowers by *Monilinia* spp., suggested by an absence of correlation between
238 blossom bight occurrence and fruit rot impact, after artificial inoculation of *M.*
239 *fructicola*, in Brazilian cultivars and selections of peach ⁷³. In fruit, *Monilinia*
240 spp. has often been considered as an opportunistic fungi that may enter in the
241 tissue only via naturally occurring entry points. Therefore, many studies have
242 focused on these entrances or employed infection tests injuring the fruit first.
243 Although in most of the cases the fungus penetrates using 'open doors',
244 (Figure 3 F), most of the species may also be able to penetrate fruit through
245 intact surface, after the establishment of latent or quiescent infections.

246 For example, the penetration of *M. fructicola* in immature apricot fruit was
247 reported to occur through wounds, stomata, (Figure 3 B and C), intact cuticle
248 or via trichoma bases, (Figure 3 A) ⁷⁴. The same way in peach, hyphae infect
249 fruits by either degrading the cuticle and epidermal tissue ⁷⁵ or directly
250 entering through pre-existing skin microcracks (Figure 3 D and E). Fungus
251 incidence is greater if the fruit has small cracks or wounds ⁷⁶. It has been
252 reported that *M. fructigena* infects fruit via wounds only, in contrasts to *M. laxa*
253 that may infect both healthy and wounded fruit ⁷⁷. Indeed, infection may
254 depend on which site is most frequently encountered by fungal germ tubes.
255 Penetration site may also depend on the developmental stage of the fruit. For

256 example, stomata are the preferred sites in the case of unripe peaches only.
257 Curtis ⁷⁸ found that apricots were penetrated through cuticle and stomata,
258 plums via stomata, and nectarines through the cuticle. Sharma and Kaul ⁷⁹
259 described the penetration of apple under laboratory conditions by *M.*
260 *fructigena* through lenticels.

261

262 3.1 Fruit susceptibility evolves along fruit development

263 The stages of development of fruit are very important to understand the
264 occurrence of BR, since the dramatic changes in fruit physiology and
265 biochemical composition are in sync with changes in the susceptibility to BR
266 infection ^{76,80,81}.

267 The first stage starts after ovule fertilization, petal fall and ends when
268 stone starts lignifying. In this stage the fruit is photosynthetically active,
269 displays intense transpiration activity, and shows the highest nutrient content
270 ⁸², resulting in a high susceptibility to BR, probably due, in part, to the fact that
271 stomata are active, and offer an entrance opportunity to the pathogen ⁷⁸.

272 The second stage, also known as “pit hardening”, is the stage most
273 resistant to infection by *Monilinia* spp. ^{76,83}. This stage is characterized by
274 intense metabolite activity of secondary compounds, like catechin, epicatechin
275 and phenolic compounds, associated with the lignification of the endocarp,
276 occurring in this stage. In order to find genes whose expression is involved in
277 the synthesis of compounds conferring pathogen resistance, Guidarelli et al
278 ⁸⁴, compared gene expression profiles obtained by microarray analysis of
279 susceptible phase (stage S1) and resistant phase (S2) RNA samples from
280 peel fruit, finding dramatic changes in the expression of phenylpropanoid and

281 jasmonate-related genes, and thus supporting a potential role of these
282 compounds in BR resistance along fruit development.

283 At the third stage, the highest cell expansion is observed and colour
284 changes from greenish to yellow to red. This stage ends with physiological
285 maturity. Stone fruits become increasingly susceptible to pathogens as they
286 mature and ripen, enabling quiescent infections to become active and new
287 infections to begin. Associated with this increased susceptibility, structural
288 changes in the fruit surface take place, such as thinning and fracturing of the
289 cuticle, changes in fruit surface chemistry (e.g. production of sugars, decline
290 of phenolic compounds and organic acids, etc.), structure and integrity of fruit
291 mesocarp ⁷⁵.

292 Notably, various works in different *Prunus* species have observed a
293 shift in the latent infection rate across the diverse stages of fruit development
294 ^{85,86,87}. However, the results vary among studies, probably due to differences
295 in methodology and cultivars used in those studies. For instance, Lou and
296 Michailides ⁸⁸ observed that pit hardening of prunes presented the lowest
297 rates of latent infections, differing from other works reporting a minimum rate
298 of latent infections at the embryo growth stage ^{85,87}.

299 3.2 Infection by direct penetration of the cuticle

300 After conidial germination, *Monilinia* species are able to develop
301 *appressoria* to establish a latent infection and ease the penetration of the
302 intact cuticle when fruit maturity conditions allow colonization ⁸⁹. This structure
303 allows adhesion of the pathogen to the surface of the host during infection ⁹⁰.
304 Direct penetration of *Monilinia* spp. is enhanced by its production of cutinases
305 ⁷⁵, whose redox-mediated over-expression results in an increased fungal

306 virulence of *M. fructigena* in stone fruit ⁹¹. More details about the infection
307 process are given in chapter 4.

308 3.3 Infection through the trichomes basis

309 A dense layer of trichomes covers the surface of the peach fruit. The
310 infection can occur in both pubescent and not-pubescent peach fruit. The role
311 of trichomes in the infection remains controversial. Indeed, trichomes may
312 protect the fruit in two ways: 1) Directly: exudates from trichome gland may
313 act as fungicide and 2) Indirectly: the high density of trichomes could prevent
314 the formation of “water film” important to spore germination. In contrast,
315 trichome basis fracture can result in epidermis crack, resulting in points for
316 fungal entrance ^{92,93}. Smith ⁹⁴ showed that removing pubescence by means of
317 brushing reduced the time of infection development, suggesting that the
318 spores could reach fruit surface more directly. Other studies ⁷⁴ affirmed that
319 *M. fructicola* is able to penetrate apricots at hair bases.. Similar results were
320 found on mature peaches ^{78,95}.

321 Finally, is not yet clear whether nectarines are more resistant or
322 susceptible to BR compared to peaches. Large variations of trichomes density
323 and length and, more generally, of fruit surface, between varieties make
324 comparisons between studies and drawing general conclusions a very hard
325 task.

326

327 3.4 Infection through stomata

328 The literature about stomata and their function on reproductive organs
329 is limited especially for drupe fruits like peaches ⁹⁶. A majority of studies
330 discuss their function and distribution in dry fruit like nuts, capsules and pod

331 fruit⁹⁷. They can occur in small numbers or are even restricted to certain parts
332 of the fruit⁹⁸. The number of stomata per fruit is determined before petal fall
333 and remains constant throughout fruit ontogeny⁹⁹. The morphology of the
334 guard cells suggests that they have the same functions, as on leaves. In early
335 stages, stomata provide aeration in the gas exchanges for the photosynthetic
336 system; however, fruit stomata are only functional to a certain extent. Due to
337 the development of the fruits, stomata can develop into lenticels and either
338 close or remain open permanently⁹⁸.

339 In mature peach fruits, the number of stomata could be insignificant
340 compared to the number of micro-cracks and may no longer be determinant
341 for pathogen susceptibility. In early fruitlets instead, the high density of
342 stomata could be one of the factors, (Figure 4) which may explain the
343 susceptibility at this early stage.

344 Fungal invasion through stomatal apertures into the substomatal
345 cavities was observed in apricots infected by *M. fructicola* under laboratory
346 conditions⁷⁴. The authors reported that the fungus enters via the stomata and
347 penetrates a guard cell through the thin walled region at the stomata pore.
348 Close examination of serial radial or tangential sections showed that in most
349 cases primary infection was through guard cells. However, in few cases the
350 lesion centre did not coincide with stomata, and initial invasion was through
351 wounds.

352 3.5 Infection through skin cracks and wounds

353 Cuticular crack is defined as the physical failure of the fruit skin, caused
354 by forces of growth as turgor pressure within the fruit cells or hydration of fruit
355 fresh acting on the skin¹⁰⁰. Cuticular cracks on nectarine fruit occur during the

356 final fruit growth stage ^{101,102,103}. Micro-cracks and cracks can develop on the
357 surface of fruit when the growth speed of the internal cells is more rapid than
358 epidermal cell growth. In this case, a time lag between fruit growth and cutin
359 deposit can occur and provoke zones of weakness that may evolve into
360 microcracks. Several factors contribute to fruit cracking, often in interactions,
361 such as unbalanced water flux into and out of the fruit, maximal elastic limit of
362 the cuticle, cuticle strain, and absence of cuticular membrane deposition.
363 Observations of the fruit skin have shown that the cracks are frequently
364 initiated around the lenticels ¹⁰⁴, (Figure 3D, 3E and Figure 5). Larger fruits
365 can present high cuticular crack densities, which may represent more than
366 10% of the fruit surface area ¹⁰¹.

367 One of the first studies on *M. laxa* penetration in micro-cracks ¹⁰⁵,
368 observed a significant number of cracks and micro-cracks organized radially
369 around lenticels and noticed that germinating conidia of *M. laxa* tended to
370 accumulate in the micro-cracks in an anarchic pattern and without apparent
371 direct attraction by micro-cracks, despite the fact that the germ tubes grew
372 inside of them. However Borve et al ¹⁰⁶, demonstrated a clear link between
373 cracking and BR in cherries, by finding significant correlations between the
374 cultivar-specific amount of micro-cracks and the resulting incidence of BR.

375 Skin wounding deprives the fruit of its main barrier to biotic stress
376 agents, as demonstrated in several reports ^{77,107}, where BR infection rates
377 obtained after infecting wounded regions of the fruit were significantly higher
378 than infecting intact fruit regions. Effect of presence of skin barrier in BR
379 resistance was investigated on apricot, peach and plum fruit, to find resistant
380 genotypes ¹⁰⁸. Injured-fruit infection developed on all fruit with quite similar

381 speed in all species. On the contrary, when uninjured fruit were infected, large
382 variability was observed between genotypes of a same species and between
383 species. These observations suggest that few resistant factors may be
384 expressed at the flesh level and that resistance factors were no more efficient
385 when the fruit was injured. However, Ogundiwin et al ¹⁰⁹, explored larger
386 genetic diversity by evaluating 81 peach genotypes by infection on wounded
387 and unwounded fruit. The authors observed variability in both cases and
388 suggested that BR resistance is associated with the pericarp or the mesocarp
389 or both, depending on the genotype ¹⁰⁹. Nonetheless, more recently the same
390 group further explored the variability of infection reaction after wounding of a
391 canning peach progeny ¹¹⁰, concluding that wounding the fruit generally
392 abrogated any resistance to brown rot. Resistance factors at the level of the
393 flesh (wounded fruit) may not provide total resistance to infection but may
394 slightly act on the speed of lesion propagation. To further explore these
395 potential factors of resistance, large trials considering a high replicate number
396 on highly contrasted germplasm panels may be needed.

397 In conclusion, it is evident that stomata, lenticels, pores, cracks and
398 microcracks offer preferential entry sites for *Monilinia* and make fungi
399 colonization easier. Number of stomata, lenticels and pores may be under
400 genetic control, but structure may be influenced by environment conditions.
401 As for cracks and microcracks, genetic determinism has not been
402 investigated, but studies have demonstrated the effect of cultural practices
403 (e.g. irrigation and thinning) on their density ¹⁰¹.

404 **4 Infection development**

405 Infection is a term that implies the entry of an organism into a host and
406 the subsequent establishment of a parasitic relationship³⁶. The process could
407 be broadly divided in three stages: pre-penetration, penetration and post-
408 penetration (Figure 6). The pre-penetration phase concerns the transport of
409 the spores from the inoculum source to the organ host that will be infected. It
410 will not be detailed here.

411 In general, fungi utilize diverse mechanisms to infect host tissue, which
412 include i) chemical sensing and oriented growth in response to mechanical
413 contact to optimally position infection structures, ii) the production of enzymes
414 to degrade host surfaces, and iii) the formation of specialized structures such
415 as *appressoria*¹¹¹. Initial events are adhesion to the cuticle and directed
416 growth of the germ tube on the plant surface. At the penetration site,
417 *appressoria* are often formed that may have melanised walls and develop
418 high turgor pressure to support the penetration process. The penetration
419 hypha accumulates components of the cytoskeleton in the tip and secretes a
420 variety of cell wall-degrading enzymes in a highly regulated fashion in order to
421 penetrate the cuticle and the plant cell wall. As cited in many articles and
422 reviewed by Rungjindamai et al¹⁴, the presence of moisture near the fruit is a
423 crucial factor for spore germination and infection development.

424 **4.1 Adhesion to the cuticle and germination**

425 Conidia and ascospores, which are the main inoculum for BR infections,
426 require free moisture for germination, which is obtained from films or droplets
427 of water and from plant exudates that accumulate on the surface of the host
428 or in damaged tissues¹¹². Germination of conidia takes about an hour in the

429 presence of free water, while ascospores require 4 to 6 hours. However the
430 germination process could sometimes last 60 hours, in the case of dried
431 spores that need time to rehydrate and reactive the protoplast³⁶.

432 4.2 Latent infection

433 Infections may remain latent when microclimatic conditions and fruit
434 growth stage are unfavourable^{36,88}. Latent infection generally happens in
435 immature fruit. A subcuticular infection begins, but growth of the pathogen
436 quickly stops. These quiescent infections may be visible or nonvisible. Along
437 fruit growth, *M. fructicola* expresses genes and proteins enabling later
438 successful infection and colonization of the fruit⁹¹. As the fruit matures, fungal
439 growth restarts and BR develops¹⁴.

440 The relationship between the numbers of conidia on the fruit surface and
441 the incidence of latent infections in orchards or after harvest has been
442 investigated for different fruit species^{77,86, 88, 89, 113}. A significant positive link
443 has been reported for peaches⁸⁵. Therefore early identification of fungal
444 infections is needed to determine pre- and post-harvest disease management
445 practices, as well as postharvest shipping strategies. In order to choose
446 targeted fungicide treatments, molecular methods to identify latent infection of
447 *Monilinia spp.* have been developed³¹.

448 4.3 *Appressorium* formation and hypha penetration

449 Formation of *appressorium* is induced by specific physical or chemical
450 cues provided by the host plant. Irrespective of whether fungi use enzymes or
451 force, or a combination of both to penetrate, *appressoria* need to adhere
452 tightly to the plant surface. *Appressorium* differentiation can be stimulated in
453 *C. gloeosporioides* by wax isolated from fruit of its host plant, avocado, but not

454 by wax isolated from other plants ¹¹⁴. Careful analyses suggested that non-
455 host wax contained inhibitors of *appressorium* development.

456 High pressure can be generated by turgor within the *appressorium* and
457 possibly also by the cytoskeleton, and pushes the hypha to penetrate through
458 the surface. Penetration is likely to be supported by enzymes that soften the
459 host cell wall. To analyse the contribution of cell wall-degrading enzymes to
460 the penetration process, Dumas et al ¹¹⁵ used the endo-polygalacturonase
461 promoter of *Colletotrichum lindemuthianum* (a necrotrophic fungus like
462 *Monilinia* spp.), to control green fluorescent protein expression. These authors
463 were able to show that the gene is expressed in *appressoria* prior to
464 penetration. Finally, *Colletotrichum lindemuthianum*, as other necrotrophic
465 fungi, required pectolytic enzymes not only for tissue maceration during *in-*
466 *planta* growth, but also to assist forceful penetration.

467 *Appressorium* formation by *M. fructicola* on fruit surfaces has been
468 related to BR incidence to fruit surface topography and hydrophobicity, as well
469 as the presence of nutrients and fruit volatiles ^{116,90}. *Appressoria* were
470 observed on the stomatal guard cell lips, and germ tubes apparently
471 perceived particular topographical features to trigger differentiation of
472 *appressoria*. Since *appressorium*-mediated penetration was observed both by
473 natural openings (stomata) as well as by direct penetration of intact cuticle
474 (through penetration pegs produced from *appressoria*), authors suggested
475 that mechanisms may be diverse. In contrast, they did not observe
476 *appressoria* on mature nectarine fruit. The authors suggested that *M.*
477 *fructicola* restrains the formation of specialized infection structures such as
478 *appressoria* to immature tissues and behaves as a saprophyte pathogen

479 when nutrients are readily accessible, as in mature fruit. Also, a role of cAMP
480 as well as calcium-calmodulin pathway was suggested in the formation of
481 *appressorium*⁹⁰.

482 4.4 *Appressoria* melanization increase pathogenicity

483 Melanins are brown-black pigments, biological macromolecules
484 composed of various types of phenolic or indolic monomers that are produced
485 by fungi and other organisms. Various fungi synthesize melanin from the
486 oxidation of tyrosine. The extracellular dark pigments produced by fungi may
487 be formed from various fungal phenols, usually named as heterogeneous
488 melanins^{112,117}. The production of melanin by microorganisms has been
489 associated with their virulence and the melanization of *appressoria* was
490 considered necessary to different fungal pathogens for infection and disease
491 development. Howard et al¹¹⁸ proposed the importance of melanization for
492 surface penetration. These authors exposed *appressoria* from the rice blast
493 fungus *Magnaporthe grisea* to solutions of high osmotic pressure and
494 observed no melanization and an inhibition of penetration of the leaves. They
495 concluded that melanization is involved in the reduction of porosity of the
496 *appressoria* wall. According to Dean¹¹⁹, this causes the locking of cytosolic
497 solutes efflux and leads to higher *appressoria* pressure.

498 Indeed, many fungal pathogens, such as *Venturia inaequalis*,
499 *Magnaporthe gray*, *Pyricularia oryzae*, and *Colletotrichum legenarium*, need
500 melanised *appressoria* to cause infection and disease development^{120,117}. De
501 Cal and colleagues has reported that a melanin-deficient mutant strain of *M.*
502 *laxa* (albino mutant) was no longer able to induce peach twig blight¹¹⁸. They
503 also observed that *M. laxa* treated with pyroquilon, an inhibitor of melanin

504 biosynthesis, could not induce peach twig blight ¹²¹. Finally, they found that *in-*
505 *vitro*, chlorogenic acid or pyroquilon added to the culture medium of *M. laxa*
506 inhibited melanization of the colony ¹²². They concluded that the ability of *M.*
507 *laxa* to produce melanin is crucial for its pathogenicity.

508 Rehnstom and Free ¹²³, however, showed that melanin-deficient mutants
509 of *M. fructicola* are able to infect nectarines, by producing lesions as large as
510 those produced by the wild type. So they concluded that melanization is not
511 required for the successful infection of host fruit. Nevertheless their presence
512 could improve the success of development of fungi and increase their
513 permanence in the field under adverse conditions.

514 4.5 pH lowering regulates the expression of pathogenicity genes.

515 Fungi are able to modify the host pH. Preliminary data on *M. laxa*, *M.*
516 *fructicola*, and also *M. fructigena* indicate that they can reduce host pH during
517 colonization of peach cvs. 'Big Top', 'Venus', and 'Tirrenia' by secreting
518 gluconic acid ⁷⁶. Analysis of the acidification process in colonized fruit showed
519 that gluconic acid was the main organic acid accumulated at the infection site
520 and under liquid-culture conditions. When compared to a nectarine cv. 'Big
521 Top' and peach cv. 'Plácido' with differing sensitivities to *M. fructicola*, a 250%
522 higher accumulation of gluconic acid was observed in the susceptible peach
523 cultivar than in the less susceptible nectarine cultivar. Under liquid conditions,
524 at pH 3.6–3.7, the relative expression of transcripts of *mfp2* and *mfp3*,
525 encoding for two polygalacturonase genes of *M. fructicola*, increased 12-fold
526 and 6-fold, respectively, suggesting the importance of acidification for the
527 secretion of pathogenicity factors by *M. fructicola*. The authors also underlined
528 the importance of acidification for the secretion of pathogenicity factors by *M.*

529 *fructicola*, suggesting that ambient pH created by the pathogen is a regulatory
530 cue that promotes pathogenicity expression. Specific genes contributing to
531 pathogenicity may be expressed as a result of the environmental pH induced
532 by the pathogen.

533 4.6 Biochemical arsenal of *Monilinia* spp.

534 Studies in the past decades ascertained the effects of fungicides on
535 fungus enzymes in buffer extracts of mycelium of *M. laxa*¹²⁴. Thus, they
536 reported large groups of enzymes like catalases, peroxidases, glutamic
537 dehydrogenases, esterases and alkaline phosphatases produced by this
538 fungus.

539 The most important enzymes produced by *Monilinia* spp. may be the
540 cutinases needed to penetrate the intact surface of fruit,^{75,91}. High levels of
541 these enzymes may result from former activation, as in the necrotroph
542 *Fusarium oxysporum*¹²⁵. In the case of *M. fructicola*, gene expression of the
543 cutinase *MfCUT1*, which is up-regulated in an oxidant environment,
544 contributes directly to the virulence of the pathogen⁹¹.

545 Cellulase has been found in all species of *Monilinia*, but its secretion
546 seems to be very restricted. The cellulase secretion was detected in *M. laxa*,
547 whereas for *M. fructigena* it was detected a trace of activity in extracts of
548 rotted pear fruits. A very weak cellulase activity for *M. fructicola* in medium
549 was found³⁶.

550 The polygalacturonic acid chain is attacked by three enzymes which
551 are secreted by all three *Monilinia* spp., namely i) endo-polygalacturonase
552 (EC 3.2.1.15), that hydrolytically attacks polygalacturonic acid, ii) pectin lyase
553 or pectin methyl-trans-eliminase, (EC 4.2.2.10), that attacks a

554 polygalacturonic acid of a high degree of esterification and iii) pectin esterase
555 or pectin methylesterase (EC 3.1.1.11) which liberates the methoxyl groups
556 from the carboxyl groups of the galacturonic acid. The optimum pH for each
557 enzyme differs for each species ³⁶. An important factor for the expression of
558 *Monilinia* spp. polygalacturonases is the presence of calcium in the
559 extracellular environment ¹²⁶. Recently, Chou et al ⁵ investigated five
560 endopolygalacturonase (endo-PG) genes in *M. fructicola*. They were
561 differentially expressed during pathogenesis and in different culture media.
562 *MfPG1* was the one mainly expressed. Gradziel and Wang ¹²⁷ observed that
563 an overexpression of *MfPG1* diminished virulence of the pathogen. Authors
564 suggested that *MfPMG1* expression could be due to the activation of the plant
565 defence by higher levels of reactive oxygen species (ROS) produced in this
566 case.

567 Among enzymes degrading neutral sugars (arabinans and galactans)
568 from the host cell wall, α -L-arabinofuranosidase (EC 3.2.1.55) from *M.*
569 *fructicola* was found to release monomeric arabinose from arabinans by
570 hydrolysing the terminal bond ¹²⁷. This enzyme is localized in the hyphae of
571 *M. fructigena* and may migrate to the plant plasmalemma and can be secreted
572 by a process of reverse pinocytosis with involvement of multi vesicular bodies
573 ¹²⁸. Other enzymes that degrade neutral sugars have been found in *M.*
574 *fructicola* as β -galactosidase, but have not been studied in details ³⁶.

575 4.7 Post-penetration

576 Once infection is established, the hyphae of the pathogen spread
577 through the host tissues and bring about the symptoms such as the browning
578 and softening of the tissue in fruit (Figure 7).

579 The spread of BR pathogens is generally intercellular. It could penetrate
580 and permeate any part of the host. Investigations by Reinganum¹²⁹ showed a
581 particular affinity of *M. laxa* for the middle lamella region. Transmission
582 electron microscopy, of *M. fructigena* attacking pear fruit also confirmed that
583 hyphae are generally intercellular, though in particular infections they become
584 occasionally intracellular and the dead protoplasts are pushed across the cell
585 lumen¹²⁴.

586 Changes in the host plasmalemma could occur even if the membrane is
587 intact. In infected tissues, its function could be drastically impaired as shown
588 by conductivity measurements resulting in leakage of sugars and amino acids
589 from cells. Subsequently, the pathogen has sources of carbon and nitrogen to
590 use. Moreover if membranes of vacuoles, mitochondria, chloroplasts or other
591 organelles have been damaged, their contents mix, following a process
592 described as decompartmentalization³⁶.

593 Endopolygalacturonases and pectin esterases activities generate low
594 molecular weight metabolites^{95,130}. These secretions cause the collapse of
595 the affected host cell^{90,130,131}. Willaman¹³², suggested that a hydrophilic gel of
596 calcium pectate is formed from pectin degraded by *M. fructicola*. This gel may
597 help the permanence of the fungus in the fruit mummy³⁶.

598 In fruit, the rate of increase in rot diameter depends on the combination
599 of environment conditions, the host genotype, the pathogen species and the
600 stages in fruit maturity¹²⁴. After a few days, conidial pustules of the fungus
601 burst through the fruit epidermis and cuticle. Apart from allowing the fungus to
602 perpetuate itself, this bursting leads to the desiccation of the host tissues and

603 often, ultimately to the formation of a mummified fruit. In the meantime, the
604 pathogen develops a stroma of dense mycelium within the host³⁶.

605 In conclusion, the infection process unfolds differently depending on the
606 growth stage of the fruit. Some steps may be extended and others avoided.
607 The fungi may deploy different strategies mobilizing specific structures (e.g.
608 appressorium), developing processes (e.g. melanization, acidification) and
609 deploying a large arsenal of enzymes. Although many works have identified
610 different elements involved in the infection process, it is still not possible to
611 fully comprehend the successive steps of the infection progress, Figure 8.

612 **5 Host factors for BR resistance/susceptibility in fruit**

613 Research has long tried to identify host factors contributing to BR
614 resistance. Byrde and Willets³⁶ listed some of them: flowering date, fruiting
615 habit, gumming of wounds for cherries, duration of flowering for apricots, cork
616 in lenticels, fiber and pentosan contents, parenchyma plugs in stomata, skin
617 thickness, and texture on ripening for plums. However authors underlined the
618 importance of caution since evidence is based on only few cultivars.

619 To date there is limited evidence on factors limiting BR in mesocarp and
620 most research has shown that BR resistance relates to fruit epidermis
621 75,116,127,133,134.

622 **5.1 Constitutive components of BR resistance: plant cuticle, a multi-** 623 **component barrier**

624 The plant cuticle is supposed to constitute an efficient mechanical and
625 chemical barrier against most of the pathogens that colonize the plant
626 surface, as a form of constitutive defence of the plant. The different layers of

627 the fruit surface (waxes, cutin, epidermis cells) and its attributes (trichomes)
628 may each play a role in this barrier, but these roles are not yet well
629 understood. To develop infection, the fungi need to pass mechanical barriers
630 corresponding to the successive barriers of fruit skin. Recent observations are
631 starting to reveal complex inter-relationships between cuticular lipids and
632 immunity, suggesting that the cuticle is not just a physical barrier, since a
633 variety of biochemical compounds localized in different layers or tissues may
634 play a role in the fruit defence to infection.

635 The first level is the epicuticular wax layer that covers the cuticle and is
636 a complex mixture of very long saturated, unbranched chain aliphatics and n-
637 alkanes, ranging in carbon number from 21 to 33, depending on the plant taxa
638 ^{135,136,137}. Waxes can form crystals that enhance water repellence and prevent
639 the formation of the film of water crucial for spore germination. If wounds
640 occur, new wax plates are formed to repair or protect the fruit.

641 However factors such as temperature, the health status of the plant
642 and the chemical treatments may interfere with this process, ⁹². In their
643 review, Reina-Pinto ¹³⁸ exposed various studies demonstrating that cuticular
644 lipids play a role as messenger molecules in plant-pathogen interactions. For
645 instance, Podila et al ¹³⁹ showed that the germination and *appressorium*
646 formation by *Colletotrichum gloeosporioides* in avocado is induced specifically
647 by the surface waxes of this host, but not by waxes from other plants ¹¹⁴. The
648 authors explained this effect by the longer-chain in fatty alcohols, the
649 presence of terpenoid components and absence of inhibitors that allow the
650 fungus to use the host surface wax to trigger germination and differentiation of
651 infection structures. Some studies reported stimulatory effects of extracted

652 cuticular waxes on the germination and differentiation of *Magnaporthe grisea*,
653 *Metarhizium anisopliae* and *Puccinia graminis f.sp. tritici*^{140,141,142}. Similarly,
654 *Blumeria graminis f.sp. hordei* germination was more rapid and greater on the
655 surfaces of intact than dewaxed barley¹⁴³

656 On the contrary, it was reported that cuticular waxes inhibit conidial
657 germination of plant pathogens, such as *Podosphaera leucotricha* on certain
658 varieties of apples¹²⁴. This evidence suggests that the different constituents
659 of waxes may play opposing roles for the pathogens. The extension of the
660 scope of the results exposed above is limited since the quantity and
661 composition of cuticular wax shows great variability among different plant
662 species, different organs of an individual plant, and/or during the ontogeny of
663 individual organs¹⁴⁴. Unfortunately, with respect to *Monilinia* spp., there is a
664 lack of information on the role of waxes in the fruit-fungi interactions and a
665 direct translation of results from other plant-pathogen couples is not valuable.
666 Further specific studies are therefore needed to decipher waxes role in
667 *Monilinia* spp. infection.

668 The cuticle is the second barrier that the fungi need to cross. This
669 structure consists of hydrocarbon polymers and cutin synthesized exclusively
670 by the epidermal cells. For example, the cuticle of *Prunus persica* fruit has
671 been characterized as a complex of structures with various protective
672 purposes. In this species, cuticle is composed of 53% cutan, 27% waxes,
673 23% cutin and 1% hydroxycinnamic acid derivatives; trichomes are covered
674 with a thin cuticular layer containing 15% waxes and 19% cutin and filled by
675 polysaccharide material (63%) containing hydroxycinnamic acid derivatives
676 and flavonoids⁹³.

677 The cuticle is structurally diverse among species, but exhibits the
678 organization of a composite material consisting of cutin, polyester that is partly
679 covered and interspersed with waxes (epicuticular and intracuticular waxes)
680 ¹⁴⁵. However, a characterization of the fruit surface of diverse varieties of
681 *Prunus*, in order to determinate cultivar-specific skin features, has not been
682 developed to date. Considering the cuticle as a structure of resistance to
683 pathogen penetration deserves caution, seeing that its proprieties are
684 dependent on qualitative and quantitative chemical composition. Indeed, a
685 complex inter-relationship between the cuticular lipids and the fungus may
686 occur, playing a molecular messenger role in interactions between plant and
687 pathogen. As well as some components of epicuticular waxes, they can act as
688 fungal pathogenicity activators or in contrast, inhibit the infection. Isaacson et
689 al ¹⁴⁶ demonstrated on tomato that cutin plays an important role in protecting
690 tissues from necrotrophic infection by *Botrytis cinerea*. According to
691 Kolattukudy et al ¹¹⁴, some pathogens sense plant surfaces thanks to cuticle
692 monomers that may be produced by basal cutinase activity of fungal spores.
693 Sensing of cutin monomers would then induce high levels of cutinase required
694 for penetration.

695 In conclusion, the cuticle is thought to be a crucial factor in the fungal
696 penetration process. However, as previously mentioned, the cuticle is not a
697 continuous layer. It may display discontinuous sites as secretory tissues,
698 trichomes, stomata and even pores that could be “open doors” for pathogen
699 colonization, as well as the presence of fractures in the epidermis.

700 The last barrier in the surface is the epidermis cell wall that could vary
701 in composition and thickness. The major substance that reinforces the cell

702 wall structure is the lignin. The process of lignification could improve the
703 resistance of the cell wall against the action of degradation enzymes; block
704 the diffusion of pathogen toxins and the diffusion of nutrients from the fruit,
705 restringing the process of colonization. Sites around the infection point could
706 also accumulate callose, suberin, tannin and pectin substances ¹⁴⁷.

707

708 5.2 Phenolic acids and their redox-mediated role in fungal inhibition

709 Early studies of peach phenolic compounds started from the
710 observation that fruit from 'Bolinha' peach cultivar, known to be resistant to
711 BR, displayed high levels of these compounds in their epidermis. This group
712 of compounds became one of the most studied for BR resistance ¹²⁷.

713 Among the phenolic compounds of the epidermis of peach fruit,
714 chlorogenic and caffeic acids have high concentrations, especially in
715 immature fruit and in fruit of peach genotypes, with a high level of resistance
716 to *M. fructicola*¹¹⁶. In cultures of *M. fructicola*, these phenolic acids did not
717 suppress spore germination or mycelia growth but they inhibited cutinase
718 activity⁷⁵. Likewise, the presence of caffeic acid in cultures prevented the
719 appearance of two major cutinase isoforms⁷⁵. In addition, a series of cinnamic
720 and benzoic acid derivatives also suppressed cutinase levels in culture ³⁸.
721 These results led the authors to suggest that chlorogenic acid and related
722 phenolics, in combination with other factors such as iron, could have a role in
723 arresting *M. fructicola* in quiescent infections ⁶³. Furthermore, they may
724 contribute to resistance by interference with the production of factors involved
725 in degradation of host polymers. Subsequent studies *in vivo* confirmed the
726 effects of caffeic acid, chlorogenic acid, or reduced glutathione on infection

727 development. Adding those compounds in conidial suspensions of *M.*
728 *fructicola* did not inhibit germination on flower petals and fruit, but inhibited
729 *appressorium* formation from germinated conidia and subsequent BR lesion
730 development ¹¹⁶.

731 Further work conducted by the same group showed that antioxidant
732 phenolic acids suppressed mRNA accumulation and enzyme activity of a
733 cutinase ¹⁴⁸. However, other antioxidant compounds also significantly
734 attenuated *M. fructicola* cutinase production, indicating a general effect of
735 antioxidants rather than a specific effect of a given phenolic compound (see
736 section 5.3) ^{91,148}.

737 Villarino et al ¹²² demonstrated that chlorogenic acid and its isomer,
738 neochlorogenic acid, can interfere with the production of melanin in *M. laxa*
739 without any effect on the growth and germination of the fungus (see section
740 4.4). ¹⁴⁹. Even though these results are interesting, the role of the different
741 phenolic compounds in limiting *Monilinia spp.* remains unsolved. Prusky and
742 Lichter ¹⁵⁰ have reviewed pathogen quiescence in post-harvest diseases and
743 discussed how fruit factors such as high acidity and phenols in unripe fruits
744 can contribute to disease resistance.

745 5.3 Active mechanisms in response to pathogen attack: defence
746 proteins

747 Although cuticle research has mainly focused on the analysis of
748 cuticular lipids, cuticular proteins may also be of importance. They are
749 referred to 'Lipid Transfer Proteins' (LTPs) and many have been shown to
750 play an important role in plant defence ¹³⁸. They specifically inhibit pathogen
751 and pest enzymes by forming complexes that block active sites or alter

752 enzyme conformations, ultimately reducing enzyme function. They include
753 defensins, amylase inhibitors, lectins, and proteinase inhibitors. Unlike simple
754 chemicals such as terpenoids, phenolics, and alkaloids, proteins require a
755 great deal of plant resources and energy to be synthesised consequently,
756 many defensive proteins are only made in significant quantities after a
757 pathogen or pest has attacked the plant. Once activated, however, defensive
758 proteins and enzymes effectively inhibit fungi.

759 On defensins in particular, Nanni et al ¹⁵¹ investigated the possible role
760 of *Ppdfn1* in peach defence against fungal pathogens. *Ppdfn1* gene
761 expression was analysed in peach tissues susceptible to *M. laxa*, such as
762 flowers and fruit, and its induction upon pathogen infection was tested. They
763 concluded that *Ppdfn1* displayed an antifungal activity through specific
764 interactions with the membrane lipids of the fungi.

765 Plants also produce hydrolytic enzymes, such as chitinases,
766 glucanases or lysozymes, in response to fungi attacks. Zemanek et al ¹⁵²
767 showed increased levels of mRNAs encoded by β -1,3-glucanase gene
768 following treatment of a peach cultivar with culture filtrates of the fungal
769 pathogen *M. fructicola*.

770 The changes in the transcriptional level of genes coding to
771 pathogenesis-related proteins (PR) has also been associated with the BR-
772 infection process in European plum fruits (*Prunus domestica* L.). It is well
773 known that some families of PR proteins are inducers of phenylpropanoid
774 accumulation and other resistance effectors ¹⁵³. El-kereamy et al ¹⁵⁴ described
775 differential expression patterns of PR-10 coding gene among two European
776 plum cultivars with contrasting BR-resistance phenotypes, as well as other

777 transcripts coding to intermediary proteins in the signalling pathway of this
778 PR. The authors observed that after *M. fructicola* artificial inoculation,
779 transcripts of PR-10 and phospholipase D-alpha (PLD α , a cell membrane-
780 phospholipid degrading enzyme, involved the signalling of stress responses)
781 remained constitutively expressed in the resistant variety (cv. 'Violette'), while
782 in the susceptible one (cv. 'Veeblue') these levels increased after pathogen
783 attack. Hydrogen peroxide concentration in fruit tissues correlated with
784 transcript pattern of these genes on both cultivars, with higher but steady
785 levels of the compound in the resistant cultivar, suggesting an inhibitor role for
786 the pathogen.

787 The same authors demonstrated the antifungal activity of PR-5, whose
788 differential expression among plum cultivars was correlated with their BR-
789 resistance. Activity was with a pattern similar to PR-10, *i.e.*, no significant
790 change in PR-5 transcript levels after infection in resistant cultivars ('Violetta'
791 and 'Stanley') and a rapid increase in susceptible genotypes ('Veeblue' and
792 'Victory'). Furthermore, the ectopic overexpression of this protein in *A.*
793 *thaliana* transformants increased resistance to *Alternaria brassicicola*, as well
794 as a higher induction of camalexin biosynthesis, and transcript abundance of
795 genes coding to phenylalanine-ammonia lyase (PAL, a central point in
796 phenylpropanoid and phytoalexin biosynthesis) and to three cytochrome P450
797 involved in the biosynthesis of some anti-fungal phenolics.¹⁵⁵ Finally, the
798 same authors,¹⁵⁶ described a very similar expression pattern after *M.*
799 *fructicola* infection in the gene coding to MYB3 transcription factor of
800 European plums, suggesting an intermediary role of this transcription factor in
801 the hormone-mediated defence responses that result in the induction of PR

802 proteins. The study of the variability of these genes, which have effects in
803 defence pathways, in *Prunus* germplasm collections has a crucial importance
804 in the generation of knowledge for the development of more resistant varieties
805 of fruit species.

806

807 5.4 ROS, oxidative stress and programmed cell death

808 The knowledge of virulence mechanisms in BR is still rudimentary;
809 however recent research reported that ROS play dual roles in plant-host
810 interactions. The production of ROS can either stimulate host resistance or
811 enhance pathogen virulence. Chiu et al ¹⁵⁷ examined the regulation of the
812 gene *MfCUT1* (that encodes the major cutinase of *M. fructicola*) by redox
813 status. Authors reported that gene expression is down-regulated by caffeic
814 acid (CA) and by the antioxidant glutathione (GSH) and up-regulated by a
815 GSH synthesis inhibitor, the buthionine sulphoximine (BSO). These results
816 indicate that changes in cellular redox status could impact virulence of BR and
817 suggested that redox cycling is related to this regulation.

818 Liu et al ¹⁵⁸ investigated the production of hydrogen peroxide, a major
819 component of ROS in peach flower petals in response to *M. fructicola* and
820 *Penicillium digitatum*, a non-host pathogen. During the interaction with the
821 host, *M. fructicola* induced hydrogen peroxide accumulation in flower petals,
822 high levels of protein carbonylation, lipid peroxidation and a significant
823 reduction of hydrogen peroxide accumulation in tissues. They also observed a
824 reduction in the incidence of BR with application of exogenous antioxidants.
825 The presence of *M. fructicola* spores at the surface of intact flower petals
826 induced gene expression and increased enzyme activity of NADPH oxidase, a

827 membrane-bound enzyme complex important to generate ROS and cell wall
828 peroxidase in host tissues. This resulted in the production of hydrogen
829 peroxide while the same tissues inoculated with a non-host pathogen did not
830 show significant responses ¹⁵⁸. These results suggested that the antioxidant
831 compounds can influence intracellular antioxidant levels in the pathogen, and
832 that changes in the redox environment may influence both gene expression
833 and the development of structures used by the pathogen to facilitate infection
834 ¹¹⁶.

835 In some cases the fruit can respond by death of cells around the point
836 of infection, the formation of phellogen at the margin of twig lesions in stone
837 and pome fruit trees ¹⁵⁹, the suberisation of walls of surrounding living cells in
838 fruit and the accumulation of phenolic compounds in cells up to 20 cells
839 around the distant site of initial infection. Despite such responses aimed at
840 limiting the spread of BR, growth of mycelium may continue although the
841 activities of some enzymes are inhibited. Several penetrations within a small
842 area would produce a greater and more obvious reaction by the host. The
843 results obtained by Jekins and Reinganum ¹⁶⁰ with *Sclerotinia fructicola* on
844 stone fruit, suggest that sometimes the host response to penetration
845 permanently inactivates the fungus.

846 The diversity of studies and results published indicates a complex
847 multifactor resistance that may involve different types of defence localized in
848 different tissues (epidermis and mesocarp). They highlighted the involvement
849 of constitutive factors (mechanical barrier), active compounds (waxes, cutins,
850 phenolic acids) as well as specific responses to the attack (proteins and

851 enzymes, ROS). However, no generic model of fruit resistance to BR has
852 been proposed.

853 **6 Breeding for BR resistance**

854 Currently, commercial cultivars are more or less sensitive to BR. The
855 peach cultivar known to have one of the highest level of resistance is the
856 Brazilian cultivar 'Bolinha'^{161,162}. Feliciano et al¹⁶³ investigated resistance in
857 peach cultivars and found that 'Bolinha' had fruit with particularly small size
858 and a thick cuticle with high phenolic content. This cultivar has been used as
859 a donor of BR resistance in conventional breeding for canning and low-chill
860 peach despite its poor fruit quality, high susceptibility to enzymatic browning,
861 reduced fruit size, and high rate of pre-harvest fruit drop^{134,164,165,155}. The
862 case of 'Bolinha' demonstrates the challenge of breeding for BR, as
863 characteristics associated with fruit resistance may conflict with commercial
864 requirements. As mentioned before, Bostock et al⁷⁵ suggested that cuticular
865 characteristics may be involved in BR resistance. Many other fruit traits
866 discussed in previous sections of this review may be implicated in host
867 resistance to BR in stone fruit. However, the statistical and genetic
868 correlations of those traits with the BR phenotype, as well as their genetic
869 basis are poorly understood.

870 Apart from cultivar 'Bolinha', from which many studies have developed
871 the knowledge about host resistance to BR in peach and stone fruit, few
872 sources of resistance have been discovered (see below) and no commercial
873 cultivar of peach with melting flesh declared to be resistant to BR has been
874 released by any *Prunus* breeding program around the world. Regardless of

875 the lack of sources of BR resistance found in the germplasm of stone fruit, this
876 trait is presently a major objective for breeding programs in different countries
877 for cherries (sour and sweet), apricots, plums and peaches. Hence,
878 deciphering the genetic control of resistance to BR remains a challenge.

879 6.1 Genetic resources, breeding programs and phenotyping strategies

880 As mentioned before, some traits associated with host resistance to BR
881 are present in cultivars or accessions of poor commercial and productive
882 quality. Identifying reliable sources of resistance to be introgressed in high
883 fruit quality genetic backgrounds is one of the main objectives of such
884 breeding programs. However, one of the first steps for the establishment of
885 breeding programs or genetic studies for a given trait is the definition of a
886 reliable measurement or phenotyping protocol, to compare afterwards the
887 phenotypic variations among a population of genetically diverse individuals
888 (cultivars, accessions or offspring from a cross), and then identify interesting
889 breeding materials in the basis of robust phenotypic data. In the case of
890 assessment of cultivar-dependent BR impact on stone fruit, there is a lack of
891 consensus in the employed experimental strategies and each laboratory has
892 adopted a particular protocol, according to its experimental capacities and /or
893 specific objectives.

894

895 6.2 Field-borne inoculum assessment

896 The simplest system to score BR resistance is to assign to each analyzed
897 accession a resistance level from a subjective scale fixed by the observer,
898 based on the disease impact caused by field-borne inoculum. Although it is
899 scarcely precise and is highly subjected to the criterion of the evaluator and

900 the environmental and climatic conditions on the experimental orchard, this
901 strategy offers a quick way to evaluate a large number of accessions. The use
902 of this strategy has been reported in the selection of numerous promising
903 accessions with relatively high BR resistance in breeding programs all over
904 the world, mostly for peach and sour cherry.

905 In the Fruit Research Institute of Cacak (Serbia), preliminary evaluation of
906 BR resistance of indigenous “vineyard” peach accession germplasm was
907 made by the use of a six-level scale, which allowed the identification of 11
908 evaluated accessions showing higher resistance level (described as
909 “Symptoms are not observed”) during three years, among a total of 75
910 genotypes evaluated ¹⁶⁶. In the same research center, but in the sour cherry
911 breeding program, ^{167,168}, a subjective scale from 1 to 9 (1 for no attack, 9 for
912 very strong attack) was used to evaluate 11 advanced selections at the final
913 step of the selection process, as well as 9 landraces from autochthonous
914 germplasm ⁷². Advanced selections showed relatively high levels of
915 resistance (score between 2 and 3), but a slightly higher diversity was found
916 in the local genotypes collection (from 1 to 4). Subjective scale scoring was
917 also used in the sour cherry breeding program of the Institute of Plant
918 Breeding in Dresden, Germany, as well as in the beginning of the peach
919 breeding program aiming to develop cultivars adapted to humid and
920 temperate climates at Embrapa in Pelotas, Brazil, from which mid- to high-
921 resistant cultivars such as ‘Olympia’ and selection ‘Conserva 947’ have been
922 generated ¹⁶⁹.

923

924 6.3 Artificial infection assessment

925 BR resistance evaluation can also be scored by artificial infection of
926 harvested fruit under laboratory conditions. This allows the control of many
927 factors that can affect the final result of BR impact in an experiment, such as
928 elimination of field-borne spores from the fruit surface, presence/absence of
929 skin barrier (wounded/unwounded fruit), spore concentration, and
930 temperature, humidity and time of incubation before BR impact measurement.
931 It also allows following infection progress by recording the diameter of the BR
932 lesion.

933 One of the first groups that started to use artificial inoculations of BR was
934 at UC Davis, within the cling peach-breeding program,^{127,170,171}. Researchers
935 considered the average rot diameter 72 hours after inoculation (10 µl drop of
936 a conidial suspension of *M. fructicola* containing 10⁵ spores/ml, on previously
937 with diluted sodium hypochloride and ethanol-disinfected fruits), as specified
938 elsewhere¹¹⁰. In this way, a large phenotyping effort has been carried out to
939 screen mature fruit for resistance to *M. fructicola* in over 4000 peach
940 genotypes from very different origins: landraces, standard canning peach
941 cultivars, advanced experimental selections with various pedigrees including
942 some with 'Bolinha' heritage as well as some interspecific hybrids generated
943 in order to introgress BR resistance from almonds. The material selected with
944 this protocol has been useful also for studies of genetic dissection of the BR
945 resistance trait in segregating populations (see "QTLs for BR resistance
946 sections").

947 As mentioned in other sections of this review, Pascal et al¹³³ evaluated
948 two screening tests for resistance to *M. laxa* in apricots (7 accessions),

949 peaches (12 accessions) and diploid plums (7 accessions of *P. salicina*, *P.*
950 *cerasifera* and interspecific hybrids between them) at INRA, Avignon, France.
951 The tests consisted of artificial inoculation of uninjured and artificially injured
952 fruit. Each fruit was inoculated with a 20 µl droplet containing conidia of *M.*
953 *laxa* at a concentration of (10^6 spores/ml). Percentage of infected fruits and
954 rot diameter progression were recorded, Figure 9. The authors observed no
955 correlation between the BR resistance rankings from the uninjured and injured
956 tests. Accordingly, they suggested that epidermal resistance and flesh
957 resistance were not linked processes. This work also highlighted high
958 variability of lesion progression within the uninjured test and very similar rot
959 spread within the injured test, suggesting that no resistance expressed at the
960 flesh levels in the tested material. In INRA-Avignon, a breeding program
961 focused on pest resistance (including resistance to BR by *M. laxa*) has
962 generated very interesting materials, such as introgression of *Prunus*
963 *dauriana* resistance to peach materials ¹⁰⁸.

964 Material from the breeding program of Embrapa-Pelotas (Brazil) has also
965 been screened with artificial inoculation, and BR resistance results on these
966 breeding materials have been reported ¹⁶². BR screenings were made by
967 monitoring the percentage of infected fruits 72 and 96 h after spraying a
968 solution (containing 10^5 spores/mL) over intact harvested fruits. The authors
969 observed a significant genetic component when comparing some selections
970 and cultivars. Interesting selections such as 'Conserva 1798', 'Conserva
971 1596', 'Conserva 1218' and 'Cascata 1493' were identified ¹⁶⁵. Authors
972 evaluated three crosses ('Conserva 672' x 'Maciel', 'Conserva 672' x 'A.334'
973 and 'Leonense' x 'Bolinha') by drop-inoculations. Broad-sense heritability was

974 estimated to be around 80%. Twelve seedlings from these three progenies
975 were determined to be of equal or better resistance than the 'Bolinha' cultivar.

976 Resistance to *Monilinia* spp. in peach cultivars for the fresh market has
977 been an important objective in the breeding program of University of Milan
978 (formerly at University of Bologna, Italy). Offspring from crosses between
979 melting flesh peaches were selected^{172,173}. In these works, mature fruits were
980 artificially sprayed with a suspension of *M. laxa* (10^5 spores/ml), and the
981 disease impact was registered as percentage of infected fruits after 5 days of
982 incubation at 25°C and 95-100% relative humidity. Several parental
983 combinations were analysed. The 'Contender' x 'Elegant Lady' F1 population
984 presented the most interesting results with individuals presenting higher levels
985 of resistance than the resistant parent (cv. 'Contender'). Besides generating
986 pre-breeding materials, this population has been useful in the genetic
987 dissection of BR resistant-related traits¹⁷⁴. This group is currently developing
988 new phenotyping strategies, based on *in planta* spray of conidial suspension
989 of *M. laxa*, aimed at increasing the capacity of sample analysis in breeding
990 programs bearing high numbers of seedlings, obtaining promising results for
991 scoring BR-resistance phenotype¹⁷⁵.

992 Studies of BR resistance evaluations in apricots by artificial inoculum
993 have been reported mostly from two breeding programs. At the Regional
994 council for agriculture of Rome (CRA-FRU, Italy), several apricot accessions
995 showing high BR resistance have been evaluated by artificial inoculation
996 procedures consisting in fruit disinfection (diluted sodium hypochlorite and
997 ethanol), inoculation with a drop of *M. laxa* conidial suspension (10^5
998 spores/ml) in two points near peduncle cavity, incubation for 7 days at 22°C

999 and registration of affected fruit percentage. Among the evaluated crosses,
1000 the authors found remarkable levels of BR resistance: selections such as
1001 '485GII37', '493C12III61' and '493 C12 VI 1' (open pollinations of cultivars
1002 'Don Gaetano', 'Fiammetta' and 'Boreale', respectively) showed 0-10% of
1003 infected fruits; while 'Don Gaetano' F2 seedlings like '493C11VIII8' or
1004 '493C11VIII26' showed very high infection rates (more than 50%). Based on
1005 the observed segregations, the authors concluded that BR resistance on the
1006 analysed crosses behave as a quantitative trait^{30,176,177}.

1007 Walter et al¹⁷⁸, tested several methods to evaluate BR in 'Sundrop' and 9
1008 accessions from the 'Clutha' series ('Sundrop' x 'Moorpark'), bred in
1009 HortResearch at Clyde research orchard (Alexandra, New Zealand). In this
1010 study, the authors analysed some infection parameters for 3 seasons: lesion
1011 area (artificial drop infections with *M. fructicola* and *M. laxa* spore suspensions
1012 in wounded and intact fruits), spore count on lesions, storage rot (natural
1013 orchard infection at room temperature and high humidity) and cuticle
1014 thickness. The authors determined that the most robust method to evaluate
1015 BR resistance in apricot was measuring lesion area on wounded, artificially
1016 infected fruits 72 h after inoculation. However they recommended combining
1017 more than one method for the evaluation of the material. Remarkably, the
1018 accession 'Clutha 14/107' showed significantly highest value of resistance to
1019 *M. fructicola* (measured as the mean of lesion area obtained in 3 seasons),
1020 the lowest quantity of produced spores per mm² lesion, a storage rot rate of
1021 less than 5% and one of the highest cuticle thickness.

1022 BR resistance was screened in several released cultivars and advanced
1023 selections from the sweet cherry breeding program at the Pacific Agri-Food

1024 Research Centre, British Columbia, Canada ¹⁷⁹. During 4 years, a total of 36
1025 genotypes were submitted to artificial inoculation (25-50 fruits triplicates per
1026 accession; ethanol and sodium hypochlorite fruit disinfection; spray of 10⁴
1027 spores/ml; incubation at 13°C and 95-97% relative humidity; BR impact
1028 assessment after 8 and 11 days after inoculation). Based on the difference in
1029 the percentage of rotten fruits between each genotype and the overall
1030 population mean, they established three resistance categories: more resistant
1031 than overall mean, close to mean and less resistant than mean. Although they
1032 identified some cultivars showing a high resistance level in two of four years
1033 (cultivars 'Staccato'TM, 'Stardust' and 'Sweetheart'), the authors stated that the
1034 observed resistance level was not enough to avoid fungicide applications in
1035 plants of these accessions and confirmed the results of Brown and Wilcox ¹⁸⁰,
1036 demonstrating that there are no sources of high level genetic resistance to BR
1037 in sweet cherry materials.

1038 Although it is difficult to find reliable sources of resistance in stone fruit
1039 species, seasonally consistent differences in the tested materials have been
1040 observed in all the works presented in this section. The existence of these
1041 differences indicates that exploring wider germplasm and using these sources
1042 to introgress resistance in cultivars of high fruit quality could result in new
1043 selections with improved BR resistance ¹⁷⁰. The 'BR resistant' cultivars and
1044 selections found up to now still have too low resistance levels to allow the
1045 suppression of fungicide application; however, the most resistant could
1046 already be cropped under integrated pest control strategies, suited to
1047 minimize exogenous chemical input in the orchard.

1048 Finally, as can be observed from the cited works, screening for BR
1049 resistance in germplasm collections and/or offspring is a very time- and effort-
1050 consuming task, and often under appreciated because results are frequently
1051 hampered by the influence that climatic conditions and agronomical practices
1052 exert in the level of resistance and pathogen strength. However, the variability
1053 observed between cultivars allowed identifying suitable materials to generate
1054 populations segregating for BR resistance and perform genetic studies for
1055 identification of genetic determinants associated to the variation in the
1056 phenotype.

1057 6.4 QTL of resistance

1058 In order to generate new cultivars with less necessity of fungicide inputs,
1059 the identification of genes or loci associated with resistance to BR would allow
1060 progressing in the incorporation of favourable alleles in breeding programs. In
1061 addition to functional studies seeking to understand the interactions between
1062 the pathogen and its host, genetic studies have been conducted to identify
1063 genomic regions associated with BR resistance. Although possible
1064 mechanisms of resistance can be inferred from these studies, their principal
1065 objective is the discovery and further incorporation of resistance alleles into
1066 breeding materials with the use of linked markers. Indeed, high-throughput
1067 molecular genetic tools and a high-quality genome sequence have been
1068 developed recently for peaches,¹⁸¹ and can now be exploited to radically
1069 improve the efficiency of disease resistance breeding in peaches, as well as
1070 in other *Prunus* species. Indeed, as commented before, breeding programs
1071 aimed at enhancing BR resistance have been impaired by time-consuming
1072 procedures for assessing this trait on field-grown segregating trees.

1073 Therefore, an important objective is the generation of new tools for the
1074 early selection of seedlings with enhanced BR resistance. Marker-assisted
1075 selection is a valuable strategy for these purposes, as it allows the early
1076 selection of seedlings bearing favourable alleles at marker loci genetically
1077 linked to genomic regions that control the trait of interest. Considering that
1078 fruit resistance to BR may be a multifactor system and that each different
1079 cultivar may hold only a little part of these factors, dealing at the same time
1080 with different sources of resistance may lead to confusion rather than to better
1081 understanding. Therefore, association studies have not been engaged and
1082 the first studies seeking for QTL of resistance had focused on bi-parental
1083 progenies stemming from a cross between a susceptible parent and a
1084 potential donor of resistance. This approach may represent the first
1085 compulsory step to identify genome regions controlling resistance. Hopefully,
1086 the comparison of detected loci between crosses may help identifying
1087 different factors of resistance coming from different donors. The final step
1088 would then be the combination of these different factors in elite genotypes to
1089 confer higher resistance.

1090 To date, two studies exploring genomic regions linked to BR-resistance
1091 have been published, both using peach host species. Martínez-García et al
1092 ¹¹⁰, performed a QTL analysis using *M. fructicola*-resistance phenotypic data
1093 of 73 seedlings from the Pop-DF progeny ('Dr. Davis' x 'F8, 1–42'), with
1094 parental accessions derived from canning peach and peach-almond back-
1095 crossing in the UC Davis breeding program ¹¹⁰. A linkage map composed by
1096 1037 SNPs segregating through the population, was used for Interval
1097 Mapping-QTL analysis. The study revealed three QTL, two of them in LG1

1098 and one in the LG4 of *Prunus* genome. The genomic region of one of the QTL
1099 in LG1 was significantly correlated with three years of phenotypic evaluation.
1100 The region included two potential candidate genes, coding for PAMP-
1101 triggered immunity, and effector-triggered immunity (ETI) proteins. SNP
1102 markers of this region are promising tools to enhance efficiency of breeding
1103 programs using similar genetic background.

1104 The second genomic study based on QTL analysis was performed
1105 using 80 melting-fleshed F1 individuals from the 'Contender' x 'Elegant Lady'
1106 cross, genotyped with a set of 89 markers (63 SSR and 26 SNP) and
1107 phenotyped for two seasons with artificial infections of *M. fructigena*, in the
1108 presence and absence of an artificial wound. The aim was to find genetic
1109 markers associated with skin and flesh resistance to BR¹⁷⁴. In this material,
1110 maturity date of seedlings correlated negatively with their BR resistance (late-
1111 maturing individuals appeared as less resistant); however, using a Multiple
1112 QTL model including maturity date as a covariate phenotype, significant
1113 genotype-phenotype associations were found between skin resistance and
1114 both M1a and EPPISF032 SSR markers (located in the LG2 and 4 of *Prunus*
1115 genome, respectively). Additionally flesh resistance was correlated with SNPs
1116 located in LG3 of peach genome, confirming the independence between
1117 genetically controlled mechanisms for skin and flesh resistance¹³².

1118 Despite the different results obtained in these two studies, probably
1119 due to differences in the different genetic background of the studied
1120 populations, the pathogenic agent employed and the different phenotyping
1121 approaches, they contribute to the literature regarding the identification of

1122 potentially useful genetic markers for assisted selection of new cultivars with
1123 enhanced BR-resistance.

1124 The research community has invested in the identification of resistance
1125 sources and the development of cultivars resistant to BR. Up to now, little
1126 progress has been made in this sense. However, notable advances in terms
1127 of phenotyping are noteworthy and the development of quantitative genetic
1128 studies may help to find ways of moving forward.

1129 **7 Conclusion**

1130 Understanding BR pathogenesis mechanisms, the biological barriers
1131 that *Prunus* fruit can offer to *Monilinia* spp. and the interaction between them,
1132 are crucial for designing phenotyping strategies able to measure resistance
1133 level in a robust way. Such approaches are needed to identify resistance
1134 sources across the *Prunus* germplasm and provide tools for breeding new
1135 hybrids with enhanced BR-resistance that, together with other alternative
1136 control strategies, could contribute to more sustainable stone fruit cropping.

1137 In this review we have collected the information available in historic and
1138 contemporary literature about the elements involved in the interaction
1139 between *Monilinia* spp. and *Prunus* fruit. We conclude that host specificity is
1140 not a strict condition for disease impact and infection development and that
1141 one of the main causes for the success of pathogen colonisation is the
1142 relatively high presence of “open doors” in some *Prunus* fruit’ epidermis,
1143 especially in peaches, cherries and plums. In the last decade, many works
1144 have identified and validated some important elements of the fungal infection
1145 and host resistance processes; nevertheless, the scientific community has not

1146 assembled these elements to generate a precise BR resistance model that
1147 explains the phenotypic diversity among *Prunus* species and their varieties.
1148 Finally, the significant influence that environment has in the infection process
1149 has been a persistent constraint that hampers a clear identification of such
1150 elements, but has to be considered in the generation of new varieties.

1151 These elements constitute valuable information and are useful in the
1152 design of new phenotyping approaches for breeding, as well as to test new
1153 alternative methods for BR control at the pre- and post-harvest stages. BR-
1154 resistant breeds and sustainable pathogen control strategies are being
1155 developed and validated^{14,9}. In the meantime, stone and pome fruit growers
1156 have the difficult task to combat damages caused by BR with lower quantities
1157 of synthetic fungicides, as recommended (or imposed) by IPM regulations and
1158 initiatives, and by adopting agronomical strategies and practices to eliminate
1159 natural inoculum sources.

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1161

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1673 **10 Figure Captions**

1674

1675 **Figure 1.** *Monilinia* spp. life cycle. Figure used with permission of Elsevier
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1677 **Figure 2.** Peach fruit infected by three different *Monilinia* species.

1678 **Figure 3.** Sites of fungi penetration: A- scanning electron microscopy
1679 examination showing the development of fungi in apricot surface 8 hours post-
1680 infection (hpi). The fungi develops on the fruit surface, twists around
1681 trichomes (t) and moves to the stoma (s) direction and trichomes basis
1682 (arrows). B - fluorescence photomicrograph 24 hpi. In this image it is possible
1683 to see a hyphae entering through an open stomata (arrows). C and E- light
1684 microscopy images showing infection on the surface of a commercial
1685 nectarine 'Magique' at maturity, coloured with Toluidine blue, 0.5%, 15 (hpi).
1686 C- Beginning of spore germination (asterisks) and penetration through
1687 stomata aperture (arrow). D - electron microscopy image shows a strong
1688 concentration and germination of spores (asterisks) fungi around the fruit
1689 cracks of cv. 'Magique' cultivar 15 hpi. E- spore germination and development
1690 of mycelium in micro crack (m) direction. F – an infection of nectarine surface
1691 at maturity coloured with Toluidine blue, 0.5%, observed with light microscopy
1692 at 15 hpi. It is possible to note the distribution of spores (asterisks) and their
1693 germination. This image illustrates a chaotic germination of spores (asterisks)
1694 and the colonization of surface by hyphae. The arrow shows the penetration
1695 of hyphae in an epidermis aperture (o). In all images fruit were infected with a
1696 drop of 10 μ L and 10^5 spore/ml⁻¹ of conidia concentration.

1697 **Figure 4.** Figure 4: Light microscopy image of surface impression of a young
1698 nectarine fruit (46 days after full bloom) showing the high density of stomata.

1699 **Figure 5.** Scanning electron microscopy of nectarine cultivar 'Magique'. The
1700 image shows the beginning of crack formation around a lenticel at maturity.

1701 **Figure 6.** The process of *Monilinia* spp infection

1702 **Figure 7.** The borderline between resistance and susceptibility to *Monilinia*
1703 *laxa* is often faint. In this figure the fungal infection, 48h after artificial
1704 inoculation on fruits from two peach varieties, are illustrated both at light (a,b,
1705 toluidine blue staining) and transmission electron microscopy (TEM, c,d) level.
1706 Both resistant BO92038071 (F1 from the cross 'Contender x Elegant Lady',
1707 left panels) and susceptible 'Elegant Lady' (right panels) present discrete
1708 fungal colonization on the epidermis with stacked hyphae (H) and conidia (C),
1709 sometimes germinating over guard cells (G). At this infection stage, the
1710 substomatal regions ¹⁰ appear digested in both fruit varieties as shown by the
1711 pink staining of pectins; however, only in the resistant fruit (a), infection is
1712 blocked, possibly by the deposition of plant phenolics (asterisks) in the
1713 adjacent cells. TEM images show that in resistant fruit hyphae, though able to
1714 digest cell walls, are almost encapsulated by electron-dense material (c,
1715 arrows) probably of phenolic origin. This material is not present in the fungal-
1716 plant interface in susceptible fruit infection (d) where cell wall matrix has been
1717 almost digested and cellulose fibrils (arrows) are completely disaggregated

1718 **Figure 8.** Main components of the biochemical warfare between *Monilinia*
1719 spp. fungi and *Prunus* fruits. Germinated spores can develop hypha that can i)
1720 enter through open doors (OPD; micro cracks, lenticels or trichomes basis), or
1721 ii) penetrate the cuticle after its degradation by fungal cutinases and
1722 subsequent *appressorium* formation. After cuticle breakdown, cell-wall
1723 degrading enzymes hydrolyze cell-wall polysaccharides through cellulases,
1724 pectinmethylesterases, exo- and endo-polygalacturonases, among others,
1725 generating dismantled tissue (DT, grey). Fungal-induced organic acid
1726 biosynthesis is another process that promotes fungal colonization. Polyphenol
1727 substances can be constitutively present or synthesized in response to
1728 pathogen colonization, amongst epicuticular waxes (EPW, light blue), cuticle
1729 (CUT, yellow), cell wall constituents or in the cytoplasm. Polyphenol
1730 substances stop hyphal colonization by creating an chemically adverse
1731 environment that results in a reduction in the gene expression of fungal
1732 cutinases or cell-wall degrading enzymes. Pathogenesis-related enzymes that
1733 constitutively are present in fruit tissues are able to activate phenylpropanoid

1734 pathway as well as peroxide emission. In some cases, cell wall strengthening
1735 by callose deposition may block the infection progress.

1736

1737 **Figure 9.** Development of brown rot 5-days post artificial infection in
1738 nectarines of cv. 'Summergrand' at maturity. Fruit were disinfected in water
1739 bath at 55°C for 40 seconds, put in acrylic plastic boxes and infected with one
1740 10µL drop at 10⁵ spores.ml⁻¹ of concentration deposited without wounding.
1741 Fruits were put in a chamber with controlled temperature (18°C) and 24°C
1742 respectively during dark (8 hours per day) and light (16 hours per day). High
1743 humidity was maintained in the closed boxes.

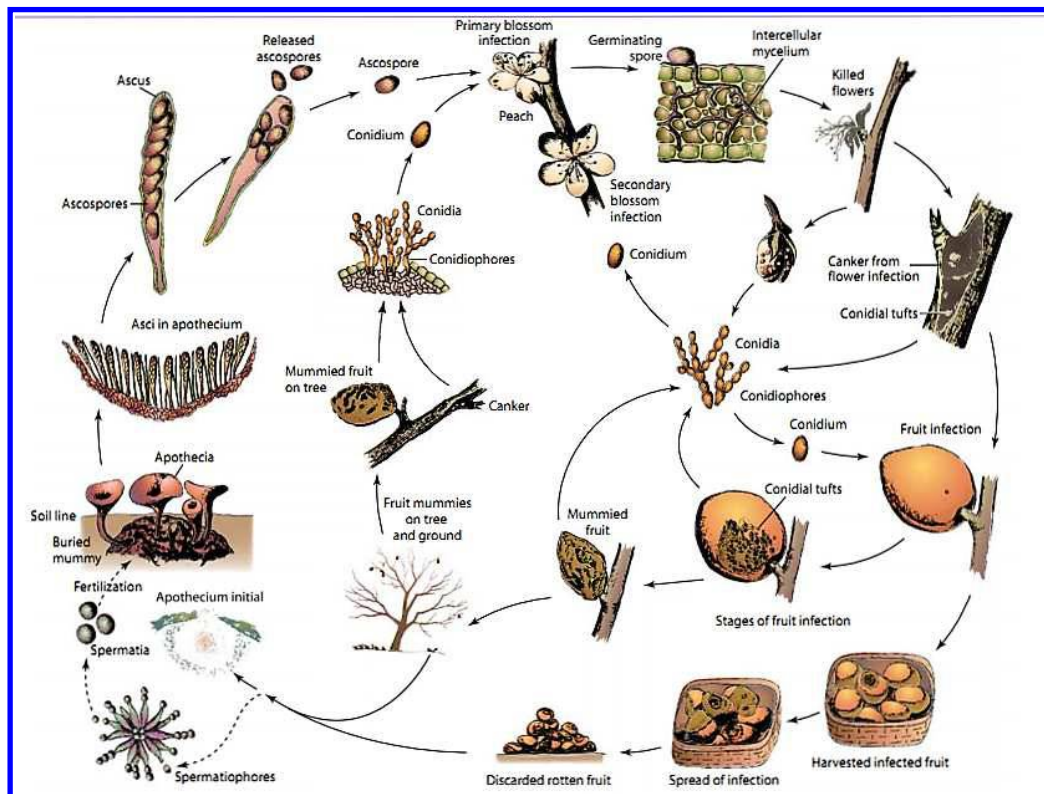
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1746 **11 Figures / Graphics**

1747

1748 Figure 1.



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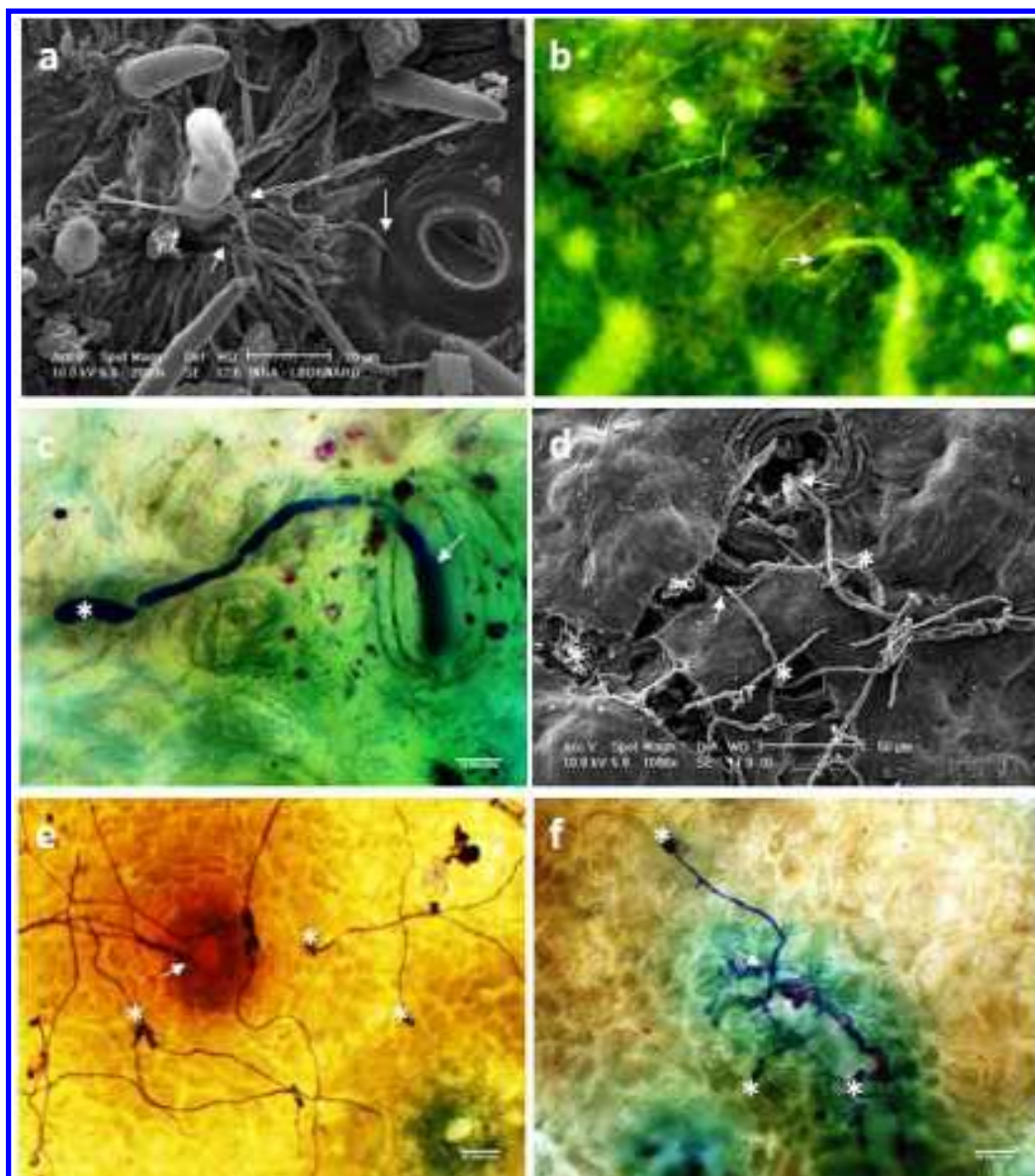
1751 Figure 2.



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1754 Figure 3.

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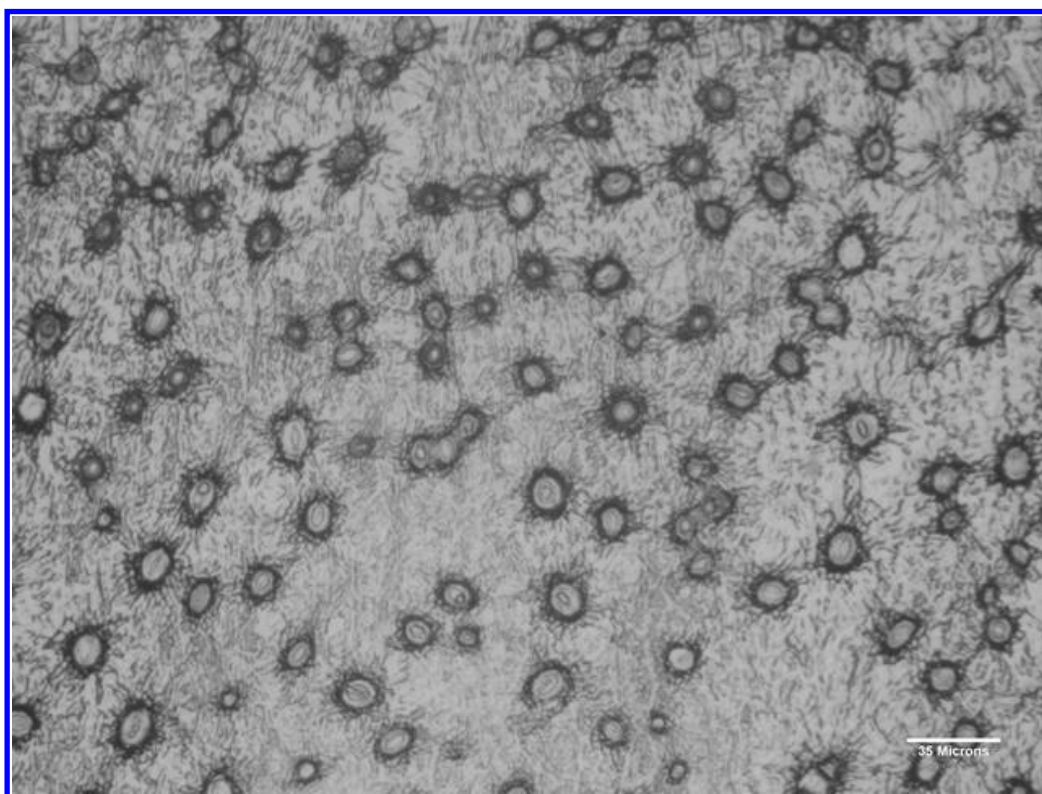


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1758 Figure 4.

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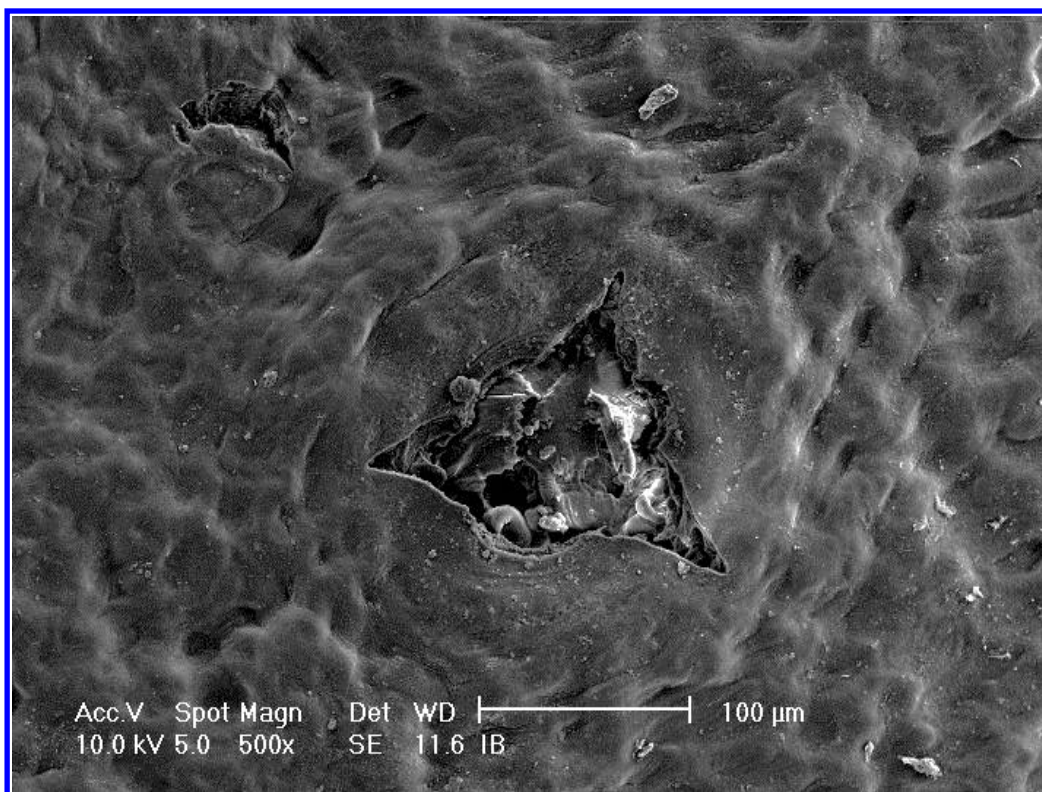
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1765 Figure 5.

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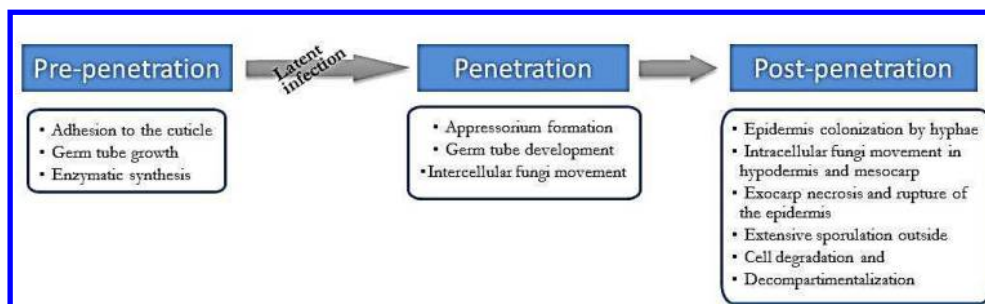


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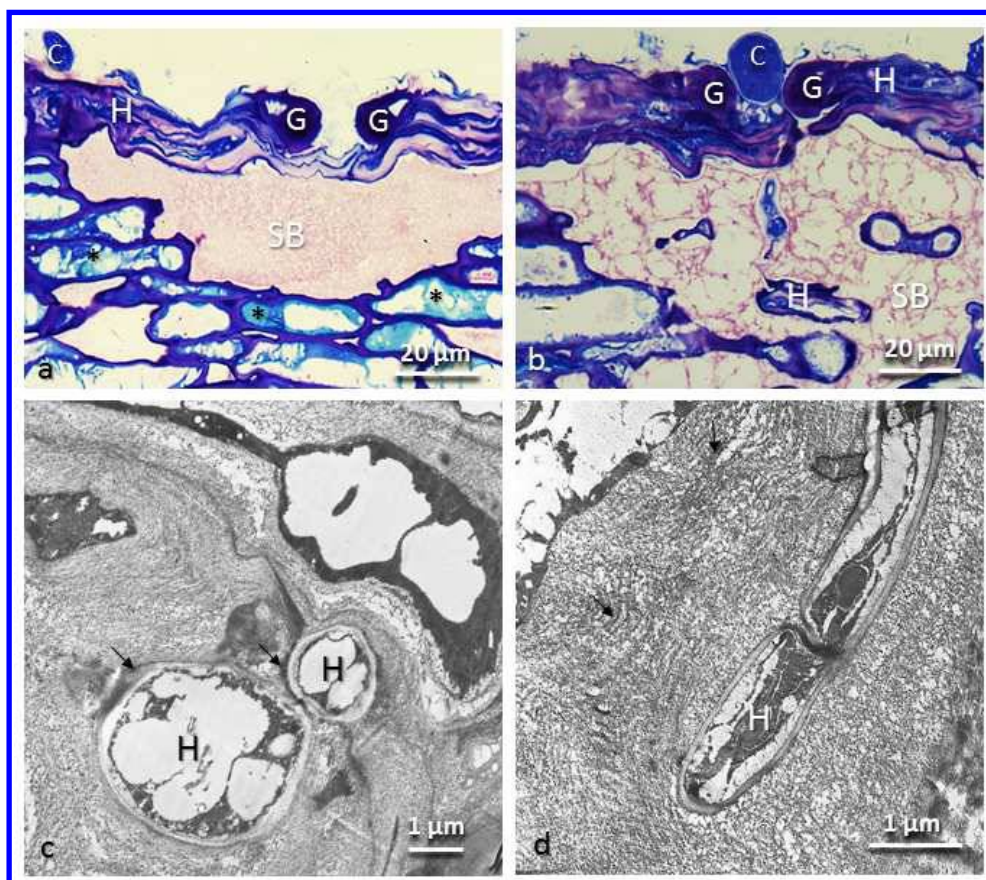
1770 Figure 6.



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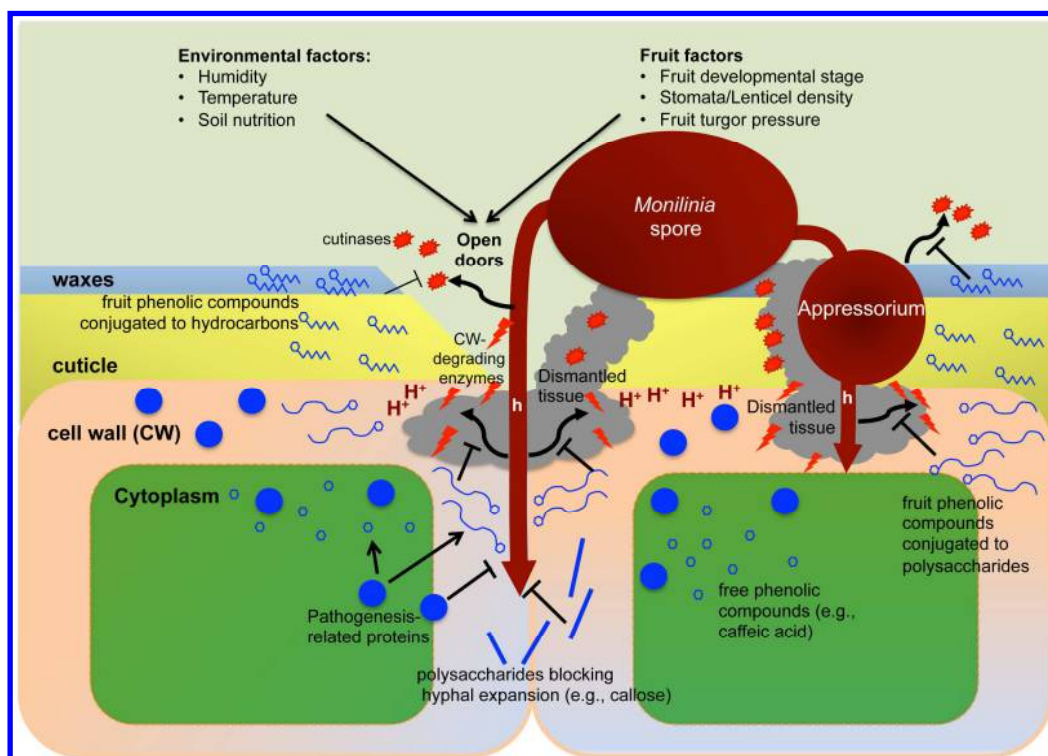
1773 Figure 7.



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1776 Figure 8.



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1778 Figure 9.



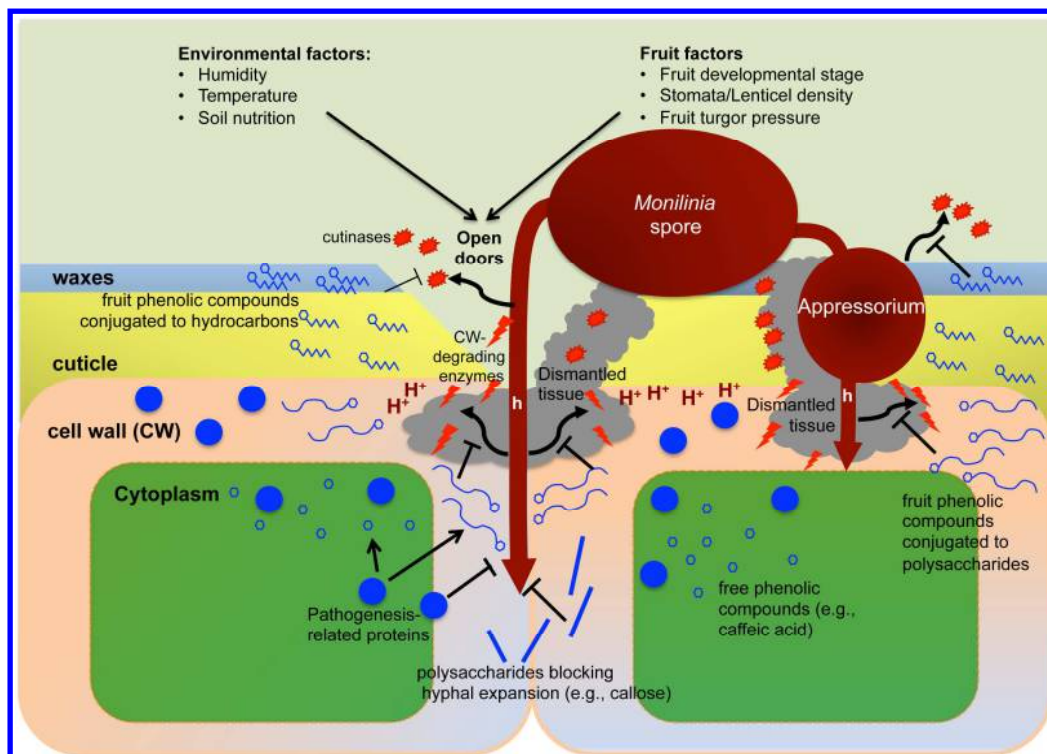
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1781 **12 Graphic for Table of Contents**

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