

Review

# Peach Brown Rot: Still in Search of an Ideal Management Option

Vitus Ikechukwu Obi <sup>1,2</sup>, Juan José Barriuso <sup>2</sup> and Yolanda Gogorcena <sup>1,\*</sup> 

<sup>1</sup> Experimental Station of Aula Dei-CSIC, Avda de Montañana 1005, 50059 Zaragoza, Spain; vitemma@live.com

<sup>2</sup> AgriFood Institute of Aragon (IA2), CITA-Universidad de Zaragoza, 50013 Zaragoza, Spain; barriuso@unizar.es

\* Correspondence: aoiz@eead.csic.es; Tel.: +34-97-671-6133

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**Abstract:** The peach is one of the most important global tree crops within the economically important Rosaceae family. The crop is threatened by numerous pests and diseases, especially fungal pathogens, in the field, in transit, and in the store. More than 50% of the global post-harvest loss has been ascribed to brown rot disease, especially in peach late-ripening varieties. In recent years, the disease has been so manifest in the orchards that some stone fruits were abandoned before harvest. In Spain, particularly, the disease has been associated with well over 60% of fruit loss after harvest. The most common management options available for the control of this disease involve agronomical, chemical, biological, and physical approaches. However, the effects of biochemical fungicides (biological and conventional fungicides), on the environment, human health, and strain fungicide resistance, tend to revise these control strategies. This review aims to comprehensively compile the information currently available on the species of the fungus *Monilinia*, which causes brown rot in peach, and the available options to control the disease. The breeding for brown rot-resistant varieties remains an ideal management option for brown rot disease control, considering the uniqueness of its sustainability in the chain of crop production.

**Keywords:** *Prunus persica*; *Monilinia* spp.; host and pathogen; stone fruits; crop protection; plant breeding

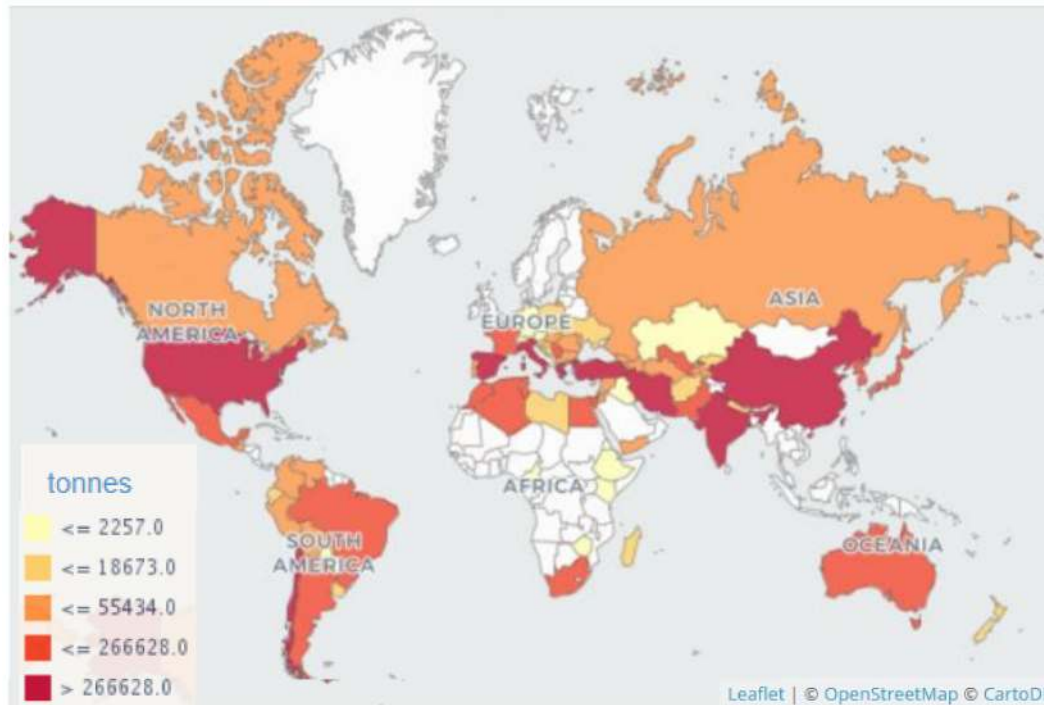
## 1. Introduction

Peaches (*Prunus persica* (L.) Batsch), nectarines (*P. persica* var. *nectarina* (Aiton) Maxim), and paraguayana (*P. persica* (L.) Batsch var. *platycarpa* L.H. Bailey) are members of the *Prunus* genus which includes hundreds of economically important fruits such as the cultivated almond (*Prunus dulcis* (Mill.) D.A. Webb), the apricot (*Prunus armeniaca* (L.)), the European plum (*Prunus domestica* (L.)), the Japanese plum (*Prunus salicina* (L.)) and the cherry (*Prunus avium* (L.)) [1].

The peach is the third most important global tree crop within the economically important Rosaceae family after apples (*Malus* spp.) and pears (*Pyrus* spp.), and its largest producer is China, followed by European countries (Spain and Italy) and the United States (Figure 1). Available information in EUROSTAT shows that in the European Union (EU) agriculture, the fruit sector weighs 6.7% of the agricultural output, peaches being the third product after apples and oranges [2].

Numerous fungal pathogens infect the peach in both the pre- and the post-harvest states. The prominent ones include *Rhizopus nigricans* Ehrenb., *Mucor* spp., *Botrytis cinerea* Pers. Fr., *Geotrichum candidum* Link ex Pers., *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., and *Monilinia* spp. [3]. However, for the purpose of this work we shall focus on the principal casual pathogens of brown rot (BR) in peaches. Species of *Monilinia* are associated with brown rot, which is the most economically

important disease of stone fruits worldwide [4,5]. Brown rot incidence (BRI) in peaches greatly varies during the fruit development [6]. Fruits are less susceptible to brown rot at the early stage of formation, becoming resistant during pit hardening, and increase susceptibility afterwards [7,8].



**Figure 1.** Global map of peach and nectarine production. Source: <http://www.fao.org/faostat/en/#data/QC/visualize> ([9]).

The causal agent of brown rot (*Monilinia* spp.) is a polycyclic pathogen [10] involving infection sequence repeated several times throughout the annual growth cycle of the host. The fungus survives the winter in mummified fruits [11], in canopy or in the ground [12], in fruit peduncles [13], in cankers on twigs, in spurs, and in branches [5,14,15]. These propagules [16] serve as sources of primary inoculum to infect blossoms, buds, and young shoots, establishing a source of secondary inoculum.

The universal annual losses from the epidemic have been estimated at 1.7 billion Euros [17]. In some cases, the disease has been associated with up to 80% of the incidence of fruit loss after harvest [18,19], mostly under favorable environmental conditions for the commencement and growth of the diseases in the orchard.

There are various control and management strategies for the brown rot epidemic in peach cultivation. These options include conventional, biological, chemical, physical, botanical, and host-resistance techniques [4]. In the present review, we will focus on peaches, their interaction with species of *Monilinia*, and the available management strategies, with a view to developing a sustainable peach-breeding scheme.

## 2. The Peach

The origin of the peach is traceable to the Western part of China in the Eastern Asian continent, where it was cultivated for 4000 years and from where it was subsequently dispersed to Europe, Africa, and America [20]. The documentation of the first cultivated peach was recorded in Chinese manuscripts as early as the 10th Century BC. In China, the species presents the greatest richness in germplasm and has the largest collections of peach germplasm, with wild peaches still growing today [20–22].

The five most important countries for the production of peaches and nectarines are shown in Figure 1 [9]. China was the leader in production (14.47 MT (million tons)), followed by Spain (1.53 MT), Italy (1.43 MT), USA (0.93 MT), and Greece (0.85 MT). The cultivation area in the world revealed that China is the leader with 838,768 ha and in the EU-28, Spain is the largest (86,896 ha), followed by Italy (69,005 ha) and Greece (44,271 ha). In the EU, the cultivation and production are concentrated in the countries of the Mediterranean region, including Spain, Italy, Greece, and France, owing to the fact that the potential risk of damage from frost [23] is less than in the countries of the Northern part of Europe. Hence, most plant disease models are known to use different climatic variables and operate at a different spatial and temporal scale from the global climate models [24].

### 2.1. Geography and Ecological Requirements of Peaches

Geographically, global commerce has brought peach tree cultivation into both the Northern and Southern hemispheres [20] which experience contrasting summers and winters allowing for year-round availability. Peach trees require wet winters and hot dry summers and will not flourish in Oceanic climates [20].

Oftentimes, the cropping practices employed in a region for the production of peaches are principally determined by the different environments, water and nutritional requirements [20]. These plantation crops, though cultivated mainly in temperate zones, between 30° and 45° latitude N and S, are not very resistant to the cold and they require up to 400 to 800 cold hours for flowering and have a good fruit set [20]. Minimum winter temperatures and spring frosts are important limiting factors in quality peach production. In temperate zones, flower bud death due to chilling injury induced or triggered by excessive cold during shooting or flowering [20]. Therefore, peaches cannot be grown successfully where temperatures normally fall to  $-23\text{ }^{\circ}\text{C}$  and  $-26\text{ }^{\circ}\text{C}$  [20]. On the other hand, they do not grow satisfactorily where winters are too mild, and most varieties require some winter chilling to induce them to burst into growth after the annual dormant period.

Edaphically, the peach grows best on well-drained sandy or gravelly loams. Hence, the peach seedling is susceptible to both calcareous and waterlogged soils [20,25]. Nevertheless, the search for iron chlorosis-tolerant rootstocks using peach–almond hybrids has led to a selection of highly vigorous rootstocks such as GF 677 [26], which were widely adopted in the Mediterranean basin countries. Later, other rootstocks tolerant to calcareous and waterlogged soils have been released [27,28]. Nitrogen-rich soils exceptionally support the performance of peach crops [29] when soil acidity is maintained above pH 6.0. On the contrary, a soil pH below 5.5 is deleterious to peach tree growth, fruit yield and size, and tree longevity [30].

### 2.2. Botany and Susceptibility of Peaches to Infection by *Monilinia* spp.

The peaches could be classified according to the flesh type (non-melting or melting), flesh color (yellow, orange, white, or yellow/orange), fruit shape (round or platicarpa), fruit type (peach or nectarine), and stone type (clingstone or freestone) [31]. Among the available varieties of peaches, the yellow-fleshed varieties, such as the famous Elberta, Redhaven, and Halford, are preferred in North America (USA), while both yellow- and white-fleshed types are popular in Europe [20,32]. Most yellow-fleshed peaches are clingstone varieties, while white-fleshed peaches fall into the freestone category [31]. The greatest difference between the two is really about texture and taste. The Spanish peach industry, hitherto, was based on yellow, non-melting fleshed and clingstone types; however, the replacement of the Spanish traditional varieties by introduced ones, mostly from North America, has promoted melting flesh cultivars [32].

Peach trees under commercial cultivation (Figure 2) are usually kept between three and four meters [33]. Peach trees are self-pollinating and have an impressive blossoming. Flowers are borne in the leaf axils along the shoots of the previous season's growth. The five petals, usually pink or pink-salmon [31], five sepals and three whorls of stamens are fused on the hypanthium, that forms the base of the flower that can be showy or non-showy [31]. The fruit is a large drupe with a thin

epidermis, a pulpy mesocarp, and a woody endocarp containing the seed, more or less globose, with a longitudinal groove and a cavity around the peduncle. Ordinarily, the skin of most ripe peaches is downy or fuzzy; however, the nectarines are a class of peaches with smooth skins [33].

The degree of susceptibility to infection by *Monilinia* spp. is variable throughout fruit development. The susceptibility is high during the early stages of fruit development, decreases during the green fruit or pit hardening stage, and increases again during the ripening period [8,34]. Phenologically, peach fruit development generally undergoes four stages (S-I to S-IV, Figure 2f–g) from flowering to maturation: Fruit set (S-I), characterized by cell division and elongation, also referred to as the exponential growth phase; pit hardening (S-II), when the endocarp hardens to form the stone and scarcely increases the fruit size; pre-climacteric phase (S-III), which is another exponential growth phase, with a resumption of rapid cell division and fruit size enlargement; the climacteric stage (S-IV), with final cell division, cell expansion, and ripening/maturation [35,36].



**Figure 2.** Phenological growth of peach trees from one season to the other (bare orchard to commercial maturity) at the Aula Dei peach germplasm collection. (a) Peach orchard in the autumn-winter, (b) Flowering peach orchard at early spring, (c) Flower buds (receptacle, sepals, and petals intact) (E), (d) Flower in blossom (petals, stamens and pistil exposed) (F), (e) Flower petals and stamen shrinking and about to fall off (G), (f) Fruit setting stage (S-I), (g) Pit hardening (S-II), (h) Physiologically matured peach fruit (S-III), (i). Commercially matured peach fruit (S-IV). Capital letters in brackets: phenological growth stages according to the Baggliolini codes (c–e) and fruit growth (f–i) [35,36].

Peach fruit nutrients have been found to be at their peak in the first stages of fruit formation and gradually reduce as the fruit develops [37]. Therefore, the period of highest brown rot susceptibility conversely coincides with the lowest peach nutrient contents.

### 2.3. Economic Significance of Peaches in EU-28

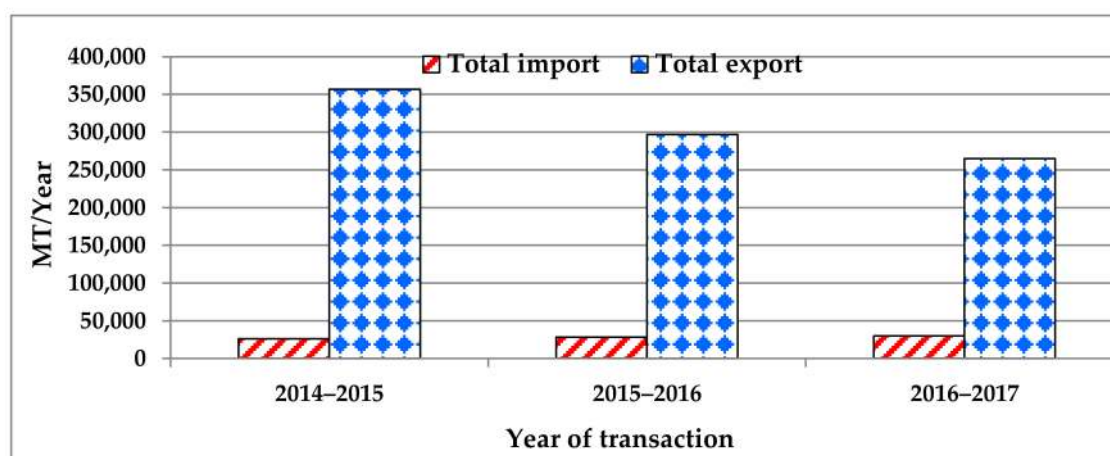
The production of peaches and nectarines in the campaign 2017–2018 for the main EU-28 producers is estimated at 4 million tons (MT), 6% higher compared to the previous cropping season 2016–2017 (Table 1).

**Table 1.** Production and estimation of peaches and nectarines (in tons) in the EU-28 main producing countries in different campaigns.

Country	2015–2016	2016–2017	2017–2018
Spain	1,581,510	1,475,849	1,487,444
Italy	1,408,504	1,262,127	1,362,749
Greece	777,160	788,120	910,000
France	217,146	207,004	214,800

Source: United States Department of Agriculture (USDA) [38], Spanish Ministry of Agriculture (MAPAMA) [39].

This production is attributable to an expected increase in most of the major producers; hence, the EU has remained a net exporter of peaches and nectarines, with total exports largely exceeding imports for three seasons running (Figure 3). However, there was a steady drop in the EU export from the 2014–2015 to the 2016–2017 campaign (Figure 3), undoubtedly suggesting an increase in the percentage of internal consumers of this precious product within the EU-28 during this period.



**Figure 3.** Total import and export of EU-28 of fresh peaches and nectarines during three seasons. (2014–2017). Source: USDA [38]. Metric tons (MT = 1000 kg)

According to a special global report, Spain has become, in the last four seasons, the largest peach and nectarine producer in the EU-28 [38]. The reasons for this feat were attributed to factors including a steady performance from the country's most important regions (Aragón, Cataluña, and Murcia) together with improved productivity in other regions and an increase of early and mid-season peaches, mainly due to good flowering and fruit set and the introduction of newer varieties in recent years [38]. According to the Spanish Ministry of Agriculture (MAPAMA) [39], peach and nectarine production in Spain for 2017–2018 is projected to reach almost 1.487 MT, accounting for an almost 40% share of the total EU-28 peach and nectarine production. This is 0.7% higher compared to the previous season and is due to favorable weather conditions that resulted in a production characterized by very good quality and calibers.

Incidentally, the optimum peach commodity production is hindered by the brown rot epidemic, with significant economic consequences [40]. In recent times, orchards have been abandoned before harvest because of the severity of this disease [4]. Consequently, the universal annual losses from the epidemic have been estimated at 1.7 billion Euros [17]. In Spain, particularly, the disease has been associated with as high as 80% of fruit loss after the harvest [3], mostly under favorable environmental conditions for the commencement and growth of the diseases in the orchard.

### 3. Brown Rot

#### 3.1. *Monilinia* spp.

*Monilinia* belongs to the group of necrotrophic fungi (Ascomycota) in the order of Helotiales (Leotiales), a large family of inoperculate discomycetes which includes both human and plant pathogens [41]. The teleomorph genus is *Sclerotinia* spp. (position in classification: Sclerotiniaceae, Helotiales, Leotiomyetidae, Leotiomyetes, Pezizomycotina, Ascomycota) [42]. The species of *Monilinia* are among the major causal organisms of brown rot disease in various orchard tree crops including: (a) Stone fruits [4,40,41,43,44], such as apricots [45,46], peaches [5,8,47,48], nectarines [48], cherries [49], and plums [45]; (b) Almonds [50] occasionally; and (c) Some pome fruits [51,52], such as pears [51], apples [53], and quinces [54].

*Monilinia laxa* (Aderhold and Ruhland) honey is one of the most important species of *Monilinia* globally associated with the brown rot in stone and pome fruits [4]. *Monilinia fructigena* (Honey), *Monilinia fructicola* (G. Winter) [5] and *Monilinia polystroma* (G. Leeuwen) [48,55] are other important species. The disease is highly destructive for peaches from fruit formation to storage, but additional losses are caused by the blighting of flowers and twigs.

Hitherto, *M. laxa* and *M. fructigena* were reported to be the two most important fungi causing the disease in peaches, particularly in Spain until 2006. Then, *M. fructicola* was detected for the first time in peach orchards in the Ebro valley, Lerida, Spain [5], and spread, displacing *M. laxa*, a supposedly indigenous pathogen [56], and reaching the same level of diffusion [56]. Nowadays, in Spain, both *Monilinia* species (*M. laxa* (Aderhold and Ruhland) and *M. fructicola* (G. Winter) Honey) coexist in the field [4,47]. It can be inferred that *M. laxa* and *M. fructicola* have similar epidemiological physiognomies [57], considering such an inherent ecological coexistence. Incidentally, the epidemiology and management of *M. fructicola* have been most extensively studied, whereas the equally important *M. laxa* has received less attention [4].

Changes in the frequency of occurrence of different fungal pathogen species may be due to fungicide resistance. Egüen et al. [18] suggested that fungicide resistance of the *M. fructicola* population is co-acting with other factors such as an adaptation in the pathogen, changing the frequency of occurrence of the three *Monilinia* species in Spain. The displacement of *M. laxa* by *M. fructicola* in Spain has also been attributed to its conjugational potency related to sexual exchange and ability to produce ascospores from pseudosclerotial mummified fruits and their gradual process of a sexual propagation [56]. *M. laxa* is not known to produce apothecia [56], while *M. fructicola* does, from which ascospores can easily be disseminated in the spring for possible infection in the fruiting season [58].

#### 3.2. Geographical Distribution of the Species of *Monilinia*

The occurrence and distribution of the species of *Monilinia*, (Figure 4) are global, having been detected in virtually all the continents of the world [4,40] where potential hosts are cultivated. Their presence is established especially when peach fruits are moved (imported) from one country of origin to another [4]. Presently, six closely related species of brown rot fungi have been reported, particularly in stone and pome fruit, including *Monilinia laxa* (Aderhold and Ruhland) Honey, *M. fructicola* (G. Winter) Honey, *M. fructigena* (Honey), *M. polystroma* (G.C.M. van Leeuwen), *M. yunnanensis* (M.J. Hu and C.X. Luo), and *M. mumeicola* (Y. Harada, Y. Sasaki, and T. Sano) [40,59,60].

The geographic distribution of these species differs across the world (Figure 4). Hence, *M. laxa* and *M. fructicola* are found more globally distributed [4,40,59,60] across the six continents of the world. *M. fructigena* is mostly restricted to the European and Asian countries [4,59,60] although its presence has also been reported in Africa (Morocco and Egypt). *M. fructigena* was a quarantine pathogen for Canada, the USA, Australia, and New Zealand but now it is not present there (Figure 4). *M. polystroma* [55] has been reported in Serbia [61], Japan, Hungary, China, Croatia, and Slovenia [59]; *M. mumecola* in Japan and China [59] and *M. yunnanensis* [62] is only domiciled in China [59]. Consequently, it is no longer relevant to affirm that the different species of *Monilinia* are distributed in specific regions considering the obvious ubiquitous pattern of spread.



**Figure 4.** Global map showing the present continental distribution of the species of *Monilinia* spp. Source: <https://www.cabi.org/isc/> ([63]).

### 3.3. Life Cycle of Species of *Monilinia*

The species of *Monilinia*, as a polycyclic pathogen [10], produces numerous secondary cycles throughout the annual growth cycle of the host (Figure 5). The fungus survives winter (and is transmitted from year to year) in several structures, such as mummified fruits [11,64,65], in canopy or on the ground [12], fruit peduncles [13], cankers on twigs, spurs, and branches [14,15]. These propagule-infested materials, according to Gell et al. [16], serve as sources of primary inoculum, and, when weather conditions are suitable, spores can infect blossoms, buds, and young shoots, thus establishing a source of secondary inoculum [16].

Thus far, the main primary inoculum in Spanish orchards is the mycelium and conidia present in the mummies found in affected trees or on the orchard floor [66]. It has also been shown that there is a positive correlation between the number of mummies in the trees and the incidence of post-harvest fruit rots [16,66].

It is also very important to note here that brown rot propagules are practically everywhere during the fruit-ripening period. In addition, infection is almost certain to occur if the weather is moist for long periods [13] and if the fruit skin is bruised [67].

Brown rot is spread by the dispersal of *Monilinia* propagules through other microorganisms [20], wind and water [68], insects, birds, and man [69]. They are also transferred by rain/overhead irrigation splashes [13]. Finally, insect and hail wounds, fruit cracking, limb rubs, twig punctures, and a variety of picking and packing injuries are predisposing factors that greatly increase fruit losses due to brown rot [70]. Hence, a clarion call for an adequate orchard sanitary [13,71] observation at all times, especially at the onset of spring. Young uninjured fruits are thus, always fairly, safe from infection. Special care

should be taken during harvesting and packing to prevent puncturing or bruising of the ripe fruit. In addition, wild or neglected stone fruit trees that serve as collateral reservoirs for the disease must always be removed [70].

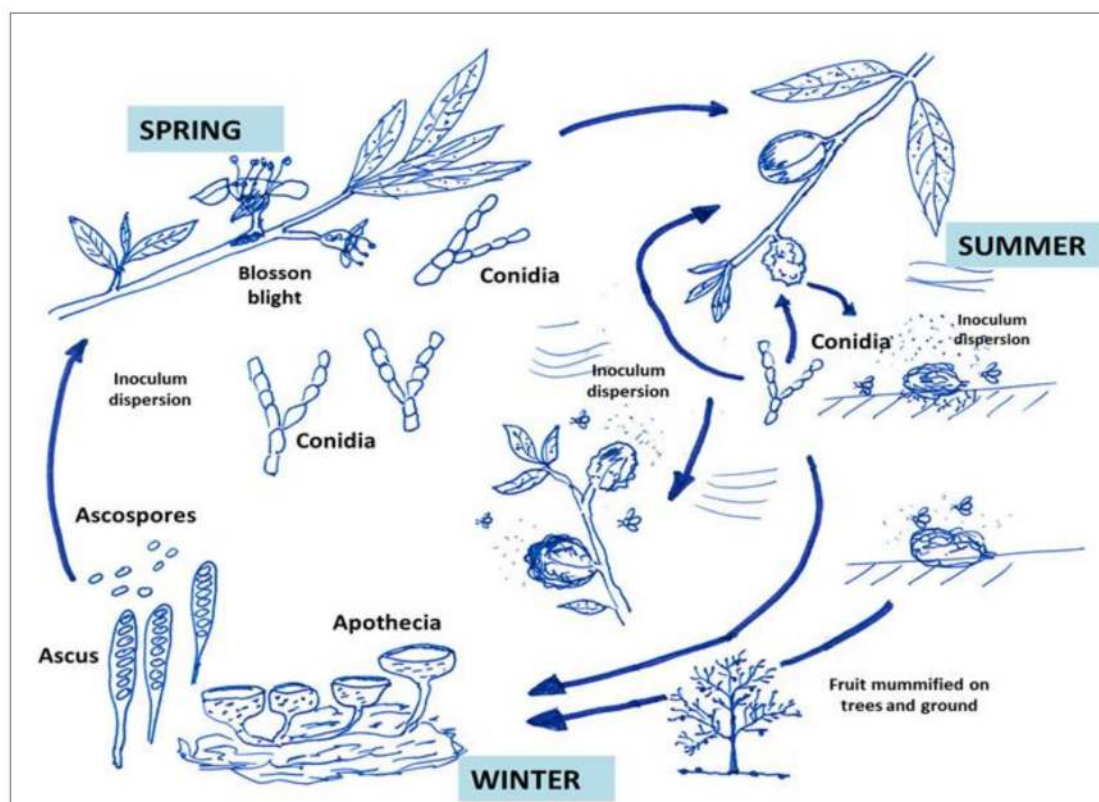


Figure 5. Brown rot disease cycle.

For *M. fructicola*, an occasional source of inoculum can be sexual spores (ascospores) [4]. Around the blossoming time, a mummified fruit produces up to 20 or more small, tan, cup-like structures on slender stalks that are called apothecia (sg. apothecium) (Figure 5). As an apothecium matures, it becomes thicker, and the cup opens to a bowl-like disc 3 to 12 mm in diameter across the top. The inner surface of each of these bowl-like discs is lined with thousands of spore-containing sacs (asci). At this stage, the slightest disturbance or air movement will cause an apothecium to effectively discharge millions of spores. If a film of water (either from dew, rain, or irrigation—especially overhead irrigation) is present for five hours or longer, the spores can germinate and penetrate the plant [4].

Infected blossoms soon wilt, and tan-gray tufts, composed of masses of another type of asexual spore (conidia), develop on the outside of the flower shuck [66]. If the infected blossom does not drop off, the fungus soon grows through the pedicel on the twig, initiating a canker. Masses of conidia are soon produced on the newly cankered twig surface during moist periods throughout the early part of the fruit development period (Figure 5). When entering the summer period, spores are easily detached and, just like the ascospores, are mainly wind-borne. Then, the brown rot disease cycle continues as indicated in Figure 5. However, when conditions (weather) are unfavorable, the infection can remain latent [72] until fruit maturity, the optimal time of disease development [8,57,73].

*M. laxa*, as a mycelium, overwinters in twig cankers, blighted blossoms parts, peduncles, and mummified peach fruits in the canopy or on the ground [12]. In addition, in the spring, mycelia propagules begin to sporulate and produce abundant conidia which initiate infections on close contact with susceptible tissues, such as blossoms, spurs, and twigs [74]. Fruits are susceptible to *M. laxa*



infection particularly in the time of fruit maturation, which may lead to a disease epidemic by harvest and infected mummified fruits [74].

### 3.4. Ecological Requirements of *Monilinia* Species

Epiphytologically, the knowledge of the ecological requirements of *Monilinia* species is essential in the development of a predictive model to comprehend the epidemiology of brown rot and provide adequate disease management strategies [75,76]. Important abiotic factors determining the potential for conidia germination and the growth of *Monilinia* propagules on the host exterior include temperature (T), water availability (water activity  $a_w$ ), and wetting period (W) [77,78]. The effects of these factors on *Monilinia* pathogenicity combine with those exerted by the moisture content of the spore, the age of the spore, and the inoculum concentration. According to Casals et al. [78], more than 80% of viable conidia of *M. fructicola* and *M. fructigena* can germinate at 25 °C and 0.99  $a_w$  within 2 h, whereas those of *M. laxa* require 4 h. The three species can germinate at a temperature range from 0 to 35 °C under 0.99–0.95  $a_w$ . The optimum temperatures for *M. fructicola* and *M. laxa* were determined at 24.5 and 19.8 °C, respectively [79]. The estimated maximum temperature for lesion development is higher for *M. fructicola* (30 °C) than for *M. laxa* (10 °C), inferring that *M. fructicola* is favored by a warmer weather compared to *M. laxa*. Hence, Bernat et al. [57] reported that *M. fructicola* is better adapted to high temperatures, whereas *M. laxa* is better adapted to low temperatures. These authors observed that, under optimal conditions, *M. laxa* is as aggressive as *M. fructicola* on peach fruits. *M. laxa*, unlike the others (*M. fructicola* and *M. fructigena*), has the potential to germinate in the absence of free water ( $a_w$ ) in the host, which makes this fungus relatively more virulent. The minimum germination temperatures estimated for *M. fructicola* and *M. laxa* were 4.7 and 0 °C, respectively [78]; nevertheless, the conidia of *M. laxa* have been reported to germinate even at −4 °C [75].

Though the lowest storage temperature for stone fruit is 0 °C, the conidia of the species of *Monilinia*, in general, could have the potential to germinate at a temperature range from 0 to 35 °C of 2 h from the initial exposure in vitro, especially when the value of free water ( $a_w$ ) or relative humidity (RH) under the equilibrium condition is under 0.99–0.90  $a_w$ . Hence, the pathogen on the peach skin can germinate under 0–40 °C at 100–80% RH [66]. However, an optimum temperature range for brown rot (moniliosis) initiation in peaches at the commercial stage is 22.5–25 °C, at which more than 79% of fruits could be infected under a wetting period of 12 h minimum [77].

### 3.5. Characterization and Identification of *Monilinia* Species

*Monilinia* species, including *M. laxa* (Aderhold and Ruhland) Honey, *M. fructigena* (Honey), *M. fructicola* (G. Winter), and *M. polystroma* (G. Leeuwen) appear difficult to differentiate from one another. However, a relative distinction is possible through the use of “CMM”, which is the observation and combination of cultural, morphological, and molecular methods [80]. The main characteristics of the morphological variance in cultures (classical) of *M. laxa* and the other three related EU-28 *Monilinia* species are presented in Table 2.

#### 3.5.1. Classical Methods

As indicated in Table 2, classical quantitative or qualitative characterizations and identification in species of *Monilinia* are possible by combining morphological data, such as conidial dimensions and the length of the germ tube [81], with cultural physiognomies [82], such as growth rate, growth pattern, and colony color index (CCI) [83].

Though with some similarities, in germ tube per conidia and colony colors, particularly between *M. laxa* and *M. fructicola*, there are peculiar pronounced characteristics in the culture of the former (*M. laxa*) that distinguish it from the others, including the formation of the smallest conidia (11–13 × 8–9.5 μm), a short and twisted germ tube, a shorter germ tube (150–350 μm) at 22 °C after more than 18 h of incubation (Table 2).

**Table 2.** Main differences in culture between *Monilinia laxa* and the other three associated EU-28 *Monilinia* species.

Characteristics/Pathogen	<i>M. laxa</i>	<i>M. fructicola</i>	<i>M. fructigena</i>	<i>M. polystroma</i>	Source
Conidia dimension	11–13 µm × 8–9.5 µm	12.5–14.5 µm × 8–10 µm	17.5–20.5 µm × 10.5–12.5 µm	13–17 µm × 9–10.5 µm	EPPO Bull [80]; van Leeuwen et al. [55]
Number of germ tube	1/conidia	1/conidia	2/conidia	2/conidia	EPPO Bull [80]; van Leeuwen et al. [55]
Form of germ tube	Short and twisted	Long and straight	Long and straight	Long and straight	EPPO Bull [80]; van Leeuwen et al. [55]
Size description	Smaller	Larger	Similar to <i>M. laxa</i>	Similar to <i>M. fructigena</i>	EPPO Bull [80]; van Leeuwen et al. [55]
Length of germ tube (>18 h at 22 °C)	150–350 µm	750–900 µm	600–900 µm	700–1000 µm	EPPO Bull [80]; van Leeuwen et al. [55]
Sporulation	Delayed and sparse	Quick, intense and abundant	Sparse	Sparse	EPPO Bull [80]; van Leeuwen et al. [55]
Sporulation range *	0–3.7	2.8–5.3	-	na	Hu et al. [62]
Mean sporulation *	1.8	3.9	na	na	Hu et al. [62]
Colony color	Hazel/Isabelline (greyish-brown)	Hazel/ Isabelline (greenish-brown)	Pale luteous (yellowish/creamy)	Pale luteous (yellowish/creamy)	EPPO Bull. [83]; Petróczy et al. [61]; Petróczy et al. [84]
Mycelium in distinct layers/colony rosetted	Resetting (mycelium in distinct layers on top of each)	No/rare	On distinct tufts; rings of aerial mycelium	Intense formation of black, stromatal plates initiated after 10–12 days incubation	van Leeuwen et al. [55]; EPPO Bull. [80]; Petróczy et al. [84]
Colony rosette with black arcs	Yes	No	No	No	EPPO Bull [80]; van Leeuwen et al. [55]
Concentric ring of spores	No	Yes	Sometimes	Sometimes	van Leeuwen et al. [55]
Colony margins	Serrulated/lobed	Not lobed but entire	Not lobed but entire	Not lobed but entire	van Leeuwen et al. [55]; Petróczy et al. [84]
Range of colony growth rate (mm/24 h)	2–11	9–20	0–12	nd	de Cal et al. [81]; van Leeuwen et al. [55]
Mean colony growth rate (mm/24 h) (in continuous darkness)	6	13	3.7	7	EPPO Bull [83]; Hu et al. [62]; Petróczy et al. [84]
Growth rating scale	Low	High	Low-moderate	Moderate	van Leeuwen et al. [55]

\* Log-transformed number of conidia per cm, (Hu et al.) [62]; nd = not detected, na = not available.

On potato dextrose agar (PDA), the colonies of *M. laxa* are greyish-brown, while the colonies of *M. fructigena* are yellowish or creamy (Table 2). In addition, colony formation in *M. laxa* is in distinct layers on top of each other (resetting) with black curvatures and never associated with a concentric ring of spores as observed for *Monilinia fructicola*. In addition, in *M. laxa*, the colony margins are serrated or lobed with low growth rating scale (Figure 6).

In general, the identification in culture of *Monilinia* species is often difficult because their appearance varies from isolate to isolate within the same species [85]. For this reason, the aforementioned quantitative methods do not allow an effective screening; therefore, there is a need for standardized conditions (molecular methods) starting with pure cultures.



**Figure 6.** Pure cultures on potato dextrose agar (PDA) showing the morphologies of the three major species of *Monilinia* at 10 days (22 °C) of incubation: (a) *M. laxa*, (b) *M. fructicola*, and (c) *M. fructigena*. (Photograph Courtesy APS Press). Reproduced with permission from Compendium of Stone Fruit Diseases, 1995, American Phytopathological Society, St. Paul, MN, USA.). <https://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/BrownRotStoneFruits.aspx> [86].

### 3.5.2. Molecular Methods

Since the first decade of this century, several methods of molecular characterization to distinguish *Monilinia* species [4,40,85,87,88] exist. Most of the molecular methods for characterization or disease detection include the Polymerase Chain Reaction (PCR), which is an in vitro, primer-directed, enzymatic reaction capable of exponential amplification of DNA. A recent review published in 2017 [89] revealed that the molecular techniques used for *Monilinia* characterization include many PCR variants [PCR, nested PCR (nPCR), cooperative PCR (Co-PCR), multiplex PCR (M-PCR), real-time PCR (RT-PCR), and DNA fingerprinting] and fluorescence in situ hybridization (FISH), which are all based on DNA analysis.

The method using *Monilinia*-specific DNA primers of the internal transcribed spacer (ITS region) was employed by Forster and Adaskaveg [90] in the detection of early brown rot infections in cherry fruits. With the same technique (ITS), Ioos and Frey [91] used the endpoint PCR to study the genomic variation within *M. laxa*, *M. fructigena*, and *M. fructicola* and designed primers to successfully achieve results directly on the diseased fruits. This method is considered the conventional PCR standard test (EPPO Bulletin 2015). A species-specific detection of *M. fructicola* from California stone fruits and flowers was developed by Boehm et al. [92] using the PCR technique in 2001. Côté et al., in 2004 [85], worked on the characterization and identification of *M. fructigena*, *M. fructicola*, *M. laxa*, and *M. polystroma* using a multiplex PCR. Later in 2007, Gell et al. [87] utilized two different PCR approaches for the universal diagnosis of brown rot and identification of *Monilinia* spp. in stone fruit trees combining a set of universal primers with the inclusion of an internal control for the diagnosis of

brown rot caused by the three more important species. More recently, the RT-PCR method developed by Brouwershaven et al. [88] was validated against all four brown rot-causing *Monilinia* species (*M. laxa*, *M. fructicola*, *M. fructigena*, and *M. polystroma*). In 2016, Guinet et al. [93] used a multiplex real-time PCR (RT-PCR) to detect and discriminate the three common species of *Monilinia* (*M. laxa*, *M. fructicola*, and *M. fructigena*) on *Prunus* and *Malus*. Other authors, [94] applying high-resolution melting (HRM) techniques, distinguished six different species of *Monilinia* in peach (*M. laxa*, *M. fructicola*, *M. fructigena*, *M. mumecola*, *M. lithartiana*, and *M. yunnanensis*) by analyzing the melting curve of amplicons of two universal primer pairs.

Recently, Garcia-Benitez [95] compared the overnight freezing-incubation technique (ONFIT) and the modified RT-PCR quantitative polymerase chain reaction (qPCR)-based methods of van Brouwershaven et al. [88] to detect latent brown rot infections and, subsequently, distinguish between the *Monilinia* spp. in flowers and peach fruits. The same authors [96] later validated the method to test performance accuracy, analytical specificity, sensitivity, repeatability, and reproducibility, as defined by standard PM7/98 of the European Plant Protection Organization (EPPO) for detection of *Monilinia* spp., demonstrating that was more sensitive, reliable, and quicker than ONFIT for detecting a latent brown rot infection.

The merits of DNA-based detection methods include reliability, timesaving, and higher sensitivity and specificity, when compared to the traditional and serological assays techniques employed during the processes of artificial cultivation [93,95]. For example, the molecular technique of multiplex RT-PCR assay (One step) developed in 2016 by Guinet et al. [93] is efficient and prompt in characterizing the three major species of *Monilinia* responsible for brown rot (*M. laxa*, *M. fructicola*, and *M. fructigena*). These authors also inferred that the exceptional reliability of their results is of paramount importance in the framework of phytosanitary regulations, considering that the performance data were generated, and the assay was fully validated in accordance to the EPPO guidelines [72].

In summary, molecular biology-based methods are progressively providing the means for a timely identification of quarantine plant pathogens including some species of *Monilinia*. The methods do not necessitate the isolation of the particular species of *Monilinia* and, therefore, significantly accelerate the identification process compared with methods based on quantitative characteristics. Finally, these methods can potentially be improved to directly and specifically identify the species infecting peaches. Their prominence, no doubt, is also necessitated by the inefficient classical screening systems, however, there are limitations associated with molecular assays

In the last decade, several protocols could not distinguish some *Monilinia* species, although modern RT-PCR has been modified to overcome this [59,95]. The methods of PCR primers and protocols for *M. fructicola* as documented by Förster and Adaskaveg [90], Boehm et al. [92], and Ma et al. [97], though discriminating *M. fructicola* from *M. laxa*, have not been validated for distinguishing *M. fructicola* from *M. fructigena*.

The characterization methods of Ioos and Frey [91], Miessner and Stammler [98], and Hily et al. [99] reliably differentiated three species of *Monilinia* (*M. fructigena*, *M. fructicola*, and *M. laxa*), but were practically unable to distinguish *M. fructigena* from *M. yunnanensis*. Similarly, the methods developed by Ioos and Frey [91] and Ma et al. [97,100] did not discriminate between *M. mumecola* and *M. laxa*. In addition, the method developed by Hily et al. [99] did not differentiate *M. mumecola* from *M. fructicola*, and the methods of Miessner and Stammler [98] and Hily et al. [99] also could not discriminate between *M. yunnanensis* and *M. mumecola*. Moreover, some investigators [96] in their study of RT-PCR detection of latent *Monilinia* spp. infection in nectarine flowers and fruits could only prevent cross-detection by stringently including an allelic discrimination step (with an extra cost) in the qPCR runs, to enable the differentiation between *M. fructicola* and *M. laxa* [95].

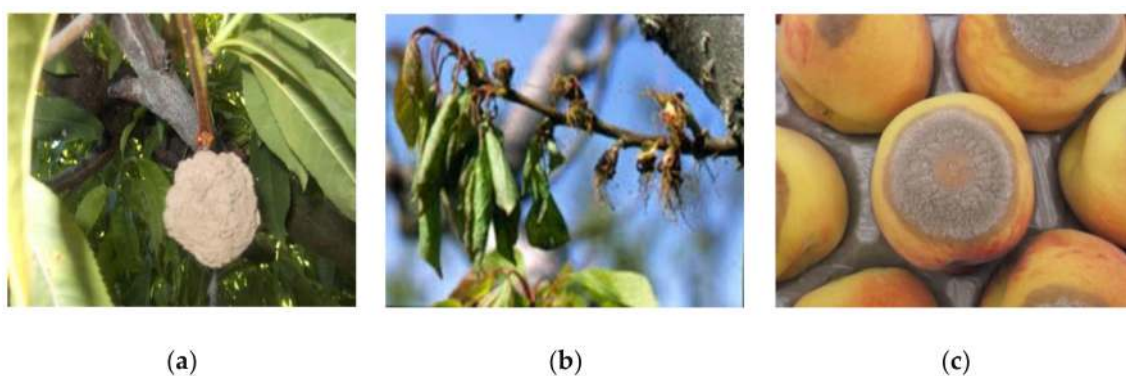
Finally, results from multiplexed and quadruplexed PCR [101] in particular, have demonstrated that this molecular assay is sensitive and specific. Other investigators [102,103] have recommended that for optimum performance and accurate identification, particularly when using PCR tests, and regardless of the DNA extraction method, chemicals' and thermocyclers manufacturers, or staining

methods, there is the exigency for a careful selection of species-specific primer pairs for the correct molecular diagnosis of *Monilinia* species. In all, it has been recommended [96] that additional research of new primers and probes for the characterization of *Monilinia* species should be conducted to make the method more transferable among qPCR platforms and laboratories [96]. We refer to Raja et al. [104] for more information on the merits and demerits of the use of “molecular tools” in fungi detection and identification.

#### 4. Host–Pathogen Interactions

Brown rot is the pathologic result of a parasitic interaction between the species of *Monilinia* and the peach [105]. In this association, and depending on the region of first contact (blossom, spur, twig, branch, fruit), the pathogen initiates and promotes rot in preharvest and after harvest [87,106]. Hence, the pathogen’s activity on the host is highly destructive from the flowering stage, through the peach formation stage, to storage [107], thereby creating an infection chain, as shown in Figure 7. Brown rot is a polycyclic epidemic [10]; hence, various secondary or monocyclic components of the brown rot infection sequence are generated throughout the annual growth cycle of the host. This biological proficiency conversely causes a grave impairment of the harvest, storage, and commercial shelf life of the product [87,108]. Interestingly, as it is found in any pathogen–host association, the growth and development of brown rot is influenced by different physicochemical conditions, such as temperature and water activity [109], light, aeration and pressure [110], pH, and titratable acidity of the fruits [8,111,112].

During such impending epiphytotics, the aforementioned physicochemical factors influence the microbial activity, determining either the growth and reproduction or the inhibition of activity and the inactivation of the pathogen. In particular, the pH and titratable acidity (TA) are interrelated concepts of organic acids [113,114] controlling physicochemical factors that act in an additive and interactive mode to inhibit the pathogen’s metabolic pathways [112].



**Figure 7.** Infection chain of *Monilinia laxa* in host peach. (a) Mummified peach; (b) blighted blossoms; (c) infected peach fruits.

In peach fruits, acidity is an important genetic quality trait [113] which influences both perception of sourness and sweetness found in varying proportions depending on the cultivar and the ripening stage [115]. The influence of pH and TA on fungal–host interactions is documented [8,116]. Some fungal species prefer neutral to slightly alkaline conditions [110]. However, the *Monilinia* species, in general, are acidophilic and therefore prefer acidic conditions for their growth [8,110]. Results by Holb [111] have shown that healthy peach fruits are quite acidic (pH < 3.5), but the pH levels rapidly increase in infected fruits reaching values of 4.6–5.4 depending on the cultivar and fungus isolate. On the contrary, other authors have found that species of *Monilinia* can acidify the host tissue of peaches and nectarines from pH values of 4.50 and 4.45, to pH values of 3.75 and 3.90, respectively [117].

## 5. Management Strategies to Control Brown Rot in Peaches

The weather conditions have been reported to influence the percentage of latent infections in peach and nectarine flowers and fruits [73] and remain a significant factor that must be considered in the effective management of this disease. This is a pathological situation where the infected fruit can remain asymptomatic (latent), and visual decay symptoms only develop during the late ripening period and after harvesting [57], particularly when there is a favorable ambient condition. Most stone fruits with a latent brown rot infection caused by *Monilinia* do not develop visible signs of disease until their arrival at the market or consumers' home. A rapid, accurate, and reliable [118] detection of *Monilinia latent* infections [95] is recommended to prevent and control the dispersion of *Monilinia* spp. in infected localities and non-infected countries [72].

There are other control and potential management options available for the brown rot epidemic in peaches, which include agronomical practices, biological, chemical, physical and biofungicides treatments, and host resistance [4].

### 5.1. Agronomical Management

Agronomical/cultural management is one of the first methods in all disease control, and in case of BR one of the most effective. Sanitation by prompt removal and adequate destruction of all infected parts (spurs, twigs and branches, mummified fruits, etc.) in winter, helps break the life cycle of the disease [16] in individual trees and the entire orchard as whole, and has been reported to keep brown rot below damaging levels. It is important to rake up and remove any fallen fruit or debris from under trees [13]. Prune trees to remove fruit mummies from the trees immediately after harvest or during the dormant season [70]. Pruning trees occasionally also helps to improve air circulation and add to fruit quality [113]. It is also advisable to use Tanglefoot® Pruning Sealer to seal all cuts and wounds and protect against insects [70], and disease organisms [20]. Avoid rain irrigation but instead use drip system under the canopy to keep from wetting blossoms, foliage and fruit [4]. Timely application to avoid rapid depletion of fungicide residue. In a commercial setting, all fruit not harvested or affected must be removed from the orchard, preventing to become mummies, thereby minimizing overwintering inoculum for next year's crop. Possible artificial fruit injury must be avoided. Hence thinned green fruits [119], undetached but injured green fruits are all potential source of inoculum and must be removed and adequately destroyed [120]. Tillage operations such as harrowing also help in destruction and burial of remnant propagules on the orchard. Carry out regular weeding and removal of wild or neglected stone fruit trees that serve as reservoirs for the disease [70]. Finally, it is very important to avoid excess water and Nitrogen application [20].

### 5.2. Biological Control Agents

Evidence abounds on the practical and biological control possibilities for diseases of the *Monilinia* species (Table 3) [121,122]. Biological control also refers to the use of formulations of living organisms (biofungicides) to control the activity of plant pathogenic fungi and bacteria [123].

An example of the use of the biological control agents (BCA) is EPS125 (*Pantoea agglomerans*, a Gram-negative bacterium) [124], which is effective in preventive treatments for the control of brown rot in several stone fruit crops, including peach. This study showed the ability of EPS125 to colonize, rapidly grow, and survive in wounds, indicating that its main mechanisms of action is mediated by cell-to-cell interactions in the absence of major toxicological effects, which constitute interesting traits for its effective use as a biofungicide in commercial conditions.

**Table 3.** Biological control agents (BCAs) against *Monilinia* spp. in peach.

Biological Agents	Formulation	Target Pathogen	Disease	Application Phase	Effectiveness	Reference
<i>Penicillium frequentans</i>	<i>P. frequentans</i>	<i>Monilinia laxa</i> (Aderh. & Ruhl.) Honey	Twig blight	Preharvest	Effective and practical	de Cal et al. [121]
<i>Penicillium purpurogenum</i>	strain 828	<i>Monilinia laxa</i> (Aderh. & Ruhl.) Honey and <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Snyder & Hansen)	Shoot canker	Preharvest	Enhanced mycoparasitism	Larena and Melgarejo [122]
<i>Pantoea agglomerans</i>	EPS125	<i>Monilinia laxa</i> (Aderh. & Ruhl.) Honey and <i>Rhizopus stolonifer</i> (Ehrenb., Fr.)	Brown rot	Postharvest	Potentially effective	Bonaterrea et al. [124]
<i>Epicoccum nigrum</i>	Fresh conidia	<i>Monilinia</i> spp.	Brown rot	Preharvest (bloom and pre-harvest)	Reduced brown rot at post-harvest	Larena et al. [125]
<i>Bacillus subtilis</i>	CPA-8	<i>Monilinia laxa</i> (Aderh. & Ruhl.) Honey and <i>Monilinia fructicola</i> (Wint.) Honey	Brown rot	Postharvest	Effective growth inhibition achieved	Yáñez-Mendizábal et al. [126]
<i>Penicillium frequentans</i>	FOR1, FOR2 and 16 others	<i>Monilinia</i> spp.	Brown rot	Preharvest (blossom to harvest)	Good potential for development	Guijarro et al. [127]
<i>Bacillus amyloliquefaciens</i>	CPA-8	<i>Monilinia laxa</i> (Aderh. & Ruhl.) Honey and <i>Monilinia fructicola</i> (Wint.) Honey	Brown rot	Preharvest	Potential alternative against <i>Monilinia</i> spp	Gotor-Vila et al. [128]

Larena et al. [125] carried out seven field experiments in peach orchards located in three different countries (Spain, Italy, France), to develop an effective and practical method of controlling brown rot disease caused by *Monilinia* spp. by the pre-application of *Epicoccum nigrum*. The result of their work demonstrated that *E. nigrum* applications, alone or in combination with fungicides treatments, to peach trees in the field reduced post-harvest brown rot. *Bacillus* spp. are among the most recommended bacteria to use against plant diseases, including brown rot, and marketed as commercial products (QST 713 strain (Table 4) [4]. Yáñez-Mendizábal et al. [126] used *Bacillus subtilis* (CPA-8) strain to control *Monilinia* spp. in peaches and indicated that fengycin-like lipopeptides play a major role in the biological action against *Monilinia laxa* and *M. fructicola*. In addition, antagonistic microorganisms such as *Penicillium frequentans* and *Bacillus amyloliquefaciens* [127,128] are examples of practical control measures, for peach production. The potential of *B. amyloliquefaciens* CPA-8 to control *Monilinia* spp. has been tested under field conditions, and CPA-8 remained active on the treated peach fruit surface at harvest and after harvest on self [128].

According to records, the number of new bio-pesticide registrations in the EU-28 is growing steadily, but they currently represent only 2.5% of the pesticide market [4]. However, there are several issues to consider before marketing the bio-pesticides. For example, Giobbe et al. [129] found that a biofilm-forming strain of *Pichia fermentans* proved to be most effective in controlling brown rot on apple fruit but pathogenic on peach fruit when co-inoculated into artificial wounds with a phytopathogenic isolate of *M. fructicola*. The authors, therefore, emphasized the need for a thorough risk assessment before allowing any deliberate release of bio-control agents, considering the associated potential effects, such as displacement of nontarget organisms, allergenicity to humans and other animals, toxicity, pathogenicity, and genetic stability. Hence, any minimal potential biohazard is inherent to the application of any bio-control agents [129] in peach commodity.

### 5.3. Fungicide Treatments

Synthetic fungicides such as cyproconazole, iprodione, and thiophanate-methyl [18], have been used to control *Monilinia* in the orchards in conventional and integrated pest management programs depending of the country. As an example in the European Union, the main conventional products, allowed in Spain including application doses and maximum residue limit (MRL) for European countries is shown in Table 4. Only two active substances are approved for the control of this fungus in commodities post-harvest product store: fludioxonil, with two different formulations (Table 4) (fludioxonil 23% (Solution Concentrate, SC) W/V and fludioxonil 60% (SC) W/V), currently registered until 31 December 2019, and pyrimethanil 30% (GE) W/W, valid until 30 April 2019 [39]. The CODEX Alimentarius as part the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) provides users with a list of MRL tolerances by commodity, pesticide, or functional class of the database at <http://www.codexalimentarius.net/pestres/data/index.html> [130]. Details for fungicides treatments, doses and MRL allowed in the United States are also provided in Adaskaveg et al. [130].

Furthermore, only eighteen active ingredients from more than 197 bio-fungicidal formulations, registered in the national pesticide guide website of the “Ministerio de Agricultura, Pesca, Alimentación y Medio Ambiente de España”, are authorized for use in the control of the brown rot of peach in Spain (Table 4). A permission limit (deadline) for fungicide use in stone fruit production is also provided. The same fungicide can be used in both pre- and post-harvest treatment, with corresponding restrictions in each case.



**Table 4.** Chemical and biological formulations used against *Monilinia* in stone fruit production in Spain.

Formulation	Number of Commercial Products	Doses/Application	SP (Days)/MRL	Permissible Limit
Sulphur 80% + Cyproconazole 0.8% (WG) W/W	1	0.1–0.2%/pulverization	14/0.1	21/11/2018
Cyproconazole 10% (WG) W/W	1	0.01–0.02%	14/0.1	12/09/2018
Cyprodinil 37.5% + fludioxonil 25% (ESP) (WG) W/W	1	0.8 kg/ha; 1 application	7/2	30/04/2019
Copper (II) hydroxide 35% (WG) W/W	23	0.2–0.25%	NA/5	31/01/2020
Iprodione 75% (WG) W/W	1	0.1% at a maximum of 2 applications/season and less than 1 kg/ha	14/10	31/10/2018
Mancozeb 20% + Dicopper chloride trihydroxide 30% (WP) W/W	20	2.5–3 kg/ha	14/2	31/01/2020
Mancozeb 75% (WG) W/W & 80%	35	0.2% at a maximum of 4 applications/season and less than 2 kg/ha	30/2	30/01/2020
Thiophanate–methyl 50% (SC) W/V; 70% (WG) W/W	4	0.09% at 1 application per season	14/2	31/10/2019
Mancozeb 8% + Cuprocalcium sulphate 20% (WP) W/W	4	4–5 kg/ha	NA/2	31/01/2020
Myclobutanil 4.5% (EW) W/V	1	0.66–1.1%	7/0.5	31/05/2021
Dicopper chloride trihydroxide 11% + Cuprocalcium sulphate 10% (WP) W/W	1	0.35–0.55%; 1.75–5.5 kg/ha to a maximum of 7.1 kg/ha per year	NA/5	31/01/2020
Copper (I) oxide 40% (O1) W/W	12	0.65% at a maximum of 3.75 kg/ha per year	NA/5	31/01/2020
Copper (I) oxide 50% (WP) W/W & 52%	47	0.3%; 2.5 kg/ha	NA/5	31/01/2020
Copper (I) oxide 70% (WG) W/W	16	0.15%; 1.35 kg/ha	NA/5	31/01/2020
Tribasic copper sulphate 40% (WG) W/W	14	0.2–0.3%; 1–3.75 kg/ha per year	NA/5	31/01/2020
Tebuconazole 25% (WG) W/W	11	0.05–0.075%; 0.75 kg/ha	7/0.6	31/08/2020
Fenbuconazol 2.5% (EW) W/V	2	0.2–0.6%; 3 L/ha	3/0.5	30/04/2022
<i>Bacillus subtilis</i> (Strain QST 713) 15.67% ( $5.13 \times 10^{10}$ CFU/g) (WP) W/W	1	2.5–4 kg/ha	NA	30/04/2019
Total active ingredients applicable in preharvest peach and nectarine bio-fungicidal control (18)				
Fludioxonil 23% and 60% (SC) W/V	2	0.3–0.4%	NA/10	12/31/2019
Pyrimethanil 30% (GE) W/W	1	6 g/tm	NA/5	30/04/2019
Total active ingredients applicable in postharvest peach and nectarine bio-fungicidal control (2)				
Total number of commercial bio-fungicidal products allowed in Spanish peach market (197)				

SP (Security Period); MRL (maximum residue limit in EU mg/Kg); WG (water dispersible granules); S/C (suspension concentrate); W/W (weight/weight); W/V (weight/volume); ESP (Spain); NA (not applicable); WP (wetable powder); SC (suspension concentrate); EW (emulsion of oil in water); CFU (colony-forming units) tm (metric tons); Source: MAPAMA [39].

#### 5.4. Limitations in the Use of Conventional Fungicides

The increase in the demand for fresh fruit with reduced residual quantities [4] has placed into interrogation the continuous use of conventional fungicides (CF) in peaches. CF treatments often alter the micro-ecosystem and, in the long term, modify disease severity by altering the interactions among microorganisms [131]. Moreover, the toxic chemical residues pose additional ecological issues and whenever possible, integrate pest management (IPM) is recommended as a sustainable environment and cost effective crop production [132]. Furthermore, recent reports have confirmed that the differential resistance to CF in *Monilinia* spp. is evolving and could be modifying the frequency of occurrence of fungicide sensitive and fungicide-resistant *Monilinia* spp. [133]. The evolution of tolerance in species of *Monilinia* (*M. laxa*) to certain CF [134,135], the increasing cost of chemical control and post application cleanup [136], and the threat of regulatory restrictions are all yearning the need for sustainable and endearing management measures to control brown rot [137].

The regulation of CF use, however, has become stricter in EU countries, especially after the release of the European Directive 2009/128/EC [80]. There is also a global intensification in the number of countries advocating for reduction and waning regarding conventional chemical uses [40]. Particularly, the use of CF is becoming more unfashionable because of consumer demands for residue-free fruit [3]. In addition, the contamination of the environment should be avoided [138]. Finally, the steady rise in the development and occurrence of *Monilinia* strains resistant to CF, worldwide, has been reported [133,134,138–140]. With all these adverse implications it is, therefore, pertinent to search for alternatives control strategies as IPM with lasting effect, enhancing consumer acceptability, and at the same time environmentally friendly. Integration of techniques for pest and disease control and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment is becoming the preference all over the world [141].

#### 5.5. Botanical Fungicides

Plants provide a wide range of secondary metabolites and essential oils that have an array of properties, including antimicrobial, allelopathical, bioregulatory, and antioxidative properties [142–144]. This class of plant derivatives is collectively referred to as biopesticides and includes the botanical fungicides [145]. Information, both descriptive [146] and practical (Table 5) [147,148], on biopesticidal efficacy and utility against phytopathogens abounds [149,150]. Some of these bioactive substances have also been assayed in vitro and in vivo [151] and found to be potent enough against brown rot of *Monilinia* species [151,152].

**Table 5.** Botanical products used on stone fruits in the postharvest control of brown rot.

Species	Common Name	Treatment Form	Disease	Target Pathogen	Fruit Type	Effectiveness	Reference
<i>Mentha balsamea</i> Wild and <i>Ocimum basilicum</i> L.	Peppermint and sweet basil	Essential oils	Yellowish, curl, brown blotch, white and soft rots syndromes	<i>Rhizopus stolonifer</i> (Ehrenb., Fr.); <i>Monilinia fructicola</i> (Wint.) Honey; <i>Aspergillus niger</i> Vantighm	Peach	High antifungal activity	Ziedan and Farrag [148]
<i>Thymus vulgaris</i> L., <i>Eugenia caryophyllata</i> L., and 2 others	Thyme and clavero	Essential oils	Brown and gray mold rots	<i>Monilinia fructicola</i> (Wint.) Honey and <i>Botrytis cinerea</i> (Pers. Fr.)	Apricot	Good antifungal activity	Hassani et al. [150]
<i>Copernicia cerifera</i> (Mill.) wax	Carnauba palm	Wax	Brown rot and <i>Rhizopus</i> rot	<i>Monilinia fructicola</i> (G. Winter) Honey and <i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.	Plums and nectarines	Presents great potential	Goncalves et al. [151]
Apiaceae and Asteraceae families	NA	Active substances	Brown rot	<i>Monilinia fructigena</i> (Aderhold & Ruhl.) Honey	Stone fruits	Potentially effective	Ganchev [152]
<i>Ocimum basilicum</i> L., <i>Ocimum tenuiflorum</i> L. and 2 others	Basil and holy basil	Essential oils	Brown rot	<i>Monilia laxa</i> (Aderh. & Ruhl.) Honey	Peach and nectarine fruits	Potential antifungal properties	Carović-Stanko et al. [153]
<i>Ocimum basilicum</i> L., <i>Foeniculum officinale</i> var. <i>saticum</i> (Bertol.) Arcangel plus 8 others	Basil and fennel	Essential oils	Brown rot and grey mold rot	<i>Monilinia laxa</i> (Aderh. & Ruhl.) Honey and <i>Botrytis cinerea</i> (Pers. Fr.)	Stone fruits	Variability in effectiveness	Lopez-Reyes et al. [154]
<i>Thymus vulgaris</i> L. and <i>Laurus cinnamomum</i> L.	Thyme and cinnamon	Essential oil vapours	Brown rot	<i>Monilinia laxa</i> (Aderh. & Ruhl.) Honey	Peach	Effective in preventive and curative treatments	Cindi et al. [155]

In an overview, Hassani et al. [150] evaluated, *in vivo*, the antifungal activities of four different (*Thymus vulgaris* (L.), *Eugenia caryophyllata* (L.), *Cinnamomum zeylanicum* (Blume) and *Carum copticum* (L.) plant extracts against two post-harvest pathogens (*M. fructicola* and *Botrytis cinerea*) of stone fruit. Other authors [151] obtained promising results using carnauba wax in post-harvest treatments for the control of brown rot and *Rhizopus* rot in plums and nectarines in experiments carried out under conditions of elevated inoculum pressure for the mature fruits. Just recently, Cindi et al. [155] have recommended thyme oil for post-harvest handling as a biofumigant for peaches after indicating that thyme oil vapor effectively reduced the incidence of brown rot caused by *M. laxa*. These authors [155] have also suggested that thyme oil fumigation treatment can be considered as a good alternative treatment because of the low concentration effective in brown rot decay control in peaches.

Nevertheless, some authors observed some limitations in the evaluated biofungicides and made adequate recommendations accordingly. Cindi et al. [155] observed the need to conduct further studies on the effect of thyme oil fumigation on fruit quality (by evaluating sensory parameters) in naturally infected peaches after low temperature storage and in retail shelf conditions. Thyme oil influences on peach volatile compounds, such as alcohols, aldehydes, carboxylic esters, ketones, and esters, need to be investigated [155].

### 5.6. Physical Treatments

There are various options of physical control in the postharvest management of brown rot in peaches (Table 6). These include: hot water dipping [156], dry heat [138], wet heat treatment curing with chitosan or in combination with *Bacillus* CPA-8 [157], radio frequency (water immersion and air exposure) [108], and hydro-cooling [158].

Jemric et al. [156] indicated that it is possible to control post-harvest BR on peaches using hot water dipping (HWD) at 48 °C for 12 min and on nectarine using HWD at 48 °C for 6 min without a significant loss of fruit quality. However, they recommended the optimization of the method according to the cultivar to prevent the loss of acidity.

In the study of Liu et al. [138], the use of heat (wet and dry) showed that both the direct inhibition of the pathogen and the elicitation of the defense response in the fruit contributed to a significant reduction of decay in peaches. The investigators associated the control effect to the inhibition of *M. fructicola* germination and growth, intracellular reactive oxygen species (ROS) accumulation, mitochondrial impairment leading to a reduction in ATP, and induction of defense-related enzymes in peaches.

**Table 6.** Physical treatments to control brown rot in peach: conditions, period, and effects.

Treatments	Temperature	Period of Exposure	Effects	Reference
Hot water dipping (HWD)	48 °C	6/12 min	Reduced brown rot (BR) incidence and no significant loss of fruit quality	Jemric et al. [156]
Heat treatment (HT)	40 °C	5/10 min	Significant reduction in peach BR	Liu et al. [138]
Heat treatment (HT) 95% RH	50 °C	2 h	Proposed as potential strategy to control brown rot on peaches and nectarines	Casals et al. [157]
Radio frequency (RF) of dipping in hot water (HT)	60 °C	20 s	A 100% BRI reduction at 6 to 12 h after inoculation and 85.7% BRI reduction at 0 to 48 h after inoculation as compared to untreated fruit	Spadoni et al. [159]
Radio frequency (RF) at 27.12 MHz of water immersion	20 °C	9 min	Controlled brown rot without adverse external and internal damage in both peaches and nectarines	Sisquella et al. [108]
Radio frequency (RF) at 27.12 MHz of exposition in air	20 °C	18 min	Brown rot incidence significantly reduced in both peaches and nectarines of different fruit size	Sisquella et al. [108]
Radio frequency (RF) at 27.12 MHz of water immersion	40 °C	4.5 min	Reduced BRI in stone fruits inoculated (0–48 h) before treatment and at all maturity levels evaluated in both peaches and nectarines without impaired fruit quality	Sisquella et al. [160]
Hydro cooling (HC) and water dump (WD)	4 °C	30 s/10 min	Reduced brown rot incidence by 50–77% when treated at 2/24 h of fruit harvest	Bernat et al. [158]

Casals et al. [157] used heat to protect peaches and nectarines from brown rot by exposing the fruits to 50 °C and 95–99% relative humidity (RH) for 2 h, which should markedly eradicate the pre-existing (from the field) *Monilinia* spp. infections, in combination with the application of chitosan at 20 °C for 1 min or the antagonist *B. subtilis* strain CPA-8. Other authors [159], combining hot water treatment with radio frequency (RF), after inoculating the fruit with conidia of *Monilinia laxa*, found BRI reduction. On the basis of these positive results, the authors recommended the techniques as a new alternative for BRI reduction in peaches.

Sisquella et al. [108] used RF (27.12 MHz) treatment to control brown rot in stone fruits either with immersion in water for 9 min or with exposure to air for 18 min as potential post-harvest alternatives for brown rot control in peaches and nectarines (Table 6). Later [160], they confirmed that the RF treatment, particularly with water immersion at 40 °C for 4.5 min, is very promising and has commercial potential for the post-harvest control of brown rot on peaches and nectarines. Furthermore, they reported a significantly reduced brown rot incidence (BRI) in the naturally infected fruit, from 92% in control fruit to less than 26% in peaches, and complete brown rot control in nectarines [160]. However, before the commercial application of this treatment technique, these authors rightly suggested that “it is necessary to design specific equipment for water immersion to determine the economic cost of the treatment”. Finally, Bernat et al. [158] applied hydro-cooling and water dump techniques to reduce BRI on peach fruit with recent infections (2 or 24 h before treatment). The techniques were able to reduce BR incidence in comparison to direct storage at 0 °C, but not when the infections were already established ( $\geq 48$  h before treatment).

### 5.7. Host Resistance and Genetic Management

Because of the increasing concern about the effects of biochemical fungicides (BCAs and conventional fungicides) on the environment [129] and human health [106] and about strain fungicide resistance [131], there is still a need for alternative treatments, based for instance on host resistance, which is considered one of the most cost-effective and environmentally safe strategies for disease control. Breeding for host resistance is a preventive decision measures in crop protection management. Apart from the pre-harvest issues, the length of conservation and commercial shelf life of peaches are also negatively influenced by post-harvest diseases principally associated with the brown rot, BR [108]. Nowadays, in the management of crop production, for effective and sustainable disease control, protective measures (prophylactic) are often preferred to curative measures with chemicals (chemotherapeutic). Consequently, breeders (Brazil, California, Italy, USA, France, and Spain), either individually or associated with pathologists, have concentrated their efforts on obtaining new cultivars resistant to the BR pathogen. Nonetheless, reports of resistance (in Mexican and Brazilian peaches) or tolerance (in peaches from Florida, New Jersey, and Harrow programs) to fruit brown rot in peaches exist [40,161].

Breeding for disease resistance is one of the most challenging objectives for crop improvement because disease expression is tetrahedral: Resistance is simultaneously influenced by agent, host, environment, and human management [162]. Screening for BR resistance in germplasm collections is a very time- and effort-consuming task [40]. Notwithstanding, different methodologies and varying protocols have continued to be assayed in breeding programs to evaluate the genetic resistance of stone fruit crops to fungi [40,48,163,164].

The ‘Bolinha’ peach variety, of Brazilian origin, presents a good resistance mechanism and less susceptibility to brown rot than other varieties and, although this variety possesses relatively poor quality characteristics, it has been observed that the resistance to this disease is transmitted to its descendancy [40,58,163]. The lack of extensive studies on *M. laxa*, in particular [4], especially on the use of tolerant varieties or genotypes with good quality characteristics in BR management and crop improvement, calls for academic and practical attention to that area.

The use of commercial varieties or genotypes with some level of disease resistance remains one of the surest and long-lasting tolerant alternatives within disease protection and improvement

management techniques in crop cultivation [20,165]. Host tolerance to plant pathogens is important for cost-effective and environmentally safe strategies for BR management. In line with this, according to Gell et al. [87], the use of tolerant cultivars for crop improvement is the topmost principle of crop protection, as this allows plants and plant products to be protected (prophylactic) from disease epidemics [166,167] and not to require cures from diseases (chemotherapeutic). Among other factors, the cultivar genotype significantly influences rot incidence and severity in stone fruits [168] and, therefore, represents an ideal modifiable element for disease control [16]. A lasting prophylactic treatment of peaches using *M. laxa*-tolerant cultivars means prevention of the pathogenic problems starting from the orchard.

Interestingly, in recent times, phytochemicals from plants and plant organs, including fruits, have drawn increasing attention due to their potent antioxidant properties and their marked effects on the prevention of various oxidative and stress-associated diseases [169,170]. In addition, several other studies also point to an active involvement of these phytochemicals in the protective reactions of crops against phytopathogens including fungi, bacteria, and viruses [40,171–173]. It is essential to note here that there are many studies showing that certain phenolic compounds present in peaches [174] have inhibitory effects *in vitro* and *in vivo* against *Monilinia* spp. [34,40]. Phenolic compounds, including caffeic and chlorogenic acids, may inhibit *M. fructicola* growth and reduce the lesion size [34]. Hence, an increase in susceptibility to brown rot infection has been associated with a concomitant decline in the concentrations of these antioxidant compounds especially in maturing fruits [175].

Breeding programs all over the world, especially the ones located in humid areas, have disease resistance as one of their top priorities, in part because the consumers' concern about chemical residues on fruits and vegetables has increased considerably. In this respect, tolerant genotypes will allow a sustainable control with zero residues in fruits and decreasing disease problems in the products during storage, leading to increased economic benefits. The total absence of treatment residues due to the use of prophylactic tolerant peach cultivars is environmentally friendly [176]. However, disease-resistant varieties are not readily available for many fruit crops [177], including commercial peach cultivars [20]. Developing peach cultivars tolerant to *M. laxa* requires, first of all, the identification of existing tolerant and susceptible genotypes by screening the germplasm [178].

It has been reported that, although a greater number of commercial stone fruit cultivars are susceptible to *Monilinia* spp. [51,179], there could exist genetic disease control elements [180,181] to be introgressed in high-quality fruit's genetic backgrounds [40]. Hence, the relative tolerance or susceptibility of fruits to disease has often been used for selecting disease-resistant genotypes for subsequent peach breeding. In addition, considering the recent drive for alternative technologies effective to control post-harvest diseases [177,182,183], in particular of stone fruits [3], any documentation of composites inhibiting BR development would have an influence on the breeding schemes and could be particularly useful to maintain fruit quality after harvesting, in the peach market. Having into account that the genetic control for the tolerance to *Monilinia* spp. in peach is complex, quantitative and multi-genic it would be of relevance to find candidate genes for resistance in peach. With the available version 2 of *Prunus persica* whole genome assembly and annotation [184] and new techniques such as GBS (Genome by Sequencing) we can search for new markers to fine mapping these quantitative regions or assay GWAs (Genome Wide Association).

#### 5.7.1. In Situ and Ex Situ Screening Methods to Evaluate Brown Rot Tolerance

There are two major known systems for screening a germplasm for susceptibility or tolerance to a disease or pathogen. These systems are the *in situ*/field method, which mostly encompasses an evaluation of flowers, bud, twigs, and shoots, and the *ex situ*/laboratory method, which is mostly an assessment on the commercial fruit derived from a germplasm. A researcher, therefore, can screen these phylogenetic properties in peach, especially in the *ex situ* evaluation by: (a) assessment of lesion development on wounded, injured, or bruised fruit [185]; (b) assessment of lesion development on intact, uninjured, or unbruised fruit [45,185]; (c) assessment of spore production

from determined lesions [46]; (d) assessment of post-harvest biodegradation from natural and latent infection; (e) assessment of cuticle thickness and firmness of commercial ripe fruits [46]. Table 7 shows some research in the ex situ evaluation of brown rot susceptibility in stone fruits. Some researchers [45,181,185] inoculated both bruised and intact fruit to screen for brown rot resistance in clingstone peach germplasm. The uninjured fruit inoculation test (UFIT) and artificially injured fruit inoculation test (AIFIT) were utilized [185] to evaluate the resistance to *M. fructicola* in peaches, and to *M. laxa* in apricots, plums, and peaches [45]. Walter et al. [46] adopted the assessment for lesion development, spore production, storage performance, and cuticle thickness and firmness in screening apricot fruits for resistance to brown rot caused by *Monilinia* spp. Many advantages exist in support of the ex situ over the in situ method: the manipulation of the fruit is easier, the inoculum load is centrally placed on the fruit cheeks, randomly including mature and immature sides [48], and the pathogenic factors, such as lesion and fungi colonization, are adequately evaluated on each inoculated fruit. Furthermore, the ex situ methodology facilitates the post-inoculation evaluation of qualities such as firmness and soluble solid contents (SSC). In addition, the ex situ method enables the washing and disinfection of the fruits, making them pure and uncontaminated for screening against targeted pathogens. However, the artificial inoculation on unwounded fruits, though found to be a reliable method in evaluating brown rot resistance ex situ [8,48], was reported to be not only lengthy and laborious but also affected by season and year variability [20,161].

Other different parts have been used to study the genetic resistance to pathogens of plant parts, such as flowers in apricots [186] and fruits in peaches [180,181,187]. Moreover, the evaluations have consistently been performed in situ with attached fruits, in natural ambient situations in the field on peaches and nectarines [180,188], or ex situ on apples [189] and stone fruits [40,190,191], involving detached apricots [45,46] and peaches, under controlled conditions [48,190,192]. Considering the fact that several mechanisms are known to be involved in BR resistance, including phenolic concentration, thickness of the epidermis, and flesh texture, the selection should be simultaneously based on all known and unknown components. Hence, some authors [46,179] have highlighted certain imperative factors to be considered when breeding for disease resistance as: (i) the adequate knowledge of the pathogenic agent, including its virulence; (ii) the knowledge of the availability, diversity, and types of genetic resistance within the breeding program as well as within the species in exam and its close relatives; (iii) the need to handle, develop, and improve the screening and phenotyping methods, including the accurate selection of the appropriate environment for the exhibition of resistance to allow its accurate tracking.

The overriding importance of the third point is underscored by Thomidis [73] who stressed that the knowledge and consideration of host specificity/non-specificity in disease management is paramount in the selection and preparation of new orchard sites and in the choice of tree species to be planted.

#### 5.7.2. Procedures for Spore Production and Inoculation in Lieu of Brown Rot Susceptibility Screening

Many different procedures have been adopted in the quest to have *Monilinia* spores and mycelia for the purpose of artificial inoculation (Table 7). Hence, *Monilinia* isolates could be grown on PDA Petri dishes directly from infected organs, i.e., fruits, mummies, twigs [193], or already prepared cultures. In addition, there are numerous methodologies describing partially similar, and in some cases divergent, protocols concerning spores concentration, inoculum load, and associated variables for screening BR susceptibility in stone fruits, peach and nectarine in particular. All the techniques presented in Table 7 were accomplished ex situ (in the laboratory controlled environment) with different inoculum densities and loads [190,191].

Several studies have investigated tolerance to BR in existing phenotypes for peach breeding purposes [178,185,190,192], and, in most cases, the relationships with the fruit quality traits [187] have not been ignored [40,45,46]. Apart from peaches and nectarines [40,192], other stone fruit germplasm that are being investigated include apricots [45,46], plums [45] and cherries [49,191].

**Table 7.** Assessment conditions to screen ex situ brown rot (*Monilinia* spp.) susceptibility in stone fruits. References, host inoculation, spores production, and parameters recorded.

Authors	Fruit Type	Number of Fruit	Method of Inoculation	Fruit cheek	Source of Inoculum	Inoculum Density (cfu)	Inoculum Load	Incubation Period	Temperature/RH of Incubation	Susceptibility Variables
Biggs and Northover [190]	Peach	NA	UFIT	Randomly	PDA culture	$10^6$ – $10^3$ mL <sup>-1</sup>	30 µL (30,000 to 30 spores)	144 h	20 °C/60–95%	Disease severity score
Northover and Biggs [191]	Cherry	10	UFIT	Suture	PDA culture	$10^6$ – $10^3$ mL <sup>-1</sup>	30 µL (30,000 to 30 spores)	144 h	20 °C/60–95%	% BRI, lesion diameter
Gradziel and Wang [185]	Peach	16	UFIT/AIFIT	Most matured	PDA culture	$2 \times 10^4$ mL <sup>-1</sup>	10 µL (200 spores)	72 h	22 °C–25 °C/95%	Lesion diameter
Pascal et al. [45]	Peach, Plum Apricot	10	UFIT/AIFIT	Randomly	Natural fruit	$10^6$ mL <sup>-1</sup>	20 µL (20,000 spores)	240 h/120 h	23 °C	% BRI, lesion diameter
Bassi et al. [192]	Peach	15	UFIT	Randomly	NA	$10^5$ mL <sup>-1</sup>	NA	168 h	25 ± 2 °C/95–100%	% BRI, lesion diameter
Walter et al. [46]	Apricot	8	UFIT/AIFIT	Randomly	Natural fruit	$1.5 \times 10^4$ mL <sup>-1</sup>	30 µL (450 spores)	48 h/120 h	Ambient temperature/lightly misted with dH <sub>2</sub> O	Lesion area, spore counts, storage rot and cuticle thickness
Pacheco et al. [181]	Peach	10	UFIT/AIFIT	Sun-exposed fruit cheek	Peach fruit	$5 \times 10^6$ mL <sup>-1</sup>	10 µL (50,000 spores).	120 h	25 °C/high RH	% BRI, average rot diameter by scores
Obi et al. [48]	Peach	20	UFIT	Randomly	Peach fruit	$25 \times 10^3$ mL <sup>-1</sup>	25 µL (625 spores)	120 h	23 °C/50–60%	Lesion diameter, colonization diameter, % BRI, disease severity

Abbreviations: NA (Not available); cfu (Colony forming units); RH (Relative humidity); dH<sub>2</sub>O (distilled water); % (percentage); BRI (brown rot incidence); UFIT (Uninjured fruit inoculation test in epidermis [45]); AIFIT (Artificially injured fruit inoculation test in mesocarp [45]); PDA (Potato dextrose agar).



The parameters usually considered in such tests comprise: the extent of necrosis and intensity of sporulation [190], the percentage of fruit infection and lesion development [191], the extension of the lesion area, the spore count, the storage rot and cuticle thickness [46]. Blighted flowers, twigs, and shoots have also been used [106,194]. However, the use of the fruit in susceptibility screenings is widely used among stone fruits [46,48]. In this case, the BRI (percentage of fruits with lesion) is determined [48,195].

## 6. Conclusions

Despite the importance of brown rot, there has been relatively little work done on the development of BR-resistant peach fruit cultivars, which is probably due to the lack of collaborative tendencies among the specialist actors involved, including farmers, breeders and phytopathologists. The lack of national projects for breeding and in general peach management (cultivation, marketing, innovations), in addition to the low price and scarce gain for the farmer, are limiting the investment for research in *Monilinia* spp. in many countries. Anyway, the search for phenotyping protocols to accurately characterize and evaluate brown rot infections is a mission that should always be encouraged by both breeders and pathologists in crop breeding programs. This review has clearly described the characteristic variance of the three most economically important species of *Monilinia* (*M. laxa*, *M. fructicola*, *M. fructigena*), in addition to *M. polystroma*, and the chemical formulations used for brown rot management in Spain, indicating also their permission limits. The continued increased demand of healthy fruit by consumers and the environmental concerns regarding the use of pesticides and associated ecomicrobial destitution require a sustainable alternative measure to combat brown rot in the peach market. In recent times, several studies have investigated tolerance to brown rot in existing peach phenotypes for peach breeding purposes and, in most cases, its relationships with the fruit quality traits. These studies have often described the use of host-resistant cultivars as a prophylactic measure rather than a chemotherapeutic strategy for brown rot management. If adequately and effectively combined with other alternative control schemes (Integrated Pest Management) and biological strategies, host resistance could promote a more sustainable peach fruit farming in the future.

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