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Growth and aggressiveness factors affecting *Monilinia* spp. survival peaches

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ABSTRACT

Brown rot of stone fruit is caused by three species of *Monilinia*, *Monilinia laxa*, *M. fructigena*, and *M. fructicola*. Eleven components of 20 different isolates of each of the three *Monilinia* species were analyzed to determine distinct aggressiveness and growth characteristics among the three fungi. *M. fructicola* showed the greatest lesion diameter, and the lowest incubation and latency period on fruit postharvest, however isolates of *M. fructigena* exhibited less aggressiveness components. Five growth characteristics of *M. fructicola* could be used to distinguish *M. fructicola* from the other two species. The dendrogram generated from only the presence of sclerotia and lesion length on infected fruit separated the 60 isolates into two clusters ($r = 0.93$). One cluster was composed of the *M. laxa* and *M. fructigena* isolates and the other cluster comprised the *M. fructicola* isolates. However, the dendrogram generated based on the presence of stromata and sclerotia in the same colony of the three species when they were grown on potato dextrose agar, and the lesion diameter on fruit infected with each species separated the 60 isolates into three clusters ($r = 0.81$). Each cluster comprised the isolates of each of three *Monilinia* spp. We discussed the effect of *M. fructicola* growth and aggressiveness differences on the displacement of *M. laxa* and *M. fructigena* by *M. fructicola* recorded in Spanish peach orchards and their effect on brown rot at postharvest.

1. Introduction

Brown rot caused by *Monilinia laxa* (Aderh et Rulh) Honey, *M. fructicola* (Wint.) Honey, and *M. fructigena* Honey in Whetzel (Byrde and Willetts 1977), is an economically important disease of commercial stone fruit production and storage. These three brown rot fungi can infect both the fruit, causing substantial preharvest and postharvest losses, and the blossoms and twigs of stone fruit trees, causing blossom and twig blight (Byrde and Willetts 1977). Postharvest losses are typically more severe than preharvest losses, and routinely occur during storage and transport, in some cases even affecting fruit at the processing stage (Hong et al. 1997).

Until 2006, peach brown rot in Spain was caused either by *M. laxa* or *M. fructigena* only (De Cal and Melgarejo 1999; Gell et al. 2009), with *M. laxa* the most prevalent (85–90%) (Larena et al. 2005). In 2006, *M. fructicola* was detected in peach orchards in the Ebro Valley, Lleida, Spain (De Cal et al. 2009). Since 2010, *M. fructicola* has displaced *M. fructigena* and co-existed with *M. laxa* in the similar relative frequency as *M. laxa* in three Ebro Valley peach orchards (Villarino et al. 2013). Six years after its first detection, the relative frequency of *M. fructicola* (absolute frequency normalized) increased on stored fruit, latent infections, blighted shoots, pruned branches, and mummified fruit on the trees (Villarino et al. 2013). However, the relative frequency of *M. laxa* in commercial orchards decreased progressively, especially on harvested fruit (Villarino et al. 2013). Co-existence between *M. fructicola* and *M. laxa* has been previously reported in Michigan and California (USA), where the two pathogens have somewhat different niches at early or late-season (Boehm et al. 2001), although *M. fructicola* is the most prevalent brown rot species in these states of USA.

M. fructicola can be distinguished from *M. laxa* by growth characteristics such as colony shape, colour of its conidia, absence of hyphal anastomoses between germinating conidia, germ tube extension of the conidia before germ tube branching, and also by VCG (De Cal and Melgarejo 1999; Mordue 1979a, 1979b; Penrose et al. 1976). *M. fructicola* produces conidia and spermatia more abundantly than *M. fructigena*, and its conidia size and hyphal diameter are smaller than those of *M. fructigena* (Mordue 1979a, 1979c). *M. fructigena* can be distinguished from *M. laxa* by its colony margins, the different conidial colour, and the length of conidial germ tubes (De Cal and Melgarejo 1999; Mordue 1979b, 1979c). Microconidia can be found on decaying fruit infected with *M. fructicola* and in cultures of *M. laxa* and *M. fructicola* which are older than 30 days (Ogawa et al. 1975). Irregular stromatal crusts and discoid sclerotia may develop on the agar surface or within the medium as colonies age. Of the three fungi, the apothecia of *M. fructicola* can be produced from pseudosclerotial mummified fruit under field (Biggs and Northover 1985; Byrde and Willetts 1977; Holtz et al. 1998) or laboratory conditions (Baxter et al. 1974; De Cal et al. 2014; Holtz et al. 1998; Willetts and Harada 1984). In contrast, the apothecia of *M. fructigena* and *M. laxa* are not found in the field (Janisiewicz et al. 2013; Villarino et al. 2010) and have not yet been

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produced under laboratory conditions (De Cal et al. 2014; Willetts and Harada 1984). Infection efficiency, latent period, spore production rate, infection period, and lesion size were considered as components of the pathogen aggressiveness, although these characteristics could vary between isolates within a species (Pariaud et al. 2009). Infection and sporulation of *Monilinia* spp. could also be influenced by the saprophytic and pathogenic fitness of each species and climatic conditions.

The main objective of this study was to compare the growth and aggressiveness factors of the three *Monilinia* spp. in culture media and on infected fruit at postharvest, in order to identify any features which could be related to their survival and to the displacement of *M. laxa* and *M. fructigena* by *M. fructicola* in Spanish peach orchards.

2. Materials and methods

2.1. Fungal isolates

Sixty isolates (20 each of *M. fructicola*, *M. fructigena*, and *M. laxa*) from different stone fruit from across the world were studied. The host, geographical origin, year, and number of isolates are listed in Table 1. Isolation and culturing of the 60 isolates was done on potato dextrose agar (PDA) (Difco Laboratories) supplemented with 0.5 g streptomycin sulphate/l (Lab. Reig Jofré S.A., Barcelona, Spain). Forty-four isolates were collected from different cultivars of peaches and nectarines in the Ebro Valley, Spain. Sixteen isolates were sent from France, Portugal, New Zealand, Australia, Japan, and South Africa.

The isolates were identified as *M. fructicola*, *M. fructigena*, or *M. laxa*, using their growth characteristics (De Cal and Melgarejo 1999) and a PCR-based assay (Gell et al. 2007). In order to investigate the 60 isolates in the collection, each isolate was stored at −80 °C in 20% glycerol (long-term storage) and at 4 °C on PDA slants in the dark (short-term storage). The isolates were grown on PDA at 22–25 °C in the dark for mycelium and spore production.

2.2. Aggressiveness components

Aggressiveness of each isolate of all *Monilinia* spp. was tested on four nectarine fruit, cv. `Big Top´ which is an early variety with high productivity (Badenes et al. 1999). The nectarines were sanitized by immersing each fruit in a 1% NaOCl solution for 5 min, and then in 70% ethanol for one minute. After two rinses in sterile distilled water (SDW), three puncture wounds were made in the skin of each fruit and 25 μl conidial suspension (10^4 conidia/ml SDW) were put on each wound. The inoculated fruit were then incubated at 22 ± 2 °C for 7 days under fluorescent lighting (100 μE/m^2s with a 16-h photoperiod) in humidity chambers that were lined with moist paper (Guijarro et al. 2008).

Each fruit was visually examined daily for symptoms of brown rot, and the incidence of brown rot caused by each *Monilinia* isolate was recorded (Guijarro et al. 2008). The percentage of fruit with brown rot symptoms (% brown rot incidence), the incubation period (the time interval between infection inoculation and the onset of symptom from that infection) and latency period (the time interval between infection inoculation and the onset of sporulation from that infection) (Pariaud et al. 2009) were recorded for each infected fruit. The daily lesion length (diameter in mm/day) was calculated from the individual measurements of the lesion's diameter on fruit that were made on each day of the 7-day incubation using regression analysis. To determine

Table 1
Geographical origin, host, year, and number of *Monilinia* isolates.

Species	Geographic origin	Host	Isolation year	Number of isolates
M. fructicola	USA	Plum	1994	1
	Bellegarde, Balandrán, France	Peach	2001	1
	Albesa, Lleida, Spain	Peach	2006	1
	Alfarràs, Lleida, Spain	Peach	2006	3
	Sudanell, Lleida, Spain	Nectarine	2006	2
	Lleida, Spain	Nectarine	2006	1
	Alfarràs, Lleida, Spain	Peach	2007	7
	Lleida, Spain	Peach	2007	3
	Hamilton, New Zealand	-	2008	1
M. fructigena	Australia	*Prunus* spp.	1971	1
	Japan	Apple	1995	1
	Portugal	Quince	1996	2
	A Coruña, Spain	Plum	1996	1
	Jubia, A Coruña, Spain	Apple	1996	1
	Jubia, A Coruña, Spain	Plum	1996	1
	Lleida, Spain	Nectarine	1998	2
	Bellegarde, Balandrán, France	Peach	2001	6
	Lleida, Spain	Peach	2006	1
	Albesa, Lleida, Spain	Peach	2006	2
	Alfarràs, Lleida, Spain	Peach	2006	1
	Sudanell, Lleida, Spain	Nectarine	2006	1
M. laxa	South Africa	Peach	1996	1
	Cehegin, Murcia, Spain	Apricot	1996	1
	Bellegarde, Balandrán, France	Peach	2001	1
	Albesa, Lleida, Spain	Peach	2006	2
	Alfarràs, Lleida, Spain	Peach	2006	3
	Sudanell, Lleida, Spain	Nectarine	2006	1
	Serós, Lleida, Spain	Nectarine	2006	1
	Riba-roja d'Ebre, Tarragona, Spain	Peach	2006	1
	Zaidi, Lleida, Spain	Peach	2006	1
	Zaidi, Lleida, Spain	Nectarine	2006	1
	Lleida, Spain	Peach	2007	1
	Alfarràs, Lleida, Spain	Peach	2007	1
	Gimenells, Lleida, Spain	Peach	2007	1
	Sudanell, Lleida, Spain	Nectarine	2007	2
	Riba-roja d'Ebre, Tarragona, Spain	Peach	2007	1
	Albesa, Lleida, Spain	Peach	2008	1

(−) no information.