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Conidial density of *Monilinia* spp. on peach fruit surfaces in relation to the incidences of latent infections and brown rot

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Abstract To evaluate the effect of conidial density of *Monilinia* spp. on the fruit surface on the incidence of latent infection and brown rot in peaches, eleven field surveys were performed in commercial orchards located in Cataluña, Spain over four growing seasons from 2002 to 2005, and nine surveys were conducted to determine the sources of overwintered *Monilinia* spp. inoculum. There was a significant positive relationship (r=0.69) between the numbers of conidia of *Monilinia* spp. on the fruit surface and the incidence of latent infections, but not with brown rot at harvest. Although mummified fruit, twigs and pits have been identified as being able to carry the pathogen from year to year in peaches grown in Spanish orchards, no relationships between any of these sources and the numbers of conidia on the fruit surface, or incidence of latent infection or brown rot were found. The effect of temperature (T), solar radiation (SR), rainfall (R) and wind speed (WS) on the area under the number of conidia of *Monilinia* spp. curve (AUncC) on peach surfaces was analysed. Regression analysis revealed that T, SR, R, and WS could account for 99% of the total variation in the area of the AUncC on peach surfaces. Thus, in order to reduce the incidence of latent infection and brown rot it is essential not only to remove the sources of primary inoculum but also to reduce the number of *Monilinia* spp. conidia on the fruit surface. Furthermore, the sources of airborne conidia of *Monilinia* spp. should be taken into consideration in disease management programmes in Spain.

Keywords Brown rot · Disease management · Epidemiology · Latent infection · *M. laxa* · *M. fructigena*

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Introduction

Monilinia laxa and *M. fructigena* are the causal agents of brown rot of peaches and their anamorphs are the only form encountered in Spanish peach orchards (M.-Sagasta 1977; Larena et al. 2005; Guijarro et al. 2007). Overwintering of the fungi has been well described and the fungus persists in the infected tissues of the peach tree (fruit mummies, fruit peduncles, leaf scars, buds, cankers on twigs and branches) or in the orchard soil (mummified fruit, pits, twigs and branches) (Byrde and Willetts 1977; Landgraf and Zehr 1982; Biggs and Northover 1985). Under favourable conditions, the mycelium sporulates and produces conidia that constitute the source of primary inoculum that infects blossoms in the early spring (Byrde and Willetts 1977; Biggs and Northover

1985). Because the relative importance of each inoculum source can differ in different crop areas (Sutton and Clayton 1972; Landgraf and Zehr 1982), it is important to investigate the main sources of primary inoculum in Spanish peach orchards.

Secondary inoculum can arise from any infected tissue in which the moisture content is sufficient for conidial sporulation (Landgraf and Zehr 1982). Depending on the climatic conditions, several generations may occur during the growing season. These conidia infect fruit and may cause either brown rot under favourable climatic conditions or remain latent when climatic conditions are unfavourable. When these conditions become favourable for disease expression, brown peach rot then develops (Byrde and Willetts 1977; Emery et al. 2000). In addition to environmental influences, disease expression is dependent upon the stage of fruit growth (Luo and Michailides 2001a; Xu et al. 2007).

The incidence of latent infections caused by *Monilinia* spp. and its relationship to brown rot has been studied by several authors. Xu et al. (2007) documented the existence of a high correlation between brown rot caused by *M. laxa and M. fructigena* and the incidence of latent infection in cherries. In the case of *M. fructicola*, several studies have reported on the existence of a positive correlation between the incidence of latent infection in immature stone fruit and the incidence of fruit rot at harvest and post-harvest (Northover and Cerkauskas 1994; Emery et al. 2000; Luo and Michailides 2001a). In Spain, latent infections caused by *Monilinia* spp. have been detected recently in peach orchards, and Gell et al. (2008) found that there was a relationship between the incidence of these latent infections and the incidence of brown rot at post-harvest.

Spore traps have been used to measure the density of airborne conidia of *Monilinia* spp. in apple (Van Leeuwen et al. 2000; Xu et al. 2001; Holb 2008) and stone fruit orchards (Corbin et al. 1968; Luo et al. 2007). Spore density in the air was related to the incidence of brown rot infection of the fruit (Van Leeuwen et al. 2000; Luo et al. 2007; Holb 2008). The viability of airborne conidia is dependent upon temperature, relatively humidity (RH), wind speed, the amount of rainfall (Holb 2008) and the intensity of ultraviolet radiation (Rotem et al. 1985). Airborne conidia of *M. fructigena* deposited on the apple surfaces can remain viable on the fruit surface for as long as 20 days when relatively low temperatures and high RH prevail and infect fruit (Xu et al. 2001). When weather conditions become favourable, immature stone fruit on trees can also be infected from airborne conidia (Luo et al. 2005), especially when the fruit ripens (Van Leeuwen et al. 2000; Holb 2008). However, the exact contribution of deposited conidia to the incidence of latent infection and the occurrence of brown rot is not yet known.

We undertook an investigation to study some epidemiological aspects of primary inoculum and the airborne conidia of *Monilinia* spp. in relation to the incidence of latent infection and post-harvest brown rot. Specifically, the aims of this study were: (1) to identify the main sources of primary inoculum for peach orchards in Spain and to correlate them with conidial numbers on peach surfaces; (2) to monitor conidial numbers on flowers and fruit throughout the duration of the crop season; (3) to determine the relationship between the number of *Monilinia* spp. conidia on peach surfaces and the incidence of latent infections or brown rot; and (4) to model the relationships between temperature, wind speed, the intensity of solar radiation, and rainfall and conidial numbers on peach surfaces.

Materials and methods

Orchard sites and experimental design

The incidence of post-harvest brown rot and latent infections caused by *Monilinia* spp. was determined from 11 surveys carried out in six different commercial peach orchards over four growing seasons from 2002 to 2005 determining the number of conidia on peach surfaces (Table 1). These orchards were located in Vinebre (Universal Transverse Mercator Coordinate, UTM: 297930, 4562145), Torre-Forns (UTM: 297330, 4613000), Gimenells (UTM: 296537, 4616547), Sudanell (UTM: 297230, 4603615), Alfarrás (UTM: 298197, 4634045), and Albesa (UTM: 305302, 4624899) Cataluña, Spain (Table 1). Sources of primary inoculum were determined from nine surveys carried out in these orchards except for the one in Vinebre. Cultivars from two varieties, namely *Prunus persica* (peach) and *Prunus persica* var. *nucipersica* (nectarines), were used because they have different embryo growth, pit hardening and harvest

Table 1 Stone fruit orchards where experiments were conducted and corresponding information on cultivars and growth stages

Orchards	Year	Fruit varieties	Fruit cultivar	Sampling dates						
				Pop corn BBCH=55	Shuck split BBCH=71	Pit hardening BBCH=76	Embryo growth BBCH=79	30 days before harvest BBCH=81	7 days before harvest BBCH=86	Harvest
Vinebre	2002	Peach	Summer Lady	–	–	27/05	16/06	1/08	–	27/08
Alfarrás	2002	Nectarine	Caldesi 2020	–	–	27/05	–	8/08	2/09	9/09
Torre-Forns	2003	Peach	Catherine	12/03	23/04	14/05	11/06	–	6/07	13/07
Gimenells	2003	Peach	O'Henry	12/03	23/04	14/05	11/06	21/07	17/08	24/08
Sudanell	2003	Nectarine	Autum Free	11/03	23/04	13/05	12/06	31/07	29/08	4/09
Albesa	2004	Peach	Roig d'Albesa	17/03	20/04	9/06	13/07	23/08	–	24/09
Alfarrás	2004	Nectarine	Caldesi 2020	17/03	20/04	9/06	13/07	18/08	–	24/09
Sudanell	2004	Nectarine	Autum Free	17/03	20/04	2/06	13/07	18/08	–	24/09
Albesa	2005	Peach	Roig d'Albesa	24/03	20/04	16/06	12/07	11/08	–	14/09
Alfarrás	2005	Nectarine	Caldesi 2020	23/03	20/04	7/06	12/07	11/08	–	14/09
Sudanell	2005	Nectarine	Autum Free	23/03	20/04	1/06	12/07	11/08	–	6/09

BBCH general scale from Biologische Bundesantalt, Bundessortenamt and CHemische Industrie, Germany (Meier et al. 1994)

date (Table 1). Fruit from the early-maturing cultivars were harvested before September whereas fruit from the late-maturing cultivars were harvested during September (Table 1).

Three adjacent trees were used as a single replicate, and ten replicates were selected randomly in each orchard. Management of these trees followed standard commercial practices, except that they were not sprayed with fungicides.

Determination of primary inoculum sources

Orchards were sampled intensively on 18 February 2003, 7 March 2004, and 7 February 2005 to record the location of overwintered *Monilinia* spp. inoculum (Table 1). All the mummified fruit that had overwintered in the trees, aborted fruit, cankers, and necrotic twigs were collected from each orchard, and taken to the laboratory. In addition, ten asymptomatic twigs were collected from each replicate in each orchard. Twenty 80 cm diam circles were marked on the ground under randomly selected trees in each orchard in order to record the location of overwintered *Monilinia* spp. inoculum on the ground. All mummified fruit, pits and pruning twigs in each circular area were collected.

In order to determine the overwintered *Monilinia* spp. inoculum on the collected specimens, each specimen was cut in pieces no longer than 5 cm and then surface-disinfected by dipping them first in 70% ethanol for 20 s, and then in a solution of 0.5% NaOCl and 0.05% Tween 20 for 4 min (Sauer and Burroughs 1986). Each sample was then rinsed for 1 min in sterile distilled water (SDW) before being placed on potato dextrose agar (PDA, Difco, Detroit, MI, USA) supplemented with streptomycin (0.5 g l^{-1}), and incubated for 5 days in the dark at 20–25°C. Samples were assessed for visible conidiophores and conidia of *Monilinia* spp. at the end of the incubation period. Percentage of samples exhibiting sporulation of *Monilinia* spp. after incubation were recorded.

Quantification of peach surface conidia and determination of the incidence of latent infections

The number of *Monilinia* spp. conidia on the peach surface, and the presence/absence of latent infections were determined in the orchards during each crop season by sampling at each of the five growth stages:

popcorn (BBCH=55) (BBCH general scale from Biologische Bundesantalt, Bundessortenamt and CHemische Industrie, Germany) (Meier et al. 1994), shuck split (BBCH=71), pit hardening (BBCH=76), embryo growth (BBCH=79), 30 (BBCH=81) and 7 days before harvest (BBCH=86) (Table 1). Ten blossoms at popcorn or ten fruit without visible signs of infection with *Monilinia* spp. per replicate were picked from orchards at each sampling date and then sent to the laboratory. At the laboratory, the blossoms or fruit were suspended first in containers of SDW. The containers were then shaken for 30 min at 150 rpm in a rotary shaker at room temperature, then centrifuged for 10 min at 14,040×*g* (Certomat RM, B. Braun, Biotech International Diessel GmbH&Co., Melsungen, Germany), and the resultant pellet was then re-suspended in 5 ml SDW. The number of conidia of *Monilinia* spp. were counted in a haematocytometer under a light microscope (×100) and expressed as number of conidia per flower or fruit.

To estimate the incidence of latent infections on each sample of either flowers or fruit, ten flowers or ten fruit from each growth stage were surface-disinfected by dipping each specimen in 70% ethanol for 20 s, and 0.5% NaOCl and 0.05% Tween 20 for 4 min after being washed in SDW, as previously described (Sauer and Burroughs 1986). After surface-disinfection, they were then rinsed for 1 min in SDW. Disinfected flowers or fruit were frozen at −20°C for 24 h (Michailides et al. 1996) after which they were incubated in humidity chambers lined with moist paper, at 22±2°C under a fluorescent light (100 μE m^{-2} s^{-1}, 16 h photoperiod) for 7 days. Latent infection with *Monilinia* spp. was recorded following the appearance of brown rotted tissue showing sporulation on the fruit and the incidence then calculated.

Determination of the incidence of post-harvest brown rot

The incidence of post-harvest brown rot in each orchard was determined from ten fruit per replicate that had been picked at harvest. Each fruit was incubated in humidity chambers lined with moist paper at 22±2°C under a fluorescent light (100 μE m^{-2} s^{-1}, 16 h photoperiod) for 7 days. The fruit was assessed for signs of brown rot after incubation and the assessment was made visually, and microscopically, when necessary.

Monitoring environmental variables

Temperature (*T*, degree Celsius), relative humidity (RH, percent), wind speed (WS, per millisecond), rainfall (*R*, millimeter), and the intensity of solar radiation (SR, megajoules per square meter) were recorded using automated weather-monitoring equipment placed close to each orchard. The proximity of this equipment to field experiment locations ranged from approximately 500 m to 5 km. The average daily *T*, RH, WS, *R*, and SR were then calculated on each day at each orchard.

Data analysis

The mean percentages of the specimens on which sporulation of *Monilinia* spp. was detected (tree mummified fruit, necrotic twigs, asymptomatic twigs, ground mummified fruit, pits and pruning twigs) were calculated from the data pooled from all orchards. The significance of the differences between the means was determined using the Kruskal–Wallis test and the level of significance was set at 5%.

The relationship between the number of conidia and incidence of latent infection and time were plotted; the area under dynamic curves of conidia (AUncC) and latent infection (AULIC), were calculated by trapezoidal integration (Campbell and Madden 1990).

Incidence of post-harvest brown rot (*z*), AUncC, and AULIC were analysed independently by analysis of variance and contrast among means between varieties, and early and late cultivars with the *F* test at significance levels of 5% (Snedecor and Cochram 1980). Data on the incidence of brown rot were arcsine-transformed before analysis.

Correlation analyses to examine the relationship between *z*, AUncC, and AULIC were performed on the pooled data from all experiments using Statgraphics Plus for Windows v. 4.1 (StatPoint Inc. Herndon, VA, USA.) (Snedecor and Cochram 1980). Each point in the analysis was the average value of ten replicates. Data on the incidence of brown rot were arcsine-transformed before analysis. In addition, correlation analyses were conducted to explore relationships between the sources of primary inoculum and *z*, AUncC, and AULIC.

The equation AUncC=*f*(*T*, HR, WS, *R*, SR,) was used to investigate the relationship of *T*, RH, WS, *R*, and SR with AUncC, using pooled data from the eleven field surveys. In this equation, *f*(*T*, HR, WS, *R*, SR) is a

linear function of the terms: T, RH, WS, R, SR, TRH, TWS, TR, TSR, T^2, RH^2, WS^2, R^2, T^2, SR^2, T^3, T^2RH, T^2WS, T^2R, T^2SR, TRHWS, TRHR, TRHSR, TWSR, TWSSR, TRSR, HRWSR, HRWSSR, HRRSR, WSRSR, HR^3, HR^2WS, HR^2R, HR^2SR, WS^3, WS^2R, WS^2SR, R^3, R^2SR, and SR^3. Data were fitted to the equation by the Model Regression Selection option in Statgraphics Plus for Windows v. 4.1 (StatPoint, Inc. Herndon, VA, USA). The selection was performed on the basis of the significance of each estimated parameter: the coefficient of determination, the adjusted coefficient of determination, the Mallow's C_p coefficient (which evaluates the fit of regression model by the squared distance between its predictions and the true values), the Durbin–Watson statistic (a test for serial correlation in the residuals of a least squares regression analysis), and the normal distribution of residuals (Jacome and Schuh 1992).

Results

Primary inoculum

Monilinia spp. were isolated from some peach tree (mummified fruit, necrotic and asymptomatic twigs) and ground (mummified fruit, pits, and pruning twigs) specimens after the winter (Table 2). However, *Monilinia* spp. were not isolated from aborted fruit and cankers. Apothecia were not found in any of the orchards. Trees in six of the nine surveys had mummified fruit still hanging and in some of them the average number of mummified fruit per tree (replicate) was <1 (Table 2). Sporulation of *Monilinia* spp. occurred in 66% of the mummified fruit collected from the trees after 5 days of incubation in the dark at 20–25°C on PDA plus streptomycin growth medium, whereas sporulation was observed in <10% of the other specimens (Table 3).

Quantifying peach surface conidia

The numbers of *Monilinia* spp. conidia on peach surfaces in each orchard and year are shown in Fig. 1. The average number of conidia on peach surfaces increased rapidly during the period between pit hardening and 7 days before harvest, and reached 10^4 and 10^5 conidia per fruit surface in 2002 and 2003, respectively. The average number of conidia on peach surfaces ranged from 10^3 to 10^4 conidia per peach surface in 2004 and 2005, or was $<10^3$ conidia per peach fruit surface seven days before harvest in 2005.

Table 4 shows the data of AUncC, AULIC and z in the different orchards. The AUncCs were significantly different from each other when the different years were compared (Table 4). The highest AUncC was obtained

Table 2 The mean number of the samples from which *Monilinia* spp. were isolated and corresponding standard errors

Orchards/year	Tree samples[a]			Ground samples[a]		
	Mummies	Necrotic twigs	Asymptomatic twigs	Mummies	Pits	Pruning twigs
Torre Forns 2003	0±0 (–)[a]	2.7±0.2 (0)	10±0 (1)	0±0 (–)	0.2±0.2 (50)	3.4±1.0 (1.7)
Gimenells 2003	0±0 (–)	1.2±0.5 (4)	10±0 (0)	0±0 (–)	0±0 (–)	0±0 (–)
Sudanell 2003	0.1±0.1 (100)	1.3±0.1 (20)	–[b]	–	–	–
Albesa 2004	0±0 (–)	0.1±0.1 (0)	10±0 (0.7)	0±0 (–)	9.7±4.7 (0)	28.7±4.3 (0)
Alfarrás 2004	1±0.3 (100)	1.3±0.7 (33.3)	10±0 (0.25)	0.2±0.1 (50)	1.4±0.6 (0)	2.4±1.1 (2.8)
Sudanell 2004	2.1±0.8 (66.4)	0.8±0.3 (0)	10±0 (1)	0±0 (–)	2.1±0.7 (0)	0.5±0.5 (0)
Albesa 2005	0.6±0.4 (33.3)	1.4±0.3 (0)	10±0 (0)	1.2±0.8 (0)	8.4±4.1 (0)	20.2±4.0 (0)
Alfarrás 2005	7.7±3.3 (56.1)	2.8±0.5 (5.5)	10±0 (0)	1.0±0.5 (0)	7.7±3.6 (1.4)	4.0±1.7 (0)
Sudanell 2005	4.8±0.6 (64.5)	3.1±0.4 (5.5)	10±0 (0)	0.1±0.1 (0)	0.6±0.3 (0)	0.5±0.3 (0)

[a] Samples were taken from the trees or collected from the orchard ground in nine surveys carried out in Spanish orchards on 18 February in 2003, 7 March in 2004, and 7 February in 2005. Three adjacent trees were used as a single replicate and ten replicates were randomly assigned in each orchard. Twenty circles of 80 cm diam were marked on the ground under randomly selected trees in each orchard. Data in brackets are the percentage of samples exhibiting sporulation of *Monilinia* spp. after 5 days of incubation in the dark at 20–25°C

[b] – not available

Table 3 Mean and standard errors of specimens collected from orchards showing sporulation of *Monilinia* spp. after incubation

Location	Samples	% sporulation *Monilinia*
Tree	Tree mummfied fruit	65.9±6.5 b
	Necrotic twigs	7.0±2.7 a
	Asymptomatic twigs	0.4±0.2 a
Ground	Ground mummified fruit	10.0±10.0 a
	Pits	1.4±1.2 a
	Pruning twigs	0.5±0.4 a

Specimens were collected from the trees and ground of Spanish orchards during the nine surveys conducted on 18 February in 2003, 7 March in 2004, and 7 February in 2005. Means followed by the same letter are not significantly different by Kruskal–Wallis test (P=0.05)

for Alfarrás 2002 and Sudanell 2003, followed by Vinebre 2002 and Gimenells 2003. The contrast analysis for AUncC, AULIC and z of different varieties (peach or nectarines), and cultivars (early or late cultivars) after pooling the data from all orchards showed no significant differences when the AUncCs from early cultivars were compared with those of the late cultivars. No significant differences in the AUncCs were found between peaches and nectarines.

Incidence of latent infection and post-harvest brown rot

In all orchards, the patterns of incidence of latent infection were similar: there was a slight increase in the incidence of latent infection during pit hardening and then a rapid increase a few days before harvest, whereas the incidence at the embryo growth stage was low (Fig. 2). Significant differences were obtained when the AULICs of the late cultivars were compared with those of the early cultivars (late > early) and when those of nectarines were compared with those of peaches (nectarines > peaches) in the contrast analysis. Significant differences were also obtained when the AULICs of the different experimental years were compared: the highest AULIC was obtained for Alfarrás 2002 (Table 4). No latent infections were recorded in 2005 (Table 4).

Significant differences were obtained on the incidence of brown rot at post-harvest (Table 4). The highest incidence of brown rot occurred in Alfarrás (2002) followed by Sudanell 2004 and Alfarrás 2004 (Table 4).

When the data from the 11 surveys were pooled, significant correlations were observed between AUncC and AULIC (r=0.68, P=0.022) and between AULIC and z (r=0.87, P=0.0005). However, the correlation between z and AUncC was not significant (r=0.56, P=0.074).

Several plausible models were obtained to relate T, HR, WS, SR, and R to AUncC. The following equation was selected to best describe AUncC as a function of T, SR, R, and WS because it has the highest adjusted determination coefficient and the lowest Mallow's C_p coefficient.

$$(1)\ AUncC = \underset{(2.0\times10^{6})}{-1.9\times10^{7}} \underset{(4.9\times10^{4})}{-4.4\times10^{5}\times SR\times R\times WS} + \underset{(5.1\times10^{3})}{5.6\times10^{4}\times T\times SR\times R} + \underset{(3.9\times10^{4})}{2.5\times10^{5}\times WS^{2}\times SR} - \underset{(3.3\times10^{4})}{9.8\times10^{4}\times WS^{2}\times T}$$
$$(R^{2}=0.986)(R^{2}*=0.967),\ C_p=1.07,\quad DW=2.31$$

The standard errors of the estimates were significant ($P \leq 0.05$) and are shown in parentheses below the corresponding parameters.

Discussion

We have demonstrated the effect of conidial density of *Monilinia* spp. on the fruit surface on the incidence of latent infection and brown rot in peach and nectarine orchards in Spain.

Mummified fruit and twigs on peach trees, and mummified fruit, pits and pruning twigs on the ground have been identified as overwintering inoculum sources in Spanish peach orchards. However, in other regions such as South Carolina, Landgraf and Zehr (1982) reported that conidial production in overwintered peach mummified fruit, cankers and fruit peduncles was not great in number and infrequent, and that ascospores and conidia produced by *M. fructicola* on infected wild plums were the main sources of primary inoculum. Apothecia of *M.*

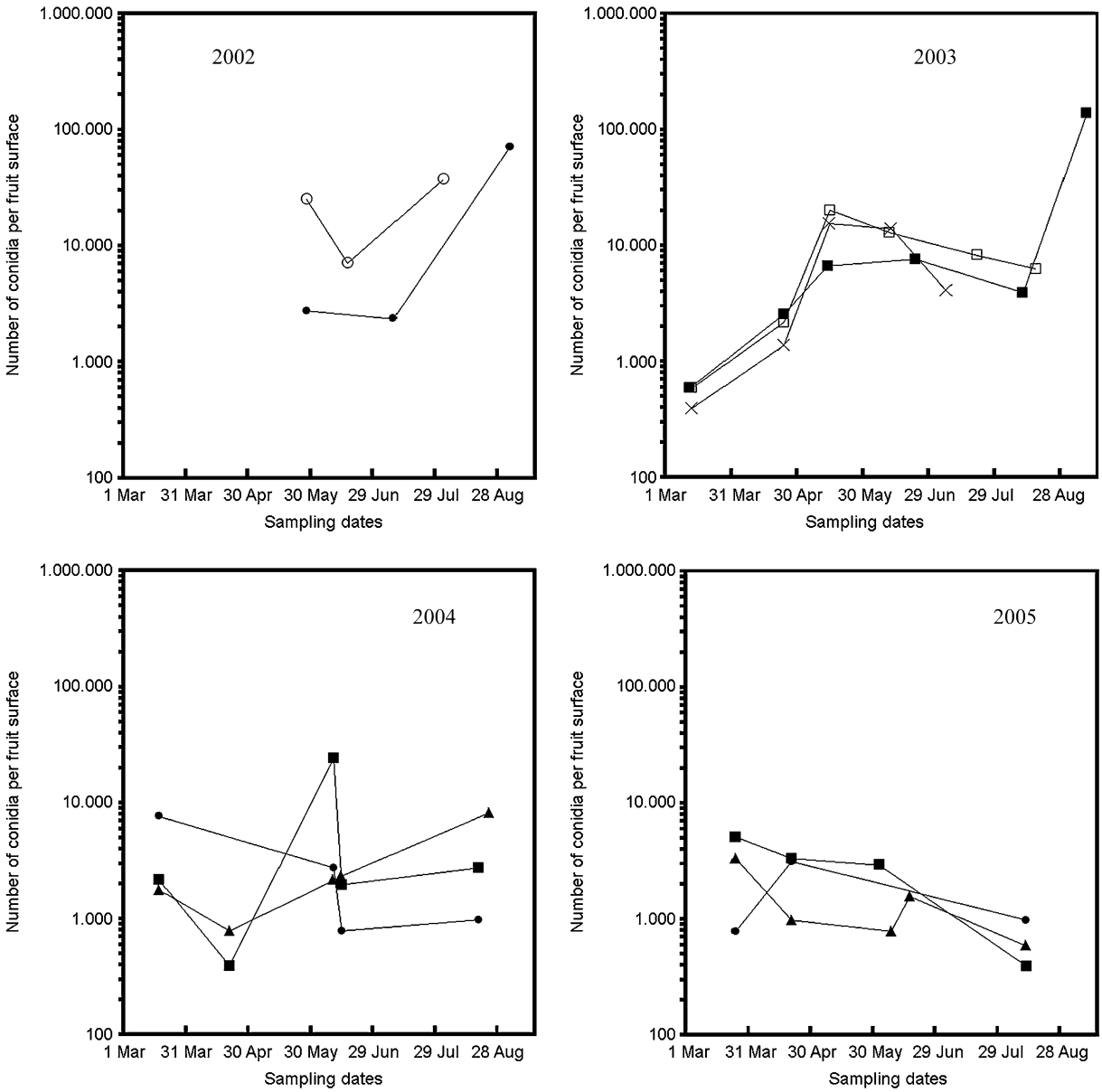

Fig. 1 Population dynamics of *Monilinia* spp. (number of conidia per flower or fruit) on peach surfaces that were recovered from pink blossom to harvest for 2002, 2003, 2004, and 2005 in 11 surveys in Cataluña, Spain: Vinebre (*open circles*), Alfarrás (*closed circles*), Torre-Forns (*multiplication symbols*), Gimenells (*open squares*), Sudanell (*closed squares*), and Albesa (*closed triangle*). The numbers of *Monilinia* spp. conidia were counted in a haematocytometer under a light microscope (×100) and are displayed on a logarithmic scale. Each point is the mean of ten trees with ten flowers or ten fruit per tree

fructigena and *M. laxa* are rarely found in the field and have not been produced in culture (Ogawa et al. 1995). Because *M. laxa* is the most prevalent species that has been isolated from brown rot in peach fruit (98% of the identified isolates) in Spanish orchards (Gell et al. 2008), it was not expected to find any apothecia in these orchards. In Ontario peach orchards, Biggs and Northover (1985) reported that sporulation on fruit peduncles, twig cankers, and necrotic twigs are sources of primary inoculum for infection of blossoms, and that non-abscised, aborted fruit, and thinned fruit on the orchard floor can also

Table 4 Values for area under the number of conidia progress curve (AUncC), area under latent infection progress curve (AULIC), and the incidence of brown rot at post-harvest (*z*) in 11 surveys in Spanish orchards between 2002 and 2005

Orchard	AUncC×10^6	AULIC	*z*
Vinebre 2002	4.87 cd	0 a	2.0 (2.65) a
Alfarrás 2002	9.08 e	922 b	65.0 (54.48) d
Torre-Forns 2003	2.62 abc	0 a	0.0 (0.0) a
Gimenells 2003	4.08 bcd	0 a	3.0 (5.53) a
Sudanell 2003	6.85 de	41 a	12.0 (12.92) ab
Albesa 2004	1.43 ab	59 a	11.0 (14.82) ab
Alfarrás 2004	0.68 ab	103 a	23.0 (25.24) b
Sudanell 2004	2.51 abc	92 a	44.0 (42.64) c
Albesa 2005	0.39 a	0 a	2.0 (3.68) a
Alfarrás 2005	0.37 a	0 a	2.0 (3.68) a
Sudanell 2005	0.65 ab	0 a	1.0 (1.84) a
MSE[a]	6.9×10^6	10,679	(152.72)

Data are the mean of ten replications, with three trees per replication. Data in brackets are subjected to arcsinsqrt transformation to improve the homogeneity of variances before analysis; analysis was made with transformed data. Means followed by the same letter in each column are not significantly different (P=0.05) by Student Newman multiple range test

[a] MS_{within}=mean squared error

contribute to fruit infections that occur later in the growing season. However, we have been unable to isolate *Monilinia* spp from aborted fruit or twig cankers in Spanish peach orchards.

The present results suggest that the conidia of *Monilinia* spp. on flowers or fruit surfaces in Spanish peach orchards could not have originated from mummified fruit and blighted or necrotic twigs. Brown rot of peaches caused by *Monilinia* spp. is a polycyclic disease (Byrde and Willetts 1977) for which the disease forecast should be based on the amount of secondary inoculum or the number of secondary cycles (Fry 1982). In this regard, excellent control of brown rot caused by *M. fructicola* has been obtained after minimising the numbers of overwintering sources in peach trees in other regions (Landgraf and Zehr 1982). Therefore, it would be better to remove mummified fruit and necrotic twigs from trees, as well as collecting mummified fruit, pits and pruning twigs from the ground, to reduce the sources of primary inoculum of *Monilinia* spp. in Spanish peach orchards.

Although significant differences were recorded between growing seasons, we found that the number of conidia of *Monilinia* spp. on peach fruit surfaces increased rapidly during pit hardening and seven days before harvest and reached a count that ranged between $10^{3–4}$ and $10^{4–5}$ conidia per fruit surface. Other workers have reported that the number of

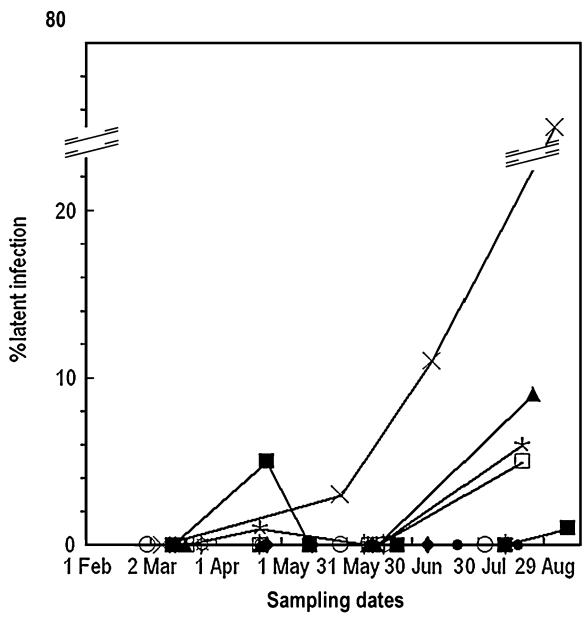

Fig. 2 Incidence of latent infection caused by *Monilinia* spp. on peach surfaces from pink blossom to harvest from 2002 to 2005 in 11 surveys in Cataluña, Spain: Vinebre02 (*open circles*), Alfarrás02 (*multiplication symbols*), Torre-Forns03 (*closed diamonds*), Gimenells03 (*closed circles*), Sudanell03 (*closed squares*), Alfarrás04 (*open squares*), Albesa04 (*closed triangle*), Sudanell04 (*asterisks*), Alfarrás05 (*open diamonds*), Albesa05 (*plus symbols*), and Sudanell05 (*inverted closed triangles*). Data are the mean of ten replicates with ten flowers or ten fruits per replicate

airborne conidia of *M. laxa* and *M. fructigena* increased as apricots and apples ripen (Corbin et al. 1968; Holb 2008). Furthermore, we have previously reported an increased frequency of latent infections and brown rot infections in peaches caused by *Monilinia* spp. as the fruit ripen (Gell et al. 2008).

A positive relationship between the incidence of latent infection caused by *Monilinia* spp. on stone fruit and the incidence of fruit rot at post-harvest in different climatic and crop conditions has been reported previously (Northover and Cerkauskas 1994; Emery et al. 2000; Luo and Michailides 2001a, 2001b; Luo and Michailides 2003; Xu et al. 2007, Gell et al. 2008). In addition, some studies have related the air spore densities of *M. fructicola* in stone fruit orchards to the incidence of fruit brown rot (Luo et al. 2005). Here, we found a significant relationship between the numbers of conidia on peach surfaces and the incidence of latent infections, but not with the incidence of brown rot at post-harvest. Many of the *Monilinia* conidia that were found on the fruit surface could be the cause of latent infections when climatic conditions are unfavourable. However, it is reasonable to assume that some of these conidia will be the cause of fruit rot when the climatic conditions are or become favourable for disease expression. It would be interesting to study correlations between the spore density at different periods of time in the season and incidence of latent infection and brown rot at post-harvest; further experiments are in progress.

The dynamics of individual populations within the epiphytic community are determined by rates of immigration, emigration, growth and death and all these factors are strongly influenced by the physical environment, chemical treatment, temperature, humidity, wind, rainfall and solar radiation (Kinkel 1997). The numbers of conidia of *Monilinia* spp. on the fruit surfaces were similar for the different peach and nectarine cultivars at each sampling date. However, we found that the incidences of latent and brown rot infections were significantly higher on nectarines than on peaches. This could be due to differences in the topography of their fruit surfaces, as suggested by Lee and Bostock (2006). They reported that the number of appressoria produced by conidial germlings of *M. fructicola* depended to a large degree on the stage of nectarine fruit development and that their formation by the pathogen was regulated by the topography of the plant surface.

The dynamics of spore density of *Monilinia* spp. on peach surfaces were affected by different weather factors. Specifically, temperature, solar radiation, wind speed, and rainfall were identified as important climatic factors that affected the AUncC of *Monilinia* spp. (*M. laxa* and *M. fructigena*) in Spanish peach orchards. However, Gell et al. (2008) demonstrated that temperature and wetness duration were the two important weather factors that were correlated to the incidence of latent infections caused by *M. laxa* and *M. fructigena* in Spanish peach orchards. Positive relationships of temperature and wind speed with airborne conidia have been reported, while a negative correlation between spore density and RH has also been documented for *M. fructigena* in apple orchards (Holb, 2008), and *M. laxa* in apricot orchards (Corbin et al. 1968).

The main finding from this study is that in order to reduce the incidence of latent infection and brown rot, it is essential not only to remove the sources of primary inoculum but to also reduce the number of *Monilinia* spp. conidia on the fruit surface. Furthermore, the sources of airborne conidia of *Monilinia* spp. that are deposited on fruit surfaces should be taken into consideration in disease management programmes in Spain.

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References

Biggs, A. R., & Northover, J. (1985). Inoculum sources for *Monilinia fructicola* in Ontario peach orchards. *Canadian Journal of Plant Pathology*, *7*, 302–307.

Byrde, R. J., & Willetts, H. J. (1977). *The brown rot fungi of fruit—their biology and control*. Oxford: Pergamon.

Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. New York: Wiley-Interscience.

Corbin, J. B., Ogawa, J. M., & Schultz, H. B. (1968). Fluctuations in numbers of *Monilinia laxa* conidia in an apricot orchard during the 1966 season. *Phytopathology*, *58*, 1387–1394.

Emery, K. M., Michailides, T. J., & Scherm, H. (2000). Incidence of latent infection of immature peach fruit by *Monilinia fructicola* and relationship to brown rot in Georgia. *Plant Disease*, *84*, 853–857. doi:10.1094/PDIS.2000.84.8.853.

Fry, W. E. (1982). *Principles of plant disease management*. New York: Academic Press.

Gell, I., De Cal, A., Torres, R., Usall, J., & Melgarejo, P. (2008). Relationship between the incidence of latent infections caused by *Monilinia* spp. and the incidence of brown rot of peach fruit: factors affecting latent infection. *European Journal of Plant Pathology*, *121*, 487–498. doi:10.1007/s10658-008-9268-3

Guijarro, B., Melgarejo, P., Torres, R., Lamarca, N., Usall, J., & De Cal, A. (2007). Effects of different biological formulations of *Penicillium frequentans* on brown rot of peach and nectarine. *Biological Control*, *42*, 86–96. doi:10.1016/j.biocontrol.2007.03.014.

Holb, I. J. (2008). Monitoring conidial density of *Monilinia fructigena* in the air in relation to brown rot development in integrated and organic apple orchards. *European Journal of Plant Pathology*, *120*, 397–408. doi:10.1007/s10658-007-9233-6.

Jacome, L. H., & Schuh, W. (1992). Effects of leaf wetness duration and temperature on development of black sigatoka disease on banana infected by *Mycosphaerella fijiensis* var. *difformis*. *Phytopathology*, *82*, 515–520. doi:10.1094/Phyto-82-515.

Kinkel, L. L. (1997). Microbial population dynamics on leaves. *Annual Review of Phytopathology*, *35*, 327–347. doi:10.1146/annurev.phyto.35.1.327.

Landgraf, F. A., & Zehr, E. I. (1982). Inoculum sources for *Monilinia fructicola* in South Carolina peach orchards. *Phytopathology*, *72*, 185–190.

Larena, I., Torres, R., De Cal, A., Liñan, M., Melgarejo, P., Domenichini, P., et al. (2005). Biological control of postharvest brown rot (*Monilinia* spp.) of peaches by field applications of *Epicoccum nigrum*. *Biological Control*, *32*, 305–310. doi:10.1016/j.biocontrol.2004.10.010.

Lee, M. H., & Bostock, R. M. (2006). Induction, regulation, and role in pathogenesis of appressoria in *Monilinia fructicola*. *Phytopathology*, *96*, 1072–1080. doi:10.1094/PHYTO-96-1072.

Luo, Y., & Michailides, T. J. (2001a). Factors affecting latent infection of prune fruit by *Monilinia fructicola*. *Phytopathology*, *91*, 864–872. doi:10.1094/PHYTO.2001.91.9.864.

Luo, Y., & Michailides, T. J. (2001b). Risk analysis for latent infection of prune by *Monilinia fructicola* in California. *Phytopathology*, *91*, 1197–1208. doi:10.1094/PHYTO.2001.91.12.1197.

Luo, Y., & Michailides, T. J. (2003). Threshold conditions that lead latent infection to prune fruit rot caused by *Monilinia fructicola*. *Phytopathology*, *93*, 102–111. doi:10.1094/PHYTO.2003.93.1.102.

Luo, Y., Ma, Z., Reyes, H. C., Morgan, D., & Michailides, T. J. (2007). Quantification of airborne spores of *Monilinia fructicola* in stone fruit orchards of California using real-time PCR. *European Journal of Plant Pathology*, *118*, 145–154. doi:10.1007/s10658-007-9124-x.

Luo, Y., Michailides, T. J., Morgan, P. D., Krueger, W. H., & Buchner, R. P. (2005). Inoculum dynamics, fruit infection, and development of brown rot in prune orchards in California. *Phytopathology*, *95*, 1132–1136. doi:10.1094/PHYTO-95-1132.

M.-Sagasta, E. (1977). *Monilinia* disease. *EPPO Bulletin*, *7*, 105–116.

Meier, U., Graf, H., Hess, M., Kennel, W., Klose, R., Mappes, D., et al. (1994). Phänologische Entwick-lungsstadien des Kernobstes (*Malus domestica* Borkh. und *Pyrus communis* L.), des Steinobstes (Prunus-Arten), der Johannisbeere (Ribes-Arten) und der Erdbeere (*Fragaria* x *ananassa* Duch.). *Nachrichtenbl Deut Pflanzenschutzd*, *46*, 141–153.

Michailides, T. J., Morgan, D. P., Felts, D., & Krueger, W. (1996). Ecology and epidemiology of prune brown rot and new control strategies. In *Prune research report and index of prune research* (pp. 109–123). Pleasanton: California Prune Board.

Northover, J., & Cerkauskas, R. F. (1994). Detection and significance of symptomless latent infections of *Monilinia fructicola* in plums. *Canadian Journal of Plant Pathology*, *16*, 30–36.

Ogawa, J. M., Zehr, E. I., & Biggs, A. R. (1995). Brown Rot. In M. Ogawa, E. I. Zehr, G. W. Bird, D. F. Ritchie, K. Uriu, & J. K. Uyemoto (Eds.), *Compendium of stone fruit diseases* (pp. 7–10). St. Paul, Minnesota: APS Press.

Rotem, J., Wooding, B., & Aylor, D. E. (1985). The role of solar radiation, especially ultraviolet, in the mortality of fungal spores. *Phytopathology*, *75*, 510–514.

Sauer, D. B., & Burroughs, R. (1986). Disinfection of seed surfaces with sodium hypochlorite. *Phytopathology*, *76*, 745–749.

Snedecor, G. W., & Cochram, W. G. (1980). *Statistical methods, 7th Ed*. Ames: University Press.

Sutton, T. B., & Clayton, C. N. (1972). Role and survival of *Monilinia fructicola* in blighted peach branches. *Phytopathology*, *62*, 1369–1373.

Van Leeuwen, G. C. M., Stein, A., Holb, I. J., & Jeger, M. J. (2000). Yield loss in apple caused by *Monilinia fructigena* (Aderh. & Ruhl.) Honey, and spatio-temporal dynamics of disease development. *European Journal of Plant Pathology*, *106*, 519–528. doi:10.1023/A:1008701315200.

Xu, X. -M., Bertone, C., & Berrie, A. (2007). Effects of wounding, fruit age and wetness duration on the development of cherry brown rot in the UK. *Plant Pathology*, *56*, 114–119.

Xu, X. -M., Robinson, J. D., Berrie, A. M., & Harris, D. C. (2001). Spatio-temporal dynamics of brown rot (*Monilinia fructigena*) on apple and pear. *Plant Pathology*, *50*, 569–578. doi:10.1046/j.1365-3059.2001.00602.x.