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Relationship between the incidence of latent infections caused by *Monilinia* spp. and the incidence of brown rot of peach fruit: factors affecting latent infection

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Abstract Five field experiments were performed in commercial orchards located in Lleida (Spain) over three growing seasons, 2000–2002, in order to estimate the relationship between the incidence of latent infection caused by *Monilinia* spp. in peaches and the incidence of post-harvest brown rot. No latent infection was recorded at popcorn and the maximum incidence occurred pre-harvest; in some orchards a second peak was detected during the pit hardening period. *Monilinia laxa* is the most prevalent species isolated from peaches with brown rot. There was a positive correlation between the incidence of latent infection and that of post-harvest brown rot. The average incidence of latent infection during the crop season explained 55% of the total variation in the incidence of post-harvest brown rot. The effect of temperature (*T*) and duration of wetness (*W*) on the incidence of latent infection in peach and nectarine orchards was analysed using multiple regression. The regression analysis indicated that *T* and *W* jointly explained 83% of the total variation in the incidence of latent infection. The model predicts no latent infections when $T<8$°C, and >22 h wetness are required when $T=8$°C but only 5 h at 25°C are necessary for latent infection to occur. The incidence of brown rot and latent infection of peaches caused by *M. laxa* under controlled experimental conditions were also affected by *T* and *W*, as well as by fruit maturity and inoculum concentration. Latent infections were produced in fruit when *T* was not suitable for the development of brown rot symptoms. In these experiments more than 4–5 h of daily wetness were required after embryo growth in fruit sprayed to run-off with an inoculum concentration higher than 10^4 conidia ml^{-1} of *M. laxa* for brown rot and latent infections to develop. The fitted model obtained from the field data was able to predict the observed data obtained under controlled environmental conditions.

Keywords Epidemiology · Disease management · *M. laxa* · *M. fructigena* · *M. fructicola*

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Introduction

In Mediterranean areas of Europe, brown rot in peaches (*Prunus persica*) and nectarines (*P. persica* var. *nectarina*) is caused by *Monilinia laxa* and *Monilinia fructigena* (De Cal and Melgarejo 1999). A third species, *Monilinia fructicola* causes brown rot in India, Japan, the Republic of Korea, Oceania, and North and South America and is in the A2 list of

quarantine organisms for Europe (organisms present in the EPPO region, but contained, under official control) (EPPO 2007). These fungi cause losses by infecting blossom, flowers, and fruit during the pre-harvest, harvest, and post-harvest periods (Larena et al. 2005). Post-harvest losses are typically more severe, especially when conditions are favourable for disease development; in some cases 80–85% of a crop may be lost (Hong et al. 1998; Larena et al. 2005).

The most important cause of post-harvest brown rot in peaches and nectarines in Spain and Italy is *M. laxa* (Larena et al. 2005; Tian and Bertolini 1999), followed by *M. fructigena* (isolated from 10% to 15% of fruit affected by brown rot) (Larena et al. 2005). The anamorphs of *M. laxa* and *M. fructigena* are dominant as inoculum sources (Byrde and Willetts 1977). Conidia produced in overwintered fruit mummies, fruit stalks, scars and buds, as well as in cankerous lesions (Byrde and Willetts 1977) act as primary inoculum sources and cause blossom blight and fruit rot in the spring (Byrde and Willetts 1977). When microclimatic conditions are unfavourable, infections may remain latent until conditions become favourable for disease expression, at which point fruit rot ensues (Byrde and Willetts 1977). In addition to weather conditions, fruit growth stage also plays an important role in disease expression (Luo and Michailides 2001b; Xu et al. 2007).

Latent infections caused by *M. laxa* have been detected in nectarines and plums, although the incidence does not show a strong correlation with that of brown rot (Fourie and Holz 2003a), except in the case of ripened fruit (Fourie and Holz 2003b). However, Xu et al. (2007) showed a high correlation between the incidence of visual rotting caused by *M. laxa* and *M. fructigena* and latent infection in cherries. In the case of *M. fructicola*, a number of studies have reported a positive correlation between the incidence of latent infection in immature plum and nectarine and the incidence of fruit rot at harvest and during the post-harvest period (Emery et al. 2000; Luo and Michailides 2001a; Northover and Cerkauskas 1994). Fruit with latent infection may also become mummified and serve as a source of primary inoculum the following spring (Luo and Michailides 2001a). These findings demonstrate the contribution of latent infection to the development of brown rot.

Temperature and the duration of wetness are considered to be the most important factors that affect infection by *M. fructicola* (Biggs and Northover 1988b; Luo and Michailides 2001a, b; 2003). The incidence of latent infection with *M. fructicola* in peaches and plums shows a positive linear and/or exponential relationship with increasing duration of wetness at different growth stages (Luo and Michailides 2001a, b). Tamm and Flückiger (1993) and Tamm et al. (1995) reported the effect of temperature, duration of wetness and phenological stage on blossom blight caused by *M. laxa* in sweet cherry in controlled environmental studies, and developed a non-linear model to estimate the incidence of blossom blight infection as a function of temperature and duration of wetness. However, no effects of the period of wetness were observed on infection of cherries of different ages (Xu et al. 2007).

No information is available on the effect of temperature, duration of wetness or inoculum concentration on the incidence of latent infections with *M. laxa* or *M. fructigena* in peach and nectarine fruit. Quantitative relationships between latent infection and environmental conditions at different fruit developmental stages are needed to develop a disease risk assessment system. The objectives of this study were to determine (1) the relationship between the incidence of latent infection of fruit and that of post-harvest fruit rot, and (2) the quantitative relationships between the incidence of latent infection and inoculum concentration, temperature and duration of wetness at different fruit development stages. A multiple regression model was developed to describe the relationships between temperature and duration of wetness and the incidence of latent infections. This model was evaluated using data obtained from experiments conducted in a controlled environment.

Materials and methods

Field experiments

To evaluate the incidence of post-harvest brown rot and the incidence of latent infection at different growth stages, five field experiments were performed in commercial orchards located in Lleida (Spain) over three growing seasons, between 2000 and 2002. The cultivars grown in the orchards were nectarine cv. Caldesi 20–20 in Alfarras (2001 and 2002), and peach cv. Rojo de Albesa in Albesa (2000 and 2001) and in

Termens (2000). Three consecutive 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 treated with fungicides. The incidence of latent infection was estimated in these trees throughout each cropping season. Samples were obtained at the following growth stages and dates: popcorn (BBCH=55; 3/15/2000, 3/13/2001, and 3/6/2002), pit hardening (BBCH=76; 5/29/2000, 5/24/2001, and 5/27/2002), embryo growth (BBCH=79; 7/21/2000, 7/4/2001, and 7/8/2002), and 7 days before harvest (BBCH=86; 9/8/2000, 8/31/2001, and 9/2/2002) (Meier et al. 1994). Ten blossoms or fruit per tree without visible signs of infection with *Monilinia* spp. were picked from orchards and sent to the laboratory on each sampling date. Fruit was surface-disinfected by dipping in 70% ethanol for 20 s, and then in a solution of 0.5% NaOCl and 0.05% Tween 20 for 4 min. The fruit was then rinsed for 1 min in sterile distilled water (SDW). The disinfected fruit was dipped in a solution of 6 g l^{-1} of paraquat (filter sterilized 1,1′-dimethyl-4,4′-bipyridinium dichloride, Gramoxone Extra N 20%, ICI-Zeltia, England) for 1 min. The paraquat-treated fruit was rinsed for 3 min in SDW. It was then incubated in humid chambers, lined with moist paper, at 22±2°C with fluorescent lighting at 100 μE m^{-2} s^{-1}, with a 16-h photoperiod for 7 to 15 days. Latent infection with *Monilinia* spp. was recorded following the appearance on the peaches of brown rotted tissue showing sporulation.

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

Temperature (°C), and average relative humidity (% RH) were recorded using automated weather-monitoring equipment placed close to each orchard. The proximity of this equipment to the field experiment locations ranged from approximately 500 m to 5 km. In the initial step the average daily temperature (*T*) and the daily duration of wetness (*W*, hours) were calculated for each day and each orchard. The daily duration of wetness was estimated as a constant threshold value for a RH of 87%; however, this value was extended to an arbitrary value for the change in RH over time (Wichink Kruit et al. 2004). For hourly RH<70%, surfaces were assumed to be dry; for 70%≤RH≤87%, the peach surface was assumed to be wet when RH increased by more than 6% in 60 min, and dry when RH decreased by more than 4% in 60 min; and for RH>87%, the surfaces were assumed to be wet. In the second step average daily temperature and daily duration of wetness were calculated for each orchard and for each period (1st January to popcorn, popcorn to pit hardening, pit hardening to embryo growth, and embryo growth to 7 days before harvest).

Experiments under controlled environmental conditions

Two experiments were conducted on branches carrying blossoms or fruit taken from a nectarine (cv. Autumn Free) orchard in Sudanell (Lleida, Spain) in 2004 and 2005. No chemical treatments were applied to the orchard during the cropping season. Samples were taken at six growth stages: popcorn (BBCH=55; 3/25/2004, 3/21/2005), shuck split (BBCH=71; 4/20/2004, 5/4/2005), pit hardening (BBCH=76; 6/2/2004, 6/1/2005), embryo growth (BBCH=79; 6/13/2004, 6/12/2005), and 30 days (BBCH=81; 8/17/2004, 8/11/2005) and 7 days before harvest (BBCH=86; 8/27/2004, 9/2/2005). Nectarine samples (branches bearing blossoms or fruit) in each growth stage were picked from the orchard and taken to the laboratory.

In 2004, individual branches carrying fifteen uniform blossoms each at popcorn (BBCH=55), or fifteen uniform small fruit each at shuck split (BBCH=71) or pit hardening (BBCH=76) were taken. Groups of three branches were placed in plastic cups (height×top diameter=11×15 cm) containing sterile vermiculite (Termita, Asfaltex, S.A., Barcelona, Spain) and sprayed to run-off with a conidial suspension of *M. laxa* (ATCC number 66106). Two conidial suspensions of *M. laxa* (10^6 and 10^4 conidia ml^{-1}) were tested, and sterile water was sprayed onto similar branches as a non-inoculated control. To maintain high humidity, cups were filled with water, and the inoculated branches were covered with plastic bags (75×40×33 cm) attached to the bottom of the cups. The cups were placed in an incubator at 23°C with fluorescent light at 100 μE m^{-2} s^{-1}, with a 16-h photoperiod. The

plastic bags were removed after 0, 4, 8 and 12 h of wetness. The cups with the branches were incubated for 7 days. Each branch was treated as a replicate, and three replicates were used for each treatment of inoculum concentration and duration of wetness at each growth stage. At the later stages of embryo growth (BBCH=79), and 30 days (BBCH=81) and 7 days (BBCH=86) before harvest, individual branches carrying five uniform fruit each were taken; the five fruit were separated from each branch and placed on plastic trays. Fruit were sprayed to run-off with sterile water or with one of the two conidial suspensions of *M. laxa*, as described above. To maintain high humidity, trays were lined with moist paper and then covered with plastic film. The trays were placed in an incubator under the same conditions as described above. The plastic films were removed after 0, 4, 8 and 12 h of wetness. The trays were incubated for 7 days. Each tray containing five fruit was treated as a replicate, and three replicates were used for each treatment of inoculum concentration and duration of wetness at embryo growth, and 30 and 7 days before harvest.

The same experiments were conducted in 2005, but the durations of wetness assayed were 0, 4, 6, and 18 h; the inoculum concentrations were the same as those used in 2004. Each treatment group of inoculum concentration and duration of wetness at each growth stage was placed in an incubator at 10°C or 23°C with fluorescent lighting at 100 μE m^{-2} s^{-1}, with a 16-h photoperiod for 7 days. Therefore, three replicates (three branches with 15 blossoms or small fruit; or 3 trays with 5 fruit) were used for each treatment group of inoculum concentration, temperature and duration of wetness at each growth stage.

The occurrence of flowers and fruit with brown rot symptoms was recorded in each replicate after 7 days of incubation. Flowers and fruit without visible signs of infection with *M. laxa* were tested for the presence of latent infection as described for the field experiments.

Data analysis

The incidence of latent infection was calculated for each orchard and sampling date, and a latent infection curve (LIC) was obtained for each experimental unit. The area under the latent infection curve (AULIC), in units of percent-days, was calculated by trapezoidal integration (Campbell and Madden 1990). Using the combined data from all experiments, correlation and regression analyses were performed using Statgraphics Plus for Windows v. 4.1 (StatPoint, Inc. Herndon, VA.) to analyse the relationship between the incidence of samples with visible brown rot symptoms and the incidence of latent infection at each sampling date, AULIC and mean incidence of latent infection (Snedecor and Cochram 1980). Twenty points were used for regression analysis (four sampling dates × five orchards). Each point was the mean of ten replicates (trees). Data for brown rot and latent infection were arcsine transformed before analysis.

The model arcsine $y=f(T, W)$ was used to investigate the relationship between the effect of temperature (T) and duration of wetness (W) on the incidence of latent infection (y), using data pooled from the five field assays. In this model, $f(T, W)$ is a linear function of the terms T, W, TW, T^2, W^2, T^3, T^2W, W^3, TW^2, T^3W, TW^3, and T^2W^2. Regression analysis was used to estimate these parameters. Data were fitted to the model using the Model Regression Selection process of Statgraphics Plus for Windows v. 4.1 (StatPoint, Inc. Herndon, VA.). Model selection was performed on the basis of the significance of the estimated parameters, the adjusted coefficient of determination, the mean absolute error (average of the absolute values of the residuals, Mallow's C_p values (a comparison of total mean squared error to the true error variance), the Durbin–Watson statistic (a test for serial correlation in the residuals of a least-squares regression analysis), and the distribution of residuals (Jacome and Schuh 1992). The resulting equation was used to generate response surfaces using the G3D procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC, USA).

Data on the incidence of brown rot and the incidence of latent infection from the controlled environment experiments were arcsine transformed before analysis. Analysis of variance (ANOVA) was applied (Snedecor and Cochram 1980) to determine significance of the treatment effects: duration of wetness, temperature, inoculum concentration and growth stage using the generalised linear models (GLM) procedure of Statgraphics Plus for Windows v. 4.1 (StatPoint, Inc. Herndon, VA.). The equation used was $y_{ijklm} = \mu + S_i + I_j + W_k + T_l + \mathrm{SI}_{ij} + \mathrm{SW}_{ik} + \mathrm{ST}_{il} + \mathrm{IW}_{jk} + \mathrm{IT}_{jl} + \mathrm{WT}_{kl} + \mathrm{SIW}_{ijk} + \mathrm{SIT}_{ijl} + \mathrm{SWT}_{ikl} + \mathrm{IWT}_{jwl} + \mathrm{SIWT}_{ijkl} + e_{m(ijkl)}$, where y was the incidence arcsine % of latent infection, and S the

growth stage, I the inoculum concentration, W the duration of wetness, and T the temperature (2005 only) were fixed factors. SI, SW, ST, IW, IT, WT, SIW, SIT, SWT, IWT, SIWT were the interactions among combinations of corresponding factors; μ represented a 'grand mean', and e was the experimental error. Because neither brown rot nor latent infection was observed in samples in the popcorn, shuck split, pit hardening or embryo growth stages, data from these stages were not included in the analysis.

The data on the incidence of latent infection from the controlled environment experiment were used to evaluate the linear model arcsine $y=f(T, W)$ described above. The model was evaluated using the Calibration Model Analysis procedure of Statgraphics Plus for Windows v. 4.1 (StatPoint, Inc. Herndon, VA.). The calibration model analysis was used to identify a calibration line that fits the data; it allows selection from several transformations when the points do not follow a straight line. The test for lack of fit is designed to determine whether the selected model is adequate to describe the observed data, or whether an alternative model should be used. The test for lack of fit was performed by comparing the variability of model residuals to the variability between observations at replicate values from the controlled environment experiment. Significant lack of fit shows that the specified model does not adequately fit the response.

Results

Field experiments

No latent infection was recorded at the popcorn stage in any of the orchards. The incidence of latent infection increased during pit hardening (this increase was not significant in ALB01 or ALF02), and remained fairly constant until embryo growth, except for ALB00, where it decreased. The incidence of latent infection increased at the pre-harvest stage in all orchards (this increase was not significant in TER00 or ALB01) (Fig. 1). The average incidence of latent infection, the AULIC, and the incidence of post-harvest brown rot varied among orchards and years (Table 1). The highest values were recorded in Albesa in 2000, Alfarrás in 2001, and Alfarrás in 2002. More than 60 isolates were recovered from diseased fruit and identified as *M. laxa*, and one as *M. fructigena*.

The AULIC showed a significant correlation with the average incidence of latent infection and with the incidence of latent infection at the different sampling dates (Table 2). In addition, the average incidence of latent infection showed correlation with the incidence of latent infection recorded at the different sampling dates (Table 2).

The incidence of latent infection at embryo growth, the incidence 7 days before harvest, the average

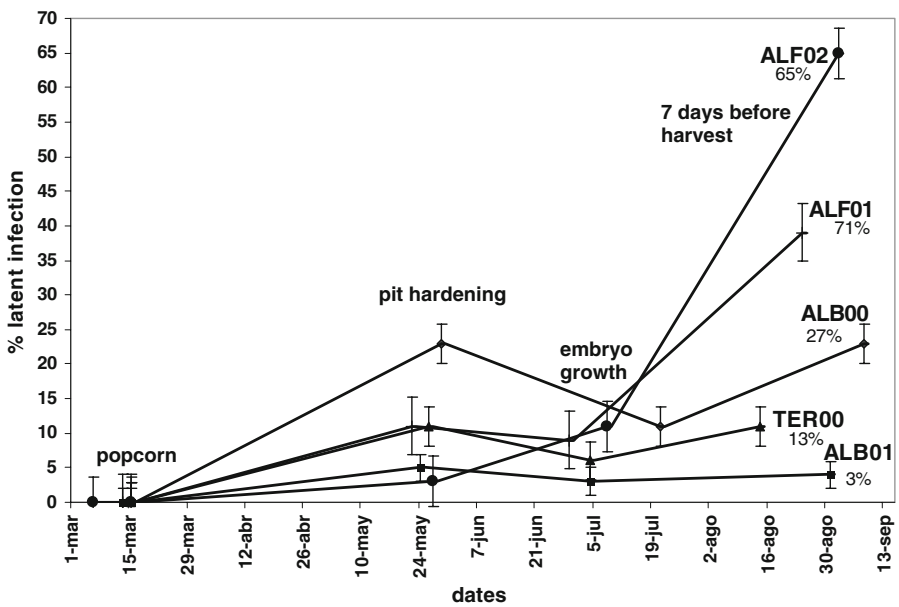

Fig. 1 Percentage of latent infections (mean±standard error) caused by *Monilinia* sp. in five field experiments, Albesa 2000 (*ALB00*), Termens 2000 (*TER00*), Albesa 2001 (*ALB01*), Alfarras 2001 (*ALF01*), and Alfarras 2002 (*ALF02*) performed in commercial orchards located in Lleida (Spain) over three growing seasons from 2000 to 2002. Post-harvest data are shown for each orchard name at the *right-hand side* of the graph

Table 1 Disease variables associated with infection by *Monilinia* spp. in peach and nectarine orchards in Lleida (Spain)

Orchard and year	Fruit	Average incidence of latent infection (%)[a]	AULIC	Incidence of post-harvest fruit rot (%)[b]
Albesa 2000	Peach	14	262	27
Termens 2000	Peach	7	129	13
Albesa 2001	Peach	3	55	3
Alfarrás 2001	Nectarine	15	220	71
Alfarrás 2002	Nectarine	20	255	65

Data are the mean of 10 replicates in each orchard.

AULIC: area under the latent infection curve (from popcorn to 7 days before harvest)

[a] The average incidence of latent infection (%) throughout the crop season estimated for four growth stages: popcorn (BBCH=55), pit hardening (BBCH=76), embryo growth (BBCH=79), and 7 days before harvest (BBCH=86).

[b] Incidence of post-harvest brown rot determined after incubation for 7 days at 23°C with fluorescent lighting at 100 μE m^{-2} s^{-1}, 16 h photoperiod.

incidence of latent infection, and the AULIC showed significant correlation with the incidence of post-harvest brown rot (Table 2). The strongest correlation was obtained between the average incidence of latent infection and the incidence of post-harvest brown rot (r=0.74). The incidence of post-harvest brown rot (z) could be predicted by the average incidence of latent infection ($\tilde{y}$) (R^2=0.55, P<0.01) using the equation:

$$\underset{}{\text{Arcsine}\, z} = \underset{(3.77)}{-0.39} + \underset{(0.45)}{3.48}\,\text{Arcsine}\,\tilde{y} \quad (1)$$

Table 2 Correlation coefficients between disease variables associated with infection by *Monilinia* spp. in five orchards in Lleida (Spain)

	Incidence of latent infection at pit hardening (%)	Incidence of latent infection at embryo growth (%)	Incidence of latent infection at 7 days pre-harvest (%)	Average incidence of latent infection (%)[a]	AULIC	Incidence of post-harvest fruit rot (%)[b]
Incidence of latent infection at pit hardening (%)	1.0 (<0.05)	0.46 (<0.05)	−0.10 (0.48)	0.40 (<0.05)	0.65 (<0.05)	0.05 (0.75)
Incidence of latent infection at embryo growth (%)		1.0 (<0.05)	0.31 (<0.05)	0.70 (<0.05)	0.79 (<0.05)	0.51 (<0.05)
Incidence of latent infection at 7 days pre-harvest (%)			1.0 (<0.05)	0.82 (<0.05)	0.62 (<0.05)	0.69 (<0.05)
Average incidence of latent infection (%)				1.0 (<0.05)	0.95 (<0.05)	0.74 (<0.05)
AULIC					1.0 (<0.05)	0.63 (<0.05)
Incidence of post-harvest fruit rot (%)						1.0 (<0.05)

Data are the mean of 10 replicates in each orchard. Data for the incidence of latent infection and the incidence of post-harvest fruit rot were arcsine transformed before correlation analysis. Values are correlation coefficients and corresponding P values (in parentheses).

AULIC: area under the latent infection curve (from popcorn to 7 days pre-harvest)

[a] Average incidence of latent infection (%) throughout the crop season estimated in four growth stages: popcorn (BBCH=55), pit hardening (BBCH=76), embryo growth (BBCH=79), and 7 days pre-harvest (BBCH=86).

[b] Incidence of post-harvest brown rot determined after incubation for 7 days at 23°C with fluorescent lighting at 100 μE m^{-2} s^{-1}, 16 h photoperiod.

where z is the incidence of post-harvest brown rot and $\tilde{y}$ is the average incidence of latent infection and the standard error of parameter estimates are given in parentheses.

The following linear regression model best described the incidence of latent infection (y) as a function of temperature and duration of wetness:

$$\underset{(4.138)(0.081)}{\text{Arcsine}(y) = 12.53} - \underset{(0.0004)}{0.35WT} + \underset{(0.00006)}{0.002T^2W^2} + 0.00019T^3W \quad (2)$$

This model explained 83.37% of the variability in the incidence of latent infection. The adjusted coefficient of determination (R^2*) of 0.80, the mean absolute error of 2.82, the standard deviation of the residuals of 4.34, C_p of 2.07, the Durbin–Watson statistic of 2.32, and the normal distribution of residuals (not shown) indicated a good fit of the model. The incidence of latent infection predicted by equation (2) in relation to average temperature (T) and duration of wetness (W) is shown in Fig. 2. No latent infection developed at temperatures lower than 8°C. To develop latent infections when T= 8°C>22 h of W was required, while only 5 h of W was needed at 25°C. One hundred percent of latent infection was predicted by the model for various combinations of T and W: 12°C–24 h, 13°C–23 h, 14°C–22 h, 15°C–20 h, 16°C–19 h, 17°C–18 h, 18°C–17 h, 19°C–16 h, 20°C–15 h, 21°C–14 h, 22°C–13 h, 23°C–12 h, 24°C–12 h, and 25°C–11 h. With T>17°C and maximum W (24 h), or maximum T with W>15 h, no latent infections occurred, except for T>20°C and W=22 to 24 h.

Controlled experiments

The three factors (growth stage, inoculum concentration, and duration of wetness) were tested using ANOVA for their effects on the incidence of latent infection. All their main effects and the two/three-way interactions, were significant (P<0.05) for the 2004 experiment. The results were similar for the 2005 experiment except that temperature (P=0.33), the two-way interaction between growth stage and duration of wetness (P=0.42), and the three-way interaction between inoculum concentration, growth stage and duration of wetness (P=0.57) were not statistically significant.

The incidence of latent infection recorded in non-inoculated fruit before harvest was <20% (Fig. 3a to d). Latent infections developed in fruit collected at 7 and 30 days before harvest, sprayed with a conidial suspension of *M. laxa* and incubated at 10°C or 23°C (Fig. 3). The highest incidence of latent infection was observed on fruit collected 7 days before harvest, sprayed with 10^6 conidia ml^{-1} of *M. laxa* and incubated at 10°C with >4 h of wetness (Fig. 3f);

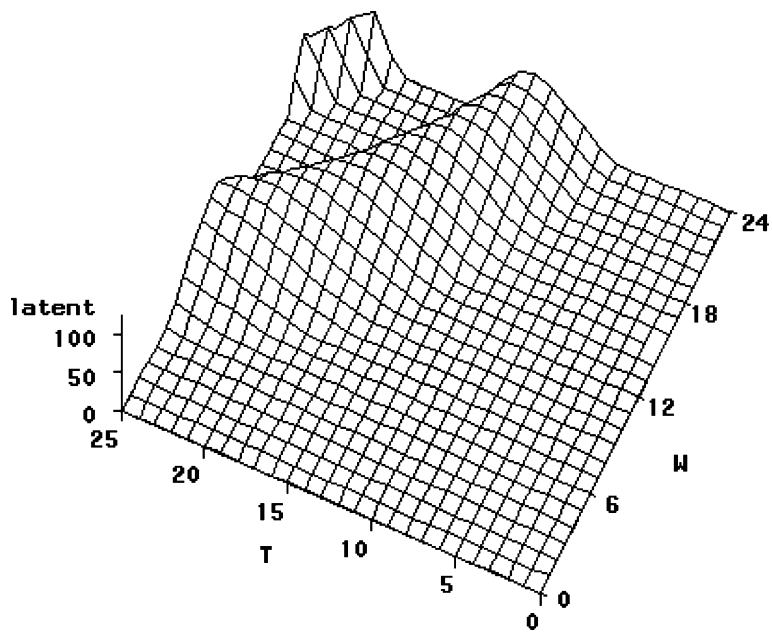

Fig. 2 Response surfaces predicting the incidence of latent infection (%) in peach and nectarine fruit at a range of durations of wetness (W) and temperatures (T). The surface was generated using Eq. 2

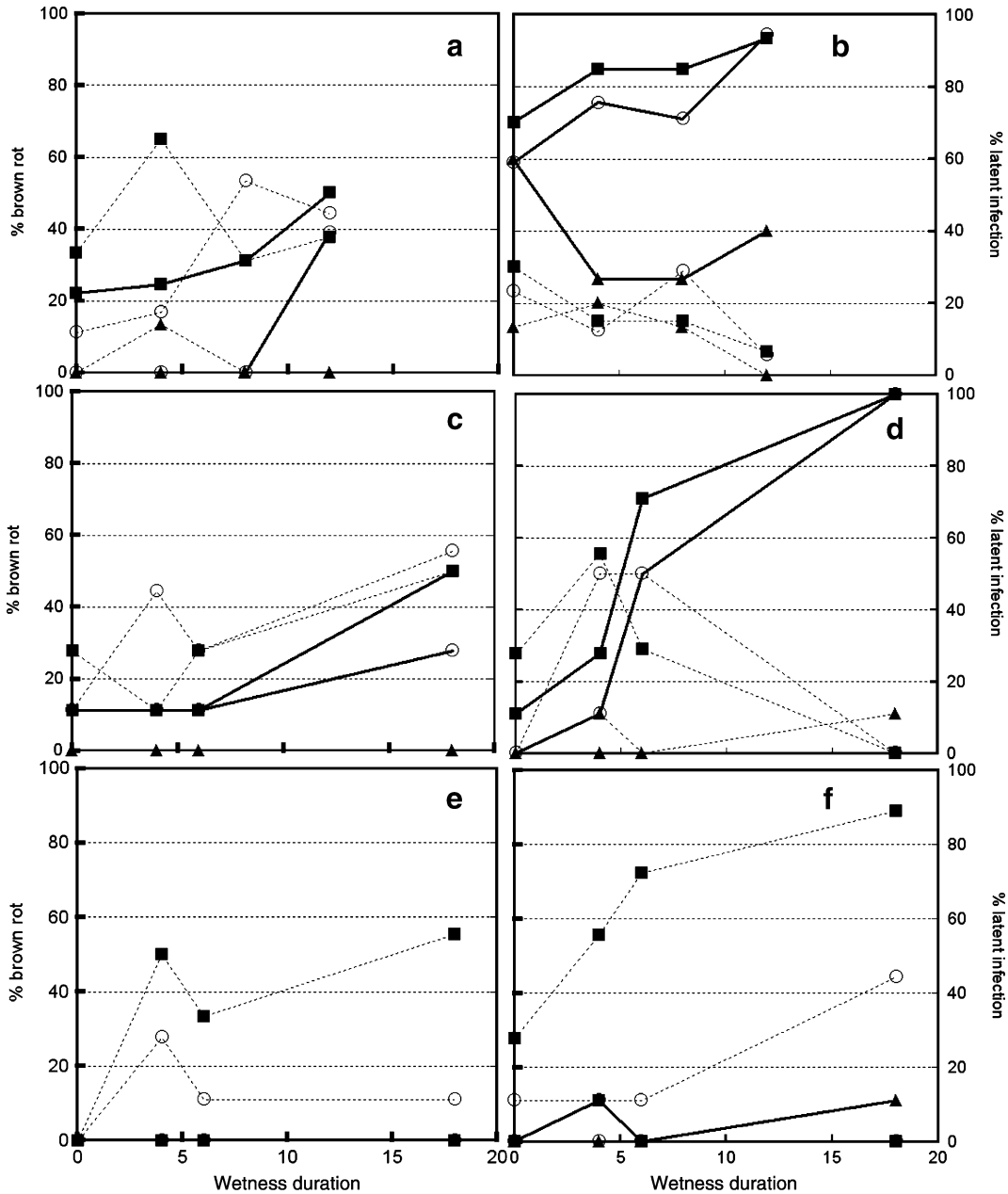

Fig. 3 Incidence of brown rot (%) (*solid lines*) and latent infection (%) (*dashed lines*) caused by *Monilinia laxa* as a function of duration of wetness (0, 4, 6, 8, 12 and 18 h) at three inoculum concentrations (0 [*closed triangles*], 10^4 [*open circles*] and 10^6 [*closed squares*] conidia ml^{-1}) on fruit (cv. Autumn Free) in controlled environment experiments. The incidence of brown rot and latent infection were assessed at two growth stages: BBCH=81 (**a**, **c**, and **e**), and BBCH=86 (**b**, **d**, and **f**) after 7 days of incubation at 23°C in 2004 (**a** and **b**) and in 2005 (**c** and **d**), or at 10°C in 2005 (**e** and **f**). BBCH=81: growth stage with fruit 30 days before harvest. BBCH=86: growth stage with fruit 7 days before harvest. Three replicates with five fruit per replicate were used for each treatment of temperature-inoculum concentration-wetness and growth stage

however visible brown rot symptoms did not develop in these fruit. The higher the incidence of brown rot recorded in fruit, the fewer latent infections were observed (Fig. 3b and d). The incidence of latent infection on fruit collected at 30 days before harvest and incubated at 23°C with 12–18 h of wetness was higher than that observed on fruit collected 7 days before harvest (Fig. 3a to d).

ANOVA of the incidence of brown rot showed significant ($P<0.05$) effects for all three factors and their interactions, except for the two-way interaction between growth stage and duration of wetness ($P=0.67$) and the three-way interaction in the 2004 experiment ($P=0.17$). For the 2005 experiment, all factors and interactions were significant ($P<0.05$).

Non-inoculated fruit did not develop brown rot, except for fruit picked 7 days before harvest in the 2004 experiment (Fig. 3b). A high percentage of brown rot was observed on fruit sprayed with conidial suspensions of *M. laxa* and incubated at 23°C (Fig. 3a to d), especially fruit collected 7 days before harvest exposed to the longest duration of wetness (Fig. 3b and d). The incidence of brown rot recorded on fruit sprayed with 10^4 conidia ml^{-1} of *M. laxa* was lower than that on fruit sprayed with 10^6 conidia ml^{-1} when the fruit were collected 30 and 7 days before harvest (Fig. 3a to d). An incidence of brown rot >50% was observed on nectarines collected 7 days before harvest, sprayed with 10^6 conidia ml^{-1} of *M. laxa*, and incubated at 23°C with >5 h of wetness. When inoculated fruit were incubated at 10°C brown rot did not develop (Fig. 3e), or the incidence was <10% (Fig. 3f).

The linear model fitted to describe the relationship between the observed and predicted incidence of latent infection was:

$$\underset{(3.45)(0.04)}{\text{Arcsine}(y) = 17.31 + 0.21\begin{pmatrix} 12.53 - 0.35WT + \\ 0.002T^2W^2 + \\ 0.00019T^3W \end{pmatrix}} \quad (3)$$

All the parameters in the equation were statistically significant ($P\leq0.01$) with low standard errors (given in parentheses). The relationship explained 52% of the variability in the observed incidence of latent infections. The *P* value for lack of fit in the ANOVA was 0.64, which indicated that the model was adequate to describe the observed data. Figure 4 shows a plot of the observed versus the predicted values. The closer the points lie to the diagonal line, the better the model predicts the observed data.

Discussion

We have demonstrated a positive relationship between the incidence of latent infection by *Monilinia* spp. and the incidence of post-harvest brown rot in peach and nectarine orchards in Spain. The most prevalent species isolated from brown rot in peach fruit (>98% of the identified isolates) was *M. laxa*. *Monilinia fructigena* is mainly associated with brown rot of pome fruit (van Leeuwen et al. 2000). Positive relationships between incidences of latent infection and brown rot at harvest or during the post-harvest period have been previously described for other species and hosts such as *M. fructicola* and peaches (Emery et al. 2000), prunes (Luo and Michailides 2001a, b; 2003), and plums (Northover and Cerkauskas 1994), and *M. laxa* and cherries (Xu et al. 2007). Under the conditions of this study, a 5–10% average incidence of latent infection during the blossom to pre-harvest periods may lead to more than 16–33% of post-

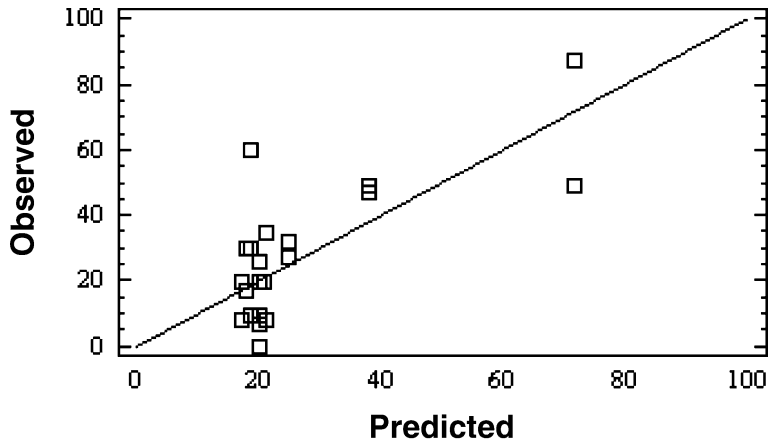

Fig. 4 Observed incidence (%) of latent infections of peach based on Eq. 2 versus the incidence of latent infection predicted from experiments in a controlled environment

harvest brown rot. This is not an acceptable level for post-harvest disease incidence in commercial peaches.

The pattern of latent infection observed in peaches and nectarines in Spanish orchards has some similarities to that in prunes infected with *M. fructicola*, especially during the pre-harvest period (Biggs and Northover 1988a; Luo and Michailides 2001b, 2003). In these studies, no latent infections were observed at popcorn, and the maximum incidence occurred during the pre-harvest; in some orchards a second peak was detected at pit hardening. Average incidence of latent infection showed a positive correlation with the incidence of latent infections throughout the season.

As reported previously (Northover and Cerkauskas 1994; Luo and Michailides 2001a), regression analysis (R^2=0.83) suggests that temperature and duration of wetness are important climatic variables that affect the incidence of latent infection caused by *Monilinia* spp. (*M. laxa* and *M. fructigena*) in Spanish field experiments. A similar model has been used to describe the severity of disease caused by *Colletotrichum acutatum* on strawberry plants (Wilson et al. 1990) as a function of temperature and duration of wetness under controlled conditions. These analyses showed that longer periods of wetness are required if temperatures are low, and vice versa. The behaviour of the model is bimodal with two peaks, but only the first peak has practical significance for Mediterranean conditions. The second peak occurred under unusual conditions for the culture of peach trees: it is unusual to have temperatures of >20°C together with >20 h of wetness in the Mediterranean climate of the temperate zone (36° to 46° latitude) (Edwards 1987).

Experiments were conducted under controlled conditions in order to understand the influence of temperature and the duration of wetness on the incidence of latent infections and fruit rot at different stages of development and using different concentrations of inoculum. Interactions among these factors were also observed. Temperature was an important factor that affected the incidence of brown rot. At temperatures <10°C, a 15% incidence of post-harvest brown rot was observed. The highest incidence of brown rot was observed on nectarines inoculated by *M. laxa*, collected 7 days before harvest and incubated at 23°C with a duration of wetness >12 h. Gupta and Agarwala (1990) and Tian and Bertolini (1999) also observed a direct correlation between temperature and rotting of peach fruit caused by *M. laxa.* In these experiments, peach fruit could be infected by the fungus at a range of temperatures between 5°C and 35°C; rotting increased with an increase in temperature and reached the maximum at 25°C: there was no rotting at 40°C. The growth stage also plays a role in disease expression. No brown rot was observed in the first nectarine growth stages in experiments performed under controlled environmental conditions. Previous results obtained with nectarine fruit infected by *M. laxa* indicated that fruit at the pit hardening stage was resistant to penetration and disease expression (Fourie and Holz 2003a). Resistance has been observed to decrease with increasing fruit maturity; furthermore, increased wetness and duration of wetness resulted in large increases in penetration and disease expression in ripening fruit (Fourie and Holz 2003a). In the case of *M. fructigena* infections of apples, Xu and Robinson (2000) observed that the incidence of brown rot was affected greatly by fruit maturity, while the effect of duration of wetness was small. Biggs and Northover (1988b) also reported that peach fruit became increasingly susceptible to infection by *M. fructicola* approximately 2 weeks before full ripeness.

Under controlled experiments latent infections were observed on fruit when conditions were not conducive to development of visible brown rot, namely, low temperatures (10°C) at 7 and 30 days before harvest, and warm temperatures (23°C) with immature fruit (30 days before harvest). Under these conditions latent infections were most likely to develop on nectarines inoculated by *M. laxa*, depending on the duration of wetness. The incidence of latent infections increased with longer duration of wetness. The highest percentage of latent infections was observed on nectarines sprayed with 10^6 *M. laxa* conidia ml^{-1}, collected 7 days before harvest and incubated at 10°C with a duration of wetness >15 h. No latent infections were observed in the first nectarine growth stages (popcorn) in controlled environment experiments. Similar results were obtained by Fourie and Holz (2003b) in plum fruit infected by *M. laxa*: fruit 2 weeks before harvest developed disease only after periods of wetness >12 h. However, in the case of prunes infected with *M. fructicola*, the incidence of latent infection increased exponentially with increased duration of wetness, and linearly with increased inoculum concentration for most bloom and fruit development

stages (Luo and Michailides 2001b), confirming similar studies on detached peach fruit (Biggs and Northover 1988a; Emery et al. 2000).

In the current study, models derived from field-collected data were validated with data obtained from controlled-environment experiments (using data from 30 and 7 days before harvest). A stage-dependent impact of wetness×temperature on incidence of infection was demonstrated. This model related the incidence of latent infection only to temperature and the duration of wetness but did not include other factors such as phenological stage, inoculum concentration, and host variables. However, in the study performed under controlled conditions it was demonstrated that with increased ripening, a shorter duration of wetness is required to achieve equivalent levels of infection. More complex models could be developed in the future, taking into account other factors. However, the results of the calibration of field-collected data using controlled environment experimental studies confirmed that the simplification of the model is satisfactory.

Present results demonstrate that latent infection should be taken into consideration in disease management programmes in Spain. Although brown rot may not be severe at harvest, it could develop later because of the high incidence of latent infection. A control strategy should be based on estimation of the risk of latent infection, assessed on the basis of the effects of temperature and duration of wetness. The quantitative relationships of the incidence of latent infection with temperature and duration of wetness could be used to develop a risk assessment and disease prediction system for brown rot. To increase the prediction accuracy, this model should be evaluated across other geographical locations.

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