Influence of temperature on decay, mycelium development and sporodochia production caused by Monilinia fructicola and M. laxa on stone fruits

M. Bernat [a], J. Segarra [b], X.-M. Xu [c], C. Casals [a], J. Usall [a *]

[a]IRTA, XaRTA-Postharvest, Edifici Fruitcentre, Parc Científic i Tecnològic Agroalimentari de Lleida, Parc de Gardeny, 25003 Lleida, Catalonia, Spain

[b]Department of Crop and Forest Sciences, University of Lleida, Avda. Rovira Roure, 191, 25198 Lleida, Catalonia, Spain

[c] East Malling Research, New Road, East Malling, Kent ME19 6BJ, UK

Abstract

Brown rot on peaches and nectarines caused by *Monilinia* spp. results in significant economic losses in Europe. Experiments were conducted to study the effects of temperature (0-33ºC) on the temporal dynamics of decay and mycelium development and the subsequent sporulation on peaches and nectarine fruit infected by *M. laxa* and *M. fructicola*. The rates of decay and mycelium development increased with temperature from 0ºC to 25ºC for both *Monilinia* species. At 0 ºC, decay was faster for *M. laxa* (0.20 cm^2 $days^{-1}$) than for *M. fructicola* (0.07 cm^2 $days^{-1}$); indeed, *M. laxa* was able to develop mycelia and sporodochia, but *M. fructicola* was not. At 4 and 20 ºC, there were no differences in decay and mycelia development between the two *Monilinia* species. When temperature increased from 25 to 33 ºC, the rates of fungal decay and mycelium development decreased. At 30 and 33 ºC, *M. fructicola* decayed faster (0.94 and 1.2 cm^2 $days^{-1}$, respectively) than *M. laxa* (0.78 and 0.74 cm^2 $days^{-1}$, respectively) and could develop mycelia and produce sporodochia, whereas *M. laxa* failed at 33 ºC. These results indicated that *M. fructicola* is better adapted to high temperatures, whereas *M. laxa* is better adapted to low temperatures. These results can be used to predict the relative importance of the two species during the season at a given site and to improve management strategies for brown rot in areas where both species are present.

1. Introduction

Brown rot caused by *Monilinia* spp. can result in significant economic losses worldwide both in peaches [*Prunus persica* (L.) Batsch.] and nectarines [*Prunus*

persica var *nectarine* (Ait) Maxim.] (Bryde and Willetts, 1977). In the EU, brown rot on stone fruit is caused primarily by *M. laxa* (Aderhold and Ruhland) Honey, and recently, *M. fructicola* (G. Winter) has become more important in southern Europe (Villarino et al., 2013). Catalonia is the most important region for stone fruit production in Spain, supplying 47% of peaches and 38% of nectarines marked for export (DAAM, 2013).

M. laxa and *M. fructicola* overwinter primarily as mycelia on mummified fruits; in early spring when the weather conditions become favourable, overwintered mycelia sporulate on mummified fruit and produce conidia, which are dispersed primarily by wind (Bryde and Willetts, 1977). Conidia can infect blossoms and both immature and mature fruit (Biggs and Northover, 1985, Gell et al., 2009). Healthy fruit infected by both of the species usually remain asymptomatic (latent), and visual decay symptoms only develop during the late ripening period and post-harvest (Gell et al., 2008, Luo et al., 2001, Luo and Michailides, 2003). Although fruit infection occurs primarily in orchards, pre- and postharvest control is often not effective due to the limited use of fungicides at orchards and the ability of *Monilinia* spp. to become resistant to fungicides (Larena et al., 2005). During cold storage, latent infections become symptomatic and decay spreads by contact with adjacent fruits. In addition, sporulation may occur on rotten fruit, leading to secondary inocula infecting healthy fruits in the storage. In addition, the infected tissue of decaying fruits may remain adhered to containers, which can become a source of inoculum in packing houses and cold storage (Tian and Bertolini, 1999).

Temperature and wetness duration have been reported as the two most important abiotic factors influencing conidial germination (Casals et al., 2010, Xu et al., 2001), fruit infection (Biggs and Northover, 1988, Phillips, 1984, Corbin, 1962), rot development and sporulation (Bannon et al., 2008, Gell et al., 2008). Although many studies have been carried out to determine the effect of temperature on brown rot infection, colonization, and sporulation on stone fruits, the effect is usually studied within a narrow range of temperature and with only a single species included in a given study. In addition, many studies on *Monilinia* behaviour have been conducted on Petri dishes. However, the mycelium area on fruit may not be the same as the decay area, and mycelium development may not lead to sporulation.

Tamm and Flückiger (1993) reported that the optimum temperature for the growth of *M. laxa* is 25 ºC, but it is even able to grow below 0 ºC *in vitro*. *M. fructicola* grows faster and sporulates more abundantly than *M. laxa* when the temperature is in the range of 15-25 ºC (Bryde and Willetts, 1977, De Cal and

Melgarejo, 1999). Villarino et al. (2010) reported that *M. fructicola* is more virulent and has a greater fitness than *M. laxa* because it has a higher percentage of conidium germination and forms longer germ tubes. Although *M. laxa* sporulates at 5-10 ºC, it has shorter germ tubes (De Cal and Melgarejo, 1999).

Recently, *M. fructicola* has been established in many stone fruit production regions in Europe. To develop effective management strategies for brown rot in these areas, we need to understand the relative effect of a wide range of temperatures on *M. fructicola* and *M. laxa*. The objective of the present study was to evaluate the effect of temperature on (i) fruit decay and mycelium development and (ii) sporulation on detached peaches and nectarines inoculated with *M. laxa* and *M. fructicola* individually.

2. Materials and Methods

2.1 Fruits

Fruits from the peach variety 'Baby Gold 9' and 'Summer Rich' and nectarine variety 'Albarret' and 'Diamond Ray' were sourced from an organic orchard in Lleida (Catalonia). Fruits were picked at an optimum stage of commercial maturity, and immediately after harvest, healthy fruits of approximately the same size were selected manually for inclusion in the experiments. Fruits were immersed in 10% commercial chlorine for 1 min, rinsed with tap water for 3 min and, finally, air-dried for 24 hours before artificial inoculation.

2.2 Fungal isolates and inoculum preparation

Two fungal strains (*M. fructicola* - CPMC1, and *M. laxa* – CPML2) were isolated from decayed fruits in Lleida, and their identities were confirmed by the Department of Plant Protection, INIA (Madrid, Spain). The two strains were maintained on potato dextrose agar (PDA) medium (Biokar Diagnostic) at 4 ºC in darkness.

The two strains were sub-cultured onto PDA Petri dishes and incubated in the dark at 25 ºC for approximately 1 week. To ensure conidial production, peach and nectarine fruits were inoculated with the isolates separately. The fruits were first wounded by a sterilized steel rod (1 mm wide and 2 mm long); conidia and mycelia were then transferred from the PDA culture onto each wound site by a sterilized pipette tip. Inoculated fruits were incubated at 25 ºC and 85% RH in the dark for *M. fructicola* and in a 12-h light photoperiod for 5-7 days for *M. laxa*.

Conidia from infected fruits were scraped with a sterile loop and transferred to a test tube with 5 ml sterile distilled water added with one droplet of 80% tween. The conidial concentration for each strain was adjusted to 10^4 conidia ml^{-1} with a haemocytometer.

2.3 Inoculation and infection development

Peach and nectarine fruits were wounded by a sterilized steel rod (1 mm wide and 2 mm long) and then inoculated with 15 µL of conidial suspension as described above. Fruits were then placed in plastic trays and incubated at 0, 4, 10, 15, 20, 25, 30 and 33 ºC with ± 1 ºC for all temperatures and 85% RH in cooled or heated rooms as appropriate. The experiment was performed twice during 2012 and 2013. Each year, one variety of peaches and another variety of nectarines was selected and inoculated with *M. fructicola* and *M. laxa* separately and then incubated at the above mentioned temperatures. There were five fruits for each of four replications per treatment.

Every inoculated fruit was assessed regularly after the appearance of the first visible symptom until the fruit lost its firmness. The first symptoms of decay are visible by a brown ring around the inoculated area. Mycelium presence was determine visually when a network of fine white filaments or sporodochia appeared in the decay area, and the presence of sporodochia was determined when several masses of brown conidia appeared all together in the epidermal decay and mycelia area. At 0 ºC, the fruits were assessed on 0, 7, 14, 21, 28, 35, 42, 49, 56, 64 and 72 days after inoculation; at 4 ºC on day 0, 7, 14, 21, 28 and 35; at 10ºC on day 0, 3, 5, 7, 8, 9, 10, 11, 14, 16, 18 and 21; at 15ºC on day 0, 2, 4, 7, 8, 9, 10 and 11; at 20 ºC on day 0, 2, 3, 4, 5 and 7; at 25ºC on day 0, 2, 3 and 4; and at 30 and 33 ºC on days 0, 2, 3, 4, 5, and 7. Decay and mycelium diameters were measured with a malleable ruler to take into account the curvature of the surface of the fruit. The presence or absence of sporodochia was recorded as well.

2.4 Mathematical and statistical analysis

The data shown correspond to the experiment conducted in 2013, and the experiment performed in 2012 exhibits the same pattern. All data were analysed using R statistical software 3.1.0 (2014).

2.4.1 Analysis of decay and mycelium development

Decay and mycelium size (area, x) were calculated assuming that a surface lesion is a perfect circle. To reduce heterogeneity and ensure that the residuals follow

approximately normal distributions, the area data were transformed to the natural logarithm, i.e., ln(x+1).

The overall effect of the treatment factor on decay and fungal development was assessed with analysis of variance, in which the variety, *Monilinia* species, assessment time and temperature were treated as factors. To quantify the relationship between decay and mycelium development and temperature, the data were subjected to logistic regression analysis (Fox & Weisberg, 2010). Logistic models were chosen based on the results from the preliminary analysis (results not shown) comparing several types of nonlinear models. The logistic model is given by

$$A = \frac{f_2}{1+\exp\left[-(f_3+f_3\times t)\right]} \quad (1)$$

in which A is a variable of interest, decay or mycelium area (cm^2) in the present study, '*exp*'is the exponential function, t is the time elapsed since inoculation (day) and f_1, f_2 and f_3 are parameters to be estimated. f_2 is the asymptote, maximum decay or mycelium area (cm^2); the quotient of f_2 and f_3 [$-f_2/f_3$] is the inflection point; and f_3 is the rate of decay or mycelium development (days^{-1}). The logistic model was fitted to each combination of temperature and *Monilinia* spp. for decay development and to each combination of temperature, *Monilinia* spp. and variety for mycelium development. Then, the observed relationship between f_3 and temperature was described by a nonlinear model, which was a variant of the thermodynamic model (Wagner et al., 1984). A possible biophysical interpretation of the original four model parameters in this variant of the thermodynamic model has been proposed based on the theory of enzyme responses to temperature. This model was reparametrized to reduce the magnitudes of the parameter estimates (Xu, 1996, Xu, 1999).

$$f_3 = \frac{a_1^{\frac{T+273.2}{298}} exp\left(d_1\left(1-\frac{298}{T+273.2}\right)\right)}{1+\exp\left(b_1\left(1-\frac{c_1}{T+273.2}\right)\right)} \quad (2)$$

The observed relationship of f_2/f_3 was well described by a negative exponential model:

$$-\frac{f_2}{f_3} = a_2 exp(-b_2 T) \quad (3)$$

In both models, T (ºC) is temperature, and a_1, a_2, b_1, b_2, c_1, d_1 are parameters to be estimated. For the thermodynamic model, parameter a_1 is a scale parameter; b_1 and d_1 primarily indicate the steepness of the curve for supra-optimum and

suboptimal temperatures, respectively (smaller values → less steep); increasing c_1 leads to both increased optimum temperature and developmental rates at the optimum temperature. For the negative exponential model, a_2 is the maximum value and b_2 describes the steepness of the curve (smaller values → less steep). It should be noted that we were only mainly interested in describing the observed relationships mathematically in the present study rather than in the biological interpretation of the individual parameters.

2.4.2 Analysis of the sporulation data

The Generalized Linear Model (GLM) was used to model the incidence of rot with sporulation, assuming that residuals follow binomial distributions:

$$Logit\,(p) = \ln\left(\frac{p}{1-p}\right) = a_3 + b_3 \times x \qquad (4)$$

where p is the probability of inoculated fruit with sporulation, x is the time elapsed since inoculation (days), and a_3 and b_3 are the parameters to be estimated.

3. Results

3.1 Decay area development

Decay expansion was affected by temperature ($P < 0.001$) and *Monilinia* species ($P < 0.001$) but not by varieties of either peach or nectarine (Table 1). There were significant interactions ($P < 0.001$) between the temperature and fungal species in affecting decay development.

The rate of decay development for both *Monilinia* species increased from 0 ºC to 25 ºC and then declined with increasing temperatures (Fig. 1). There were significant differences in the rate of decay development between *M. fructicola* and *M. laxa* at 25 ºC (2.50 and 1.75 days $^{-1}$, respectively), at 0 ºC (0.07 and 0.2 days^{-1}, respectively) and at 33 ºC (1.2 and 0.8 days^{-1}, respectively). A thermodynamic model satisfactorily described the relationship between the rate expansion and temperature for each fungal species (Fig. 1 and Table 2). There were no significant differences in the relationship between peach and nectarine (Table 1). Fitted models indicated that *M. fructicola* develops slower than *M. laxa* from 0 to 17.5 ºC, and the opposite is true for temperatures from 17.5 to 33ºC. The models underestimated the development rate at 25 ºC for both species (Fig. 1), and the fitted thermodynamic model for *M. fructicola* accounted for more variation than for *M. laxa*.

Table 1. Analysis of variance of the decay and mycelia area development of *M. fructicola* and *M. laxa* on peach and nectarine fruit in relation to temperature (Temperature), incubation time (Time), *Monilinia* specie (Specie) and fruit variety (Variety).

Factor	Decay		Mycelia	
	% SS[1]	*P* > F[2]	% SS[1]	*P* > F[2]
Temperature	4.47	< 2.2e-16 *	18.91	< 2.2e-16 *
Time	43.74	< 2.2e-16 *	14.66	< 2.2e-16 *
Specie	0.40	7.344e-07 *	1.38	9.833e-06 *
Variety	0.00	0.883 NS	0.35	0.025 *
Temperature x time	41.91	< 2.2e-16 *	47.22	< 2.2e-16 *
Specie x Temperature	8.89	< 2.2e-16 *	9.48	< 2.2e-16 *
Variety x Temperature	0.17	0.153 NS	0.96	0.057 NS
Specie x Variety	0.01	0.528 NS	0.00	0.974 NS
Specie x time	0.06	0.057 NS	5.47	< 2.2e-16 *
Variety x time	0.01	0.429 NS	0.61	0.003 *

[1] *Percentage of sum of square*
[2] *P value greater than F value indicate the overall results are significant*

** Significant (P < 0.05) and NS (not significant).*

The time to 50% of the maximum decay area (incubation period) was longer at low temperatures than at intermediate temperatures and slightly shorter at 30 and 33 ºC for *Monilinia* species (Fig. 2). The shortest time was 3 days at 25 ºC, and the longest was at 0 ºC for both species. At 0 ºC, there were significant ($P < 0.05$) differences in the length of the incubation period between the two species: 49 and 33 days for *M. fructicola* and *M. laxa*, respectively. The relationship between the length of the incubation period and the temperature was described by an exponential model (Table 2 and Fig. 2). The incubation period was longer for *M. fructicola* than for *M. laxa* from 0 ºC to 15 ºC, whereas from 15 to 33 ºC, the fitted models indicated a similar incubation time for the two *Monilinia* species.

Table 3 shows the maximum decay area for *M. fructicola* and *M. laxa* at each temperature. The maximum area for *M. laxa* was smaller ($P < 0.05$) at 30 and 33 ºC than that of *M. fructicola*, and the opposite was true at 4, 10 and 25 ºC.

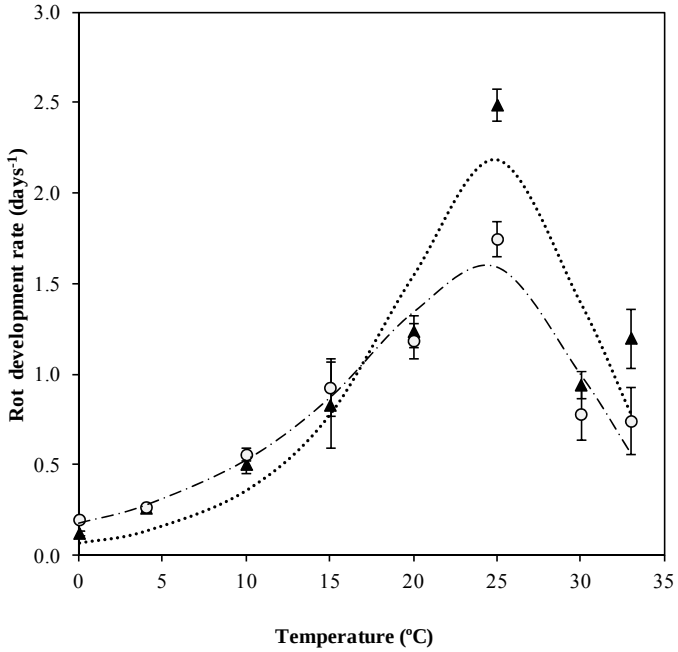

Figure 1. Relationship between temperature and the estimated decay development rate caused by *M. fructicola* (▲) and *M. laxa* (○) on fruit for each incubation temperature. Lines represent the thermodynamic models for *M. fructicola* (····) and *M. laxa* (□—□—□). The development rate (days^{-1}) is calculated as the parameter f3 of the fitted logistic model and represents the mean value of 40 fruits. The observed development rate (A [cm^2] days^{-1}) was logarithmically transformed on the natural base, i.e., ln(A+1). Bars represent the standard deviation of the means. Bars are not shown where they are smaller than the symbol size.

3.2 Mycelium area development

As for decay area development, mycelium area expansion (Table. 4) had a similar relationship with temperature, increasing from 0 ºC to the maximum when the temperature was in the range of 22-25 ºC and then decreasing with increasing temperature. There were significant differences between peaches and nectarines ($P < 0.025$) and between the two *Monilinia* species ($P < 0.001$) (Table 1); the interaction between temperature and *Monilinia* species was also significant. At 25 ºC, the mycelial development rate was greater ($P < 0.05$) on nectarines (4.4 cm^2 days^{-1} for *M. laxa* and 4.1 cm^2 days^{-1} for *M. fructicola*) than on peaches (1.6 cm^2 days^{-1} for *M. laxa* and 2.3 cm^2 days^{-1} for *M. fructicola*). At 20 ºC, the rate of mycelium development was higher for *M. fructicola* than for *M. laxa*. At 33 ºC, the mycelia of *M. laxa* could not develop, whereas the mycelia of *M. fructicola* developed faster ($P < 0.05$) on nectarines (1.5 cm^2 days^{-1}) than on peaches (0.60 cm^2 days^{-1}); however, no mycelia of *M. fructicola* were observed at 0 ºC. At 10 and 15 ºC, mycelia were not observed for either species.

Table 2. Estimated parameters of secondary negative exponential model describing the relationship of decay incubation period and temperature.

Specie	Negative Exponential model		Thermodynamic model	
	Parameters[1]	Estimate	Parameters[2]	Estimate
M. fructicola	a_2	46.42±2.82	a_1	3.34±2.28
	b_2	0.126±0.015	b_1	116.45±39.45
			c_1	299.62±4.62
			d_1	41.23±23.29
M. laxa	a_2	31.84±1.64	a_1	2.27±0.7
	b_2	0.0960±0.01	b_1	106.35±28.48
			c_1	300.37±2.52
			d_1	26.96±8.16

[*] *All the parameters in both secondary models only described the observed relationships rather than in the biological interpretation of the individual parameters.*

[1] *Parameter a_2 is the maximum value and b_2 describes the steepness of the curve from the negative exponential model.*

[2] *Parameter a_1 is a scale parameter; b_1 and d_1 primarily indicate the steepness of the curve for supra-optimum and suboptimal temperatures; increasing c_1 leads to both increased optimum temperature and developmental rates at the optimum temperature from the Thermodynamic model.*

The time required for the mycelial area to reach 50% of its maximum area was longer at low temperatures than at intermediate temperature (Table 5). The shortest time occurred (2-3 days) at 25 ºC for *Monilinia* species and the longest at 0 ºC for *M. laxa* on peaches (44 days) and nectarines (36 days).

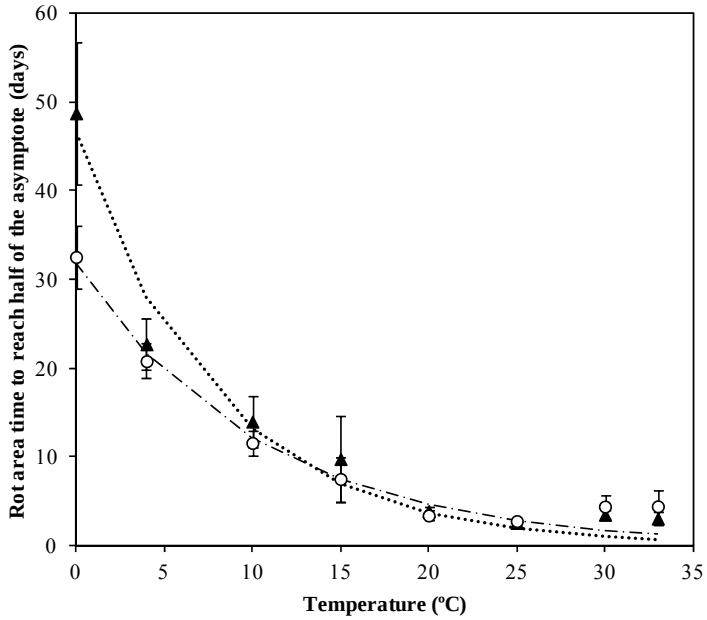

Figure 2. The estimated time for the rot area to reach half of the asymptote for *M. fructicola* (▲) and *M. laxa* (○) on fruits for each incubation temperature. Lines represent the negative exponential models for *M. fructicola* (····) and *M. laxa* (□—□—□). The incubation time (days) is calculated as the coefficient of the parameter (–f2/f3) of the logistic model and represents the mean value of 40 fruits. Bars represent the standard deviation of the means. Bars are not shown where they are smaller than the symbol size.

Maximum mycelial area for *M. fructicola* and *M. laxa* on peaches and nectarines is shown in Table 3 at each temperature. For *M. fructicola*, the maximum area was greater at 30 and 33 ºC, and the opposite was true for *M. laxa* at 0, 4, 10, 15, 20 and 25 ºC.

3.3 Production of sporodochia

The incidence of fungal sporulation on peaches and nectarines is plotted against only those temperatures at which mycelial development was observed (Fig. 3). Generally, *M. fructicola* and *M. laxa* were able to produce sporodochia at a range of temperatures on both peaches and nectarines. At 0 ºC, *M. laxa* produced sporodochia on nectarines and peaches (Fig. 3 C and D), whereas at 33ºC, only *M. fructicola* produced sporodochia (Fig. 3 A and B). Although *M. fructicola* developed mycelia on nectarines at 4 and 20 ºC, sporodochia were not produced at those temperatures but did appear at 25, 30 and 33 ºC (Fig. 3 A). In contrast, *M. fructicola* produced

sporodochia on peaches at all temperatures at which mycelia developed (4, 20, 25, 30 and 33 ºC) (Fig. 3 B). Similarly, at all temperatures at which mycelia developed, *M. laxa* produced sporodochia on nectarines (0, 4, 10, 20, 25 and 30 ºC) and peaches (0, 4, 20 and 25 ºC) with varying incidences of sporulation (Fig. 3 C and D). The lowest incidence of sporulation by *M. laxa* on peaches was at 25ºC after 5 days of incubation (Fig. 3 D).

Table 3. The estimated maximum decay and mycelium areas (cm^2) caused by *M. fructicola* and *M. laxa* on fruits for each incubation temperature (º C); these were estimated by the logistic model (parameter f_1). In fitting the logistic models, the observed area (A [cm^2]) was logarithmically transformed on the natural base, i.e. ln(A+1). The decay and mycelium area was averaged over 40 and 20 fruits, respectively, at each temperature on each assessment time before the logistical model was fitted to the data collected at each temperature.

Temperature (ºC)	Decay				Mycelia							
	M. fructicola		*M. laxa*		*M. fructicola*				*M. laxa*			
					Peach		Nectarine		Peach		Nectarine	
	Max. area	SE[1]	Max. area	SE	Max. area	SE	Max. area	SE	Max. area	SE	Max. area	SE
0	3.43	0.14	2.65	0.03	0.11	0.18	0.09	0.10	1.47	0.30	1.84	0.32
4	3.77	0.09	4.08	0.06	2.05	0.32	1.53	0.29	3.51	0.18	3.54	0.28
10	3.09	0.14	3.71	0.07	0.05	0.05	0.29	0.38	1.03	0.34	2.44	0.36
15	2.90	0.49	3.41	0.17	0.10	0.19	0.01	0.01	0.06	0.13	0.22	0.33
20	4.13	0.10	4.30	0.12	3.57	0.07	3.83	0.08	4.15	0.15	3.98	0.16
25	3.32	0.04	3.73	0.06	2.44	0.25	2.96	0.20	3.09	0.23	3.74	0.11
30	4.37	0.15	3,.5	0.41	3.99	0.21	3.80	0.12	0.30	0.20	1.73	0.28
33	3.78	0.15	1.05	0.11	2.19	0.25	4.04	0.20	0.08	0.14	0.08	0.16

[1] *Standard error of the parameter estimate*

4. Discussion

This study has for the first time modelled and compared the effects of temperature on brown rot, mycelia development and sporulation on peaches and nectarines for both *M. fructicola* and *M. laxa*, in contrast to other studies, which focussed only on a single specific aspect of brown rot development (Weaver, 1950, Xu et al., 2001, Harada, 1977, Corbin, 1962, Phillips, 1982, Tian and Bertolini, 1999, Tamm and Flückiger, 1993). We showed that *M. fructicola* is better adapted to high temperatures, whereas *M. laxa* is better adapted to low temperatures.

The optimum temperature determined in this study for brown decay and mycelial development agrees with previous studies (Biggs and Northover, 1988, Tamm and Flückiger, 1993). The optimal temperature for *M. fructicola* to produce sporodochia was approximately 25 ºC and between 20-25 ºC for *M. laxa*; however, a limited number of sporodochia was produced on peaches at 25 ºC. Corbin (1962) obtained a similar optimal temperature at 23 ºC for *M. fructicola* to produce sporodochia after 24 hours of incubation. *M. laxa* may be more influenced by other factors that were not considered in the present study, including light intensity and photoperiod. It is more difficult for *M. laxa* to produce sporodochia than *M. fructicola*, although our unpublished results suggest that *M. laxa* is able to produce an abundant amount of sporodochia in a fotoperiod chamber compared with a dark chamber on stone fruit.

Table 4. The estimated mycelium development rate (days^{-1}) caused by *M. fructicola* and *M. laxa* on nectarine and peach fruits for each incubation temperature (º C); these were estimated by the logistic model (parameter f3). In fitting the logistic models, the observed development rate (A [cm^2] days^{-1}) was logarithmically transformed on the natural base, i.e., ln(A+1). The mycelium development rate was an average of 20 fruits at each temperature for each assessment time before the logistical model was fitted to the data collected at each temperature.

Temperature (ºC)	Mycelium development							
	M. fructicola				*M. laxa*			
	Peach		Nectarine		Peach		Nectarine	
	Dev. Rate[1]	SE[2]	Dev. Rate	SE	Dev. Rate	SE	Dev. Rate	SE
0	nd	nd	nd	nd	0.107	0.016	0.254	0.049
4	0.275	0.038	0.275	0.108	0.275	0.016	0.253	0.028
10	nd	nd	nd	nd	0.534	0.080	0.412	0.042
15	nd	nd	nd	nd	nd	nd	nd	nd
20	1.867	0.155	2.018	0.243	1.507	0.159	1.395	0.162
25	2.275	0.357	4.107	0.758	1.578	0.179	4.368	0.280
30	1.083	0.222	1.167	0.159	1.339	0.226	1.339	0.359
33	0.604	0.092	1.457	0.248	nd	nd	nd	nd

[1] *Development rate*
[2] *Standard error of the parameter estimate*
nd: not determinated

Table 5. The estimated time for mycelial area to reach half of the asymptote (days) for *M. fructicola* and *M. laxa* on peach and nectarine fruits for each incubation temperatures (ºC); these were estimated by the logistic model (parameter (- f2/f3). The time for mycelial area to reach half of the asymptote was averaged of 20 fruits at each temperature on each assessment time.

Temperature (ºC)	Time for mycelial area to reach half of the asymptote							
	M. fructicola				*M. laxa*			
	Peach		Nectarine		Peach		Nectarine	
	Days	SE[1]	Max. area	SE	Max. area	SE	Max. area	SE
0	nd	nd	nd	nd	43.724	6.544	36.445	7.225
4	32.068	4.525	28.790	11.609	25.914	1.520	22.906	2.669
10	nd	nd	nd	nd	17.282	2.739	16.556	1.802
15	nd	nd	nd	nd	nd	nd	nd	nd
20	4.529	0.459	4.571	0.670	4.305	0.559	4.448	0.629
25	3.297	0.673	3.068	1.328	3.620	0.527	2.163	0.270
30	4.609	1.142	4.517	0.747	4.090	0.337	4.090	1.430
33	6.147	1.064	4.463	0.927	nd	nd	nd	nd

[1] *Standard error of the parameter estimate*
nd: not determinated

M. laxa decay developed faster at 0 ºC than *M. fructicola*. In addition, *M. fructicola* took a longer time to show first decay symptoms and was unable to produce mycelia. However, *M. fructicola* resulted in a greater decay area at the end of storage than *M. laxa*. This suggests that at 0 ºC, *M. fructicola* could continue to develop rot, while *M. laxa* develops sporodochia. Tian and Bertolini (1999) also observed larger rots of *M. laxa* at 0 ºC on nectarines than at high temperatures after 6 weeks of storage. Storing fruit after harvest as soon as possible at 0 ºC is a widely recommended management practice to suppress disease development and maintain fruit quality (Crisosto and Kader, 2014, Fraser, 1992, Brosnan and Sun, 2001). In areas such as Ebro Valley (Lleida, Spain), where both *Monilinia* species co-exist with similar frequencies (Villarino et al., 2013), *M. laxa* can lead to a secondary spread of the disease and could also produce conidia, leading to new infections in cold storage. However, *M. fructicola* could only generate secondary infections by contact.

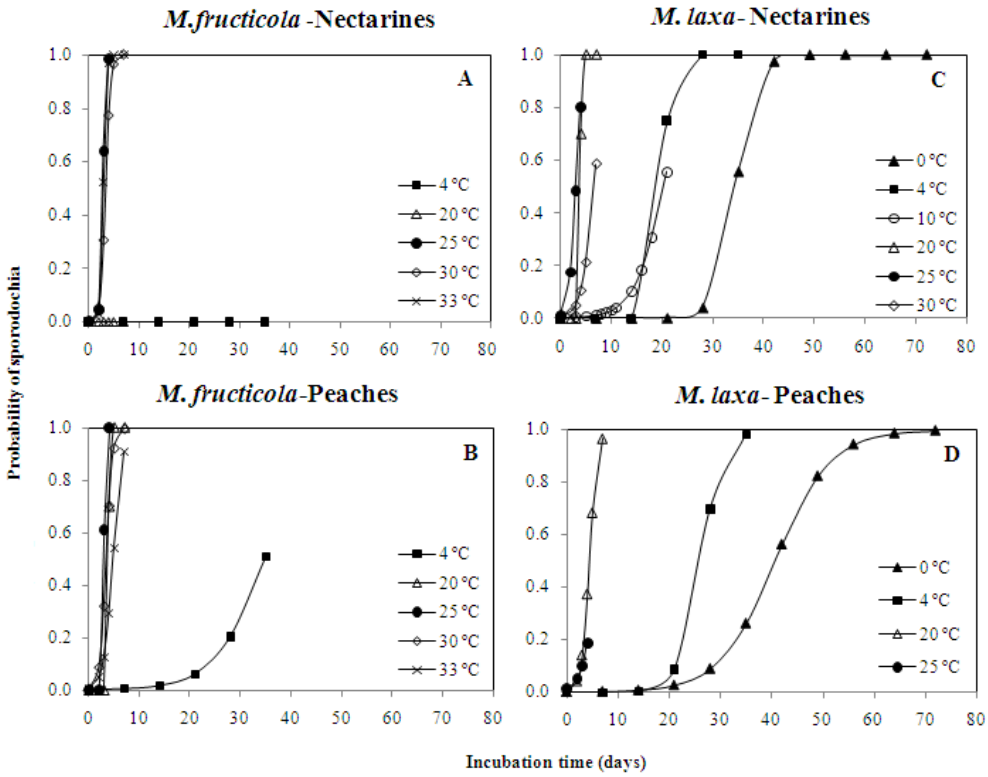

Figure 3. The estimated probability of sporodochia production from the mycelium area during the incubation time (days) at each incubation temperature on nectarines and peaches inoculated by *M. fructicola* and *M. laxa*. Each point represents the mean value of 20 fruits.

Several authors also reported that the maximum temperature for *M. fructicola* growth is between 30 and 33 ºC (Weaver, 1950, Harada, 1977). In the present study, *M. laxa* cannot develop at 33 ºC, and previously, Tamm and Flückiger (1993) reported that growth on Petri dishes declined rapidly approaching 31 ºC. Decay in the development of *M. laxa* at temperatures above 33 ºC has not been evaluated, but conidia germination has been evaluated at 35 ºC. *M. laxa* and *M. fructicola* have the ability to germinate at 35 ºC with high water activity (Casals et al., 2010, Xu and Robinson, 2000), and research has shown that moisture on the wounded surface of apples was sufficient for *M. fructigena* to germinate and infect. *Monilinia* conidia should have infected fruit in the present inoculation study, and therefore, other factors such as temperature, time and fruit are intrinsic properties that may have influenced subsequent disease development on peaches and nectarines. In the Lleida area (Catalonia, Spain), daily mean temperatures in the summer commonly exceed 25 ºC and can easily reach 30-33 ºC or higher for a few hours. Although rainfall is usually

quite low, humidity can be still high because orchards often have irrigation systems with an increasing duration and frequency of irrigation approaching the harvest. In addition, the maximum number of airborne conidia in peach orchards occurs around harvest time (Villarino et al., 2012). These spores may result in secondary infection if the fruit is susceptible to brown rot. Wetness duration and temperature are not limiting factors for *M. fructicola* infection if the fruit is susceptible (Kreidl et al., 2015). *M. fructicola* sporulates abundantly at high temperatures on both peaches and nectarines 2-3 days after inoculation. Similarly, Landgraf and Zehr (1982) achieved abundant sporulation of *M. fructicola* on peach blossoms during rainy periods. The influence of temperature and wetness duration on *M. fructicola* infection has been widely studied by several authors (Biggs and Northover, 1988, Kreidl et al., 2015, Weaver, 1950), but few studies have been carried out for *M. laxa*. Once conidia have produced an infection, rot development is influenced by temperature and humidity; however, the present research has shown that *M. laxa* development could be limited when temperatures are above 30ºC.

At 10 ºC, only *M. laxa* was able to develop mycelia and produce sporodochia on nectarines, and at 15 ºC, both species of *Monilinia* were not able to develop mycelia and produce sporodochia. This variability may explain why both thermodynamic and exponential models failed to fit the mycelium data. It is likely that the pathogens did not have sufficient time to develop mycelia and sporulate before fruit degradation (hence discarded) at 10 and 15 ºC. These results regarding the sporodochia could be due to a lack of high humidity; high humidity has been reported to favour sporulation (Hong et al., 1997, Luo et al., 2001, Xu et al., 2001).

Combining the findings of this study with those of previous works (Weaver, 1950, Xu et al., 2001, Harada, 1977, Corbin, 1962, Phillips, 1982, Tian and Bertolini, 1999, Tamm and Flückiger, 1993), it is known how *M. fructicola* and *M. laxa* develop in response to temperature during preharvest and postharvest once conidia have infected the fruit. Understanding germination, infection, decay, and mycelia and sporodochia development in brow rot disease is essential for predicting risk and deciding on strategies for disease management, in particular whether *Monilinia* species behave differently. Reducing the number of airborne conidia and therefore reducing the disease incidence in the field and postharvest could be possible with physical, chemical or biological control. The use of biological control agents requires more knowledge about the behaviours of the pathogen as well as the control agent to ensure effective control. Further studies are needed to understand how latent infections develop into visual decay and sporulate and to study the effect of fluctuating conditions on disease development under field conditions.

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