Influence of Temperature and Wetness Duration on Infection of Peach and Sweet Cherry Fruits by *Monilinia fructicola*

A. R. Biggs and J. Northover

Research scientists, Agriculture Canada, Research Station, Vineland Station, Ontario, Canada L0R 2E0.
This research was funded in part by grants from the Ontario Ministry of the Environment, Pesticides Advisory Committee, and the Ontario Ministry of Agriculture and Food, Pest Management Research and Services Committee.
We thank Herman Neufeld, Ann Curwin, Len Mancini, and Lawrie McEwan for their technical assistance, and Drs. Kerry Britton and Wayne Wilcox for reading the original manuscript.
Accepted for publication 16 May 1988 (submitted for electronic processing).

ABSTRACT

Biggs, A. R., and Northover, J. 1988. Influence of temperature and wetness duration on infection of peach and sweet cherry fruits by *Monilinia fructicola*. Phytopathology 78:1352-1356.

Nonwounded peach and sweet cherry fruits were inoculated with a conidial suspension of *Monilinia fructicola* at temperatures of 15–30 C at 2.5 C intervals, and wetness durations of 3–15 hr (peach) or 6–18 hr (cherry) at 3-hr intervals. Both peach and sweet cherry had an increased incidence of fruit infection with increased wetness duration over the range of temperatures tested. Optimum observed temperature for cherry fruit infection was 20–22.5 C, with greater than 80% infection after 15 hr of wetness. After 18 hr of wetness, infection was greater than 80% at all temperatures except 30 C. Optimum observed temperature for infection of peach fruit was 22.5–25 C. Greater than 70% infection occurred after 12 hr at all temperatures except 27.5 and 30 C. Nontransformed polynomial and logistic equations for peach and cherry, respectively, were chosen as the best regression models to describe the incidence of fruit infections as functions of temperature and wetness duration. Coefficients of determination for two sweet cherry and two peach experiments were 0.89 and 0.90, and 0.71 and 0.84, respectively. The Analytis "Beta" model was used to describe the effect of wetness duration on incubation period for sweet cherry. The incubation period decreased with increasing wetness duration. All models had significant coefficients, and the experimental runs were similar within fruit species.

Brown rot, caused by *Monilinia fructicola* (Wint.) Honey, is one of the most important diseases of peaches (*Prunus persica* (L.) Batsch) and sweet cherries (*P. avium* L.) in the temperate fruit-growing regions of North America (2). Field losses can be extensive if favorable conditions of wetness and temperature occur during the blossom period, following shuck fall, or during the preharvest and harvest period (8).

The environmental conditions that influence infection and colonization of stone fruits by *M. fructicola* have been elucidated only partially. The effects of temperature and relative humidity (RH) on blossom and fruit infection, sporulation, and incubation period have been studied (4,5,9). Clayton (3) showed that free moisture was required for spore germination to occur. Weaver (9) demonstrated that the optimum temperature for penetration of peach and cherry blossoms was 25 C and that disease severity increased with increasing RH. Corbin (4) concluded that postinfection RH did not affect the incubation period or sporulation. Incubation period was influenced by inoculum dosage, fruit maturity, and degree of skin injury, where the shortest incubation periods were observed on ripe fruit with severe skin injuries inoculated with higher inoculum levels (up to 1×10^6 spores per milliliter). No information was provided regarding the length of the wet period, the interaction of wet period with temperature, or the influence of wetness duration on the incubation period.

The objective of this study was to determine the effect of wetness duration and temperature on infection by *M. fructicola* of sweet cherry and peach fruits. The effect of temperature and wetness duration on the incubation period of *M. fructicola* on sweet cherry also was examined. Regression models were developed to quantify the relationship between disease incidence and incubation period and the environmental variables.

MATERIALS AND METHODS

Fruits. Sweet cherry (*Prunus avium* L. cv. Vista) (experiment 1) and Bing (experiment 2) fruits were harvested with stems at full ripeness during the second and third weeks, respectively, in July 1985. The orchard, located in Jordan Station, Ontario, had received no pesticide applications during the growing season. Twenty-five sweet cherries, divided into five replicates, were used for each temperature/wetness combination during each of two experiments. Cultivars with different maturity dates were used for each experiment to ensure a uniform degree of ripeness between experiments. The fruit was washed in tap water and dried. The stems were cut to about 1 cm in length, and the fruit was placed, suture side up, on autoclaved 1.27-cm mesh screens placed in $23 \times 30 \times 8$ cm aluminum baking trays. Wet paper towels were placed beneath the screens to maintain high atmospheric moisture.

Peach (*Prunus persica* L. Batsch. cv. Loring) fruit was harvested in late August (experiment 1) and early September (experiment 2). The orchard, located immediately adjacent to the sweet cherry orchard, had received no fungicide applications during the growing season, although two to four applications of phosmet were made to provide control of Oriental fruit moth

(*Grapholitha molesta* Busck). Twelve peaches, divided into three replicates, were used for each temperature/wetness combination during each of two experiments. Peaches were prepared for inoculation similar to sweet cherries; however, the fruit was picked without stems and placed on inverted 4-cm-diameter autoclaved juice jar lids in paper-lined wooden boxes.

Inoculum. Spores were prepared from *M. fructicola* isolate S.4 (benomyl-sensitive) cultures grown for 10–14 days on potato dextrose agar in the dark at 20 C. Inoculum was prepared by slicing the agar culture into 10 pieces and placing in 40 ml of sterile distilled water in a 500-ml sterile flask. The flask was shaken vigorously for about 30 sec, and the resulting suspension of spores and mycelial fragments was passed through coarse filter paper (Ederol no. 261) to remove the hyphal fragments. Two additional 50-ml aliquots of water were used to rinse the remaining spores from the agar pieces. The spore suspension was shaken vigorously to break up chains of spores and then was passed through 0.22 μm Millipore filters. Spores were rinsed from the filter discs with sterile water by using a compressed air atomizer. Spore concentration was estimated with a haemocytometer, and the spore suspension adjusted to the desired inoculum concentrations by using Miller's solution (7). For peaches, the spore suspension was amended with Tween 20 to give a final concentration of 0.05%. Nonwounded fruit was inoculated with a 30-μl drop of spore suspension delivered from a 12.7-cm Pasteur pipette. Spore concentrations of 1×10^5 and 1×10^6 spores per milliliter were used for sweet cherries and peaches, respectively.

Controlled environment studies. Before inoculation, trays of fruit were preconditioned for 3 hr at the temperature at which they would be inoculated. This minimum air temperature equilibration time was determined in preliminary experiments by using a remote temperature probe connected to a micrologger (sensor 207, micrologger 21X, Campbell Scientific, Logan, UT). After inoculation, trays of fruit were sealed in plastic bags and placed into controlled temperature chambers (Lab-Line Ambi Hi Low Chambers, Melross Park, IL) at 15 to 30 C, at 2.5 C intervals, without light. Wetness periods were defined by the length of time that the inoculum droplet was on the fruit surface. After 6, 9, 12, 15, and 18 hr for sweet cherry, or 3, 6, 9, 12, and 15 hr for peach, trays were removed from the chambers. Inoculum drops were removed with absorbent paper, and the fruit was allowed to dry completely for 2–4 hr at 20 C and 60% RH. Trays were moved to a controlled environment room maintained at 20 C and >95% RH (measured with an aspirated psychrometer) and were observed daily for 7 days for disease symptoms. The fruit was rated for the presence of infection and sporulation and was assessed individually on a scale of 0–3 (0 = no infection; 1 = necrosis equal in diameter to the inoculum drop, no sporulation; 2 = necrosis larger in diameter than the inoculum drop, no sporulation; and 3 = sporulating necrotic lesion). The incubation period was calculated as the number of hr elapsed from the time of inoculation until 50% of the infected fruit exhibited sporodochia of *M. fructicola*.

Data analysis. Regression analysis was used to describe the effect of temperature (T) and wetness duration (W) on the level of infection (Y) of ripe fruit. Y represents the proportion of fruit rated as two or three using the above scale on the seventh day after inoculation. Nontransformed polynomial models and logistic models of the form

$$\ln(Y/(1-Y)) = f(T, W) \qquad (\#1)$$

were evaluated, in which $\ln(Y/(1-Y))$ is the logit of Y and $f(T, W)$ were linear combinations of the following terms: W, T, T^2, T^3, W^2, W^3, WT, WT^2, and WT^3. A generalized form of the Analytis "Beta" model was evaluated (1). The model can be written as:

$$\ln(Y) = \ln(p) + m\ln(t) + n\ln(1-t) + q\ln(W) \qquad (\#2)$$

in which $t = (T - T\text{min})/(T\text{max} - T\text{min})$ and p, m, n, and q are unknown parameters. Tmin and Tmax were set to 3 and 33 C, respectively (8). Regression models for each experiment and the combined data were evaluated by using the following criteria: significance of estimated parameters, the normal and random distribution of residuals, and coefficients of determination between observed and predicted Y values (R^2), and R^2 adjusted for degrees of freedom (R_a^2), and, when transformed Y values were used, the goodness of fit for the back-transformed predicted values and the observed Y values (R^{*2}). An F-test was used to determine if the regression results of the two experimental runs for each fruit species were significantly different.

RESULTS

Sweet cherry. There was an increase in the percentage of infected cherry fruit with increases in wetness duration over all the temperatures tested (Fig. 1). The highest percentage of infected cherries occurred at 20–22.5 C, with greater than 80% fruit infection by 15 hr (Fig. 1). After 18 hr, fruit infection was greater than 80% at all temperatures except 30 C.

A logistic model of the form

$$\ln(Y/(1-Y)) = b_o + b_1 W + b_2 T + b_3 WT + b_4 T^3 \qquad (\#3)$$

best described the incidence of sweet cherry infection as a function of temperature and wetness duration for spore inoculations. All b value estimates were significant ($P \leq 0.01$), and there was no significant difference ($P \leq 0.05$) in the regression results between the two experiments for sweet cherries. The coefficients of determination, R^2, R_a^2, and R^{*2}, for the combined cherry data were 0.93, 0.92, and 0.95, respectively (Table 1).

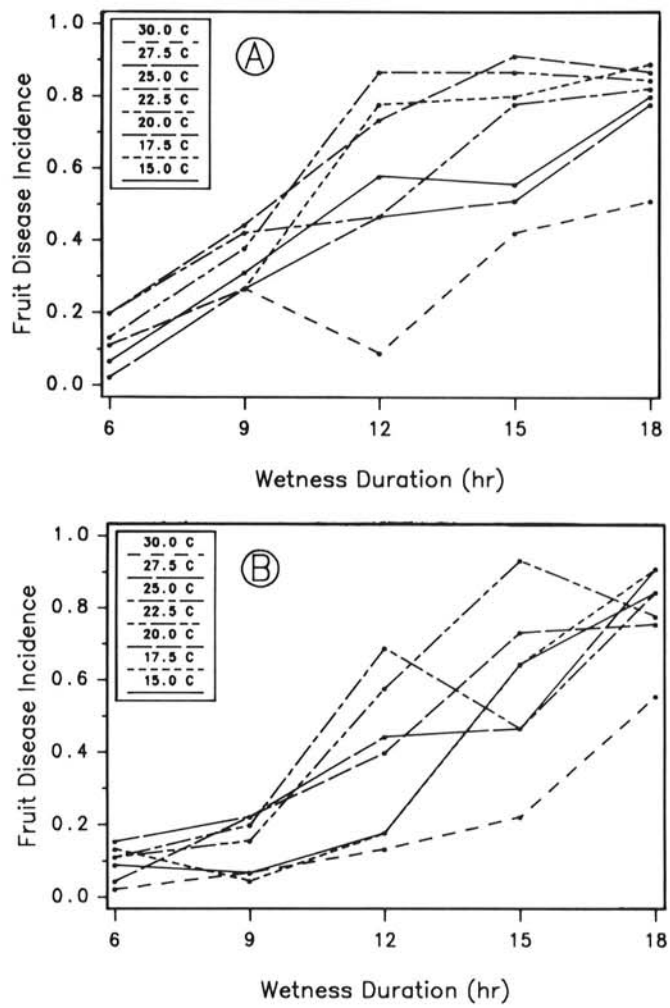

Fig. 1. Proportion of sweet cherry fruit infected by *Monilinia fructicola* for wetness durations of 6–18 hr at temperatures of 15–30 C. Results are for experiments 1 with Vista (**A**) and 2 with Bing (**B**). Each point represents the disease incidence from 25 fruit.

TABLE 1. Estimated parameters for a logistic model that describes the logit of the proportion of ripe sweet cherry fruit infected by *Monilinia fructicola* as a function of temperature (T) and wetness duration (W) at spore droplet inoculations, together with the coefficient of determination (R^2) and R^2 adjusted for degrees of freedom (R_a^2) based on logits, R^2 for the back-transformed disease-incidence levels (R^{*2}), and the standard error about the regression line (s)

	Estimated parameters[y]								
	b_0[z]	b_1	b_2	b_3	b_4	R^2	R_a^2	R^{*2}	s
Expt. 1	−11.699	0.556	0.508	−0.0104	-3.2×10^{-4}	0.884	0.867	0.912	0.517
	(1.832)	(0.110)	(0.0969)	(0.0043)	(6.1×10^{-5})				
Expt. 2	−11.482	0.468	0.491	−0.0079	-2.7×10^{-4}	0.887	0.869	0.920	0.565
	(2.023)	(0.121)	(0.107)	(0.0047)	(6.8×10^{-5})				
Combined	−11.715	0.529	0.466	−0.0073	-3.1×10^{-4}	0.929	0.916	0.953	0.415
data	(1.435)	(0.0859)	(0.0762)	(0.0033)	(4.8×10^{-5})				

[y] Estimated parameters correspond to W (b_1), T (b_2), WT (b_3), and T^3 (b_4). The numbers in parentheses are the standard deviations of the estimated parameters.
[z] b_0 is the value of the logit of disease incidence when $T = 0$ and $W = 0$.

TABLE 2. Estimated parameters for the generalized Analytis "Beta" model that describes incubation period (hr) of *M. fructicola* on sweet cherry fruit as a function of temperature and wetness duration during spore droplet inoculations, together with the coefficient of determination (R^2) and R^2 adjusted for degrees of freedom (R_a^2) for log-transformed data, R^2 for the back-transformed disease-incidence levels (R^{*2}), and the standard error about the regression line (s)

	Estimated parameters[y]							
	ln$p$[z]	m	n	q	R^2	R_a^2	R^{*2}	s
Expt. 1	6.0129	0.386	0.199	−0.306	0.776	0.753	0.757	0.0742
	(0.158)	(0.137)	(0.0645)	(0.0325)				
Expt. 2	4.907	−0.501	−0.248	−0.241	0.708	0.678	0.719	0.0710
	(0.154)	(0.135)	(0.062)	(0.0327)				
Combined	5.369	−0.173	−0.0891	−0.288	0.856	0.842	0.859	0.0492
data	(0.102)	(0.0874)	(0.0401)	(0.0215)				

[y] Estimated parameters correspond to: for (m), ln(t), where $t = (T\text{-}T\text{min})/(T\text{max}\text{-}T\text{min})$; or ($n$), ln($t$-1); and for ($q$), ln($W$). The numbers in parentheses are the standard deviations of the estimated parameters.
[z] ln(p) is the value of incubation period when $W = 1$ and $T = T$min.

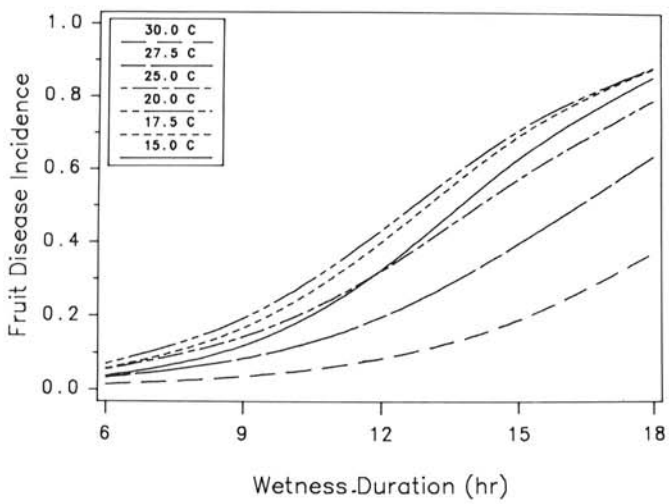

Fig. 2. Effect of wetness duration on predicted incidence of *Monilinia fructicola* infections of sweet cherry at temperatures of 15–30 C. Curve from $T = 22.5$ C was not included because it was virtually indistinguishable from the curve for $T = 17.5$ C. Curves were generated by using equation 3 with estimated parameters in Table 1 for the combined data.

There was an interaction between W and T in these experiments. The parameters b_3 and b_4 were negative in both experiments. With the logistic equation rewritten as:

$$\ln(Y/(1 - Y))\ b_0 + W(b_1 + b_3 T) + T(b_2 + b_4 T^2) \qquad (\#4)$$

it is clear that Y increases with W when b_1 is greater than $b_3 T$, and these conditions were met by our data. The predicted levels of infection (Y) versus wetness duration were plotted for the temperatures used in the inoculation experiments (Fig. 2). This figure shows that Y increased with an increase in W at all temperatures. The negative b_3 and b_4 parameters indicate that the response to T was not monotonic. The observed data and the model parameters show that Y increased with T to a maximum and then declined. Predicted optimum temperature for infection using the combined data was ≈19.6 C.

Incubation period. The generalized Analytis model was the best model to describe the influence of wetness duration on the length of the incubation period following spore drop inoculations on sweet cherry fruit (Table 2). As wetness duration increased, incubation period decreased (Fig. 3A). The predicted incubation periods were plotted against wetness duration (Fig. 3B). The influence of temperature, although small when compared with the influence of wetness duration, is seen as longer incubation periods at 15 and 30 C and shorter incubation periods at 20 and 22.5 C (Fig. 3B). The optimum temperature for the most rapid incubation period was calculated as 22.4 C.

Peach. There were increases in the percentage of infected peach fruit with increases in wetness duration over most temperatures tested (Fig. 4); however, a reduction in infection was observed after 15 hr wetness, relative to 12 hr, in one experiment. Observed optimum temperature for peach fruit infection was 22.5–25 C, with about 70% infection occurring after 12 hr wetness at all temperatures except 27.5 and 30 C (Fig. 4).

A nontransformed polynomial model best described the incidence of peach brown rot:

$$Y = b_0 + b_1 T + b_2 WT + b_3 WT^3 \qquad (\#5).$$

All b value estimates were significant ($P \leq 0.01$), and there was no significant difference ($P \leq 0.05$) in the regression analyses between the two experiments for peaches. As with cherries, there was an interaction between W and T in the peach experiments. The coefficients of determination, R^2 and R_a^2, for the combined peach data were 0.73 and 0.71, respectively (Table 3). The b_3 parameter was negative in both experiments. This did not indicate a decrease in brown rot as a function of wetness duration. With equation 5 rewritten as:

$$Y = b_0 + b_1 T + W(b_2 T + b_3 T^3) \qquad (\#6)$$

it can be seen that Y increases with W when b_2T is greater than b_3T^3, and this condition was met by our data. The predicted levels of brown rot (Y) versus wetness duration were plotted for the temperatures used in the inoculation experiments (Fig. 5), which showed that Y increased with an increase in W. The observed data and the negative b_3 parameter indicate that Y increased with T to a maximum and then declined. Predicted optimum temperature for infection using the combined data was ≈23.9 C.

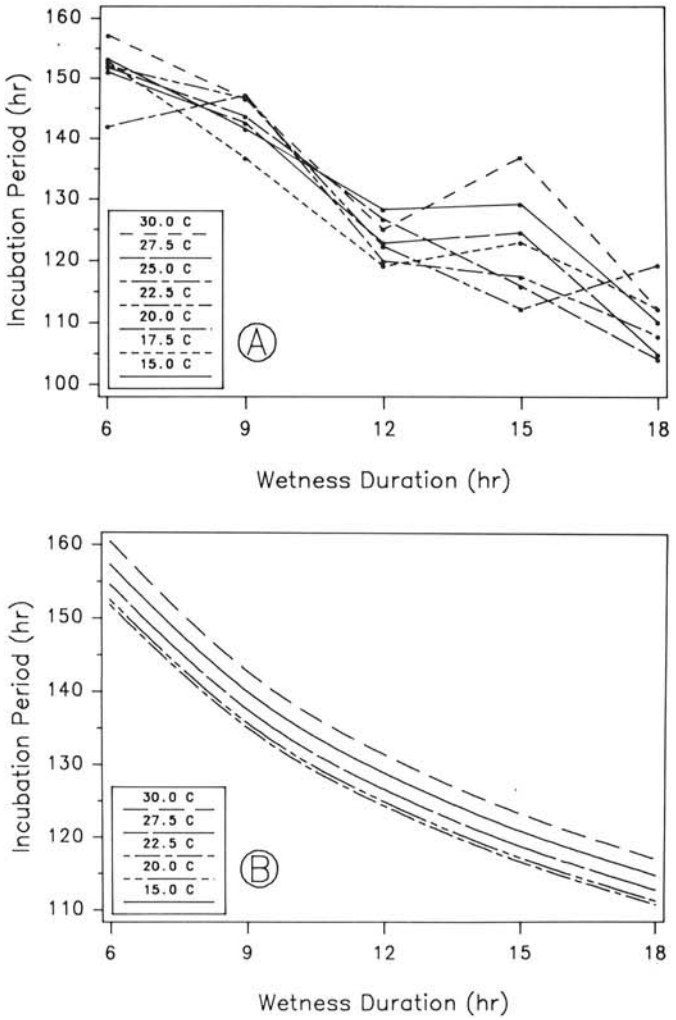

Fig. 3. Effect of wetness durations of 6–18 hr on the observed (**A**) and predicted (**B**) incubation period for *Monilinia fructicola* on sweet cherry fruit at temperatures of 15–30 C. Each point of **A** represents the incubation period mean from 50 fruit. In **B**, curves were generated by using equation 2 with estimated parameters in Table 3 for the combined data. Curves from T = 17.5 and 25 C were not included because they were virtually indistinguishable from the curves for 27.5 and 22.5 C, respectively.

The generalized Analytis model did not fit the sweet cherry nor the peach disease incidence data as well as equations 3 and 5, respectively. The Analytis model residuals had a nonrandom pattern, predicted Y at levels greater than 1.0 (100% fruit infection), and had generally lower coefficients of determination. However, for comparison with Table 1, for the combined sweet cherry data, R^2, R_a^2, and R^{*2} were 0.93, 0.92, and 0.89 for the Analytis model. Although R^2 and R_a^2 values were similar to those from the logistic model, the back-transformed predicted values from the Analytis model did not fit the observed rot values as well as those from the logistic model.

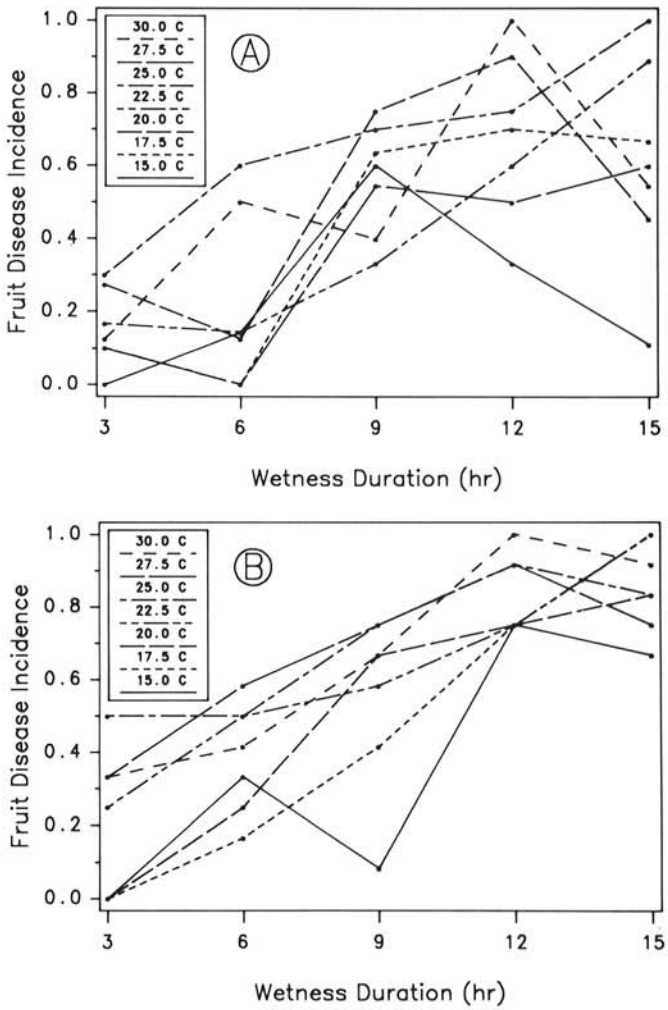

Fig. 4. Proportion of Loring peach fruit infected by *Monilinia fructicola* for wetness durations of 3–15 hr at temperatures of 15–30 C. Results are for experiments 1 (**A**) and 2 (**B**). Each point represents the disease incidence from 12 fruit.

TABLE 3. Estimated parameters for a polynomial model that describes the proportion of ripe peach fruit infected by *M. fructicola* as a function of temperature (T) and wetness duration (W) at spore droplet inoculations, together with the coefficient of determination (R^2), R^2 adjusted for degrees of freedom (R_a^2), and the standard error about the regression line (s)

	Estimated parameters[y]						
	b_o[z]	b_1	b_2	b_3	R^2	R_a^2	s
Expt. 1	−0.540	0.0241	0.00505	-4.7×10^{-6}	0.707	0.675	0.162
	(0.261)	(0.0115)	(0.00083)	(1.2×10^{-6})			
Expt. 2	−0.653	0.0313	0.00472	-3.7×10^{-6}	0.836	0.820	0.129
	(0.200)	(0.00874)	(0.00059)	(8.7×10^{-7})			
Combined	−0.546	0.0255	0.00462	-3.7×10^{-6}	0.727	0.713	0.161
data	(0.179)	(0.00784)	(0.00054)	(7.9×10^{-7})			

[y]Estimated parameters correspond to T (b_1), WT (b_2), WT^3 (b_3). The numbers in parentheses are the standard deviation of the estimated parameters.
[z]b^o is the value of Y when $T = 0$ and $W = 0$.

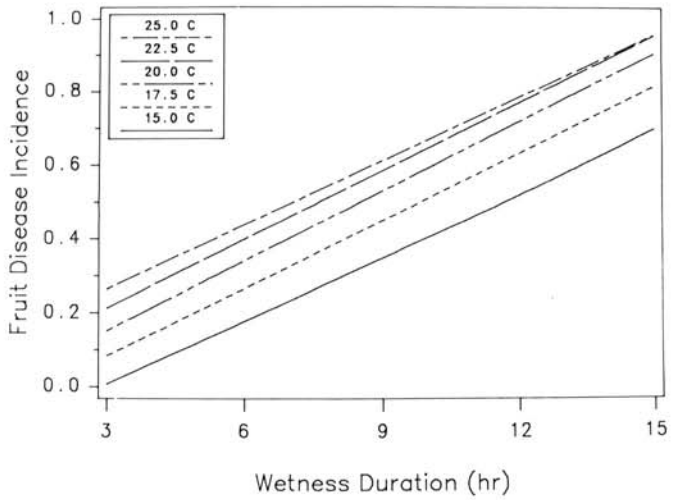

Fig. 5. Effect of wetness duration on predicted incidence of *Monilinia fructicola* infections of peach at temperatures of 15–30 C. Curves were generated using equation 5, with estimated parameters in Table 2 for the combined data. Curves from $T = 27.5$ and 30 C were not included because they were virtually indistinguishable from 25 C.

DISCUSSION

Temperature and wetness duration during conidial inoculations of mature sweet cherry and peach fruits affected fruit infection by *M. fructicola*. Equation 3 best predicted the incidence of fruit rot for sweet cherries based on W and T when inoculated with drops of spore suspension. Coefficients of determination (R^2) for the sweet cherry model were high, indicating that a major proportion of variability in logits of fruit rot incidence was accounted for by the independent variables. When R^2 was adjusted for degrees of freedom (i.e., R_a^2), the values were just slightly less than the nonadjusted values, indicating the significance of terms in the model. The high R^{*2} values for the individual experiments and the combined data indicate that the logistic model accounted for a high proportion of the variability in the observed rot incidence data.

For peach, the best models for predicting the proportion of fruit rot based on W and T utilized nontransformed Y values. Although R^2 and R_a^2 values were high, indicating that a high proportion of variance in fruit rot incidence was accounted for by wetness duration and temperature, the peach model was not as accurate as that for cherry. In comparing Figure 1 with Figure 4, it can be seen that cherry disease incidence approximately doubled when wetness duration increased from 9 to 12 hr and doubled again from 12 to 18 hr. For peach, increase in disease incidence was linear over this time period (Fig. 4). The behavior of *M. fructicola* on the different hosts may be influenced by fruit surface characteristics such as pubescence, cuticle and/or epidermal cell wall thickness, presence and distribution of stomata, or other less obvious, physiological differences such as the presence of germination inhibitors or phytoalexins (2). The greater variation observed in the peach data, relative to that for sweet cherry, may be related to the smaller sample size used for peach.

The optimum temperature for the germination of spores of *M. fructicola* has been reported as 20–25 C (9) and 21–27 C (6,10). Between 0–30 C, temperatures above or below the optimum delay germination but do not inhibit it (10). One would expect that considerable variation in temperature requirements might exist between geographic isolates of *M. fructicola*. Optimum temperatures for fruit infection had not been described. For sweet cherry and peach, our data show that infection temperature optima occur between 17.5–22.5 and 22.5–27.5 C, respectively. These temperatures are similar to those reported for blossom infection of peach and sweet cherry by Weaver (9) in Geneva, New York. The difference in temperature optima also may be related to the fruit-surface characteristics as discussed above.

Wetness duration influenced the incubation period of *M. fructicola* on sweet cherry fruit. Equation 2 best predicted incubation time based on T and W. Coefficients of determination (R^2) were high, thus indicating that a major proportion of variability in the natural logarithm of incubation time was accounted for by the independent variables. The adjusted values were just slightly less than the nonadjusted values, indicating the significance of terms in the model. The high R^{*2} values for the individual experiments and the combined data indicate that the Analytis model accounted for a high proportion of the variability in the observed incubation values. The incubation period for spore inoculations on nonwounded fruits, evaluated as a function of wetness duration and temperature, had not been described previously. Increased wetness duration probably reduced the incubation period indirectly. One suggestion is that a greater percentage of spores penetrated the fruit as wetness duration increased, thereby resulting in a larger thallus and hence more rapid conidiation relative to a shorter wet period.

In Ontario, wet periods coincident with temperatures between 15–30 C occur frequently during the July and August ripening phases for sweet cherry and peach fruits, respectively (A. R. Biggs and J. Northover, unpublished). Fungicide applications during the preharvest and harvest period are necessary if *M. fructicola* is to be controlled. There are no fungicides with postinfection or eradicative activity against *M. fructicola* registered in Canada or the United States for brown rot control during this period. Timing of fungicide applications and rates could be guided by versions of the models described herein should such a product become available. The primary economic benefit of such a strategy would be from the prevention of sporulation from established, nonsporulating lesions, thereby reducing the apparent infection rate. Field studies of temperature and wetness duration models are needed. Other factors, including cultivar susceptibility to *M. fructicola* and the potential for latent fruit infections, should also be investigated.

LITERATURE CITED

1. Bulger, M. A., Ellis, M. A., and Madden, L. V. 1987. Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence of fruit originating from infected flowers. Phytopathology 77:1225-1230.
2. Byrde, R. F. W., and Willetts, H. J. 1976. The brown rot fungi and fruits, their biology and control. Pergamon Press, New York. 171 pp.
3. Clayton, C. N. 1942. The germination of fungus spores in relation to controlled humidity. Phytopathology 32:921-943.
4. Corbin, J. B. 1963. Factors determining the length of the incubation period of *Monilinia fructicola* (Wint.). Honey in fruits of *Prunus* spp. Aust. J. Agric. Res. 14:51-60.
5. Corbin, J. B., and Cruickshank, I. A. M. 1963. Environment and sporulation in phytopathogenic fungi. V. *Monilinia fructicola* (Wint.) Honey: Effect of water relations on conidia in vivo. Aust. J. Biol. Sci. 16:99-110.
6. McCallan, S. E. A. 1930. Studies on fungicides. II. Testing protective fungicides in the laboratory. N. Y. (Cornell) Agric. Exp. Stn. Mem. No. 128.
7. Miller H. J. 1944. The use of *Venturia inaequalis* and Sclerotinia fructicola with pure chemical stimulants in slide germination tests of fungicides. (Abstr.) Phytopathology 34:1009.
8. Roberts, J. W., and Dunegan, J. C. 1932. Peach brown rot. USDA Tech. Bull. No. 328.
9. Weaver, L. O. 1950. Effect of temperature and relative humidity on occurrence of blossom blight of stone fruits. Phytopathology 40:1136-1153.
10. Wellman, R. H., and McCallan, S. E. A. 1942. An analysis of factors causing variation in spore germination tests of fungicides. IV. Time and temperature. Contrib. Boyce Thompson Inst. 12:431-449.