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Effect of conidial concentration of *Monilinia fructicola* on brown rot development in detached cherries

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Mature sweet and sour (tart) cherries, free of fungicide residues, were harvested, arranged in trays and inoculated without wounding with single 33 μL drops containing 1×10^3 to 1×10^6 conidia/mL of *Monilinia fructicola*. After 22 h wetting at 20°C, fruits were dried (2–4 h), incubated at 20°C, and evaluated daily for development of lesions and sporodochia for 8 da or 9 da post-inoculation. For sweet cherry fruits, increasing the inoculum concentration advanced initial lesion appearance from 5 da to 2 da post-inoculation, increased the incidences of fruits with lesions from 23% to 99% (9 da) and increased the proportions of fruits with sporodochia from 21% to 99% (9 da). The response pattern of ripe sour cherry fruits was very similar, with initial lesion appearance advanced from 4 da to 2 da, and the incidences of fruits with lesions and sporodochia increased from 9% to 92%, and from 9% to 91%, respectively, after 8 da postinoculation incubation. Fruits of Vista sweet cherry were five times more susceptible to infection than those of Montmorency sour cherry. Longer wetting durations of 36 h and 48 h, increased lesion and sporodochial development of Bing sweet cherry fruits, especially from low inoculum concentrations. Similar numbers of conidia applied in 3–10 μL drops induced a slightly higher incidence of lesions than when applied in 30 μL drops. The responses of the proportions of fruits with lesions or sporodochia to inoculum concentration and post-inoculation incubation time, or inoculation wetting duration, were described by polynomial models.

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Des cerises douces et des cerises acides, mûres et exemptes de résidus de fongicides, ont été récoltées, disposées sur des plateaux et inoculées, sans incision ni autre dommage, avec une goutte de 33 μL contenant 1×10^3 à 1×10^6 conidies de *Monilinia fructicola* par mL. Après 22 h d'humectation à 20°C, les cerises ont été séchées (2 à 4 h), puis incubées à 20°C. Pendant les 8 ou 9 jours suivant l'inoculation, les auteurs ont surveillé quotidiennement l'apparition de lésions et de sporodochies. Dans le cas des cerises douces, une élévation de la concentration d'inoculum réduisait de 5 à 2 jours après l'inoculation l'apparition des premières lésions, augmentait de 23 % à 99 % la proportion des fruits présentant des lésions après 9 jours et augmentait de 21 % à 99 % la proportion des fruits présentant des sporodochies après 9 jours. Les cerises acides mûres ont réagi de manière très semblable: l'apparition des premières lésions était réduite de 4 à 2 jours et, 8 jours après l'inoculation, la proportion des fruits présentant des lésions passait de 9 à 92 % tandis que celle des fruits présentant des sporodochies passait de 9 à 91 %. Les cerises douces Vista étaient cinq fois plus vulnérables à l'infection que les cerises acides Montmorency. Par ailleurs, une prolongation du temps d'humectation à 36 h et à 48 h favorisait l'apparition de lésions et de sporodochies sur les cerises douces Bing, particulièrement quand la concentration de conidies était peu élevée. De même, l'application d'un nombre semblable de conidies dans une goutte de 3 à 10 μL (plutôt que de 30 μL) entraînait une légère augmentation de la fréquence des lésions. Les auteurs décrivent au moyen de modèles polynomiaux l'effet de la concentration d'inoculum, du temps d'incubation après l'inoculation et de la durée d'humectation durant l'inoculation sur la proportion des fruits présentant des lésions ou des sporodochies.

Brown rot of sweet and sour (tart) cherries in southern Ontario is caused by *Monilinia fructicola* (Wint.) Honey. Blossom blight is often severe in sweet cherry orchards that have received inadequate fungicide protection during the bloom period. Conidial inoculum from blighted European plum blossoms is suspected of causing infection of immature plum fruits, causing a very low incidence of green fruit rot. Most of the infections remain as symptomless latent infections that develop into brown rot as the fruits ripen and after harvest (Northover & Cerkauskas 1994). A similar situation appears to occur in sweet cherry where green fruit rot and latent infections have been demonstrated (Northover unpublished, Ogawa et al. 1975, Wittig et al. 1991). Latent infections that develop into brown rot immediately prior to harvest are possibly a major source of inoculum contributing to brown rot epiphytotics of mature sweet cherries during wet weather, causing severe preharvest and postharvest fruit losses.

By comparison, blossom blight of Montmorency sour cherry is rarely noticed in Ontario orchards, although blossoms are readily infected under controlled conditions (Wilcox 1989, 1990). In Ontario, the development of brown rot epiphytotics in fungicide-free sour cherry orchards is not as rapid nor as severe as in unprotected sweet cherry orchards in the same season. The relative susceptibility of detached sweet and sour cherry fruits to *M. fructicola* (Northover & Biggs 1990) agrees well with orchard observations. The greater potential for serious losses of sweet cherries from brown rot, necessitates a more frequent application of fungicides than for sour cherries, with attendant concerns over fungicide residues on harvested fruit (Northover et al. 1986).

The infection of nonwounded stone fruits is greatly increased as inoculum levels are elevated (Biggs & Northover 1988b, Corbin 1963, Northover & Biggs 1990). Infection is favored also by wetting periods of at least 18 h, and of temperatures close to the optimum of 20.0–22.5°C (Biggs & Northover 1988a). Interactions between inoculum concentration, temperature, and wetting duration, have been described for sour cherry blossoms (Wilcox 1989). However, relatively little information has been published on the effect of inoculum concentration on the rapidity of appearance of *M. fructicola* lesions and of sporodochia on stone fruits, except for apricot (Corbin 1963).

The objectives of this study were to determine the effect of conidial concentration on the rapidity of appearance of brown rot lesions and sporodochia for ripe sweet cherries and sour cherries. For sweet cherries, we examined conidial production in relation to the inoculum applied, and the effects upon the incidences of fruit infection of extended wetting durations with lower inoculum concentrations, and of the size of the inoculum drop. An abstract of this research has been published (Biggs & Northover 1992).

Materials and methods

Source and preparation of fruits. Sweet cherries (*Prunus avium* L. 'Bing' and 'Vista') and sour cherries (*P. cerasus* L. 'Montmorency') were grown at Jordan Station, Ontario. The orchards received single applications of triforine (475 g a.i./ha) and captan (4.5 kg a.i./ha) during bloom to prevent blossom blight, but fungicides were not used later in the season. Infestations of cherry maggot (*Rhagoletis cinqulata* Loew) were minimized with two applications of azinphosmethyl (1.4 kg a.i./ha). Ripe fruits were hand-picked with stems attached and tumble-washed in tap water (22°C) for 2 min and air-dried for 1–2 h. Stems were trimmed to 6 mm and 52 fruits were arranged, with the suture-side up, on sterile galvanized screens (1.27 × 1.27 cm mesh) supported within sterile aluminum baking trays (29 × 41 × 5 cm). Each tray constituted a replicate unit.

Preparation of inoculum. Conidial inocula were prepared from colonies of a benomyl-sensitive isolate of *M. fructicola* grown for 10–14 da on potato dextrose agar (PDA) in the dark at 20°C. Conidia were suspended by vigorously shaking colony sectors in sterile water. The suspension was coarse-filtered through Ederol 261 filter paper (Binzer, Hatzfeld, Germany), and conidia were separated from the supernatant on 0.22 µm Millipore filter discs (Millipore Corp., Bedford, MA). The conidia on the discs were rinsed with 5 mL of sterile water while under low vacuum, and then were washed from the discs with sterile water or Miller's solution (0.01 g Na citrate, 0.01 g K citrate, and 20 g sucrose/L) (Miller 1944) using a sterile compressed-air glass atomizer. Initial conidial concentrations were measured with a hemacytometer, and the suspensions were adjusted to the desired concentrations with water or Miller's solution. Non-wounded fruits were individually inoculated with one 33 µL drop of conidial suspension placed on the uppermost part of the suture, delivered from a calibrated sterile Pasteur pipette. Fruits in check treatments received drops of sterile water or Miller's solution. Miller's solution was used in all except one of the experiments. Inoculum germination on PDA after 24 h at 20°C was greater than 95%, with vigorous germ tube growth.

Incubation conditions and disease assessment. Trays of inoculated fruits were kept for 22 h at 20°C in the dark, at 100% relative humidity (RH) in a lightly-misted inoculation room so that the inoculum drops did not dry. Afterwards, the trays were moved to a well-ventilated growth room where the inoculum drops dried within 2–4 h at 20°C and 60% RH. The trays were then moved to a third room maintained at 20°C and >95% RH in the dark and exposed to light only during evaluation. Fruits were examined daily for brown rot lesions and sporodochia centered on the inoculation site. Individual fruits were assessed using a scale of 0 to 3, where 0 = no visible infection; 1 = necrosis not wider than the inoculum drop; 2 = necrosis wider than the width of the inoculum drop but without sporodochia; and 3 = sporodochia present on necrotic lesion (Biggs & Northover 1988a).

When lesions arose on the fruit surface other than at the inoculation site, they were considered to be "background" field infections. When a background infection encroached on a nonlesioned, inoculated site, the fruit was discounted and the sample size was reduced by one.

Effect of inoculum concentration on the development of lesions and sporodochia. The effect of inoculum concentration was studied on Vista sweet cherry and Montmorency sour cherry using three replicates each of 52 fruits for each of 11 treatments with inoculum concentrations of 0, 1×10^3, 2×10^3, 5×10^3, 1×10^4, 2×10^4, 5×10^4, 1×10^5, 2×10^5, 5×10^5, and 1×10^6 conidia/mL of Miller's solution. The sour cherry experiment included six treatments in which the inoculum was suspended in water for comparison with the treatments using Miller's solution. These inoculum concentrations were 0, 5×10^3, 1×10^4, 2×10^4, 5×10^4, and 1×10^5 conidia/mL of water. Fruits were evaluated and individual records made on days 2 through 8 (sour cherry) or days 2 through 9 (sweet cherry), after inoculation. Because the inoculation site of some sweet cherry fruits was invaded by background infections, the sample size in each experiment was reduced to a uniform number, by using tables of random numbers.

Conidial production. The numbers of conidia produced on sporodochia on the Vista sweet cherries, in the previous experiment, were determined 8 days after inoculation. Samples of 8–10 fruits, each with sporodochia centered on the inoculation site, and without background infection, were taken at random from each replicate of each of the 10 inoculum concentration treatments. The conidia were washed from individual fruits with a fine water spray from a compressed-air atomizer. The resulting conidial suspension was weighed, vigorously shaken to break up spore chains, and the conidial concentration was determined with a hemacytometer. The results were expressed for each inoculum concentration as the mean number of conidia produced on each fruit with sporodochia, and also as the mean number of conidia produced per inoculated fruit.

Effect of wetting duration and inoculum concentration on fruit infection. The effects of inoculum concentration and the duration of wetting upon fruit infection and sporodochial development were examined using Bing sweet cherries. The inoculum concentrations employed were 1×10^3, 3×10^3, 1×10^4, 3×10^4, and 1×10^5 conidia/mL, using one 33 µL drop/fruit. The wetting durations were 12, 24, 36, and 48 h. Three replicate trays each of 52 fruits were used for each of the 20 treatments and the check (Miller's solution without conidia). The conditions of the experiment were the same as those described above.

Effect of drop size and conidial number on fruit infection. Inoculum drop sizes of 3, 10, and 30 µL, were delivered using Hamilton microsyringes. One drop was applied to each nonwounded Bing sweet cherry fruit. The conidial concentrations were adjusted so that for each drop size, 0, 30, 100, 300, or 1000 conidia were delivered to the single inoculation site/fruit. Each of the 15 treatments was applied to three replicate trays each of 52 fruits supported above damp paper towelling. Trays of inoculated fruits were enclosed in polyethylene bags for 22 h at 20°C, after which the bags were removed and the inoculum drops were dried, as described above. The fruits were incubated at 20°C and >95% RH, and evaluated daily for 6 da after inoculation. Replicate sample size was reduced to 37 fruits because of background infection. This experiment was conducted three times.

Data analysis. All data on lesion and sporodochial development were corrected for the proportions of fruits with lesion or sporodochial development in the check treatment using Abbott's formula (Healy 1952). Regression analysis was used to describe the effect of postinoculation time (T, expressed as days) and inoculum concentration (I, expressed as the $\log_{10}$ concentration of conidia/mL) on the proportion (Y) of fruits with lesions or sporodochia. Polynomial models, evaluated for their closeness to fitting the data were of the form:

$$Y = f(I,T) \qquad (1)$$

in which f(I,T) were linear combinations of the following terms: b_0, b_1I, b_2T, b_3I^2, b_4I^3, b_5T^2, b_6T^3, b_7IT, b_8IT^2, b_9IT^3, $b_{10}I^2T$, $b_{11}I^3T$, $b_{12}I^2T^2$, $b_{13}I^3T^2$, $b_{14}I^2T^3$, and $b_{15}I^3T^3$. Logistic models with the same terms were evaluated, in which Y was transformed to logits, $(\ln(Y/(1-Y))$. Variable selection for the models was accomplished with a stepwise linear regression procedure with stepwise selection (SAS Institute, Cary, NC 1989). Regression models were evaluated by using the following criteria: significance of estimated parameters, the normal and random distribution of residuals, coefficients of determination between observed and predicted Y values (R^2), and R^2 adjusted for degrees of freedom ($R_a{}^2$). When transformed Y values were used, the goodness of fit (R^{*2}) of the back-transformed predicted values with the observed Y values was calculated.

In the experiment determining the effects of inoculum concentration (I) and wetness duration (W) on lesion and sporodochial development in sweet cherry, polynomial and logistic models similar to those just described were used, where I was the $\log_{10}$ concentration of conidia/mL and W was the wetness duration in hours at 20°C.

The effect of inoculum concentration on the numbers of conidia produced on sweet cherry fruits was examined with linear regression using $\log_{10}$ transformed data. Data of the effect of drop size and conidial number on sweet cherry infection were examined by factorial analysis.

Results

Sweet cherry. *Lesion development* — There was an increase in the percentage of infected sweet cherry fruits over time with all the inoculum concentrations tested (Fig.1A). Incidences of >95% fruit infection were observed after 2 da for fruits inoculated with 1×10^6 conidia/mL and after 3, 4, and 5 da for fruits inoculated with 5×10^5, 2×10^5, and 5×10^4 conidia/mL, respectively. After 9 da incubation, fruits inoculated with conidial concentrations of less than 5×10^4 conidia/mL exhibited progressively lower incidences of infection. Lesion appearance was delayed at the lower inoculum concentrations (Fig. 1A). For example, the elapsed time for lesion appearance on 50% of fruits was 6 da for fruits inoculated with 1×10^4 conidia/mL compared with 2 da for fruits inoculated with 2×10^5 conidia/mL. Fewer than 50% of fruits inoculated with concentrations lower than 1×10^4 conidia/mL were infected after 9 da of incubation.

A polynomial equation of the form:

$$Y = b_0 + b_1I + b_2I^2 + b_3I^3 + b_4IT + b_5I^2T^2 \qquad (2)$$

best described the incidence of infection of sweet cherry fruits as a function of inoculum concentration

Table 1. Estimated parameters[w] for six polynomial models describing the proportion (Y)[x] of ripe sweet or sour cherry fruits[y] with developing lesions or sporodochia of *Monilinia fructicola*, as a function of conidial concentration (I)[x] and wetting duration (W)[x] or post-inoculation incubation time (T)[x], with coefficients of determination (R^2), R^2 adjusted for degrees of freedom (Ra^2) and the standard errors of the regression lines (s)

Equation no.[w]		Estimated parameters[w]									Coefficients		Regression
		b_0	b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_8	R^2	Ra^2	s
2	t		I	I^2	I^3	IT	I^2T^2				0.918	0.912	0.117
	p	5.440	-4.721	1.187	-0.0909	0.0473	-0.000649						
	sd	1.171	1.195	0.271	0.0201	0.00493	0.0000886						
3	t		T	I^2	I^3	T^2	IT	I^2T	I^3T	I^3T^2	0.952	0.947	0.095
	p	1.236	0.478	-0.159	0.0158	0.0204	-0.662	0.195	-0.0133	-0.000326			
	sd	0.265	0.242	0.0360	0.00522	0.00457	0.163	0.0375	0.00288	0.000038			
5	t		IW^2	I^2W	I^3W^2	I^3W^3					0.925	0.920	0.096
	p	-0.629	-1.238×10^{-4}	6.56×10^{-3}	-3.579×10^{-5}	3.943×10^{-7}							
	sd	0.0681	1.534×10^{-5}	5.41×10^{-4}	4.538×10^{-6}	6.344×10^{-8}							
6	t		I^2W	I^3W	I^3W^2	I^3W^3					0.906	0.899	0.109
	p	-0.450	3.466×10^{-3}	4.853×10^{-4}	-3.477×10^{-5}	3.177×10^{-7}							
	sd	0.0693	4.065×10^{-4}	7.613×10^{-5}	5.158×10^{-6}	6.524×10^{-8}							
7	t		IT^3	I^2T	I^2T^2						0.885	0.883	0.119
	p	-0.379	1.75×10^{-4}	1.42×10^{-2}	-1.43×10^{-3}								
	sd	0.0305	5.621×10^{-5}	8.483×10^{-4}	1.720×10^{-4}								
8	t		I	T	I^2	I^3	I^2T^2	I^3T	I^3T^3		0.908	0.905	0.105
	p	4.152	-2.452	-0.143	0.484	-0.0333	0.00228	8.658×10^{-4}	-3.973×10^{-5}				
	sd	0.959	0.665	0.0235	0.151	0.0113	3.455×10^{-4}	1.787×10^{-4}	4.455×10^{-6}				

[w] The number of the equation is that given in the text in parenthesis. For each equation, the algebraic terms are given in row t, and the numerical value of each estimated parameter (b_1-b_8) and its standard deviation are given in rows p and sd, respectively, under the appropriate column headings.

[x] Y is the proportion of fruits affected (1.00 = 100%), I is the $\log_{10}$ transformation of the conidial concentration, W is the duration of the inoculation wetting period in hours, and T is the number of days of post-inoculation incubation.

[y] The six relationships examined were: Vista sweet cherry, conidial concentration (I) versus incubation time (T) for lesion development (equation 2) and sporodochial development (equation 3); Bing sweet cherry, inoculum concentration (I) versus inoculation wetting duration (W), for lesion development (equation 5) and sporodochial development (equation 6); and Montmorency sour (tart) cherry, conidial concentration (I) versus incubation time (T) for lesion development (equation 7) and sporodochial development (equation 8).

[z] The estimated parameter b_0 is the theoretical value of Y when I = 0 and W or T = 0.

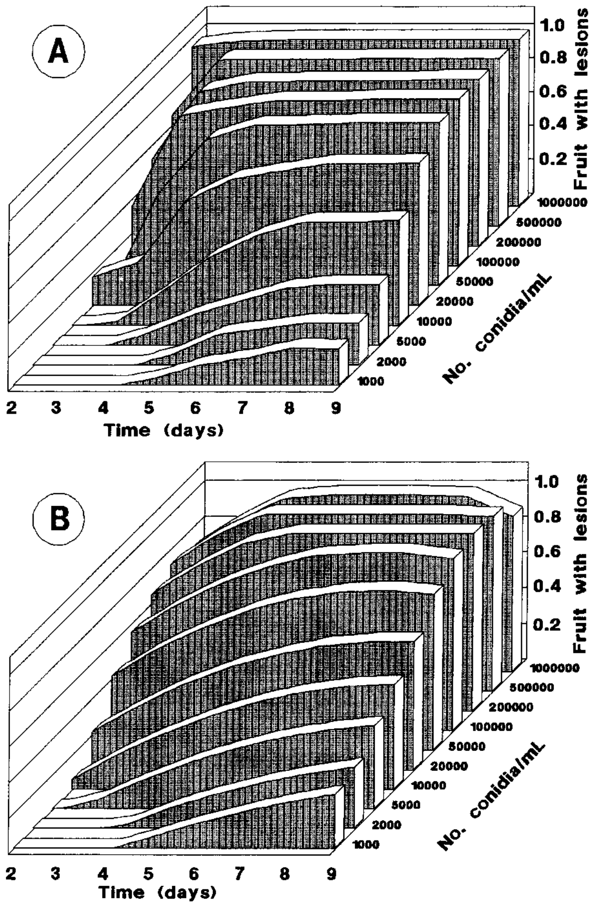

Figure 1. Effect of the number of conidia/mL of *Monilinia fructicola* in inoculum drops and incubation time (days) on the **A**) observed and **B**) predicted proportions of Vista sweet cherry fruits with necrotic lesions. In B, curves were generated by using equation 2 with the estimated parameters given in Table 1, Eq. 2.

(I) and time (T). All b value estimates were significant ($P<0.05$). The distribution of residuals was normal and random and the coefficients of determination, R^2 and R_a^2, were 0.918 and 0.912, respectively (Table 1, Eq. 2). In comparison to the polynomial equation, the best logistic model for describing the data gave a nonrandom distribution of residuals and lower coefficients of determination ($R^{*2} = 0.86$). The interaction between I and T, was shown by the terms b_4IT, and $b_5I^2T^2$. The predicted proportions of fruits with lesions (Y), from the polynomial equation, were plotted for the same inoculum concentrations and incubation times used experimentally, and are shown in Fig. 1B.

Development of sporodochia — Sporodochia were observed 3 da after inoculating fruits with concentrations $\geq 1\times10^5$ conidia/mL, and after 6 da with 1×10^3 conidia/mL (Fig. 2A). Sporodochia developed on 50% of fruits after approximately 3, 5, and 9 da following inoculation with 1×10^6, 5×10^4, and 1×10^4 conidia/mL, respectively.

A polynomial equation of the form:

$$Y = b_0 + b_1T + b_2I^2 + b_3I^3 + b_4T^2 + b_5IT + b_6I^2T + b_7I^3T + b_8I^3T^2 \qquad (3)$$

best described the proportion of inoculated fruits with sporodochia as a function of inoculum concentration and time. All b value estimates were significant ($P < 0.01$). The coefficients of determination, R^2 and R_a^2, were 0.952 and 0.947, respectively (Table 1 Eq. 3). The predicted proportions of fruits with sporodochia (fruit sporulating) for the inoculum concentrations and incubation times used in this experiment are shown in Fig. 2B. In comparison to the polynomial equation, the best logistic model for describing the data gave a nonrandom distribution of residuals and lower coefficients of

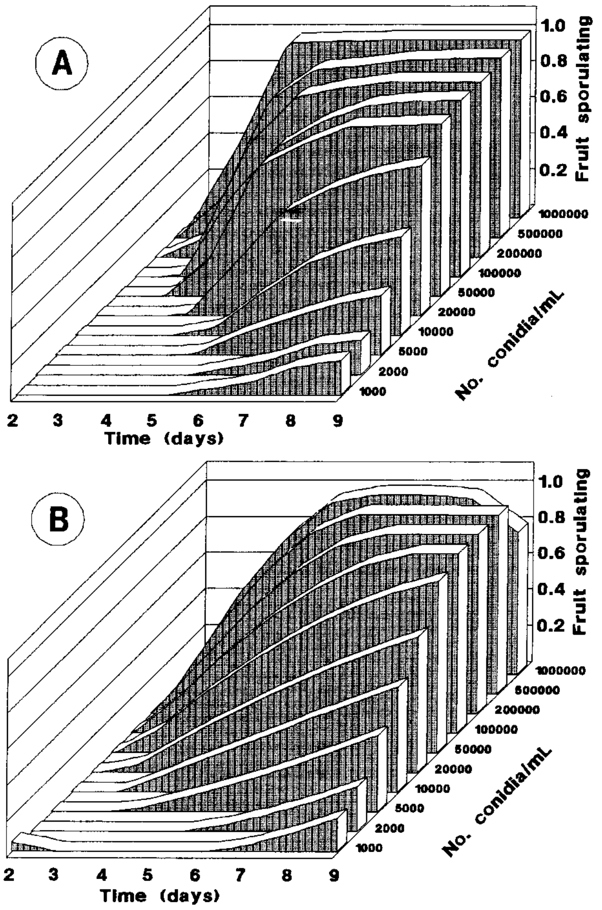

Figure 2. Effect of the number of conidia/mL of *Monilinia fructicola* in inoculum drops and incubation time (days) on the **A**) observed and **B**) predicted proportions of Vista sweet cherry fruits with sporodochia. In B, curves were generated by using equation 3 with estimated parameters in Table 1, Eq.3.

determination, 0.86 and 0.85 for R^2 and R_a^2, respectively.

Conidial production — As the number of spores applied per site (I) declined from 30 000 to 30, so the mean number of conidia produced per inoculated fruit (Y) declined from 6×10^6 to 1×10^5 (Table 2). The regression of Y against I was linearized by log/log transformation and was expressed by the equation:

$$\log_{10} Y = 4.349 + 0.546 \log_{10} I \qquad (4)$$

The reproductive factor (Rf) was calculated as the number of conidia produced per inoculated fruit divided by the number of conidia that constituted the inoculum. The Rf value ranged from 200 for 1×10^6 conidia/mL, to over 3 000 for 1×10^3 conidia/mL (Table 2, Column 6).

Effect of wetting duration and inoculum concentration on fruit infection — The incidence of brown rot on Bing sweet cherries inoculated with various conidial concentrations (I) and subjected to wetting durations (W) of 12–48 h, and incubated for 9 da is shown in Fig. 3A. A concentration of 1×10^5 conidia/mL with 12 h wetting caused 85% fruit infection, and longer wetting had little further effect. However, with a concentration of 1×10^3 conidia/mL, wetting periods of 12, 24, 36, and 48 h caused lesion development on 4, 15, 24, and 41% of fruits, respectively. A polynomial model of the form:

$$Y = b_0 + b_1 I W^2 + b_2 I^2 W + b_3 I^3 W^2 + b_4 I^3 W^3 \qquad (5)$$

best described the proportion of fruits with visible lesions as a function of inoculum concentration and wetness duration (Fig. 3B). All b value estimates were significant ($P < 0.01$) and the coefficients for

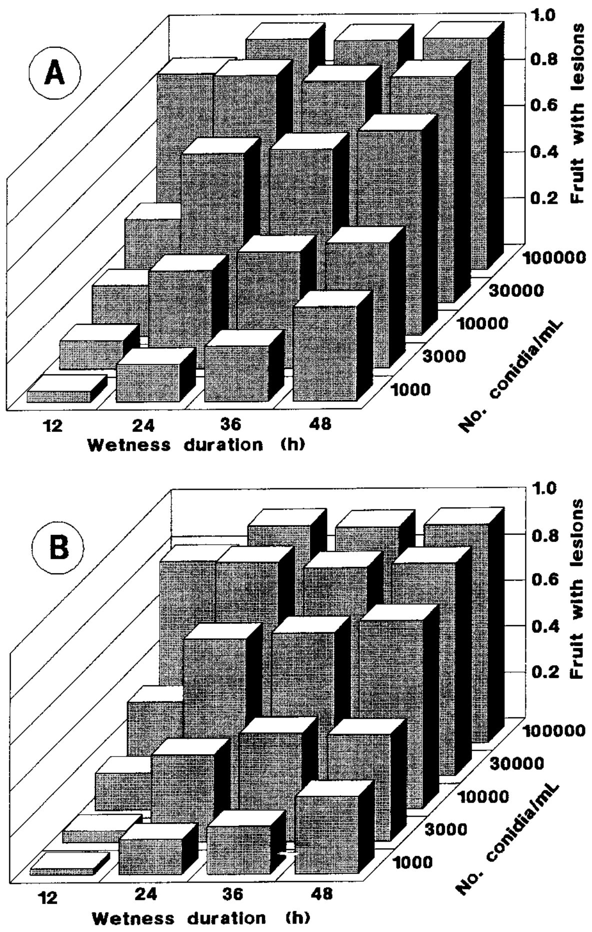

Figure 3. Effect of the number of conidia/mL of *Monilinia fructicola* in inoculum drops and wetness duration on the **A**) observed and **B**) predicted proportions of Bing sweet cherry fruits with lesions. In B, the estimates were generated by using equation 5 with estimated parameters in Table 1, Eq. 5.

Table 2. Production of conidia of *Monilinia fructicola*, 8 days after inoculation of ripe Vista sweet cherries in relation to the numbers of conidia applied to the inoculation site, and the inoculum reproductive factor

Number conidia /mL	Conidia applied /fruit (in 33μL)	Conidia/ sporulating fruit ($\times 10^5$)	Fruits sporulating day 8 (%)	Conidia/ inoculated fruit ($\times 10^5$)	Inoculum reproductive factor[1]
1×10^6	30000	61[2]	99	61	203
5×10^5	15000	25	100	25	167
2×10^5	6000	21	98	21	350
1×10^5	3000	28	98	27	900
5×10^4	1500	16	96	15	1000
2×10^4	600	17	80	14	2333
1×10^4	300	10	50	5	1667
5×10^3	150	11	29	3	2000
2×10^3	60	9	18	2	3333
1×10^3	30	4	16	1	3333

[1] Inoculum reproductive factor = no. conidia per inoculated fruit (col. 5) ÷ no. conidia applied per fruit (col. 2).

[2] Tabulated means are the numbers of conidia recovered from three replicates, each of 8–10 sporulating fruits/treatment.

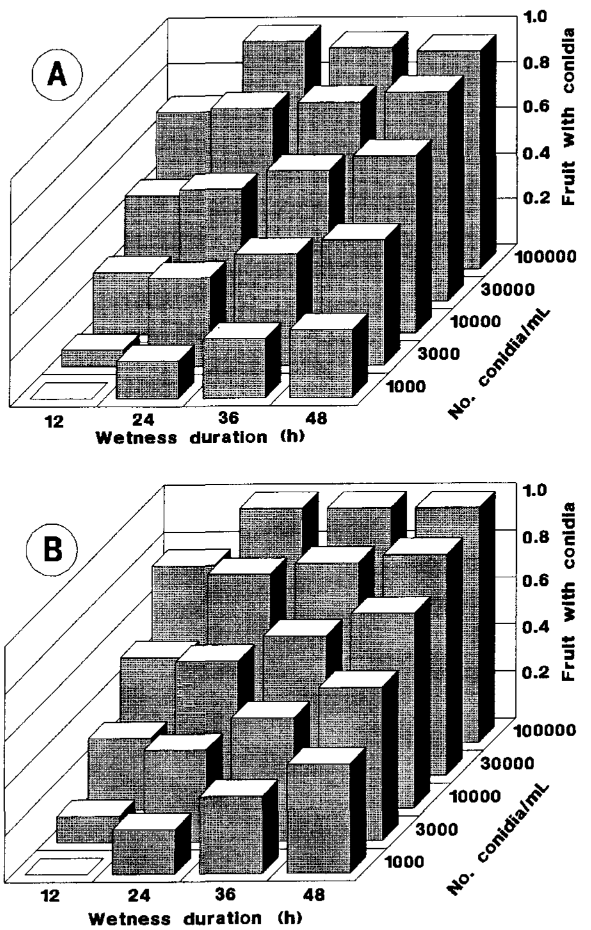

Figure 4. Effect of the number of conidia/mL of *Monilinia fructicola* in inoculum drops and wetness duration on the **A**) observed and **B**) predicted proportions of Bing sweet cherry fruits with sporodochia. In B, the estimates were generated by using equation 6 with estimated parameters in Table 1, Eq. 6.

determination, R^2 and R_a^2, were 0.925 and 0.920, respectively (Table 1 Eq. 5).

The development of sporodochia on these same fruits was similarly affected by inoculum concentration and wetness duration (Fig. 4A) and was best described by a polynomial equation of the form:

$$Y = b_0 + b_1I^2W + b_2I^3W + b_3I^3W^2 + b_4I^3W^3 \quad (6)$$

All b values were significant ($P < 0.01$) and the coefficients of determination, R^2 and R_a^2, were 0.906 and 0.899, respectively (Table 1 Eq. 6). The proportions of fruits with sporodochia, as predicted from this model, are shown in Fig. 4B.

Effect of drop size and conidial number on fruit infection — Inoculum drops of 3–10 μL, averaged over conidial number/drop, gave 53–54% fruits with lesions compared with a slightly but significantly ($P = 0.05$) lower incidence of 43% with 30 μL drops (Table 3). Averaged over drop size, numbers of 30, 100, 300, and 1000 conidia/drop resulted in 15, 31, 70, and 84% fruits with lesions, respectively. Similar results were obtained in two earlier experiments (data not presented).

Sour cherry. *Lesion development* — There was an increase in the percentage of infected sour cherry fruits with time between days 2 and 8 postinoculation, over most of the inoculum concentrations tested (Fig. 5A). Lesions appeared 2, 3, and 4 da after inoculation with conidial concentrations of 1×10^5, 2×10^4, and 1×10^3 conidia/mL of Miller's solution, respectively. Lesion development on 50% or more of fruits occurred after 3 da at inoculum concentrations equal to or greater than 2×10^5 conidia/mL, and after 4 and 5 da with concentrations of 1×10^5 and 5×10^4 conidia/mL, respectively. After incubation for 8 da, lesions occurred on 9% to 35% of fruits inoculated

Table 3. Effect of inoculum drop size and the number of conidia per drop on the percentage of Bing sweet cherries infected by *Monilinia fructicola*

	Incidence of fruit infection (%)				
	Number of conidia per drop				
Drop size (μL)	30	100	300	1000	Mean effect of drop size, averaged over conidia/drop
3	18	18	91	91	54 b[1]
10	22	45	55	90	53 b
30	6	30	65	72	43 a
Mean effect of conidia/drop averaged over drop size	15 A[1]	31 B	70 C	84 D	

[1] The analysis of nontransformed data showed significant effects for drop size ($P = 0.01$) (S) and the number of conidia/drop (N) ($P = 0.0001$) and for the S × N interaction ($P = 0.0001$). Only the main effects were examined for differences. Means in the same column or row followed by a different letter differ significantly ($P = 0.05$) by Duncan's Multiple Range Test.

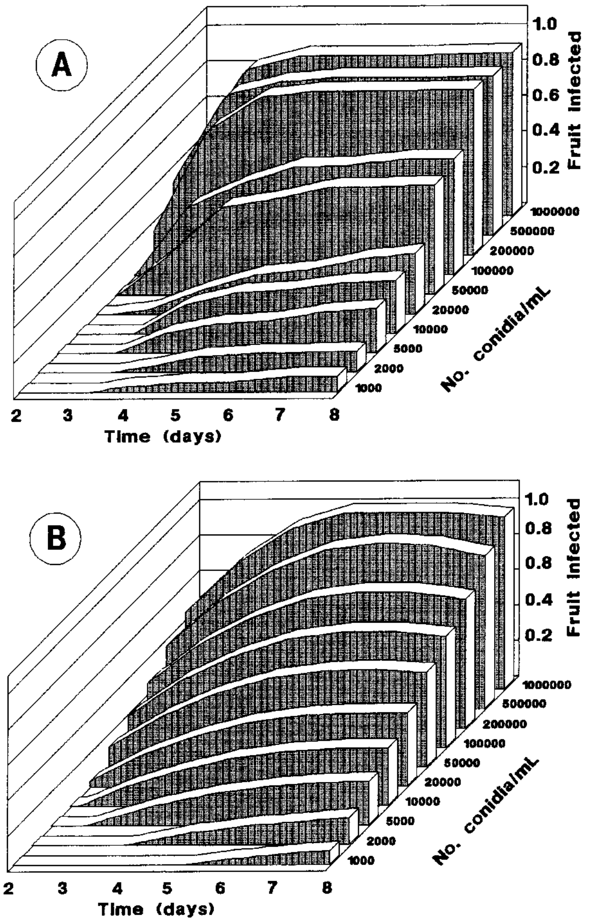

Figure 5. Effect of the number of conidia/mL of *Monilinia fructicola* in inoculum drops and incubation time (days) on the **A**) observed and **B**) predicted proportions of Montmorency sour cherry fruits with necrotic lesions. In B, curves were generated by using equation 7 with estimated parameters in Table 1, Eq. 7.

with 1×10^3 to 2×10^4 conidia/mL, respectively. A polynomial equation of the form:

$$Y = b_0 + b_1IT^3 + b_2I^2T + b_3I^2T^2 \quad (7)$$

best described the incidence of lesion development in ripe sour cherries as a function of inoculum concentration and incubation time. All b value estimates were significant ($P < 0.05$), the distribution of residuals was normal and random, and the coefficients of determination, R^2 and R_a^2, were 0.885 and 0.883, respectively (Table 1 Eq. 7). The predicted levels of infection (Y) versus postinoculation time (T) were plotted for the inoculum concentrations (I), as shown in Fig. 5B. In comparison to the polynomial equation, the best logistic model for describing the data gave a nonrandom distribution of residuals and lower coefficients of determination (R^{*2}).

Sporodochial development — Sporodochia of *M. fructicola* were observed first on sour cherries 3, 4, and 5 da after inoculation with concentrations of 2×10^5, 5×10^3, and 1×10^3 conidia/mL, respectively (Fig. 6A). The incidences of fruits with sporodochia exceeded 50% 4 da after inoculation with concentrations of 5×10^5 conidia/mL, and after 5 and 6 da with concentrations of 2×10^5 and 5×10^4 conidia/mL, respectively. After 8 da incubation, 9% to 29% of the cherries showed sporodochia following inoculation with 1×10^3 to 2×10^4 conidia/mL, respectively.

A polynomial model of the form:

$$Y = b_0 + b_1I + b_2T + b_3I^2 + b_4I^3 + b_5I^2T^2 + b_6I^3T + b_7I^3T^3 \quad (8)$$

best described the development of *M. fructicola* sporodochia on sour cherries as a function of inoculum concentration and incubation time (Table 1, Eq. 8). All b value estimates were significant ($P < 0.01$) and coefficients of determination, R^2 and R_a^2, were

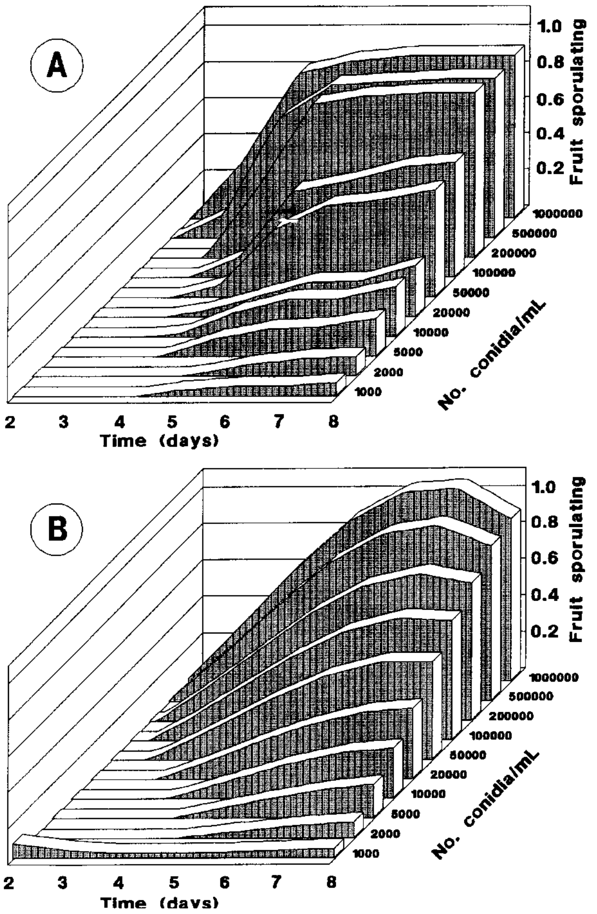

Figure 6. Effect of the number of conidia/mL of *Monilinia fructicola* in inoculum drops and incubation time (days) on the **A**) observed and **B**) predicted proportions of Montmorency sour cherry fruits with sporodochia. In B, curves were generated by using equation 8 with estimated parameters in Table 1, Eq. 8.

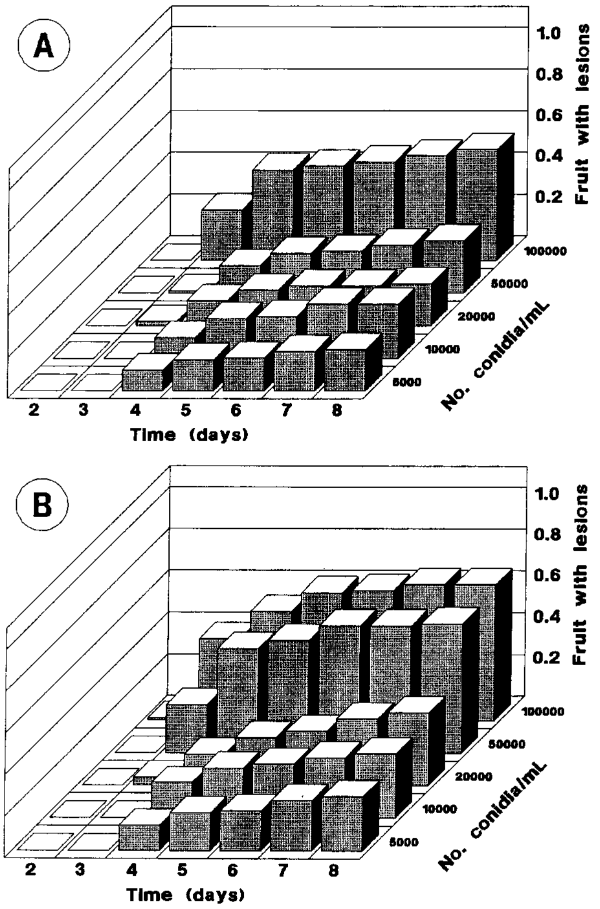

Figure 7. Effect of incubation time (days) and the number of conidia/mL of *Monilinia fructicola* suspended **A**) in water or **B**) in Miller's solution, and applied as single inoculum drops to fruits of Montmorency sour cherry, upon the proportions of fruits with lesions.

0.908 and 0.905, respectively. The predicted frequencies of sporodochial development (Y) were plotted for the inoculum concentrations (I) and days of incubation (T) used in this experiment (Fig. 6B).

Effect of Miller's solution on infection of sour cherries — The pattern of lesion development over the 8 da incubation period is shown in Fig. 7 for inoculum suspended in water (Fig. 7A) or in Miller's solution (Fig. 7B). Relative to water, Miller's solution increased the incidence of diseased fruits but had little if any effect on the rate of lesion appearance. After 4 da, the mean incidences of fruit infection were 18% with water and 29% with Miller's solution, and after 8 da, these had increased to 29% and 44%, respectively. Relative to water, Miller's solution increased the incidence of brown rot by a mean of 57% during the 4- to 8-da post-inoculation period. This agreed well with prior determinations for both sour and sweet cherries (unpublished data).

Discussion

Increases in conidial inoculum concentration gave a proportionate increase in the incidence of cherries with brown rot lesions and with sporodochia. This detailed study confirmed and extended our earlier findings obtained with a coarser range of conidial concentrations (Northover & Biggs 1990). Corbin (1963) described the earlier appearance of lesions with sporodochia on apricots with higher inoculum concentrations and showed that the incubation period (IP_{50}), the time interval between inoculation and the appearance of sporodochia on 50% of fruits, was inversely related to inoculum concentration for several species of *Prunus*. Similarly on peach fruits, elevated inoculum concentrations increased the incidence of brown rot from *M. fructicola* (Biggs & Northover 1988b) and from *M. laxa* (Aderhold & Ruhland) Honey (Fourie & Holz 1985).

In this study, detached ripe Bing and Vista sweet cherries were more susceptible than ripe Montmorency sour cherries. The inoculum concentration necessary to induce brown rot on >50% of sweet cherries, 6 or 7 da after inoculation, was 1×10^4 conidia/mL, and it was five times greater for sour cherries. This confirmed our earlier finding of a 10-times difference using a coarser range of inoculum concentrations (Northover & Biggs 1990). Brown and Wilcox (1989) found that ripe Montmorency cherries were readily infected with concentrations of only 1×10^3 conidia/mL, and that they were as susceptible as several ripe sweet cherry varieties. Brown and Wilcox used a spray inoculation method as opposed to our drop inoculation procedure, and this may have contributed to the higher incidences of infection and an absence of a differential effect between sweet and sour cherries.

Conidial production was greater on sporulating sweet cherries inoculated with high rather than low inoculum concentrations. This was attributed to the earlier and more abundant formation of sporodochia on the older and larger lesions that developed earlier from inoculation with high conidial concentrations. Contrasting fruits inoculated with 1×10^4 and 1×10^6 conidia/mL, the 100-times greater inoculum resulted in only a six times greater conidial production. The reproductive factor (Rf) with 1×10^6 conidia/mL was only one eighth of that for 1×10^4 conidia/mL, and was interpreted as a measure of saturation of inoculum at the infection site.

Extending the wetting duration to 48 h greatly favored infection of sweet cherries with low concentration of inoculum. This was especially evident with 30 conidia/drop ($1\text{x}10^3$ conidia/mL), which with 12, 24, and 48 h of wetting resulted in 4, 15, and 41% of fruits with lesions. This extended our earlier observations that were limited to a range of 6–18 h wetting duration

duration (Biggs & Northover 1988a). When rain is followed by slow drying, droplets may shrink slowly and slightly favour increased infection. In sweet cherry clusters, water or inoculum drops persist for several hours after single fruits have dried thereby extending the wetting period and possibly increasing infection. Infection through contact surfaces between cherries may also be easier than through the possibly thicker cuticle of single cherries (Biggs & Northover 1989), similar to the situation in prune clusters (Michailides & Morgan 1994). Under favorable conditions the minimum number of conidia necessary to induce infection may be lower than 30 per site, depending on the duration of wetting, cuticle thickness, and incubation time. Hall (1971) found that Millicent peach fruits could be infected with a single conidium.

The nontransformed polynomial equations fitted the data well, with better fits than the logistic models. The polynomial models are mathematical descriptions of the data and the expressions have limited biological implications. As discussed by Madden (1986), polynomial models may give predictions which reach a maximum and decline, although this makes no sense, biologically. Examples of this type were seen in the predictions for lesion development in Vista sweet cherry (Fig. 1B) and for sporodochial development in sour cherry (Fig. 6B). Furthermore, the polynomial equation describes only the observed data and cannot be used to extrapolate responses beyond the experimental limits. Accordingly, no attempt was made to calculate the least number of conidia needed to bring about a low incidence of infection at wetting durations and incubation time intervals longer than those studied. The inoculum concentrations, wetting durations and incubation times that were studied represented conditions commonly encountered in stone fruit orchards. Therefore these polynomial models have considerable utility.

Controlled studies with detached ripe fruits have helped to demonstrate the relative importance of several major factors upon the infection of mature cherries and sporodochial formation by *M. fructicola*. Because detached fruits start to senesce as soon as they are harvested, we recognize that tree-attached fruits may be slightly less susceptible to infection than those used in these studies. Miller's solution was used throughout this research to provide optimum infection conditions. On sour cherries, Miller's solution, in comparison with water, increased fruit infection by 57%, resembling similar preliminary observations with sweet cherry. Similarly, Hall (1971) found that infection occurred more rapidly with conidia suspended in malt extract than in water. Further studies might more appropriately use conidial suspensions containing nutrients and salts normally found in water redistributed from diseased fruits under orchard conditions.

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