Effects of Leaf Age and Inoculum Concentration on Infection of Sour Cherry by *Coccomyces hiemalis*

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ABSTRACT

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Effects of leaf age and inoculum concentration on infection of Montmorency cherry by conidia of *Coccomyces hiemalis* were investigated in the greenhouse. With increasing leaf age from 5 to 36 days at inoculation, there was a linear decrease in the ln ($\log_e$) of the number of leaf spot lesions per square centimeter of leaf area 11 days after inoculation with 10^5 and 10^6, but not 10^4 spores per milliliter; with leaves from 35 to 70 days old, the decrease in ln lesions per square centimeter occurred only at 10^6 spores per milliliter. No changes in the ln of numbers of lesions were observed in leaves inoculated at 103 to 126 days of age. Leaves expanded fully within 16 days of unfolding. Resistance did not increase in the same manner as leaf growth, but continued after growth was completed. With 1- to 32-day-old leaves, mean number of lesions per square centimeter of leaf at inoculation did not increase between 10^2 and 10^4, increased approximately 10-fold between 10^4 and 10^5, and increased less than 10-fold between 10^5 and 10^6 spores per milliliter. Germination on water agar was reduced at 10^6 spores per milliliter.

Additional key words: epidemiology, *Prunus cerasus*.

A system for predicting infection of sour cherry (*Prunus cerasus* L. 'Montmorency') leaves by *Coccomyces hiemalis* Higgins was described (1) and used to time fungicide applications for the control of cherry leaf spot disease in the field (2). This system is based on an environmental favorability index computed from hours of leaf wetness and average air temperature during the wet period. Most forecasting schemes (5) assume that inoculum and a susceptible host are present, and evaluate the suitability of the weather for infection or disease development. However, variations in host susceptibility or inoculum density can affect disease severity even under favorable environmental conditions (6). The environmental favorability index in the cherry leaf spot model could be modified to account for variation in host susceptibility and inoculum levels if the relationship of these variables to infection frequency were known.

The purpose of this study was to investigate the effects of leaf age and inoculum concentration on infection frequency under greenhouse conditions.

MATERIALS AND METHODS

The effects of inoculum concentration and leaf age on infection frequency were examined in seven factorial experiments conducted at different times over a 16-mo period. Experiments I and II were performed with inoculum concentrations of 10^2, 10^3, 10^4, 10^5, and 10^6 spores per milliliter and experiments III to VII were performed with 10^4, 10^5, and 10^6 spores per milliliter. Experiment I contained 1- to 32-day-old leaves, experiments II and III contained 1- to 36-day-old leaves, experiment IV contained 5- to 40-day-old leaves, experiments V and VI contained 35- to 70-day-old leaves, and experiment VII contained 103- to 126-day-old leaves. Treatments were arranged in the mist chamber in a completely randomized design with five or six replications per treatment.

Three-year-old Montmorency sour cherry trees on *Prunus mahaleb* rootstocks were grown at 16–25 C in a greenhouse. Trees with three to five shoots were maintained in 3-L cans in a mixture of sand, peat moss, and soil (1:1:1, v/v). A 20% N, 20% P_2O_3, 20% K_2O fertilizer (Robert B. Peters Co., Inc., Allentown, PA 18104) was mixed at 5.3 g/L of water and approximately 0.5 L was applied to each can biweekly. The age of a leaf was calculated from the date of unfolding; ie, when its laminar blades were separated by an angle greater than 90 degrees. All leaves unfolding within a 4-day period were assigned to an age class. Thus, 1- to 4-day-old leaves were assigned to class 1, 5- to 8-day-old leaves to class 2, etc. All trees used in an experiment had a range of leaf ages present at the time of inoculation. Leaves of appropriate ages were selected at random for use in each experiment.

Leaves were inoculated with conidial suspensions of *C. hiemalis* prepared by washing infected cherry leaves with distilled-deionized water. Concentrations of conidia in the suspensions were determined with a hemacytometer. The spore suspension was sprayed uniformly onto the undersurface of each leaf with an atomizer (The DeVilbiss Co., Somerset, PA 15501) and compressed air at a pressure of 1.4 kg/cm^2 (20 psi).

Percent germination of conidia on 2% water agar was determined in experiment I. At the time of inoculation, spores were sprayed onto agar blocks in a petri dish and incubated at 20 C for 24 or 48 hr. Germinated and ungerminated spores were counted at ×200 with a light microscope. Percent germination was determined from a total of 100 to 400 spores per inoculum concentration.

Within 1 hr after inoculation, the trees were placed in a mist chamber at 20–24 C for 48 hr. After removal from the mist chamber, the trees were placed under a cheesecloth tent on a greenhouse bench. The cheesecloth was wetted to maintain a humidity of 90–100% (as measured with a hygrothermograph) around the plants. Under these conditions, chlorotic flecks were visible 6 days after inoculation, but lesions were not counted until 11 days after inoculation.

The area of each leaf was measured with an area meter (Model LI-3000, Lambda Instrument Corp., Lincoln, NE 68504) on the day of inoculation and again 11 days later. These measurements were used to determine if leaf size and rate of expansion were constant among different leaf age classes. Leaf spot severity was assessed by counting the number of lesions per leaf and adjusting the data based on the leaf area on the day of inoculation or on the day of assessment. The data for each experiment were subjected to analysis of variance to determine if differences in disease severity between leaves could be attributed to leaf age or inoculum

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concentration and if an interaction existed between leaf age and inoculum concentration. Differences among treatment means were detected ($P = 0.05$) with the Student-Newman-Keuls' procedure.

RESULTS

Combined data from experiments I, II, III, and IV showed that leaves become more resistant with age. For leaves 5–36 days old at inoculation there was a highly significant ($P = 0.01$) decrease in the number of lesions per square centimeter of leaf area measured at assessment with increases in leaf age at inoculation. The decrease in number of lesions with increasing leaf age occurred at inoculum concentrations of 10^5 and 10^6 spores per milliliter; no significant trend was observed at 10^4 spores per milliliter (Fig. 1A). Combined data from experiments V and VI showed a highly significant decrease ($P = 0.01$) in number of lesions with increasing leaf age from 35 to 70 days when 10^6 spores per milliliter were used, but when 10^4 or 10^5 spores per milliliter were used there was no significant difference in number of lesions (Fig. 1B). For leaves 103 to 126 days old at inoculation (experiment VII), numbers of lesions did not decline significantly with increasing leaf age at any spore concentration. Means of 0.31, 1.19, and 1.42 lesions per square centimeter of leaf were obtained from inoculations with 10^4, 10^5, and 10^6 spores per milliliter, respectively.

The relationship between number of lesions and successive leaf age classes was determined by regression analyses of combined data from experiments I, II, III, and IV, and of combined data from experiments V and VI. With 5- to 36-day-old leaves, a linear relationship between ln ($\log_e$) lesions per square centimeter and leaf age accounted for 90 and 94% of the variation in lesion numbers from inoculation with 10^5 and 10^6 spores per milliliter, respectively (Fig. 2). Slopes for the two regression lines did not differ significantly ($P = 0.01$). With 35- to 70-day-old leaves, a linear relationship between ln lesions per square centimeter and leaf age accounted for 85% of the variation in numbers of lesions from inoculations with 10^6 spores per milliliter (Fig. 3). At 10^6 spores per milliliter, the slope of the regression line for 35- to 70-day-old leaves was about half the slope for 5- to 36-day-old leaves. This indicates that the rate resistance increases in older leaves is only half that of younger leaves.

Highly significant differences ($P = 0.01$) in numbers of lesions between leaves were observed in each of four experiments (I, II, III, and IV) involving leaves less than 40 days old (Table 1). Five- to 20-day-old leaves had significantly more ($P = 0.05$) leaf spot lesions than did 21- to 40-day-old leaves. Lesions per leaf did not appear to differ among 5- to 20-day-old leaves. However, when adjustments

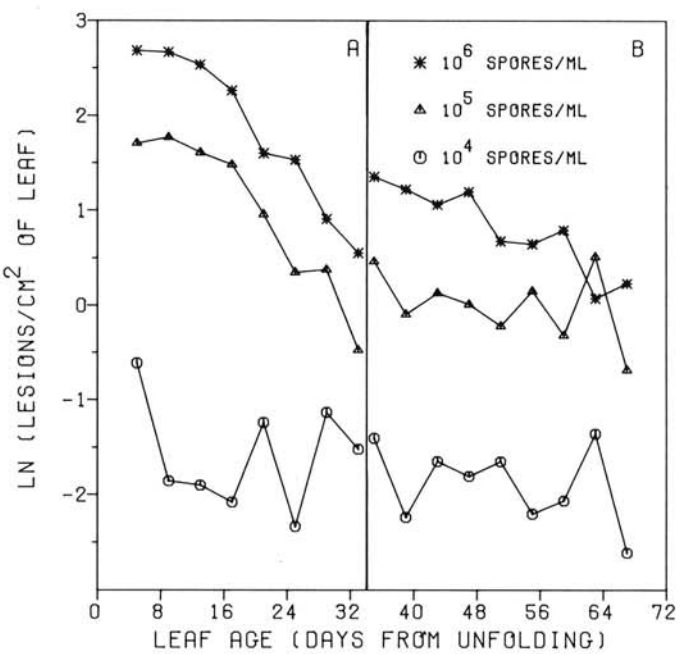

Fig. 1. Number of leaf spot lesions on Montmorency sour cherry leaves of increasing ages inoculated with *Coccomyces hiemalis* at three inoculum concentrations. Lesion numbers were adjusted for leaf area at time of assessment and each value is the average of four (A) and two (B) experiments, respectively.

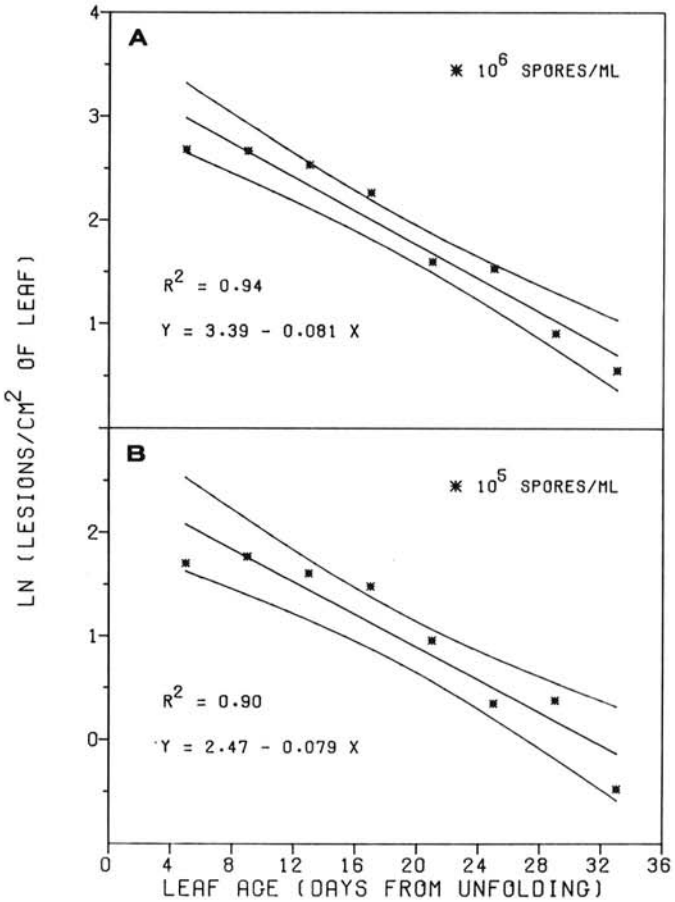

Fig. 2. Linear regression of ln of the number of leaf spot lesions per square centimeter of leaf area 11 days after inoculation on Montmorency sour cherry leaves of increasing age inoculated with *Coccomyces hiemalis* at concentrations of **A**, 10^6 spores per milliliter and **B**, 10^5 spores per milliliter vs leaf age at time of inoculation.

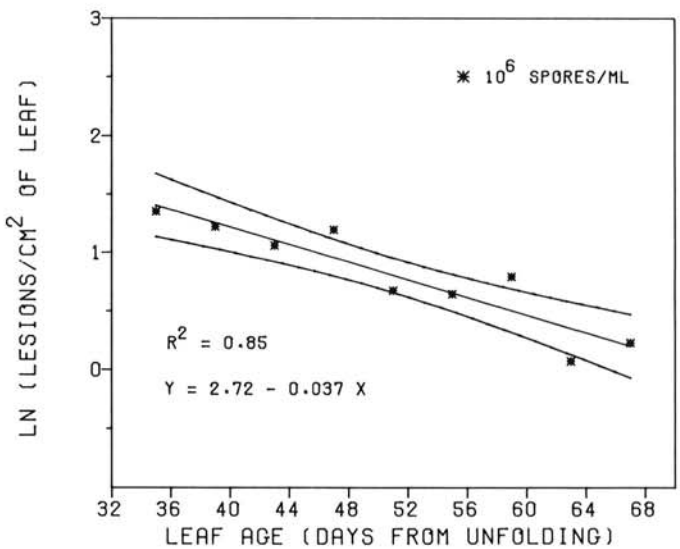

Fig. 3. Linear regression of ln of the number of leaf spot lesions per square centimeter of leaf area 11 days after inoculation on Montmorency sour cherry leaves of increasing age inoculated with *Coccomyces hiemalis* at a concentration of 10^6 spores per milliliter vs leaf age at time of inoculation.

were made for variations in leaf area at time of inoculation or at 11 days later, 5- to 8-day-old leaves had significantly higher ($P=0.05$) numbers of lesions than older leaves.

Five- to 8-day-old leaves had significantly smaller ($P = 0.05$) areas at time of inoculation than leaves 9 days or older. Leaves 13 days or older did not significantly differ ($P= 0.05$) in area at time of inoculation (Table 1). Newly unfolded leaves expanded fully within 16 days, with the expansion rate decreasing exponentially with time. Lesion numbers at 10^5 spores per milliliter declined gradually over the 36-day-period and at 10^6, lesion numbers remained high for 16 days then declined rapidly (Fig. 4).

The relationship of inoculum concentration to lesion number was examined in each experiment to determine if number of lesions was proportional to inoculum concentration as reported by Keitt et al (4). Significant differences ($P=0.05$) in numbers of lesions could be attributed to inoculum concentration in all experiments. With 1- to 32-day-old leaves (experiment I), numbers of lesions did not differ significantly ($P = 0.05$) between 10^2 and 10^4 spores per milliliter, but did increase significantly between 10^4 and 10^5 and between 10^5 and 10^6 spores per milliliter (Fig. 5). The increase from 10^5 to 10^6 was significantly less ($P = 0.05$), as determined with a t-test, than the 10-fold increase expected when a 10-fold higher inoculum concentration was applied. Spore germination on water agar was 90.8, 92.4, and 40.3% after an incubation period of 24 hr and 93.0, 93.0, and 55.7% after 48 hr for 10^4, 10^5, and 10^6 spores per milliliter, respectively.

TABLE 1. Number of leaf spot lesions, before and after adjustment for changes in leaf area, on Montmorency sour cherry leaves of different ages following inoculation with *Coccomyces hiemalis*[x]

Age of leaves (days)	Leaf area[y]		Leaf spot lesions[y]		
	Day of inoculation (cm^2)	Day 11 after inoculation (cm^2)	Number per leaf	Number per cm^2 of leaf on: Day of inoculation	Day 11 after inoculation
5–8	15 a[z]	21 a	444 b	31.0 c	22.0 c
9–12	26 b	31 ab	395 b	15.0 b	12.7 b
13–16	32 bc	35 b	454 b	14.7 b	13.4 b
17–20	37 bc	37 b	376 b	10.7 b	10.6 b
21–24	36 bc	37 b	160 a	4.4 a	4.4 a
25–28	46 c	46 b	88 a	1.8 a	1.8 a
29–32	46 c	47 b	85 a	1.8 a	1.7 a
33–36	43 c	43 b	77 a	1.9 a	1.9 a
37–40	43 c	44 b	93 a	2.4 a	2.4 a

[x]Inoculum: ~0.5 ml containing 10^5 conidia per milliliter.
[y]Means of five replications from experiment IV.
[z]Values in a column followed by the same letter do not differ significantly ($P = 0.05$) according to the Student-Newman-Keuls' procedure.

DISCUSSION

Keitt et al (4) established that cherry leaves were resistant to the leaf spot fungus prior to unfolding and that, once unfolded, leaves were susceptible and remained so throughout the season. The resistance of folded leaves is probably due to lack of mature stomates through which the pathogen normally penetrates. Our results indicate that susceptibility of leaves decreases with age and that the decrease in susceptibility of leaves is expressed more effectively against high rather than low inoculum concentrations. The ratio of number of lesions to number of spores applied decreased with increasing inoculum concentration. The nature of this decrease in infection efficiency is not known, but may be limited by the number of stomates per square centimeter and by reduced germination at higher spore concentrations.

Results of this study can be used to develop standard techniques to assess the resistance of sour cherry selections to *C. hiemalis*. For accurate assessment of resistance, a range of leaf ages should be inoculated and an inoculum concentration high enough to detect leaf age effects should be used. These techniques may allow the selection of resistant plants prior to planting in the field.

Our findings on the relationship of leaf age and inoculum concentration to lesion frequency should be incorporated into the cherry leaf spot prediction system. The environmental favorability index of this system could be modified by a relative susceptibility factor; eg, the sum of the percentages of leaves in each age class that comprise the total canopy multiplied by the relative susceptibility for that age class. Determining the stage of canopy development requires good estimates of number of emerged leaves and the area of those leaves. A model for predicting leaf emergence from cumulative degree-day data has been validated (3) and a model for estimating leaf expansion is under development by the first and third authors.

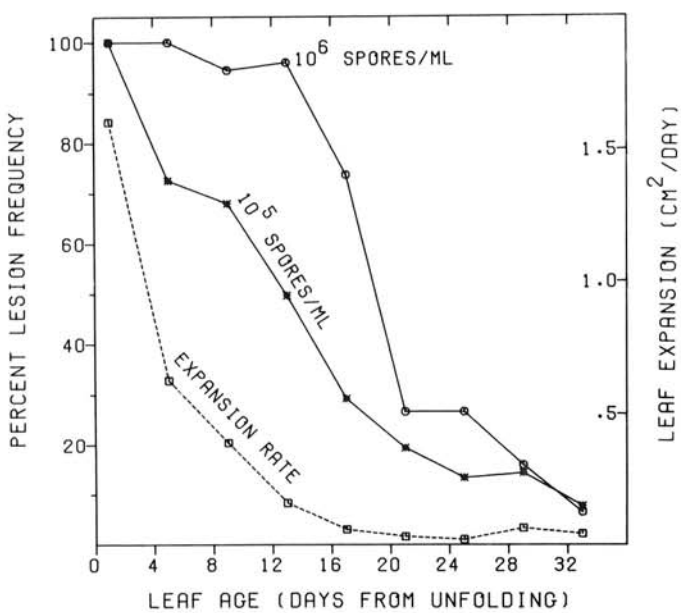

Fig. 4. Relationship of percent of maximum lesion frequency on 1- to 4-day-old Montmorency sour cherry leaves inoculated with *Coccomyces hiemalis* at 10^5 and 10^6 spores per milliliter to leaf expansion rate for 1- to 36-day-old leaves.

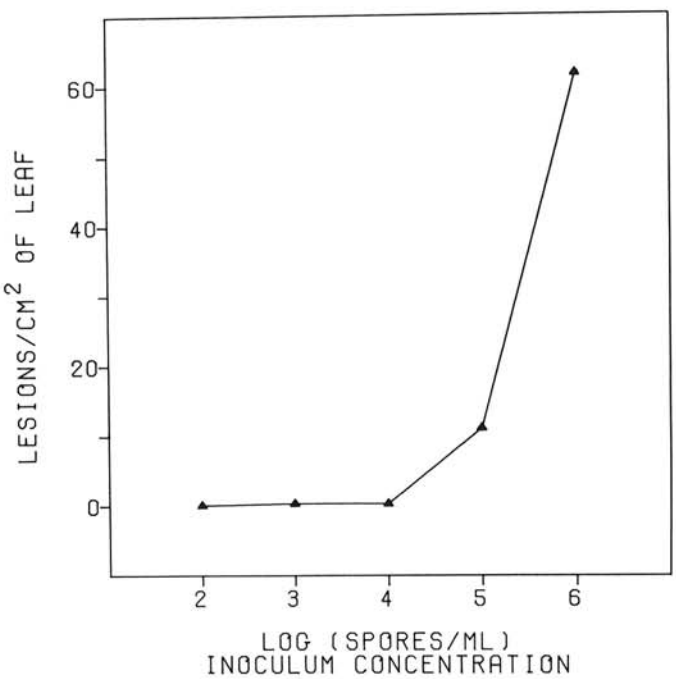

Fig. 5. Relationship of lesions per square centimeter of leaf at time of inoculation on 1- to 29-day-old Montmorency sour cherry leaves to $\log_{10}$ inoculation concentration of *Coccomyces hiemalis* conidia.

These data suggest that leaf spot control is very important early in the season because leaves are most susceptible between the time they unfold and full expansion. Fungicide control strategies should insure good coverage during the period of leaf emergence and expansion and take advantage of the fact that older leaves are less susceptible. Growers currently do not adjust fungicide applications to account for changes in resistance during the season. In seasons when control is good during canopy development, leaf spot should be less of a problem in August and September (2). Since susceptibility decreases with age, inoculum concentration in the orchard will be the key factor in determining whether leaf spot will be a problem after terminal growth ceases.

LITERATURE CITED

1. Eisensmith, S. P., and Jones, A. L. 1981. A model for detecting infection periods of *Coccomyces hiemalis* on sour cherry. Phytopathology 71:728-732.
2. Eisensmith, S. P., and Jones, A. L. 1981. Infection model for timing fungicide applications to control cherry leaf spot. Plant Dis. 65:955-958.
3. Eisensmith, S. P., Jones, A. L., and Flore, J. A. 1980. Predicting leaf emergence of 'Montmorency' sour cherry from degree-day accumulations. J. Am. Soc. Hortic. Sci. 105:75-78.
4. Keitt, G. W., Blodgett, E. C., Wilson, E. E., and Magie, R. O. 1937. The epidemiology and control of cherry leaf spot. Wisc. Agric. Exp. Stn. Res. Bull. 132. 118 pp.
5. Krause, R. A., and Massie, L. B. 1975. Predictive systems: Modern approaches to disease control. Annu. Rev. Phytopathol. 13:31-47.
6. Populer, C. 1978. Changes in host susceptibility with time. Pages 239–262 in: J. G. Horsfall and E. B. Cowling, eds. Plant Disease, An Advanced Treatise. Vol. II. How Disease Develops in Populations. Academic Press, New York. 436 pp.