Effects of Interrupted Wet Periods on Infection of Sour Cherry by *Coccomyces hiemalis*

S. P. Eisensmith, A. L. Jones, and C. E. Cress

Graduate research assistant and professor, Department of Botany and Plant Pathology and the Pesticide Research Center; and professor, Department of Crop and Soil Sciences, Michigan State University, East Lansing 48824, respectively.
Michigan Agricultural Experiment Station Journal Series Article 10108.
The authors acknowledge technical assistance by Nicholas Bolgiano.
Accepted for publication 17 September 1981.

ABSTRACT

Eisensmith, S. P., Jones, A. L., and Cress, C. E. 1982. Effects of interrupted wet periods on infection of sour cherry by *Coccomyces hiemalis*. Phytopathology 72:680-682.

Montmorency sour cherry trees inoculated with conidia of *Coccomyces hiemalis* were subjected to interrupted wet periods (IWP) and continuous wet periods (CWP) of various durations to determine the effect of dry interruptions on infection by the leaf spot fungus. Fewer lesions per square centimeter of leaf resulted from IWP than from CWP in each of four series of experiments. A trend of decreasing infection with increasing length of dry interruption was observed when initial and final wet periods were 4 and 8 hr. Infection from IWP with an initial 4-hr wet period, 1- to 48-hr dry interruptions, and a final 8-hr wet period was greater than from a 4-hr CWP, but not statistically different from an 8-hr CWP. When the dry period was 108 hr, infection was greater than from a 4-hr CWP, but less than from an 8-hr CWP. Trees allowed to dry up to 16 hr after inoculation developed less infection than trees subjected to wetting immediately after inoculation. Infection on trees given initial wet periods of less than 12 hr was less than on trees with longer initial wet periods.

Additional key words: epidemiology, *Prunus cerasus*.

We have developed a system for predicting infection of sour cherry (*Prunus cerasus* L. 'Montmorency') by *Coccomyces hiemalis* Higgins and scheduling fungicide applications to control the cherry leaf spot disease (1,2). In this prediction system we compute an environmental favorability index using hours of continuous leaf wetness from rain and average air temperature during the wet period. In practice, wet periods are not always continuous, which leads to the problem of how to interpret and predict the results of wetting periods that occur close together.

Keitt et al (4) found that interrupted wet periods (IWP) resulted in less infection of sour cherry than continuous wet periods (CWP), but they examined only extremely long (>96 hr) dry interruptions and did not include certain control treatments necessary for interpretation. Furthermore, they did not analyze the effects of leaf size, leaf age, and inoculum concentration, which affect disease severity (3).

The objectives of this study were to confirm that IWP result in less infection than CWP, and to determine if interruptions early in a wetting period reduce infection more than interruptions late in a wetting period.

MATERIALS AND METHODS

The effects of interrupted wetting on infection by the leaf spot fungus were examined in four series of experiments performed in the greenhouse with 4-yr-old Montmorency cherry trees on *Prunus mahaleb* rootstock. Trees with three to five shoots each were maintained as previously described (3). Four to 12 fully expanded, 12- to 16-day-old leaves per tree were inoculated with conidial suspensions of *C. hiemalis* with an atomizer (3). Concentrations of conidia in the suspensions were determined with the aid of a hemacytometer and were adjusted to 3 to 7×10^5 spores per milliliter.

Inoculated trees were subjected to either CWP, or to IWP consisting of an initial wet period, a dry interruption, and a final wet period. The total duration of an IWP was the time in hours from inoculation to the end of the final wet period. Trees were placed in a mist chamber in the light at 20–24 C during wet periods, and in a greenhouse with relative humidities of 40–90% and temperatures of 18–28 C during dry interruptions. The leaves dried quickly after the trees were removed from the mist chamber. Following treatment, the trees were held in the greenhouse and examined for leaf spot symptoms 11 days after inoculation.

Experiment I and experiment II were conducted to examine the effect of dry interruptions of increasing length on the level of infection. Two treatments in each experiment were 4- and 8-hr CWP; the remaining 12 treatments were arranged in a factorial design with the type of wet period (IWP or CWP) as one factor, and length of the dry interruption as the second factor. All IWP treatments consisted of an initial 4-hr wet period separated from a final 8-hr wet period by dry interruptions of various lengths. In experiment I, IWP with dry interruptions of 4, 8, 12, 16, 24, and 36 hr were compared with CWP of 16, 20, 24, 28, 36, and 48 hr, respectively. Treatments within a trial were not replicated, but experiment I was repeated four times. In experiment II, IWP with dry interruptions of 1, 2, 3, 6, 48, and 108 hr were compared with CWP of 13, 14, 15, 18, 60, and 120 hr, respectively. There were two replications of each treatment, and experiment II was repeated three times.

Experiments III and IV also were conducted to determine if a dry interruption early in a wetting period reduced infection as much as an interruption late in the wet period. Both experiments contained six treatments in a randomized complete block design; treatments in experiment III were replicated four times and those in experiment IV were replicated three times. Both experiments were repeated three times. In experiment III, trees were subjected to initial wet periods of 0, 4, 8, 12, and 16 hr; a dry interruption of 8 hr; and final wet periods of 16, 12, 8, 4, and 0 hr, respectively, to give IWP of 24 hr. A 24-hr CWP treatment served as a control. In experiment IV, trees were subjected to initial wet periods of 0, 8, 16, 24, and 32 hr; a dry interruption of 16 hr; and second wet periods of 32, 24, 16, 8, and 0 hr, respectively, to give IWP of 48 hr. The sixth treatment was a 48-hr CWP.

Disease severity was assessed by counting all lesions on the undersurface of inoculated leaves 11 days after inoculation. At the time of disease assessment, the area of each leaf was measured with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0031-949X/82/06068003/$03.00/0

an area meter (Model LI-3000, Lambda Instrument Corp., Lincoln, NE 68504). Numbers of lesions per square centimeter of leaf were calculated and subjected to analyses of variance after a logarithmic transformation to insure homogeneity of variance (5), then converted back to the original scale for tabulation. Differences among treatment means were detected using the least significant difference or Duncan's multiple range test (5).

Percent reduction in numbers of lesions per square centimeter attributed to the dry period was used to evaluate the relationship between IWP and CWP treatments, and was calculated for each IWP and CWP treatment of the same duration. In cases where the IWP mean exceeded its corresponding CWP mean, percent reduction in numbers of lesions per square centimeter was set at zero. This adjustment is possible because no statistically significant increases over the control means were found. These data were subjected to analyses of variance after arcsine square root transformation, and differences among treatment means were detected using Duncan's multiple range test (5).

TABLE 1. Effects of lengths of interrupted wet periods (IWP) or continuous wet periods (CWP) following inoculation of sour cherry with conidia of *Coccomyces hiemalis*

Wet treatment				Leaf lesions/cm² developed on trees that received:		Reduction in infection[u] (%)
IWP			CWP			
Wet (hr)	Dry (hr)	Wet (hr)	Wet (hr)	IWP	CWP	
Experiment I						
-	-	-	4	...	0.3[v]	...
-	-	-	8	...	2.7	...
4	4	8	16	4.8[v]	6.8	20.5 a[x]
4	8	8	20	6.2	14.0***[w]	50.9 a
4	12	8	24	8.4	14.0***	32.0 a
4	16	8	28	5.2	14.0***	60.5 a
4	24	8	36	3.9	14.5***	73.1 a
4	36	8	48	1.6	17.5***	84.9 a
Mean response				4.5	13.0[y]	
Experiment II						
-	-	-	4	...	0.2[z]	...
-	-	-	8	...	3.6	...
4	1	8	13	2.8[z]	4.6	31.8[x] a[x]
4	2	8	14	1.5	7.2***[w]	75.5 b
4	3	8	15	3.1	6.4*	35.2 a
4	6	8	18	2.1	7.8***	72.0 b
4	48	8	60	1.5	12.7***	87.6 bc
4	108	8	120	0.5	11.3***	97.3 c
Mean response				1.6	7.9[y]	

[u] Calculated by dividing the difference between CWP and IWP lesion numbers by the lesions per square centimeter of leaf from CWP times 100 for each dry interruption.
[v] Means of four nonreplicated trials.
[w] Mean values between IWP and CWP columns differ significantly ($P = 0.05$, *; $P = 0.001$, ***) according to the least significant difference test performed on $\log_e$-transformed data.
[x] Values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test performed on arcsine square root-transformed data.
[y] Values differ significantly ($P = 0.001$) as determined by analysis of variance of disease data subjected to $\log_e$ transformation before analysis.
[z] Means of three trials with two replications of treatments in each trial.

RESULTS

Interrupted wet periods resulted in significantly ($P = 0.001$) fewer lesions per square centimeter of leaf area than CWP (Table 1). Mean reductions in lesions for the six IWP treatments were 8.5 and 6.3 lesions per square centimeter of leaf for experiments I and II, respectively. When IWP and CWP of equal lengths were compared, IWP had significantly fewer lesions than CWP, except for the 4-hr dry interruption in experiment I and the 1-hr dry interruption in experiment II. Increasing the duration of the dry period between wet periods tended to reduce infection in experiment I, and significantly reduced infection in experiment II, except for the 3-hr dry interruption.

Infection levels in the 4- and 8-hr CWP were compared with infection in the other 12 treatments in both experiments I and II to determine if infection from an IWP can be attributed to the initial or the final wet period. Log_e of the number of lesions per square centimeter from the 4-hr CWP was significantly less ($P = 0.05$, range test not shown) than the $\log_e$ of the number of lesions for all other treatments within each experiment (Table 1). Log_e of the number of lesions from the 8-hr CWP did not differ significantly (range test not shown) from IWP treatments having dry interruptions of 1 to 48 hr in experiment I and from all but the 108-hr dry interruption in experiment II. An IWP with an initial 4-hr wet period, a 108-hr dry interruption, and a final 8-hr wet period had significantly more ($P = 0.05$) lesions than a 4-hr CWP and significantly fewer ($P = 0.05$) lesions than an 8-hr CWP.

In experiments III and IV the length of the initial and final wet periods were varied but the dry period was maintained at 8 or 16 hr (Table 2). The ranking of IWP treatment means for lesions per square centimeter of leaf and for percent reduction in infection

TABLE 2. Effects of timing of interruptions in the wet period following inoculation of sour cherry with *Coccomyces hiemalis*

Wet treatment			Lesions/cm² of leaf area				Percent reduction of infection[w]			
			Trial number			Mean response	Trial number			Mean response
Wet (hr)	Dry (hr)	Wet (hr)	1	2	3		1	2	3	
Experiment III										
24	+ 0	+ 0	2.7[x]c[y]	6.7[x]c[y]	2.4[x]b[y]	3.5 d[y]	...	...	...	...
0	+ 8	+ 16	2.1 bc	3.3 ab	1.5 a	2.2 ab	38.8[x]ab[y]	56.0[x]ns	67.2[x]b[y]	54.1 b[y]
4	+ 8	+ 12	1.3 a	2.8 a	1.4 a	1.8 a	81.3 c	64.6 ns	67.2 b	71.4 b
8	+ 8	+ 8	1.7 ab	3.9 ab	1.4 a	2.1 ab	54.5 bc	46.4 ns	70.0 b	57.2 b
12	+ 8	+ 4	2.6 c	4.4 b	1.5 a	2.6 bc	14.5 a	32.4 ns	60.1 b	34.5 a
16	+ 8	+ 0	2.7 c	4.4 b	2.6 b	3.2 cd	18.9 ab	33.0 ns	12.4 a	20.9 a
Experiment IV										
48	+ 0	+ 0	7.1[z]b[y]	6.1[z]b[y]	59.7[z]ns	13.7 b[y]	...	...	...	...
0	+ 16	+ 32	0.9 a	4.2 b	24.8 ns	4.6 a	82.7[z]b[y]	23.8[z]ns	57.1[z]b[y]	55.1 bc[y]
8	+ 16	+ 24	0.8 a	3.1 ab	30.7 ns	4.1 a	88.7 b	52.3 ns	47.5 b	64.5 c
16	+ 16	+ 16	1.2 a	1.5 a	27.8 ns	3.7 a	76.1 b	74.4 ns	37.8 ab	63.4 c
24	+ 16	+ 8	1.4 a	3.1 b	48.7 ns	6.0 a	74.4 b	51.0 ns	9.1 a	42.9 b
32	+ 16	+ 0	18.7 b	3.4 b	39.5 ns	13.6 b	2.9 a	45.8 ns	31.7 ab	23.3 a

[w] Calculated by dividing the difference between lesion numbers from the first treatment in each experiment and the remaining five treatments by the lesions per square centimeter of leaf from the first treatment times 100.
[x] Means of four replications.
[y] Values in a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test performed on transformed data.
[z] Means of three replications.

were not consistent among trials in experiments III and IV. However, an increase in infection with increased length of the initial wet period was noted in experiment III but not experiment IV. Initial wet periods of 4, 8, and 12 hr in experiment III resulted in means of 1.8, 2.1, and 2.6 lesions per square centimeter of leaf, respectively. Sixteen- and 24-hr CWP in experiment III, and 32- and 48-hr CWP in experiment IV, resulted in more infection than IWP treatments of 24 and 48 hr, respectively. Trees allowed to dry 8 or 16 hr after inoculation and subjected to wet periods of 16 or 32 hr, respectively, also had less disease than trees subjected to initial wet periods of 16 or 32 hr immediately after inoculation.

DISCUSSION

Although the four experiments used shorter dry durations than those employed by Keitt et al (4), our results support their conclusion that IWP result in less infection than CWP. Because of this, an improved predictive system is needed to account for reduced cherry leaf spot infection from IWP. In our predictive system (1), we arbitrarily treated any IWP with dry interruptions <8 hr as a CWP, and IWP with dry interruptions >8 hr as separate CWP. Our study indicates that the system's infection predictions are too severe when IWP are treated as a CWP.

Our data indicate that early (≤ 8 hr) dry interruption result in fewer lesions than dry interruptions after a long (8–16 hr) initial wet period. Moist chamber experiments by Keitt et al (4) indicate light infection from a 4-hr wet period, and increased infection with longer wet periods. The increase in infection, however, is not linear and about one-half of the infection obtained from a 70-hr CWP occurs in the first 12 hr. Dry periods after 4 and 8 hr of a wet period were more disruptive than dry periods after 12 hr of continuous wetting, although the reduction in infection after 8 hr was not statistically significant.

When wet periods are not continuous, the question arises as to how much each wet period contributes to the final incidence of infection. In our studies, trees subjected to an 8-hr final wet period 1 to 48 hr after a 4-hr initial wet period had levels of infection not different from a CWP of 8 hr. However, when Keitt et al (4) subjected trees to a 30-hr final wet period 96 to 192 hr after a 16-hr initial wet period, the infection level was equivalent to that from a 16-hr CWP. Thus, the contribution of a second wet period appears to be most important when the initial wet period is shorter than 16 hr.

The variability of our data is great. One would expect dry interruptions of 1, 3, or 4 hr to reduce infection by half as much as 2-, 6-, or 8-hr dry interruptions (Table 1). Furthermore, a tenfold increase in infection frequency among trials in experiment IV indicates that other factors strongly influence infection frequency (Table 2). Some of the variability can be attributed to the low levels of infection that result from CWP of 4 or 8 hr. A 4-hr CWP is the minimal wet period for infection under the conditions of our experiments. Fluctuating temperatures and relative humidities during the dry interruption and incubation period may have contributed to the variability among trials within experiments. Other sources of variation among trials are inoculum quality and the amount of inoculum deposited on a leaf.

To predict infection severity from IWP requires an understanding of the underlying mechanism of spore germination and penetration during interrupted wetting. Keitt et al (4) found that conidia of *C. hiemalis* on glass slides subjected to dry periods show reduced germination; and after a 12-hr wet period and 12-hr dry period, no additional germination or germ tube extension occurred upon rewetting. If the second wet period does not promote germination or germ tube extension, it may increase survival of infections initiated during the first wet period. Because leaves might be expected to provide a much better substrate than glass for spore survival, spore germination and development should be monitored on the leaf surface during IWP and CWP to establish the mechanism for increased infection upon rewetting.

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., Sjulin, T. M., Jones, A. L., and Cress, C. E. 1982. Effects of leaf age and inoculum concentration on infection of sour cherry by *Coccomyces hiemalis*. Phytopathology 72:574-577.
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. 117 pp.
5. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. 2nd ed. McGraw-Hill, New York. 633 pp.