Influence of Temperature and Wetting Period on Inoculum Production by *Monilinia fructicola* in Peach Twig Cankers

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ABSTRACT

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Overwintering cankers in peach twigs caused by the brown rot fungus, *Monilinia fructicola*, were studied to identify the relationships of wetting period and temperature on sporulation. Sporulation was observed on blighted blossoms, peduncles, abscission scars, and cankers resulting from contact with infected fruits. The frequency of sporulation on overwintered infected tissues was greater at 15 and 23°C than at 4 or 11°C. Twelve hours of wetting was sufficient at all temperatures studied (5 to 23°C) for sporulation to occur, but the number of twig cankers supporting sporulation increased with time of wetting up to 72 h. Given the additional moisture requirements for spore germination, ingress, and infection, 17 to 30 h of wetting or high humidity during bloom may be needed for blossom blight to occur unless viable conidia are already present as a result of previous wetting periods.

Additional keywords: *Prunus persica*

The mild, humid climate of the southeastern United States is especially suitable to the development of brown rot, caused by *Monilinia fructicola*, in peach (*Prunus persica* (L.) Batsch), nectarine (*P. persica* var. *nuspersica* (Suckow) D. K. Schneid.), and other stone fruits. Ripening fruits are the most susceptible developmental stage, but infections also may be numerous in opening flowers. Blossom blight resulting from such infections does not commonly result directly in serious crop loss, but conidia on the infected flowers and twigs are produced for weeks or months after the initial infections (1,2,8,9,13). These spores may be important as inoculum for ripening fruits later in the season (1,2,4,5,9).

Inoculum available to infect opening flowers includes ascospores from apothecia that develop at flowering from mummies on the ground, or conidia that form on twig cankers, peduncles, or mummies resulting from infections that occurred in previous years (1,6,8,9,13). In orchards that are well

managed, apothecia are uncommon in the Southeast (8), and conidia are presumed to initiate most blossom infections.

The importance of high humidity and moist surfaces in the development of blossom blight has been studied by Weaver (10) on peach flowers and Wilcox (11) on cherry blossoms. As little as 5 h wetting was needed for infection to occur even at temperatures well below the optimum of 24°C if the inoculum level was high (11). However, we have observed (*unpublished data*) that in South Carolina peach orchards, blossom blight seldom becomes widespread unless wet weather during bloom persists for 24 h or more. Additionally, visible sporulation was absent on twig cankers during the winter, but in late winter (February) sporodochia developed when wet weather persisted for several days. These observations imply that moisture requirements for production of conidia may be important as a contributing factor in epidemics of blossom blight. Since apothecia are not numerous in South Carolina peach orchards (8) and mummies usually are removed with winter pruning, we regard twig cankers as the principal overwintering site for *M. fructicola*. Therefore, we studied the effects of temperature and wetting periods on inoculum production on peach twig cankers caused by *M. fructicola*.

MATERIAL AND METHODS

Sample collection. To determine when first sporulation occurred in the spring, dormant twigs were harvested in 1991 and 1992 from trees in commercial peach or-

chards where many twig cankers resulted from a brown rot epidemic in preceding seasons. The cankers originated from blighted blossoms, pedicels, and cankers resulting from rotted fruit resting against a twig, which then became infected. In 1991, cv. Junegold was used. Arbitrarily selected twigs were cut randomly with hand pruners on 15 and 29 February and 8 March, approximately 4, 2, and 1 week prebloom, respectively. In 1992, twigs were cut in a similar fashion from trees of cv. Harvester on 21 and 27 February and 7 March, approximately 2, 1, and 0 week prior to bloom. Twigs collected in 1991 were from trees that had not been sprayed the previous season, whereas those used in 1992 had been sprayed with fungicides commonly used for brown rot control (propiconazole, iprodione, and wettable sulfur) the previous years. In each instance, infection by *M. fructicola* had progressed from decayed fruit or blighted flowers into the twigs. When the twigs were harvested in 1991, cankers resulting from blighted blossoms, pedicels, and contact with infected fruit were numerous.

Laboratory assays. After collection, infected twigs were placed in unsealed plastic bags in dry containers and transported at ambient temperatures of 10 to 20°C to the laboratory. Two to 24 h elapsed between collection and assay. In the laboratory, twigs were trimmed to 8 to 10 cm so that each twig had at least one canker. Twigs were placed in a plastic screen basket and misted with distilled water until runoff. Each moist twig then was placed in a screw-cap test tube measuring 1.3 × 12 cm, with the distal end of the twig in contact with moist cotton placed in the bottom of the tube prior to insertion of the twig. Each tube was inverted, drained of excess water, and capped. Wetting periods were measured from the time that twigs were moistened until the cankers were examined for sporulation. Forty and 36 twigs were used per temperature treatment on each sampling date in 1991 and 1992, respectively. The tubes were capped and placed in one of four sets of racks; each rack then was placed in an incubator preset at 5, 11, 15, or 23°C. Cankers were observed for sporulation after 12, 24, 36, 48, and 72 h of wetting at each temperature in 1991 and, in addition, at 3, 6, and 9 h in 1992. Each canker was inspected under a dissecting microscope (magnification, ×20 to ×60) at

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the time intervals stated. When necessary, twigs were removed from tubes for examination. Spore identification was based on visual observation of conidia and identification occasionally was confirmed by isolation of the fungus. When sporulation was observed, the observation was recorded and the canker was removed from the experiment.

Statistical procedures. The experiment was a randomized complete block, split plot in time design. There were three blocks (sampling times) and it was done in 2 years (1991 and 1992). Analyses were done using the SAS GLM procedure (SAS version 6.17, SAS Institute, Cary, NC) in which blocks were fitted as the random effect, temperature as the whole plot effect and hours of wetting and the interaction hours $\times$ temperature interactions as the sub-plot effects.

RESULTS

Analysis of response to temperature and wetting period. There was a highly

significant response of sporulation on twig cankers to both temperature and time of wetting in 1991 and 1992 (Table 1). In 1991, the interaction of temperature and hours of wetting was not significant, indicating that the response to hours of wetting was similar at all temperatures tested. However, in 1992, an interaction of temperature with wetting period was observed. At 5 and 11°C very little increase in sporulation occurred over the 72-h period of wetting, whereas at 23°C and especially 15°C, sporulation increased over the 72-h period (Table 2).

Frequency of sporulation. Among the 480 twig cankers assayed in 1991, 149 (31%) supported sporulation by *M. fructicola* after 72 h of wetting at temperatures between 5 and 23°C. Among the cankers supporting sporulation, most were associated with blighted blossoms or peduncles, but some also were associated with abscission scars and contact with infected fruits.

Response to wetting duration. At least 12 h of wetting was required for sporula-

tion to be observed regardless of temperature. No sporulation was visible at 3, 6, or 9 h of wetting, even at the most favorable temperature (15 or 23°C). Sporulation was visible at 12 h over the entire range of temperatures (5 to 23°C) in 1991 (Table 3), although very little occurred at 12 h at 5 or 11°C.

The frequency of sporulation on cankers was less in 1992 than in 1991 (Table 2), but the trends were similar. At least 12 h of wetting was necessary for sporulation to occur at 15 or 23°C but none was observed until 36 h of wetting at 5 or 11°C.

Sporulation response to temperature and wetting period. In 1991, the percentage of cankers with sporulation increased with time of wetting up to 72 h, but little increase was observed after 60 h at 11, 15, or 23°C (Table 3). At 5°C, the number of cankers with sporulation continued to increase slowly throughout the 72-h period. At each time interval more cankers supported sporulation at 15 or 23°C than at 5 or 11°C. Although a larger percentage of cankers supported sporulation at 23 than at 15°C (Table 3), the differences were not significant ($P = 0.05$).

In 1992, little sporulation occurred at 5 or 11°C throughout the 72-h period of wetting (Table 2) and none was observed through the first 24 h. At 15 and 23°C the percentage of cankers with sporulation was smaller than in 1991 and increased slowly throughout the 72-h wetting period (Table 2).

In both years, replicates differed significantly ($P = .0001$) in the percentage of cankers with sporulation (Table 1). In 1991, the cumulative means of sporulation for all temperatures combined were 16.9, 42.5, and 33.8% for the first, second, and third sampling times, respectively. In 1992, the cumulative means were 3.5, 9.0, and 8.3% respectively. However, in the third

Table 1. Analysis of variance of temperature and wetting period on sporulation of *Monilinia fructicola* on peach twig cankers in 1991 and 1992

Source	df	SS	Mean square	F	P > F
1991					
Replicate (Rep)	2	6,404.34	3,202.17	124.21	<0.0001
Temp	3	10,986.37	3,662.12	7.67	<0.0178
Rep*temp error (A)	6	2,864.41	477.40	18.52	<0.0001
Hours	5	4,150.43	830.09	32.20	<0.0001
Temp*hours	15	573.52	38.23	1.48	0.1582
Error (B)	40	1,031.25	25.78		
Corrected total	71	26,010.33			
1992					
Rep	2	236.22	118.11	21.57	<0.0001
Temp	3	948.06	316.02	10.23	<0.0089
Rep*temp error (A)	6	185.27	30.88	5.64	0.0004
Hours	5	288.15	57.63	10.52	<0.0001
Temp*hours	15	194.43	12.96	2.37	0.0200
Error (B)	32	175.22	5.48		
Corrected total	63	1,674.38			

Table 2. Sporulation of *Monilinia fructicola* on peach twig cankers in 1992 in response to temperature and wetting period^y

Temperature	Hours of wetting						Average
	12 ^z	24	36	48	60	72	
5	0.0 a	0.0 a	0.8 a	0.8 a	1.2 a	1.2 a	0.7 a
11	0.0 a	0.0 a	0.8 a	0.8 a	1.2 a	1.2 a	0.7 a
15	2.5 a	7.5 b	7.5 c	9.2 c	11.2 c	13.8 c	8.6 c
23	0.8 a	3.3 a	5.0 b	5.8 b	6.2 b	6.2 b	4.6 b

^y Data show percentage of cankers with sporulation at each wetting period and temperature. Each number represents an average of three replicate sampling times.

^z Means in each column followed by the same letter are not significantly different (least significant difference = 3.7, $P = 0.05$).

Table 3. Sporulation of *Monilinia fructicola* on peach twig cankers in 1991 in response to temperature and wetting period^x

Temperature (°C)	Hours of wetting						Average ^y
	12	24	36	48	60	72	
5	0.8	2.5	6.8	6.8	7.5	10.0	5.7 a
11	3.3	10.8	21.7	24.2	26.7	26.7	18.9 a
15	15.8	25.8	32.5	37.5	40.8	40.8	32.2 ab
23	19.0	31.7	37.5	44.2	45.8	46.7	37.5 b
Mean ^z	9.8 a	17.7 b	24.6 c	28.1 cd	30.2 d	31.0 d	

^x Data show percentage of cankers with sporulation at each wetting period and temperature. Each number is an average of three replicate sampling times.

^y Means followed by the same letter in the average temperature column are not significantly different (least significant difference = 17.4, $P = 0.05$).

^z Means followed by the same letter across wetting periods are not significantly different (least significant difference = 3.7, $P = 0.05$).

sampling in 1992, sporulation of *M. fructicola* at 60 and 72 h of wetting was hidden by growth of competing fungi and the actual cumulative percent may have been more than 8.3%.

DISCUSSION

Corbin and Cruickshank (3) found that for sporulation of *M. fructicola* on apricot leaves, mummies, and peduncles, moisture content of tissues was more important for sporulation than relative humidity. Constant high relative humidity resulted in absorption of moisture from the atmosphere sufficient to induce sporulation. Presoaking infected fruitlets and peduncles in water, followed by exposure to 98% relative humidity at 20°C, resulted in sporulation 12 to 16 h after the presoaking period. Results from our experiments support the report of Corbin and Cruickshank (3) that this period of exposure appears to be approximately 12 h or more and are consistent with our observations in commercial peach orchards.

In our experiments, canker surfaces remained wet for the entire 72-h duration, and influences of variable humidity were not studied. The onset of sporulation appears to be regulated to some degree by temperature and perhaps also by breaking of dormancy. Some sporulation was observed in 1991 after 12 h of wetting even at relatively low temperatures (5 and 11°C), and the frequency of sporulation increased as the time of flowering approached. The frequency of cankers supporting sporulation increased with length of the wetting period up to 48 h, but only a slight increase was evident thereafter.

In 1991, at all temperatures combined, 31% of cankers examined supported sporulation, but only 6.3% did so in 1992. We cannot account for the low percent with sporulation in 1992, but past management may have been a factor. The orchard from which infected twigs were collected in 1992 received preharvest applications of triforine and iprodione fungicides in 1991, whereas the twigs collected in 1991 had not received fungicide sprays in 1990. Some fungicides can suppress sporulation of *M. fructicola* on infected tissues (7,12), but the length of time that sporulation can be suppressed by fungicides or the fungus eliminated is unknown. However, other environmental conditions may have been

more important. In 1992, the frequency of sporulation was low on twigs collected from several widely scattered orchards, including some that did not receive fungicides in 1991 (*unpublished data*). Environmental factors that encourage or suppress sporulation may include some that affect a wide area in addition to those related to specific orchard cultural practices. Our experimental methods did not reveal whether *M. fructicola* failed to survive in cankers that did not support sporulation.

Some fundamental aspects of the biology of *M. fructicola* that might be used to develop a predictive model for blossom blight on peaches now are known, but others are still to be discovered. The time-temperature-moisture relationships first described by Weaver (10) for peach flowers were confirmed by Wilcox (11) on cherries, but Wilcox found that inoculum levels also are important. We do not have an effective way to quantify inoculum levels for *M. fructicola* in stone fruit orchards. Implementation of an effective predictive model probably must incorporate aspects of a total management program to suppress inoculum levels in and near peach orchards, including the effects of temperature and wetting on the number of conidia produced on active cankers and other sources of inoculum. Our field observations (*unpublished data*) indicate that, in well-managed orchards, fungicide sprays for blossom blight control often can be omitted if wetting periods during bloom do not exceed 24 h. However, if relative humidity or moisture levels needed for conidia to be produced on twig cankers occur just before bloom, significant amounts of inoculum might be available when the first flowers open. In Australia, conidia placed in the canopy of apricots having mature fruit remained viable for at least 4 days (5). We need to know whether conidia remain viable and infective for open flowers during short wetting periods that might occur during bloom in the humid conditions that usually prevail in the southeastern United States.

In South Carolina, daily mean temperatures commonly experienced during moist periods in the time of flowering of peaches usually are near 10 to 12°C, although cooler or warmer temperatures may occur. The comparatively slow production of

conidia at these temperatures coupled with the moisture-temperature requirements for infection (10,11) help to explain why blossom blight usually does not become severe unless high relative humidity or wetting periods persist for time intervals approaching or exceeding 24 h.

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LITERATURE CITED

1. Biggs, A. R., and Northover, J. 1985. Inoculum sources for *Monilinia fructicola* in Ontario peach orchards. *Can. J. Plant Pathol.* 7:302-307.
2. Byrd, R. F. W., and Willetts, H. J. 1976. The Brown Rot Fungi of Fruit, Their Biology and Control. Pergamon Press, New York.
3. Corbin, J. B., and Cruickshank, I. A. M. 1963. Environment and sporulation in phytopathogenic fungi. V. *Monilinia fructicola* (W.) Honey: Effect of water relation on regeneration of conidia in vivo. *Aust. J. Biol. Sci.* 16:99-110.
4. Dunegan, J. C., and Goldsworthy, M. C. 1968. The control of blossom blight and its relation to brown rot of Red Bird peaches at harvest. *Plant Dis. Rep.* 32:136-137.
5. Jenkins, P. T. 1968. The longevity of conidia of *Sclerotinia fructicola* (Wint.) Rehm under field conditions. *Aust. J. Biol. Sci.* 21:937-945.
6. Kable, P. F. 1965. The fruit peduncle as an important overwintering site of *Monilinia fructicola* in the Murrumbidgee Irrigation Area. *Aust. J. Exp. Agric. Anim. Husb.* 5:172-175.
7. Kable, P. F. 1976. Use of benzimidazole fungicides on peach twigs during late dormancy to suppress sporulation by *Monilinia fructicola*. *J. Hort. Sci.* 51:261-265.
8. Langraf, F. A., and Zehr, E. I. 1982. Inoculum sources for *Monilinia fructicola* in South Carolina peach orchards. *Phytopathology* 72:185-190.
9. Ogawa, J. M., Zehr, E. I., Bird, G. W., Ritchie, D. F., Uriu, K., and Uyemoto, J. K., eds. 1995. Compendium of Stone Fruit Diseases. American Phytopathological Society, St. Paul, MN.
10. Weaver, L. O. 1950. Effects of temperature and relative humidity on occurrence of blossom blight of stone fruits. *Phytopathology* 40:1136-1153.
11. Wilcox, W. F. 1989. Influence of environment and inoculum density on the incidence of brown rot blossom blight of sour cherry. *Phytopathology* 79:530-534.
12. Wilcox, W. F. 1990. Postinfection and antispore activity of selected fungicides in control of blossom blight of sour cherry by *Monilinia fructicola*. *Plant Dis.* 74:808-811.
13. Zehr, E. I. 1982. Control of brown rot in peach orchards. *Plant Dis.* 66:1101-1105.