Bacterial Spot of Peach as Influenced by Water Congestion, Leaf Wetness Duration, and Temperature

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ABSTRACT

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Development of bacterial spot on Suwanee peach leaves after inoculation with *Xanthomonas campestris* pv. *pruni* was studied at 24 and 30°C and after-inoculation wetness periods of 0, 6, 18, 24, and 48 h. Water congestion following leaf wetness resulting from exposure to 100% relative humidity for at least 36 h and growing plants in sandy soil were necessary for symptom development. Symptom development was slower at 24 than at 30°C. Postinoculation wetness periods greater than 18 h were necessary for extensive symptom development at 24 but not at 30°C. However, some symptom development was evident at either temperature with no leaf wetness after inoculation if leaves were water-congested. Symptoms did not develop when trees were grown in a sandy loam–vermiculite soil mixture.

Bacterial spot of peach (*Prunus persica* (L.) Batsch) and other stone fruits, caused by *Xanthomonas campestris* pv. *pruni*, is favored by continuous moisture on leaves and warm temperatures (3,4,12). Wind is important if accompanied by rainfall (3), but the contribution of wind to epidemics is not well understood. Severe epiphytotics often occur when these environmental conditions prevail. Lesions may be observed at any time from early spring until autumn if weather conditions favor the disease.

Water congestion, defined as accumulation of excessive water in intercellular spaces as a result of internal water pressures (5), and leaf wetness are requisites for development of bacterial spot (6). These conditions commonly occur during rains, dews, or periods of high relative humidity coupled with abundant water absorption by peach trees. A continuous film of water extending from the leaf surface through stomata into the substomatal chamber is necessary for ingress of *X. c.* pv. *pruni* into peach foliage (6,7). Stomata also are sites of egress from infested peach leaves (8).

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Soil texture also influences the development of water congestion and susceptibility to bacterial spot. Light, sandy, and sandy loam soils have greater aeration and porosity than fine-textured, heavy soils. These conditions seem to increase the predisposition to water congestion and susceptibility to bacterial spot (6,7). For example, Sunhigh peach trees grown in sand or sandy loam were more susceptible to bacterial spot than were those grown in loam or silt loam soils (6). Differences in apparent susceptibility of peach cultivars grown in northern versus southern New Jersey were attributed to edaphic factors (6). Likewise, cultivars that are susceptible to bacterial spot in sandy soils of the Carolinas (11) often escape severe infection when grown in the heavier soils of the Piedmont sections of the state.

Young et al. (13) found the optimum temperature for *X. c.* pv. *pruni* in vitro to be ~31°C. Growth was very slow at 10°C or below. In preliminary studies, we found that symptom development was minimal and appearance was delayed in trees inoculated and maintained at 20°C (D. P. Shepard and E. I. Zehr, *unpublished*). Infection was initiated at 20°C, but symptoms became apparent only after 12 days at ambient greenhouse temperatures (25 to 32°C). Peach trees inoculated and held at ambient temperatures of 22 to 26°C or at 30°C showed typical symptoms in 5 to 12 days. Therefore, we studied effects of water congestion, leaf wetness duration, and temperature (24 and 30°C) on the severity of bacterial spot.

MATERIALS AND METHODS

Test plants. Nodal cuttings of Suwanee peach twigs were dipped for 10 s in indolebutyric acid at 1,000 μg ml^{-1} and rooted under mist in vermiculite, or Suwanee peach trees budded on Lovell rootstock were purchased from a commercial nursery. Rooted cuttings and young trees were planted in steamed (65°C for 1 h) Lakeland sand (89% sand, 6% silt, 5% clay) in plastic pots, 11.5 × 11.5 cm. Beginning 30 days after planting, the young plants were fertilized every 2 weeks alternately with 50 ml of a solution of 45 g of $Ca(NO_3)_2$ and 15 g of $MgSO_4$ in 19 liters of water, or 45 g of 15-0-14 NPK, 12 g of superphosphate, and 2 ml of Stoller's Crop Mix (Stoller Chemicals, Houston, TX) per 19 liters of water. Commercial miticides were applied to all aerial portions of the plants as needed. Plants were grown without supplemental light in the greenhouse at ambient temperatures of 20 to 30°C.

Inoculum. An isolate of *X. c.* pv. *pruni* from apricot (courtesy of C. C. Reilly, USDA, ARS, Byron, GA) was maintained on 2.3% nutrient agar and transferred weekly. Inoculum was prepared from 48-h-old cultures grown on yeast extract–dextrose–calcium carbonate agar slants at 27°C. Bacteria suspended in sterile distilled water were adjusted to 10^7 CFU ml^{-1} turbidimetrically (60 to 64% transmission at 620 nm in a Bausch & Lomb Model 20 Spectrophotometer, Bausch & Lomb, Rochester, NY). Titer of inoculum for each experiment was determined by spreading 0.1 ml of an appropriate dilution on the surface of XPSM (2), incubating the plates at 27°C, and counting the colonies 7 days later.

Inoculation. To induce water congestion prior to inoculation, plants were transferred from the greenhouse into Percival Model E-54U-DL dew chambers (Percival Manufacturing Co., Boone, IA) at 27°C, 100% relative humidity, and a 16-h photoperiod for 48 h. Plants were approximately 60 cm tall, with 50 to 75 leaves per plant. Water congestion was observed visually as the appearance of irregular, darkened, moist patches on the abaxial leaf surface. Six fully expanded leaves per plant were selected arbitrarily for inoculation from the upper, middle, and lower portions of each tree. Inoculum was applied to the abaxial side of leaves with an artist's airbrush at 68.9 kPa. Leaves were sprayed to runoff, approximately 0.2 ml of bacterial suspension per leaf, supported during inoculation with a sterile paper towel to absorb excess inoculum. Control plants were sprayed with sterile distilled

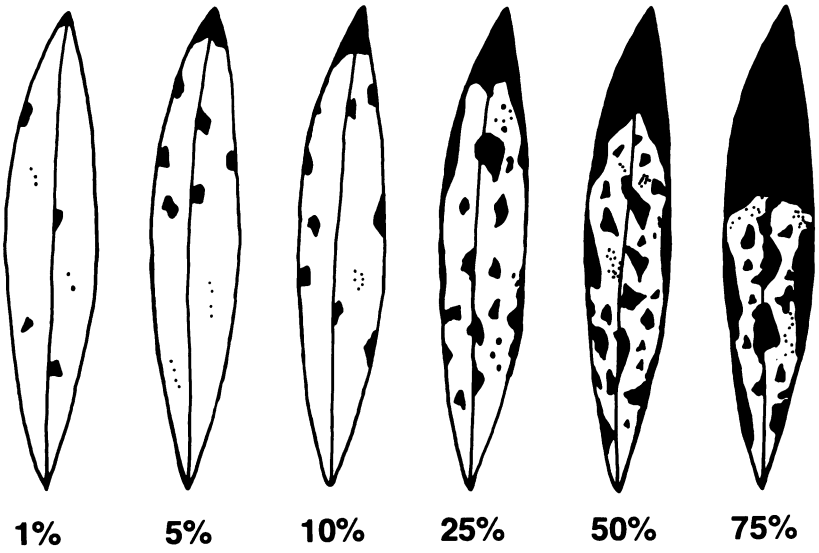

Fig. 1. Bacterial spot severity index for peach (cv. Suwanee) leaves infected by *Xanthomonas campestris* pv. *pruni*. Percentage of leaf area infected is indicated.

Table 1. Time (days) of latent period in peach (cv. Suwanee) leaves after inoculation with *Xanthomonas campestris* pv. *pruni*, as related to temperature and leaf wetness[a]

	Latent period (days)	
Leaf wetness (h)	24°C	30°C
0	14	3
6	12	3
18	12	3
24	12	3
48	10	3

[a] All plants were preconditioned with a 48-h wetting period at 27°C prior to inoculation. After inoculation, plants were incubated at the stated temperature for each wetness period then moved to dry growth chambers at the same temperature.

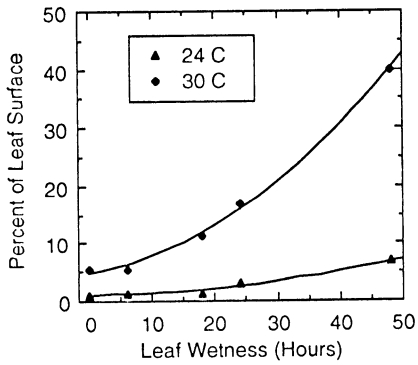

Fig. 2. Percentage of surface necrosis for peach (cv. Suwanee) leaves after inoculation with *Xanthomonas campestris* pv. *pruni* as related to leaf wetness duration at 24 and 30°C. Plants were preconditioned with a 48-h wetting period at 27°C prior to inoculation. Percentage of leaf surface necrosis was measured after 15 days.

water. After inoculation, the plants were moved into Percival dew chambers at 24 or 30°C in 100% relative humidity and darkness for 6-, 18-, 24-, or 48-h leaf wetness periods. Then the plants were transferred to Sherer Cel-7 growth chambers (Sherer-Gillett Co., Marshall, MI) at 24 or 30°C on 16-h light/8-h dark cycles for 14 days. Fluorescent lighting in growth chambers provided photosynthetically active radiation measured between 360 and 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ (LI-190SB quantum sensor, Li-Cor, Inc., Lincoln, NE).

In a preliminary experiment, symptoms on leaves included angular lesions of variable sizes, scattered pinpoint lesions, and tip and marginal necroses. Therefore, disease severity was assessed by determining the approximate percentage of leaf area that was necrotic. A scale of lesion development was devised to aid in assessing disease severity (Fig. 1). Interpolation was used to estimate levels of infection different from those shown. Plants were observed daily and symptom development was recorded. After 15 days, symptoms were measured as percent leaf area diseased.

Experimental design and analysis. A completely randomized experimental design with subsampling was used. The treatment layout was a factorial arrangement with two temperatures (24 and 30°C) and five wetness periods (0, 6, 18, 24, and 48 h) for a total of 10 treatments. The experimental unit was a single plant, with three plants per treatment. The subsample consisted of 18 leaves from those plants. The experiment was performed four times. The same experiments were done twice also using cuttings or budded trees growing in a 50% (vol/vol) sandy loam–vermiculite mix.

Analysis of variance was used to test the effects of temperature, leaf wetness, and their interactions on disease severity. The effect of wetness on disease severity was studied and quantified using second-order regression models that were fit separately for each temperature. Calculations were performed using the Statistical Analysis System (SAS).

Table 2. Analysis of variance of main and interactive effects of temperature and leaf wetness on disease severity in peach (cv. Suwanee) leaves inoculated with *Xanthomonas campestris* pv. *pruni*

Source	df	*F*
Temperature[a]	1	39.43**[b]
Leaf wetness period[c]	4	10.87**
Temperature × leaf wetness	4	5.41**
Test repetitions	3	NS

[a] 24 and 30°C.
[b] Asterisks indicate significance of F at $P \leq 0.01$. NS = not significant.
[c] 0, 6, 18, 24, and 48 h after inoculation.

RESULTS

Water congestion. Water congestion of leaves was essential for symptom development. Only scattered necrotic specks that did not enlarge developed on inoculated leaves that were not water-congested. Thirty-six to 48 h of leaf wetness were required to achieve visible water congestion when young trees were grown in sandy soil. Symptoms developed 3 to 14 days after inoculation of water-congested leaves by *X. c.* pv. *pruni*, depending on temperature (Table 1). However, when plants were grown in 50% (vol/vol) sandy loam–vermiculite blend, symptoms did not appear even with 48-h pre- and postinoculation wetting periods. In a preliminary test, inclusion of Carborundum in the inoculum also did not result in symptoms in plants growing in the soil–vermiculite mix, even after rubbing to induce injury. Plants growing in steamed or unsteamed sand remained water-congested longer in less-than-saturated air than did those in the soil–vermiculite mix.

Temperature and wetting periods. Small amounts of necrosis were observed when water-congested leaves were allowed to dry immediately after inoculation or remained wet for up to 18 h at 24°C (Fig. 2). The amount of necrosis increased if leaves after inoculation remained wet 24 to 48 h. The percent leaf surface that became necrotic at 24°C is described by the equation: $\% = 1.004 + 0.009\ h + 0.0023\ h^2$. At 30°C, however, air-drying of water-congested leaves immediately after inoculation still resulted in 5% of the leaf surface becoming necrotic (Fig. 2), and wetting periods of 6 h or more resulted in increasing amounts of necrotic tissue. The percent leaf surface becoming necrotic at 30°C is described by the equation: $\% = 4.683 + 0.207\ h + 0.011\ h^2$. Tip and marginal necrosis was observed on all leaves 3 days after inoculation and was most severe on the youngest leaves.

Lesions developed more slowly at 24 than at 30°C (Table 1) and did not enlarge

after appearance. At 30°C, lesions did not enlarge after appearance if no wetting period followed inoculation; but if 6 h or more of wetting followed inoculation, necrotic areas near the leaf margins spread inward, enlarging the area of necrotic tissue.

Water congestion was necessary for symptom development. Time and temperature were important after water congestion was visible. Statistical comparisons (Table 2) showed significant differences ($P \leq 0.01$) between temperatures of 24 and 30°C, length of leaf wetness period after inoculation, and temperature × leaf wetness interaction. Differences among repetitions of the experiment were not significant.

DISCUSSION

In peach orchards, infections by *X. c.* pv. *pruni* often are initiated early in spring and continue through the summer. The sporadic occurrence of the disease is often attributed to variable frequency of rainy, warm weather. We show here that rates of disease development are related to leaf wetting periods and temperature. Symptoms developed after only 3 days at 30°C, but not for 10 to 14 days at 24°C. Daytime temperatures may average 24°C as early as the middle of April in South Carolina and often approach 30°C in May. More commonly, temperatures reach or exceed this value from June through August. Rolfs (9) found that the incubation period varied from 7 to 15 days in warm weather to between 20 and 25 days in cold weather, but he did not induce water congestion before moistening leaves with a bacterial suspension.

Disease severity and incidence were much greater at 30°C than at 24°C for all leaf wetness periods. A large difference was not expected because the disease is common when daytime temperatures range from 20 to 30°C. Rolfs (9) cited 20 to 28°C as the optimum temperature range for the bacteria in the orchard. In the laboratory, optimal temperatures for *X. c.* pv. *pruni* have been reported as 24 to 28°C (9) and 31°C (13).

Exposure to 100% relative humidity for 24 or 48 h immediately after inoculation resulted in much greater disease severity than was observed with shorter wetness exposures. Using a rain machine, Daines (3) found that the number of infections increased with the duration of rain, and these results are consistent with our findings.

Peach leaves in a water-saturated condition may undergo changes in cell membrane permeability leading to leakage of nutrients into intercellular spaces (12). Presumably, *X. c.* pv. *pruni* may use these nutrients as a substrate for growth. Young's (12) suggestion that naturally occurring host defenses may be altered in water-soaked tissues is supported by a recent study (1) showing that water congestion altered the protein pattern in a susceptible cultivar (Babygold 7), but not in a resistant cultivar (Nemaguard). Perhaps preformed inhibitors of bacterial growth in leaves are rendered ineffective through dilution in saturated tissues or to chemical changes in response to water congestion.

The failure of bacterial spot to develop when trees were planted in a soil–vermiculite mixture in our tests is consistent with our observations in the field and reports in literature (3,6,7) that sandy soil increases the severity of the disease. Bacterial spot in South Carolina is much more severe in sandy and sandy loam soils of the Ridge than in the heavier soils of the Piedmont. However, bacterial spot may become severe in the Piedmont also if soils during the growing season remain wet for extended periods.

Given the temperatures that prevail in South Carolina during late spring and summer months, some degree of bacterial spot probably can be expected when peach leaves are wet for 36 h or more. Perhaps even shorter wetting periods are sufficient if they are frequent, given that *X. c.* pv. *pruni* may be found on symptomless leaves (10). These criteria perhaps should be interpreted as tentative because we did not test isolates of *X. c.* pv. *pruni* from several sources or locations. Effects of interrupted periods of wetting when the bacteria are present have not been studied. The severity of infection will depend on temperature, length of the wetting period, soil type, and susceptibility of the cultivar. More precise definitions of these criteria are needed to develop a forecasting model for bacterial spot.

ACKNOWLEDGMENT

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