Wind and Wind-Generated Sand Injury as Factors in Infection of Pepper by *Xanthomonas campestris* pv. *vesicatoria*

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ABSTRACT

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Wind and windblown sand were examined for their role in infection of pepper (*Capsicum annuum*) by the bacterial spot pathogen (*Xanthomonas campestris* pv. *vesicatoria*). At four inoculum concentrations from 10^5 to 10^8 cfu/ml, significantly more lesions occurred on Jupiter plants wounded by simulated windblown sand than on nonwounded plants. There were also more lesions on pepper plants when leaves rubbed against each other in simulated windstorms than when no abrasion occurred. Numbers of lesions doubled after 10-min exposure to an 8-m/sec windstorm or sandstorm in a wind tunnel. Many more lesions developed on plants that were wounded prior to arrival of splash-dispersed bacteria. At 15 cm from the impact site, 2.1 times more lesions developed on sand-injured plants than on unwounded ones, and at 46 cm, this difference increased to 7.5 times. Greater increases in disease associated with wounding occurred on Early Calwonder than on Ssupersweet 860 and Jupiter. Wounding did not affect disease levels on resistant cultigen PR 9008-321.

Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, continues to be one of the most serious diseases affecting production of pepper (*Capsicum annuum* L.) in southern Florida (7). The current disease management strategy includes application of crop protection chemicals on a regular basis. However, chemical control is often marginal, especially during warm, rainy weather. Changes in the relative abundance of races of the pathogen have hampered attempts to manage this disease by host plant resistance (9).

Vakili (14) showed that wounding was an important factor in the infection of tomato by *X. c. vesicatoria*. Field observations support the view that wounding may also be important in infection of pepper by this pathogen, although several questions relative to the role of wounding in infection of pepper by *X. c. vesicatoria* remain unanswered. Specific objectives of this study were to determine the relationship between wounding and inoculum concentration in disease severity, to examine the role of injury from wind-associated leaf rub in infection, and to evaluate the disease severity on several cultigens subjected to wounding predisposition.

MATERIALS AND METHODS

Growth of plants. Pepper plants, cv. Jupiter, were used in all experiments unless otherwise noted. Plants were grown in an air-conditioned greenhouse with a maximum temperature of 26–29 C. Minimum temperatures varied with ambient conditions and were usually in the range of 18–22 C. For most of the year, the house was covered with polypropylene shading providing 30% light attenuation so that a typical light intensity was 755 $\mu E \cdot m^{-2} \cdot sec^{-1}$.

Seeds were germinated in cells in Styrofoam trays containing a commercial potting mix (Metro-Mix 300, W. R. Grace & Co., Cambridge, Massachusetts) and transplanted approximately 3 wk later into square 9-cm plastic pots. Two days after transplanting, each pot received 30 g of a slow-release fertilizer (Osmocote 20-20-20, Sierra Chemical Co., Milpitas, California). After inoculation, plants were watered at the pot rim to prevent splash dispersal of bacteria.

Inoculum preparation. Bacteria were grown for 72 hr on nutrient agar amended with 0.5% (w/v) glucose. Plates were flooded with sterile, buffered saline (SBS) (6), and resultant suspensions were adjusted turbidimetrically to approximately 3.0×10^8 colony-forming units (cfu)/ml. Tenfold dilutions in SBS were used to adjust inoculum concentrations as needed. Strains P109 and P437 were used in experiments. These strains were isolated from pepper leaf lesions in 1989 and 1990, respectively, and both were identified as race 1 of *X. c. vesicatoria* (9). Permanent cultures were maintained as turbid suspensions in 15% aqueous glycerol solution at −70 C (12).

Wounding and inoculum concentration. In three preliminary experiments, four plants sprayed with SBS or sandblasted and sprayed with SBS were included as controls. Since no lesions were ever observed on control plants (data not shown), SBS treatments were omitted from subsequent tests.

Tenfold dilutions of strain P109 were used to prepare five inoculum concentrations from 3.0×10^4 through 3.0×10^8 cfu/ml. Eight plants in the five- to six-true-leaf stage were inoculated with each concentration of inoculum. Four of these plants were sandblasted just prior to application of bacteria and four were not. Sand (grade 20-30, Standard Sand and Silica Co., Miami, Florida) was autoclaved and air-dried. A hand-held garden fertilizer spreader was filled to capacity (approximately 1,100 ml of sand) and set to the widest orifice (yielding the greatest sand volume). Sand was discharged during two 360-degree passes around each test plant. The distance from the discharge plate of the spreader to the plants was about 22 cm. Additional sand was added to the spreader frequently to keep the level at or near capacity. Suspensions of inoculum were applied with a nonpressurized atomizer until runoff. Plants were arranged in a completely randomized design and enclosed in plastic bags for 4 days after inoculation. Planting sticks were used in the summertime to keep bags from touching plants. Twelve days after treatment, bacterial spot lesions were counted, with each plant considered a replication. The number of lesions per plant was regressed on concentration. Slopes of regression lines were compared with a *t* test using computed pooled residual mean squares. This experiment was performed three times.

Injury from wind and windblown sand. The possibility that leaves rubbing together alone could result in increased disease was tested in greenhouse experiments using a household fan to simulate the high winds frequently encountered in southern Florida production areas. Experimental units were groups of six pepper plants in the four- to five-true-leaf stage spaced closely together in two rows on a greenhouse bench. Experiments consisted of four treatments. In one treatment, plants were inoculated without wind exposure. In the other three treatments, inoculum was atomized onto plants after wounding. In one case, sand was gently placed on leaves by hand. Most of the sand that adhered settled in the proximal areas of blades and in the depressions associated with the larger veins. Care was taken to keep injury from

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the sand to a minimum. The third treatment consisted of exposing plants for 5 min to the wind generated by the highest setting (equivalent to about 8.0 m/sec as measured by an anemometer) of a three-speed household fan. The two tandem rows of plants were perpendicular to the direction of air flow and 0.61 m from the fan. The fan was held off the bench at a height about that of the rim of the pots. It was oscillated from front to back to increase movement and contact between leaves and to simulate the uneven, gusty winds that occur in grower fields. In the final treatment, plants were gently covered with sand and then exposed to wind as described above. Most of the sand was blown off the leaves within the first 30 sec of the 5-min wind exposure. Immediately after the treatment, a 3.0×10^7 cfu/ml suspension of strain P437 was atomized onto plants. Individual plants were kept in plastic bags for the first 4 days after inoculation. Each of the treatments was replicated three times and arranged in a randomized complete block on the greenhouse bench. Disease development was assessed 18 days after inoculation and is expressed as the number of lesions on the adaxial surface of leaves on all plants in an experimental unit. This experiment was performed three times.

In another experiment, a wind tunnel was constructed in the greenhouse to study the direct effect of sustained exposure of plants to wind-driven sand. The tunnel body was a section of aluminum culvert pipe 61 cm in diameter and 1.22 m in length. Wind was provided from a household fan (wind velocity was 8.0 m/sec) placed on the bench at one end of the tunnel. Two rows of three potted plants each were placed at the other end of the tunnel. The pots were touching so that considerable contact occurred between foliage on adjacent plants. Wind injury was achieved by exposing plants to 10-min of a constant airstream.

Other plants were subjected to a steady, 10-min stream of windblown sand. For this treatment, a fine sand field soil (Osceola fine sand series) from pepper production areas near Delray Beach, Florida, was autoclaved and air-dried. A metal tray filled with 10 kg of sand was placed just inside the fan side of the wind tunnel. The airstream from the fan was concentrated in the confines of the tunnel and readily lifted the sand. Wind-borne sand particles were generated in abundance, as evidenced by the accumulation of sand on the bench beyond the target plants. The reservoir in the tray was replenished with sterile sand after each replication.

Immediately after wounding, plants were inoculated with a 10^7 cfu/ml suspension of strain P437 and kept in plastic bags for four days. A set of plants inoculated in the same manner, but without wind-related injury, was used for comparison. Treatments were replicated three times in a randomized complete block design. Lesions were counted 18 days later. This experiment was repeated once.

Splash dispersal of bacteria onto wounded plants. To simulate the inoculation of plants in the field, a splash dispersal experiment was performed in the laboratory. A 25-ml titration burette was anchored in the laboratory ceiling 2.1 m above a 9-cm-diameter watch glass filled with 30 ml of a 10^8 cfu/ml suspension of strain P437. Splash-dispersed inoculum was generated by discharging distilled water at a rate of 120 drops per minute from the burette into the watch glass. Six potted plants were positioned circumferentially at 15, 30, and 46 cm around the impact site. Half of the plants were wounded with sand from the fertilizer spreader as already described, and half were not wounded. The entire procedure was repeated three times; the watch glass and burette were recharged between each replication. Plants were bagged, removed to the greenhouse, and maintained as described previously until lesions were counted 14 days later. The experiment was repeated once.

Cultigen responses to wounding. Four cultigens were compared for responses to wounding. In the field, two of these cultigens, Jupiter and Ssupersweet 860, are highly susceptible to bacterial spot. Early Calwonder exhibits some disease tolerance, and PR 9008-321 is resistant to races 1 and 2 of the pathogen. Sixteen plants of each cultigen at the four-true-leaf stage (half of which were wounded with sand discharged from the hand-held fertilizer spreader) were misted with strain P437. Plants were bagged and arranged in a completely randomized design on the greenhouse bench. Twelve days later, the number of lesions per plant was recorded, and an estimate was made of the percentage of diseased foliage. This experiment was repeated once.

Data analysis. Results were similar for repeated trials, and data from representative experiments are shown. Lesion data and percentage data were converted to $\log(x + 1)$ and arcsine square root equivalents, respectively, before analysis. Analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC). Specific comparisons were made using a series of preplanned single-degree-of-freedom orthogonal contrasts as suggested by Swallow (13).

RESULTS

Wounding and inoculum concentration. With the exception of the 10^4 cfu/ml concentration, highly significant increases in the number of bacterial spot lesions were associated with sandblast wounding (t test, $P \leq 0.008$). A sharper rise in the number of lesions per plant with concentration occurred when plants were sandblasted before inoculation. The slopes of the regression lines for number of lesions per plant on inoculum concentration were significantly different ($P \leq 0.01$) (Fig. 1), based on a t test of pooled residual mean squares.

Injury from wind and windblown sand. The abrasion associated with leaves rubbing together in the wind increased the incidence of infection. A comparison of treatments with (2,148 lesions) and without (1,490 lesions) wind exposure before inoculation was significant at $P \leq 0.01$. When sand was carefully placed on the surface, only a slight increase in lesion numbers was recorded (1,801 vs. 1,490 lesions). However, if leaves were exposed to the airstream from the fan, lesion numbers were about twice (3,061 lesions) those of plants receiving inoculum misting alone (1,490 lesions). When rubbing leaf surfaces were covered in part by sand, bacterial spot ratings increased. In the fan-exposure treatments, this increase was an additional 150 more lesions per plant than the wind-only treatment (contrast significant at $P \leq 0.05$).

Enhanced infection of pepper in simulated windstorms was also evident in the wind tunnel experiments. Contrasts comparing plants in the wind tunnel with those inoculated with *X. c. vesicatoria* alone (724 lesions) as well as windblown sand (1,456 lesions) versus wind alone (903 lesions) were both significant at $P \leq 0.05$.

Splash dispersal onto wounded plants. More lesions developed on plants

Table 1. Role of wounding in infection of pepper by splash-dispersed *Xanthomonas campestris* pv. *vesicatoria*[a]

Plant distance from splash droplets (cm)	No. of lesions per six plants		Probability of $P > t$[b]
	Sandblasted	Not wounded	
15	907	435	0.02
30	499	150	0.02
46	468	62	0.04

[a] Six pepper plants, cv. Jupiter, placed circumferentially around a watch glass filled with a 2.0×10^8 cfu/ml suspension of *X. c. vesicatoria*, strain P437, at each of the three radii. Splash dispersion was generated by discharging water from a burette suspended from the ceiling 2.1 m above the watch glass (ground zero). Half of the plants at each radius were wounded with sand immediately prior to inoculation.

[b] Analysis based on Student's t test, three replications per treatment.

Table 2. Responses of four pepper cultigens to inoculation with *Xanthomonas campestris* pv. *vesicatoria* with and without wounding by sandblasting[a]

	Lesions per plant				Percent diseased foliage			
Cultigen	Wounded	Not wounded	$P \geq F$[b]	Ratio of wounded to not wounded	Wounded	Not wounded	$P \geq F$[b]	Ratio of wounded to not wounded
Early Calwonder	386	44	0.0001	8.8	44.0	2.0	0.001	22.0
Jupiter	185	88	0.014	2.1	49.4	24.6	0.005	2.0
PR 9008-321	61	16	0.017	3.8	4.2	2.6	0.28	1.6
Ssupersweet 860	157	33	0.001	4.8	30.0	2.4	0.0002	12.5

[a] Based on eight plants of each cultigen either wounded by sandblasting or not wounded and misted with a 2.0×10^7 cfu/ml suspension of strain P437.

[b] Analysis of variance showed strong interaction between cultigen and wounding treatment. Therefore, wounding effects were tested for each cultigen separately.

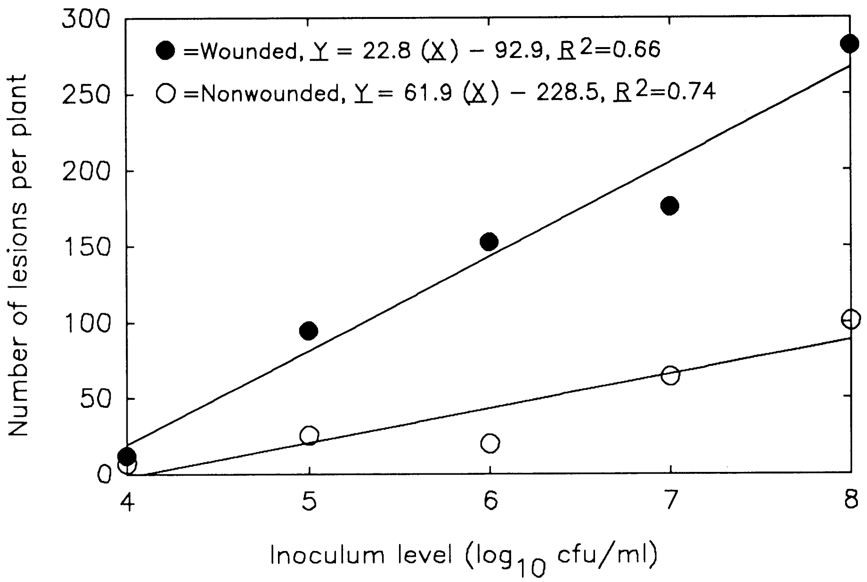

Fig. 1. Regression lines of relationships between number of lesions per plant and inoculum concentration, with and without wounding prior to inoculation. Slopes of regression lines were compared with a *t* test using pooled residual mean squares. Slopes were significantly different ($P \leq 0.01$); computed $t = 45.2$, tabular $t = 9.9$.

wounded prior to arrival of splashed-dispersed pathogen cells than plants that were not wounded (Table 1). The differences between wounded and unwounded plants became considerably larger as the distance from the impact site of the simulated rain drops increased. At 15 cm, there were 2.1 times more lesions on sand-injured plants; at 46 cm, this difference increased to 7.5 times. Whether plants were wounded or not, lesion numbers dropped off rapidly as distance from the impact site increased.

Cultigen responses to wounding. There was a strong interaction between wounding and cultigen in the analysis of variance, leading us to conclude that disease development was enhanced by preinoculation sandblasting on some cultigens more than others. Early Calwonder was particularly affected by preinoculation sandblasting (Table 2). An average of 8.8 times more lesions and a diseased foliage rating 22 times higher were recorded on wounded Early Calwonder plants. Infection of Ssupersweet 860 was also significantly increased by wounding prior to inoculation. On Jupiter, both numbers of lesions and percent diseased foliage on wounded plants were only about twice those for nonwounded plants.

Although PR 9008-321 is described as resistant to races 1 and 2 of *X. c. vesicatoria*, some lesions were observed on most plants. However, lesion numbers were much lower than in other cultigens. In addition, PR 9008-321 was the only cultigen for which wounding did not significantly increase ratings for percent diseased foliage (Table 2).

DISCUSSION

Wounding clearly plays a significant role in the infection of pepper by *X. c. vesicatoria*. We confirm the conclusion of Vakili (14) that wounds probably are more important than natural openings for entry of *X. c. vesicatoria* into its hosts under field conditions. As inoculum dosage increases, numbers of lesions increase more rapidly in wounded than in nonwounded plants. It may be that the number of sites for pathogen entry (natural openings and wounds) are limiting at higher inoculum concentrations. This could explain the increased disease response of wounded plants to higher inoculum concentrations (Fig. 1).

The largest increases in disease ratings occurred when pepper leaves were abraded with sand. In wind tunnel experiments, use of wind velocities that often occur in southern Florida readily lifted and dispersed a typical, sandy field soil. Exposure of pepper plants to the impingement of these airborne sand particles for only 10 min resulted in a doubling of the number of bacterial spot lesions.

What may be of greater significance is that leaves that simply rubbed together in an airstream had significantly more lesions than plants inoculated in still air. Daft and Leben (2) observed that the injury associated with rubbing of soybean leaves is important in the epidemiology of bacterial blight. Most likely, the rubbing of leaves results in broken trichomes that serve as portals for pathogen entry (3,4). Getz et al (3) reported that exposed trichome bases created by the shearing off of trichomes were important entry sites for *Pseudomonas syringae* pv. *tomato* in young tomato fruit. Moreover, the environment in these broken trichome bases may be favorable for multiplication of *X. c. vesicatoria* to levels sufficient to cause disease (5,10). However, wind exposure may also affect stomatal status or the microenvironment on the phylloplane (1).

The most dramatic increases in bacterial spot associated with sand injury were observed in the splash dispersal experiment. This experimental design probably provides the best simulation of inoculation under natural conditions. Splashing during wind and rain storms is important in the epidemiology of bacterial diseases. However, much larger numbers of lesions were observed on plants wounded prior to arrival of contaminated "raindrops" (up to seven times at the distance farthest from the impact site). We suggest that the injury associated with windblown sand, especially that which occurs in the 10-15-min period of gusty winds that often immediately precedes thunderstorms, is likely very important in the epidemiology of bacterial spot of pepper.

Some cultigens of pepper were relatively more affected by wounding than others. Increases in disease ratings associated with wounding were higher for Early Calwonder and Ssupersweet 860 than they were for Jupiter and PR 9008-321. It may be that some cultigens that look promising when screened without wounding will not prove as desirable under field conditions when severely predisposed by wounding.

The importance of wounding should be taken into account when recommendations are developed for integrated management of bacterial spot (7). Handling of plants should be kept to a minimum in order to reduce wounding as well as to control mechanical transmission (8). Some growers in southern Florida already use windbreaks in fields. These usually consist of strips of tall grasses such as sugarcane intercropped with pepper every 12 rows or so. Historically, windbreaks have been employed primarily to reduce insect damage, especially that associated with aphid-transmitted viruses (11). The utility of these windbreaks for reduction of bacterial spot needs to be evaluated in the field.

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LITERATURE CITED

1. Blakeman, J. P., ed. 1981. Microbial Ecology of the Phylloplane. Academic Press, London. 502 pp.
2. Daft, G. C., and Leben, C. 1972. Bacterial blight of soybeans: Epidemiology of blight outbreaks. Phytopathology 62:57-62.
3. Getz, S., Fulbright, D. W., and Stephens, C. T. 1983. Scanning electron microscopy of infection sites and lesion development on tomato fruit infected with *Pseudomonas syringae* pv. *tomato*. Phytopathology 73:39-43.
4. Layne, R. E. C. 1967. Foliar trichomes and their importance as infection sites for *Corynebacterium michiganense* on tomato. Phytopathology 57:981-985.
5. Leben, C. 1981. How plant pathogenic bacteria survive. Plant Dis. 65:633-637.
6. Leben, C., Daft, G. C., and Schmitthenner, A. F. 1968. Bacterial blight of soybeans: Population levels of *Pseudomonas glycinea* in relation to symptom development. Phytopathology 58:1143-1146.
7. Pohronezny, K., ed. 1989. The impact of integrated pest management on selected vegetable crops in Florida. Fla. Agric. Exp. Stn. Bull. 875. 72 pp.
8. Pohronezny, K., Moss, M. A., Dankers, W., and Schenk, J. 1990. Dispersal and management of *Xanthomonas campestris* pv. *vesicatoria* during thinning of direct-seeded tomato. Plant Dis. 74:800-805.
9. Pohronezny, K., Stall, R. E., Canteros, B. I., Kegley, M., Datnoff, L. E., and Subramanya, R. 1992. Sudden shift in the prevalent race of *Xanthomonas campestris* pv. *vesicatoria* in pepper fields in southern Florida. Plant Dis. 76:118-120.
10. Schneider, R. W., and Grogan, R. G. 1977. Tomato leaf trichomes, a habitat for resident populations of *Pseudomonas tomato*. Phytopathology 67:898-902.
11. Simons, J. N. 1957. Effects of insecticides and physical barriers on field spread of pepper veinbanding mosaic virus. Phytopathology 47:139-145.
12. Sleesman, J. P., and Leben, C. 1978. Preserving phytopathogenic bacteria at 70C or with silica gel. Plant Dis. Rep. 62:910-913.
13. Swallow, W. H. 1984. Those overworked and oft-misused mean separation procedures—Duncan's, LSD, etc. Plant Dis. 68:919-921.
14. Vakili, N. G. 1967. Importance of wounds in bacterial spot (*Xanthomonas vesicatoria*) of tomatoes in the field. Phytopathology 57:1099-1103.