Tomato Leaf Trichomes, a Habitat for Resident Populations of Pseudomonas tomato

R. W. Schneider and R. G. Grogan

Department of Plant Pathology, University of California, Davis, CA 95616. Present address of senior author: Department of Plant Pathology, University of California, Berkeley, CA 94720.

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ABSTRACT

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A large portion of the resident populations of *Pseudomonas tomato* on tomato leaves was not killed by surface sterilization with sodium hypochlorite, indicating that many of the bacteria on the leaf surface were not exposed. Microscopic examination of trichomes on noninjured, inoculated leaves revealed that many of the apparently intact but nonliving trichomes were colonized by bacteria. Injury of the basal cells of trichomes, followed immediately by inoculation, resulted in more lesion production than in noninjured controls, but only when leaf surfaces were maintained dry after inoculation. However, if inoculation was delayed for 24 hr after trichomes were injured, lesion production was not enhanced. Tomato mutants, deficient in leaf hairs, supported relatively small resident populations under dry conditions, and the resident populations did not increase as much as in pubescent mutants after the leaves were exposed to free moisture.

Additional key words: *Lycopersicon esculentum*, tomato bacterial speck.

We reported (10) that *Pseudomonas tomato* (*P. syringae*), the incitant of bacterial speck of tomato (*Lycopersicon esculentum* Merr.), survives during extended periods of hot, dry conditions as a leaf resident, sensu Leben (8). Subsequently, under favorable conditions of temperature and moisture, this resident population multiplied and lesions were produced (10).

It seemed likely that survival of the resident population on the leaf surface would be enhanced and possibly dependent upon availability of a protective habitat. Habitats on the leaf which might provide increased protection are the depressions between epidermal cells (8), substomatal chambers, and trichomes, of which there are three types in tomato: glandular, short, and long multicellular hairs (4). Layne (7) and Kontaxis (6) reported that trichomes provided sites for infection of tomato leaves by *Corynebacterium michiganense*. This study was conducted to determine whether trichomes are involved in survival and increase of the resident population of *P. tomato*, and whether tomato mutants with fewer trichomes differ from pubescent cultivars in ability to support resident populations.

MATERIALS AND METHODS

Effect of surface sterilization and wet and dry incubation on resident populations.—Inoculum of *P. tomato* was prepared and plants were inoculated as previously described (10). Briefly, bacteria were washed from King's Medium B agar slant cultures, and suspensions were adjusted by dilution with distilled water to a concentration of about 10^4 cells/ml. A glass chromatography sprayer was used to apply the suspension as a fine mist over the noninjured upper and lower leaf surfaces of 8-wk-old tomato plants (cultivar 145-B-7879) until small droplets were apparent; leaves were not water-soaked during inoculation. Plants were allowed to dry at 26 C and 10% relative humidity (RH) for 3 hr before placement in a growth chamber at 25 C and 40-60% RH with continuous light provided by fluorescent and incandescent bulbs (4,800 lux). At 48 hr after inoculation, five (one-half) of the plants were moved to a lighted mist chamber at 25 C for 24 hr and then were returned to the dry chamber. Noninoculated control plants were treated similarly except that sterile water was used instead of inoculum.

Immediately after plants had dried following inoculation, and at 24-hr intervals thereafter, five leaflets from the three youngest fully expanded leaves from each of the five plants were sampled for populations of *P. tomato* as previously described (10) except that comparisons were made between the resident populations on nonsterilized leaves and on comparable leaves after surface sterilization. Leaves were surface-sterilized by immersion and agitation for 20 sec in 0.525% sodium hypochlorite containing 0.15 ml Tergitol NPX (Sigma Chemical Co.) per liter. All of 25 fluorescent pseudomonad colonies selected at random from the many colonies on dilution plates made from inoculated plants were pathogenic on tomato. In contrast, only four colonies (all nonpathogenic) developed on comparable dilution plates made from noninoculated control plants.

Leaves from this experiment also were used for

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microscopic examination for bacteria on leaf surfaces and in trichomes. Sections of leaflets (about 150 μm thick) were cut with a razor blade, and placed in a drop of water on a microscope slide, and covered with a cover slip. By use of the vertical and horizontal calibrations on the microscope stage, repeated observations of the same trichomes could be made for up to 24 hr.

Effect of leaf injury on lesion development.—The upper or lower surfaces of leaflets of fully expanded leaves of 8-wk-old tomato plants (cultivar 145-B-7879) were injured by gently sliding an empty 30-ml beaker across the leaflet surfaces. The pressure produced by the weight of the beaker was about 1.8 g/cm^2. Microscopic examination of leaf surfaces after injury revealed that, of approximately 550 trichomes that were observed, less than 10 and 1% of the long and short hairs, respectively, were visibly damaged. Injury usually was evident as rupture of one or more of the basal trichome cells which are a part of the epidermal cell layer. Other epidermal cells apparently were not injured.

Four experiments comprising various sequences of injury, inoculation, and incubation for 24 hr at 25 C were conducted at about 1-wk intervals as follows: (i) injury followed immediately by inoculation and incubation at 70 to 80% RH (dry), or with free moisture maintained on the leaf surfaces for 24 hr (wet). Free moisture was maintained by atomizing the plants with sterile water and enclosing them in plastic chambers; (ii) inoculation, dry incubation, and injury followed by either dry or wet incubation; (iii) inoculation, wet incubation, and injury followed by either dry or wet incubation; and (iv) injury, dry incubation, and inoculation followed by either wet or dry incubation. The lower or upper surfaces of eight leaflets on each of 10 plants were inoculated in each experiment. The number of lesions/cm^2 of leaf area for all treatments was determined 5 days after inoculation as described previously. Comparisons were made (t-test) between inoculations made on the upper and lower leaf surfaces and between injured and noninjured opposite leaflets on the same leaf in each experiment. Comparisons of results among the four experiments were not attempted.

Trichomes as habitats for the resident population.—Four tomato mutants, two of which were deficient in long, short, or glandular trichomes, and

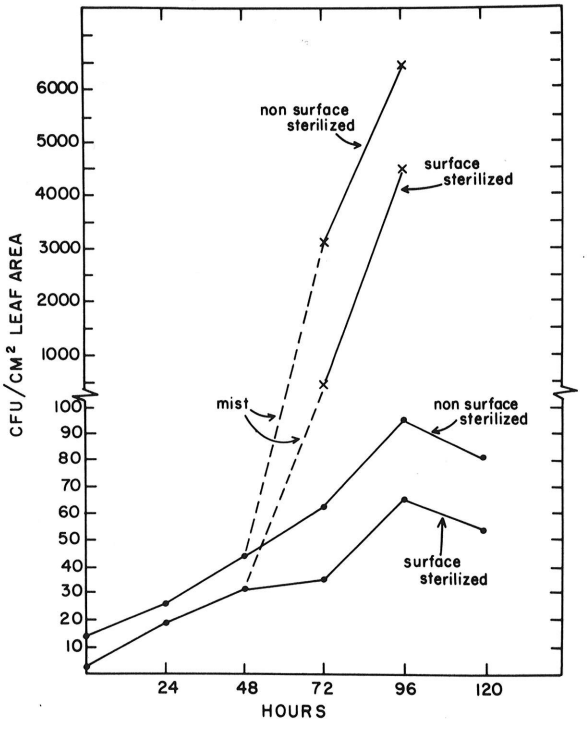

Fig. 1. Comparison of effects of surface sterilization and exposure to free moisture (dotted lines) or dry incubation (solid lines) on resident populations of *Pseudomonas tomato* on inoculated, noninjured tomato leaves. Plants were inoculated by gently spraying with a bacterial suspension (10^4 cells/ml). The leaves of some plants were surface-sterilized by immersion for 20 sec in 0.525 sodium hypochlorite containing 0.15 Tergitol NPX surfactant, and some plants (sterilized and nonsterilized) were incubated continuously at 25 C and 40-60% relative humidity (dry) whereas others were placed (after 48 hr) in a mist chamber at 25 C for 24 hr (wet). Immediately after plants had dried following inoculation, and at 24-hr intervals thereafter, leaflets were sampled for populations of *P. tomato*.

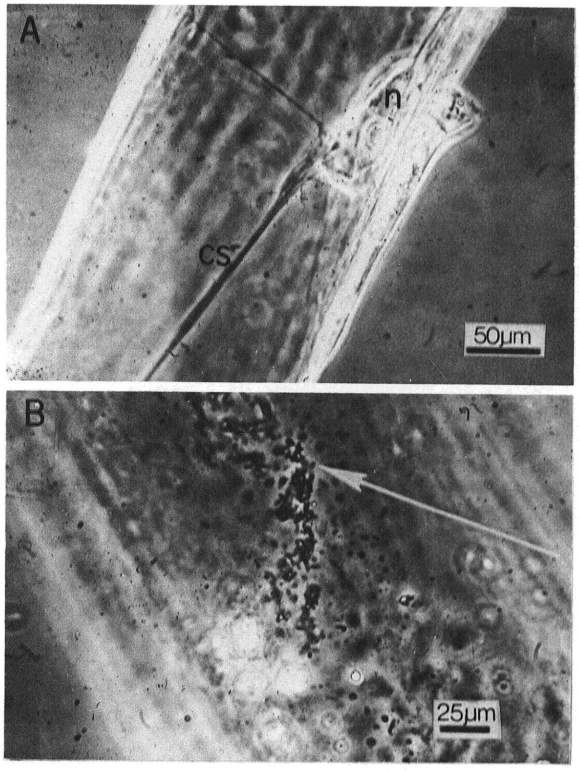

Fig. 2-(A,B). Phase contrast micrographs of tomato leaf trichomes, noninfected and infected with *Pseudomonas tomato*; **A)** portion of a noninfected, living tomato leaf trichome in which the nucleus (N) and cytoplasmic strands (CS) are evident. **B)** trichome from a noninjured, inoculated leaf incubated in 70 to 80% relative humidity (dry) for 24 hr and then misted for the next 24 hr; micrograph was made after wet incubation for 8 hr. Note the bacterial cells (arrow) embedded in the gelatinous remains of the cytoplasm.

cultivar 145-B-7879 were compared to assess the role of trichomes as habitats for the resident population. Densities of leaf hairs on the upper and lower leaf surfaces were determined by examination of five microscope fields (×200 magnification) on each of five leaflets. Sixteen plants of each mutant and the cultivar were inoculated as described previously and divided into two groups of eight plants each. One group was maintained under continual mist (wet incubation) for 24 hr and then incubated on an open greenhouse bench (18 to 24 C, 30 to 60% RH) for an additional 3 days (dry incubation). After inoculation, the second group of plants was incubated dry for 3 days, then exposed to continual mist for 24 hr. Samples were taken for determination of leaf populations after inoculation, after the 3-day dry period, and after exposure to mist for 24 hr. Each plant was considered a replication and disks (1.2 cm diam) from each of five mature leaflets per plant were combined for each sample.

RESULTS AND DISCUSSION

Effect of surface sterilization and wet and dry incubation on resident leaf populations.—About 70% of the leaf population of *P. tomato* survived the surface sterilization (Fig. 1). This protected population increased with time at about the same rate as the population on nonsterilized leaves which either were misted or maintained dry. This result indicates that most of the bacteria were in one or more habitats which excluded sodium hypochlorite. Substomatal chambers probably were not the habitat of the protected resident population because our method of inoculation, which did not watersoak the leaves, decreased direct penetration via stomata (9); furthermore, no symptoms developed within 1 wk after inoculation on noninjured leaves which had been incubated dry. Lesions developed, however, within 4 days after these plants were exposed to mist. It seemed unlikely that exposed leaf surfaces or depressions between epidermal cells would afford protection from the disinfectant which contained a wetting agent. Thus, we surmized that trichomes were the most likely protective habitat.

Microscopic examination of trichomes.—Microscopic examination of leaves from inoculated and noninoculated plants revealed that trichomes are short-lived, but remain attached, a trait shared with many other plant species (2, 4, 5). Living trichomes had intact nuclei and streaming cytoplasmic strands (Fig. 2-A). Bacteria were not observed in living or glandular trichomes of inoculated or noninoculated plants. The presence of phenolic compounds in glandular trichomes (1) may be involved in preventing these structures from becoming infected by *P. tomato*. The trichomes were highly vacuolated after death and became hydrated almost immediately upon contact with free moisture. Within 1 hr after hydration, remains of the cytoplasm were swollen and appeared gelatinous.

Microscopic observation of 100 trichomes on each of four inoculated plants revealed that more than 90% of the trichomes apparently were nonliving; they were characterized by the absence of both nuclei and streaming cytoplasmic strands. Bacteria were observed in approximately 17% of the nonliving trichomes after incubation in water on a microscope slide for 24 hr (Fig. 2-B). They were also seen in trichomes, though in fewer numbers, immediately after leaves were removed from

TABLE 1. Effect of various sequences of inoculation, leaf injury, incubation (wet or dry), and inoculation of upper or lower leaf surfaces on the number of leaf lesions caused by *Pseudomonas tomato* on plants of tomato cultivar 145-B-7879

		Lesions per cm^2 of leaf surface inoculated[a]					
		Upper			Lower		
		Moisture regime of incubation[b]					
Expt. no.	Sequence of treatments[b]	Wet		Dry	Wet		Dry
i	a) inj, inoc	1.4		0.8	2.9		3.4
				*			
	b) inoc	1.2	*[d]	0.0	3.0	*	0.4
ii	a) inoc, (24 hr dry), inj	1.5		0.7	4.9		3.3
		*			*		*
	b) inoc, (24 hr dry)	0.0		0.1	2.5		0.8
iii	a) inoc, (24 hr wet), inj	4.0		3.1	4.8		4.1
	b) inoc, (24 hr wet)	3.5		3.2	4.5		3.8
iv	a) inj (24 hr dry) inoc	0.6		0.2	5.6	*	2.5
	b) inoc	0.9		0.0	5.4	*	2.5

[a]Total numbers per mm^2 on the upper and lower leaf surfaces were: (i) trichomes, 22.4 and 92.5, respectively; and (ii) stomata 132 and 302, respectively.

[b]Treatments consisted of various sequences of injury (inj) inflicted by sliding an empty glass beaker across the leaf surface, inoculation (inoc) by gently atomizing a suspension of bacteria (10^4 cells/ml) on the leaves, and 24-hr incubation periods at 70 to 80% relative humidity (dry) or in a moist chamber (wet). In experiment (i), injury was followed immediately by inoculation; in all others there was a 24-hr delay before the treatments indicated by the "wet" or "dry" incubation periods were begun.

[c]Numbers of lesions were determined 5 days after inoculation.

[d]Asterisks between numbers indicate significant differences ($P = 0.05$) as determined by t-tests. The four experiments were conducted at about 1-wk intervals.

plants. Their presence was confirmed after 24 hr of incubation because they had multiplied and could be differentiated readily from plant cellular components which resembled bacteria. The proportion of nonliving trichomes on noninoculated plants was about the same as on the inoculated, but only a few of them (1-2%) were colonized with bacteria. The identity of these bacteria was not determined, but random tests of isolates for pathogenicity on tomato and the lack of lesion development on any noninoculated control plants indicated that they were not *P. tomato*.

Effect of leaf injury on lesion development.—In all of the different treatments in the leaf-injury experiments, the lower surface of leaflets was more susceptible to infection (Table 1). This probably resulted from the presence of more stomata and trichomes (two and four times, respectively) on the lower than on the upper surface of the tomato cultivar used in this experiment (Table 2). Thus, there were more sites for penetration and infection.

If plants were kept wet for 24 hr immediately after inoculation, the numbers of lesions that developed on injured and noninjured leaves were about equal [Table 1, experiments (i)a, (i)b, (iii)a, and (iii)b]. This indicates that with wet incubation penetration probably occurred mostly through stomata, and that injury of trichomes accounted for an insignificant proportion of the infection sites that developed into lesions. However, if plants were kept dry after inoculation, significantly more lesions were produced on the newly injured than on noninjured leaves [Table 1, experiments (i)a, (i)b, (ii)a, and (ii)b]. The few lesions (not significantly different from zero) that developed on plants exposed to the dry treatments may have resulted from injuries caused inadvertently.

Injury followed by a 24-hr dry period prior to inoculation did not result in increased lesion production [Table 1, experiments (iv)a and (iv)b]. Possibly during the time between injury and inoculation, the wounds had healed or the injured living trichomes had died and become desiccated. If so, they would be similar to the nonliving trichomes on noninjured leaves which supported development of the resident population (Fig. 1), but usually did not function as sites for lesion production during dry incubation.

The results from these experiments indicate that lesions produced during or following wet incubation can develop after penetration and infection of injured basal cells or through stomata, but that most infections probably occurred through stomata. In contrast, when inoculation was followed by continuous dry incubation, very few lesions developed unless leaves were artificially injured just before or after inoculation. We think that these lesions resulted from penetration and infection of newly injured basal cells. The microscopic observations and the results presented in Fig. 1 show that artificial leaf injury was not a requisite for infection of nonliving trichomes. Although the sites of entry of the bacteria into these trichomes were not determined, it seems probable either that there were natural openings in the nonliving trichomes or they must have been damaged to provide openings for infection. These cells, however, were only hydrated for a short time while leaves were wet following inoculation. Thus, under dry conditions, the resident population of *P. tomato* cells supported by the nonliving trichomes either did not multiply sufficiently or was not able to penetrate the epidermal layer and produce lesions owing to lack of free moisture. We observed that the injury treatment damaged the basal cells of some trichomes and thus infection of living cells in the epidermal layer could result if inoculum was applied immediately before or after injury. However, further work will be required to determine whether this explanation is valid.

Trichomes as habitats for the resident population.—The mean number of lesions for each of five 6-wk-old plants for mutants *h*, *af*, *od*, and *LPG*/+ and cultivar 145-B-7879 at 5 days after inoculation with 10^4 cells/ml and incubation in a mist chamber for 18 hr was 101.2, 86.2, 85.6, 97.0, and 104.2, respectively. The lack of statistically significant differences ($P = 0.05$) in numbers of lesions indicated that there were no apparent differences in resistance to infection among the mutants. Tomato mutants, *od* and *LPG*/+ that lack one or more types of trichomes, supported relatively low levels of resident populations when incubated dry after

TABLE 2. Effect of various postinoculation incubation conditions on resident populations of *Pseudomonas tomato* on noninjured leaves of tomato cultivar 145-B-7879 and four trichome-mutants of tomato

Mutant or cultivar	Total trichomes per mm² on both leaf surfaces[a]	Initial colony forming units per cm²/leaf area[b]	Percentage of initial population after indicated treatments			
			Wet then dry[c]		Dry then wet[d]	
			Wet	Dry	Wet	Dry
h	149.3 (5.2)[e]	17.0 (5.1)	566 (92)	255 (71)	118 (66)	759 (278)
af	37.4 (2.7)	38.7 (7.2)	274 (56)	280 (44)	88 (43)	475 (150)
od	12.5 (1.20)	14.7 (4.3)	633 (123)	36 (15)	0	34 (18)
LPG/+	0.7 (0)	18.0 (9.2)	687 (171)	26 (11)	7 (3)	17 (10)
145-B-7879	93.5 (7.2)	15.7 (3.0)	539 (90)	694 (194)	81 (30)	760 (368)

[a]Trichomes were counted in five microscope fields (×200 magnification) on each of five leaves.

[b]Samples were taken immediately after leaves apparently had dried following inoculation.

[c]After inoculation of both leaf surfaces by spraying gently with 10^4 cells/ml suspension, plants were exposed for 24 hr to continual mist (wet) then incubated on an open greenhouse bench for 3 days (dry).

[d]After inoculation of both leaf surfaces, plants were incubated on an open greenhouse bench for 3 days (dry), then exposed for 24 hr to continual mist (wet).

[e]Standard deviation.

inoculation (Table 2). Mutant *h*, which lacks only long hairs, and mutant *af*, which has very few short hairs on the upper leaf surface, supported resident populations comparable to that of cultivar 145-B-7879. In these mutants, the resident populations survived the dry period, and resumed multiplication upon rewetting. However, mutants *od* and *LPG*/+, which have no long hairs, and very few short hairs on either leaf surface, supported only a small resident population on leaves that had dried, and there was only a relatively small increase in the population following exposure to free moisture after the 3-day dry period. However, both of these mutants supported populations of bacteria comparable to cultivar 145-B-7879 during the initial wet period, indicating that there were no bacteriostatic or bactericidal agents involved in the drastic reductions in numbers of bacteria on the hairless mutants during drying.

These results indicate that tomato leaf trichomes serve as a major habitat for survival of resident populations of *P. tomato* during dry conditions. The report by Hass and Rotem (3) concerning the survival of *P. lachrymans* (the cause of angular leaf spot of cucumber) also indicates that trichomes probably are important for survival of this pathogen on dry leaf surfaces. Survival was better on the pubescent leaves of cucumber and potato and was least on the glabrous leaves of pear. Furthermore, they stated that the superior survival on cucumber during dry incubations resulted from bacterial multiplication because lesions eventually were produced. Unfortunately, however, they did not determine the sites of multiplication.

The elimination of hairs possibly can be exploited by developing cultivars which may escape bacterial speck infection under conditions which require survival of a resident population. This type of resistance would not be effective, however, when long-term survival and infection by the resident population are not essential. For instance, splash-dispersal of inoculum by rain or irrigation from infested soil or from existing lesions to water-soaked leaves would result in infection of hairless mutants because the bacteria could infect immediately through stomata.

LITERATURE CITED

1. BECKMAN, C. H., W. C. MUELLER, and W. E. MC HARDY. 1972. The localization of stored phenols in plant hairs. Physiol. Plant Pathol. 2:69-74.
2. ESAU, K. 1967. Plant anatomy. John Wiley and Sons, New York. 767 p.
3. HAAS, J. H., and J. ROTEM. 1976. Pseudomonas lachrymans adsorption, survival, and infectivity following precision inoculation of leaves. Phytopathology 66:992-997.
4. HAYWARD, H. E. 1938. The structure of economic plants. MacMillan, New York. 674 p.
5. JOHNSON, H. B. 1975. Plant pubescence: an ecological perspective. Bot. Rev. 41:233-258.
6. KONTAXIS, D. G. 1962. Leaf trichomes as avenues for infection by Corynebacterium michiganense. Phytopathology 52:1306-1307.
7. LAYNE, R. E. C. 1967. Foliar trichomes and their importance as infection sites for Corynebacterium michiganense on tomato. Phytopathology 57:981-985.
8. LEBEN, C. 1974. Survival of plant pathogenic bacteria. Ohio Agric. Res. Dev. Cent., Spec. Circ. 100. 21 p.
9. PANOPOULOS, N. J., and M. N. SCHROTH. 1974. Role of flagellar motility in the invasion of bean leaves by Pseudomonas phaseolicola. Phytopathology 64:1389-1397.
10. SCHNEIDER, R. W., and R. G. GROGAN. 1977. Bacterial speck of tomato: sources of inoculum and establishment of a resident population. Phytopathology 67:388-394.