Role of Nonhost Species as Alternate Inoculum Sources of *Xanthomonas phaseoli*

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ABSTRACT

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Rifampin-resistant mutants R15-1 of *Xanthomonas phaseoli* (*Xp*, cause of bean common bacterial blight) and R17 of *X. phaseoli* var. *fuscans* (*Xpf*, cause of bean fuscous bacterial blight) were used in studies on the role of weeds and other nonhost species in *Xp* and *Xpf* epidemiology. *Xp* grew epiphytically on leaves of various nonhost crop and weed species, and viable populations were recovered up to 21 days after bacteria were placed on leaf surfaces. Reciprocal secondary spread between susceptible bean and lambsquarters (*Chenopodium album*) and pigweed (*Amaranthus retroflexus*) occurred within 12 days after inoculation.

Debris from diseased plants has always been considered a possible source for seasonal carry-over of plant pathogenic bacteria. Recent studies have shown that phytopathogenic bacteria can survive in protected positions on healthy leaves of host as well as nonhost plants (2–5,9,15, 17). The ability of plant pathogenic bacteria to grow epiphytically in and/or on susceptible and resistant plant tissue may be of epidemiologic importance by serving to build up inoculum before infection. Such growth may provide pathogen cells for dissemination and for season-to-season survival (13). Ercolani et al (5) recovered *Pseudomonas syringae* throughout the year from leaf surfaces of healthy *Vicia villosa* (hairy vetch) and correlated natural outbreaks of bean brown spot with the epiphytes on nonsusceptible hairy vetch. Isaka (8) reported that *Xanthomonas oryzae* was able to overwinter on various weeds growing in rice fields, and Laub and Stall (11) suggested that *X. vesicatoria* may be disseminated to weeds, survive as a resident through the summer period, and serve as source of inoculum to tomato and pepper plants. Recently, Latorre and Jones (10) reported that *P. syringae* was isolated from weeds and infected sour cherry leaves; weed populations of the pathogen were considered a source of inoculum for bacterial canker.

Little information is available on the possible role of weeds in the survival and dissemination of *Xp* and *Xpf*. Gardner (6) and Sabet et al (16) suggested that different isolates of *Xp* may infect a number of weeds under natural conditions. Schuster (19,20) reported that *Xp* overwintered in bean and weed debris under Nebraska field conditions.

We now report the results of studies on 1) epiphytic populations of *Xp* on leaves of various nonhost plant species and 2) reciprocal spread of *Xp* between weeds and susceptible bean.

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MATERIALS AND METHODS

Field experiments were done at the Botany and Plant Pathology Research Farm, Michigan State University, East Lansing, during the 1977 and 1978 growing seasons. In greenhouse experiments, plants were grown under controlled conditions of temperature (27 ± 2 C) and illumination (daylight supplemented with 14 hr of fluorescent lighting) in a standard soil mixture in 16-cm diameter clay plots and were watered alternately as needed with Rapid-Gro (1 tsp per 2 L of water) and tap water.

Bacterial isolates. Naturally occurring mutants R15-1 of *X. phaseoli* and R17 of *X. phaseoli* var. *fuscans* resistant to rifampin were obtained by conventional selective plating methods (14) and were found to possess virulence equivalent to the parental wild types (*Xp* 15 and *Xpf* 17, Michigan isolates).

Inoculation techniques. Bacterial cells were washed from plates of 2-day-old YCA (10 g of yeast extract, 2.5 g of calcium carbonate, and 15 g of agar per 1,000 ml of distilled water) cultures incubated at room temperature (24 ± 1 C) and suspended in sterile distilled water at concentrations of 1.0×10^7–2.0×10^8 cells per milliliter of R15-1 or R17. Inoculum was applied to plants in the vegetative stage of development by gently spraying with a DeVilbiss sprayer (in the greenhouse) or with a knapsack sprayer (in the field); inoculum was applied to runoff on the lower and upper leaf surfaces or by water-soaking the leaves (18).

Determination of bacterial populations. Epiphytic growth of *Xp* (R15-1) on leaves was determined at intervals after inoculation by means of leaf-impression cultures. Direct leaf prints were made by gently pressing the upper and lower surfaces of leaves for 1 min onto plates of YCA medium supplemented with 50 μg per milliliter of rifampin. Bacterial growth was evaluated by estimating the percentage of the leaf-print area covered with bacteria after 72 hr of incubation at room temperature. Multiplication and spread of *Xp* (R15-1) or *Xpf* (R17) were monitored at intervals after inoculation. Populations of viable bacterial cells were assayed from 21 randomly sampled leaflets replicated three times by mincing the tissue in 0.01 M phosphate buffer, pH 7.2. After appropriate serial dilutions, suspensions were plated on YCA medium supplemented with 50 μg per milliliter of rifampin and 25 μg per milliliter of cycloheximide. Colonies were counted after 4 days of incubation at room temperature. Populations of blight bacteria were expressed as number of colony-forming units (CFU) per 100 cm^2 of leaf tissue (approximate average area of one leaf) or CFU per gram dry weight of tissue.

RESULTS

Epiphytic growth of *Xp* (R15-1) on nonhost species as determined by leaf prints on YCA medium. High bacterial populations were detected on leaves of susceptible bean cultivars Seafarer and Tuscola, resistant genotype P597 (*Phaseolus acutifolius*), soybean (cultivar Hark), cowpea (cultivar Mississippi Silver), sugar beet (US-20), and pigweed. Populations in the last five species tended to decline about 12 days after inoculation

Table 1. Epiphytic growth of *Xanthomonas phaseoli* (R15-1) on different host and nonhost species as determined by leaf prints on YCA-rifampin medium[a]

	Days after inoculation								
Cultivar and species	**1 hr**	**1**	**3**	**6**	**9**	**12**	**15**	**18**	**21**
Seafarer (*Phaseolus vulgaris*)	++++[b]	++++	++++	++++	++++	+++	(+++)[c]	(+++)	(+++)
Tuscola (*P. vulgaris*)	++++	++++	++++	++++	++++	+++	(+++)	(+++)	(+++)
P597 (*P. acutifolius*)	++++	++++	++++	+++	++	++	++	++	+
Hark (*Glycine max*)	++++	++++	++++	++	++	+	+	+	+
Mississippi Silver (*Vigna unguiculata*)	++++	++++	++++	+++	+++	++	++	+	+
WG-4A (*Zea mays*)	++++	++	+	+	+	+	+	+	−
US-20 (*Beta vulgaris*)	++++	+++	++	++	++	+	+	+	+
Chenopodium album	++++	++	+	+	+	+	+	+	+
Amaranthus retroflexus	++++	++++	++++	++	+	+	+	+	+

[a]Leaves were sprayed to runoff with a 2×10^8 cells/ml suspension of R15-1.
[b]++++ = 75% of leaf-print area covered with bacterial growth after 72 hr of incubation, +++ = 50–75%, ++ = 25–50%, + = 25%, − = 0%. Estimates are average of three experiments.
[c]Parentheses indicate macroscopic disease symptoms.

Table 2. Population of R15-1 (*Xanthomonas phaseoli*) and R17 (*X. phaseoli* var. *fuscans*) in dry leaf tissue of greenhouse-grown plants[a]

		CFU/g dry leaf tissue[b]	
Cultivar	**Species**	**R15-1**	**R17**
Tepary (Arizona-Buff)	*Phaseolus acutifolius*	9.4×10^7	1.2×10^7
P597	*P. acutifolius*	6.4×10^8	1.7×10^7
MSU-51319	*P. vulgaris*	1.4×10^8	6.1×10^7
Tara	*P. vulgaris*	6.8×10^8	5.8×10^7
Seafarer	*P. vulgaris*	6.2×10^8	6.0×10^7
Hark	*Glycine max*	6.0×10^8	6.1×10^7
Mississippi Silver	*Vigna unguiculata*	2.2×10^7	1.5×10^7
US-20	*Beta vulgaris*	3.2×10^5	1.0×10^4
WG-4A	*Zea mays*	1.2×10^5	3.3×10^3
Lambsquarters	*Chenopodium album*	2.0×10^6	1.8×10^6
Pigweed	*Amaranthus retroflexus*	2.6×10^6	1.0×10^4
Black nightshade	*Solanum nigrum*	1.2×10^6	7.5×10^3
Ragweed	*Ambrosia artemisiifolia*	3.6×10^4	2.0×10^4
Barnyard grass	*Echinochloa crusgalli*	2.1×10^4	6.7×10^4

[a]30- to 35-day-old plants were inoculated by water-soaking leaf tissue with a 5×10^7 cells/ml suspension of R15-1 and R17. Leaves were harvested 22 days after inoculation and dried at room temperature (22–24 C).
[b]Number of viable bacterial cells (CFU) of R15-1 and R17 was determined in dry leaf tissues after 14 days of storage at room temperature.

Table 3. Reciprocal secondary spread of *Xanthomonas phaseoli* (R15-1) from susceptible bean genotype (Tuscola) to *Chenopodium album* (lambsquarters) and *Amaranthus retroflexus* (pigweed) under field conditions

		CFU/100 cm² leaf area, days after inoculation[a]				
Genotype	**Replicates**	**1**	**6**	**12**	**18**	**24**
Tuscola, inoculated	1	2.7×10^4	1.0×10^6	(7.8×10^7)[b]	(1.1×10^8)	(5.0×10^7)
	2	1.7×10^4	9.2×10^5	(8.4×10^7)	(1.2×10^8)	(1.0×10^8)
	3	1.6×10^4	1.2×10^6	(9.8×10^7)	(1.2×10^8)	(4.6×10^7)
	4	1.1×10^4	1.3×10^6	(3.9×10^7)	(7.5×10^7)	(7.6×10^7)
	$\bar{x}$	1.8×10^4	1.1×10^6	7.5×10^7	1.1×10^8	6.8×10^7
Weeds, noninoculated	1	0.0	0.0	3.7×10^2	2.1×10^5	2.5×10^3
	2	0.0	0.0	0.0	3.1×10^4	3.9×10^3
	3	0.0	0.0	4.2×10^2	7.2×10^4	3.7×10^3
	4	0.0	0.0	8.4×10^2	1.1×10^4	1.4×10^3
	$\bar{x}$	0.0	0.0	4.1×10^2	8.1×10^4	2.9×10^3
Weeds, inoculated	1	1.4×10^3	7.7×10^4	1.2×10^6	2.7×10^4	7.6×10^4
	2	3.4×10^3	1.0×10^5	1.3×10^5	2.5×10^4	5.5×10^4
	3	6.8×10^2	6.9×10^4	1.3×10^6	3.8×10^4	6.3×10^4
	4	3.4×10^2	1.1×10^5	1.6×10^6	1.8×10^4	4.2×10^4
	$\bar{x}$	2.2×10^3	8.9×10^4	1.4×10^6	2.7×10^4	5.9×10^4
Tuscola, noninoculated	1	0.0	0.0	3.0×10^4	(1.8×10^7)	(7.6×10^4)
	2	0.0	0.0	3.2×10^4	(1.2×10^7)	(7.5×10^6)
	3	0.0	0.0	3.2×10^4	(1.1×10^7)	(2.8×10^7)
	4	0.0	0.0	1.7×10^4	1.6×10^3	(2.9×10^7)
	$\bar{x}$	0.0	0.0	2.8×10^7	1.0×10^7	(5.1×10^7)

[a]Plants were inoculated at day 0 by gentle spraying to runoff with a 1.0×10^7 cells/ml suspension of R15-1.
[b]Parentheses indicate macroscopic disease symptoms.

(Table 1). On corn (WG-4A) and lambsquarters, bacterial populations tended to decline the third day after inoculation but remained at detectable amounts 18 and 21 days after inoculation, respectively. At that time, lambsquarters leaves were almost senescent. Bacteria were detected on the upper and lower surfaces of the leaves; concentrations were higher on the lower surface. Pathogenicity of R15-1 isolated 21 days after inoculation from each of the different materials studied was tested by inoculating a susceptible bean cultivar; no change in the virulence of the isolate was observed.

Survival of *Xp* (R15-1) and *Xpf* (R17) in dry tissues of nonhost species. High populations of R15-1 and R17 were recovered 36 days after leaf inoculation (water-soaking) from almost all the species studied (Table 2). R15-1 and R17 isolated from each of the dry tissue samples were pathogenic, as tested by host inoculations. Weighed samples of these dry leaf tissues were used to study overseason bacterial survival (1).

Reciprocal secondary spread of *Xp* between susceptible bean cultivar and weeds. *Xp* multiplied in inoculated leaves of weeds and bean plants; growth rates in weeds were lower (Table 3). A substantial proportion of the total *Xp* population on weeds was detected on leaf surfaces, as determined by direct leaf prints on the rifampin-selective medium. Secondary spread to noninoculated beans and weeds was first detected after several days of heavy rains, 12 days after inoculation. At that time, *Xp* had reached exponential growth in both bean and weed leaves, with average populations of 7.5×10^7 and 1.4×10^6 bacterial cells per leaf, respectively.

DISCUSSION

The increase of a pathogen in the absence of symptoms in susceptible and resistant tissue may be of epidemiologic importance by serving to build up inoculum before infection or as a source of inoculum for secondary spread and also by providing pathogen cells that survive unfavorable conditions. On the basis of work with *X. vesicatoria*, Leben (12) suggested that pathogenic bacteria possessed a "resident phase" in their life cycle; this was defined as the capacity for multiplication on the surface parts of healthy tissue. Several studies subsequently confirmed that a number of bacterial plant pathogens possess a resident phase, which may be associated with leaves, buds, or flowers of host or nonhost plants (4,7,9,11,17). More recently, Leben (13) proposed to expand the term "resident" to include all types of associations of microflora with healthy plants, including the surface and interior of plants, above and below ground.

The results obtained in our study suggest that leaves of nonhost plants may support epiphytic multiplication of blight bacteria and that the bacterium may possess a resident phase in its life cycle. To what extent the epiphytic capability of bean blight bacteria is epidemiologically important for the disease under field conditions remains to be determined.

Secondary spread of *Xp* between blight-susceptible beans and weeds indicates that inoculum is available for dissemination early after colonization of the plants, suggesting that secondary spread, primarily due to rain splashing, occurs in the field before symptom expression. Although epiphytic growth of *Xp* on weed plants has been suggested (6,16), our results indicate that the bacteria may be a resident on weed species. The inherent ability of leaves of host and nonhost species to support epiphytic growth of blight bacteria may be of importance under Michigan bean-growing conditions.

In Latin America, particularly in the tropics, environmental conditions allow more than one successive crop during the year. Also, beans are frequently cultivated in association with other crops, and heavy weed infestations are common problems in bean fields. Weeds, as well as associated crops such as corn, could function as important sources of blight bacterial inoculum in tropical and semitropical bean-production regions.

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