Seasonal Variation in Populations of Pathogenic Pseudomonads on Soybean Leaves

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Scientific Journal Series Paper 11,599 of the Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul 55108.
Accepted for publication 11 May 1981.

ABSTRACT

Mew, T. W., and Kennedy, B. W. 1982. Seasonal variation in populations of pathogenic pseudomonads on soybean leaves. Phytopathology 72: 103-105.

In each of two seasons, greater numbers of pathogens could be washed from symptomless soybean leaves approximately 6 wk after planting than earlier or later in the season. Numbers of both plant-pathogenic and nonpathogenic bacteria declined as leaves approached senescence. Early in the growing season, pathogenic races within the population tended to be specific to the cultivar from which they were isolated; late in the season, percentages of isolates pathogenic to a number of cultivars increased and cultivar-specific components of the total population decreased.

Additional key words: Pseudomonas glycinea, Glycine max, epiphytes.

Bacterial blight of soybean (*Glycine max* L.) caused by *Pseudomonas syringae* pv. *glycinea* (4) (hereafter called "pv. *glycinea*") is a chronic leaf-spot disease in major production areas of the USA; the pathogen seldom invades vascular tissues and rarely is markedly destructive. In view of the apparent ease of dispersal of bacteria from plant to plant once inoculum is present, the study reported here was initiated to investigate the natural occurrence, at various stages of plant growth, of races of pv. *glycinea* on leaves of cultivars of differing genetic origin grown in close proximity. No inoculum was applied. It was assumed that races occurring naturally on (or in) the seed of a given cultivar would become dispersed generally in the canopy of all cultivars. Since the natural occurrence of epiphytic growth of this pathogen on soybean leaves in the field, especially of races on resistant cultivars, is unclear, symptomless leaves were used for all assays to minimize selection for pathogenic races capable of causing lesions on any particular genotype assayed. Plots were located on land cropped to either soybean or cereal grains in previous seasons.

In other areas of the midwest, the bacterium can be readily isolated from rainwater that drops from infected soybean leaves in the field (3), can colonize surfaces of soybean buds (8), and is inherently capable of multiplying on leaf surfaces (6,10). A similar (yet pathologically distinct) bacterium often occurs on buds, pollinated flowers, and young pods of healthy soybean plants in the field (7). Aerosols of the pathogen occur naturally around the foliage of infected plants during rain or sprinkler irrigation (13) and artificially created aerosols can be responsible for infection or initiation of epiphytic growth in the greenhouse (12). Several races have been reported in Minnesota (1), yet rarely has more than one race been found in a single commercial field.

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0031-949X/82/01010303/$03.00/0

MATERIALS AND METHODS

Soybean cultivars Acme, Chippewa-64, and Merit were selected for detailed studies because they represent genotypes with varying agronomic characters and also had contrasting reactions to a number of pathogenic races of pv. *glycinea* (1). In some studies, garden bean (*Phaseolus vulgaris* L. 'Bush Blue Lake'), and lima bean (*Phaseolus limensis* Macf. 'Fordhook 242') also were used. Each cultivar was assayed for the presence of fluorescent pseudomonads by washing leaves for 2 hr and estimating numbers of bacteria in the aqueous extract via plate counts on SDP, a selective and differential medium that we modified (10) from Kado and Heskett (5).

In all field studies, soybeans were planted in a completely randomized split plot design with four replicates per treatment. Main blocks consisted of the three cultivars in rows 8.6 m long and 76 cm apart. Snap bean cultivar Bush Blue Lake was sown between blocks and in border rows. Reading on severity of naturally occurring blight disease was made at intervals by counting numbers

of bacterial blight lesions on trifoliolate leaves in descending order from terminal buds.

At approximately 1-wk intervals beginning 3 wk after planting, 20 symptomless trifoliolate leaves (second from terminal buds) were picked from randomly selected plants, transported in plastic bags to the laboratory within 30 min, weighed, washed in sterile distilled water plus 0.01% Tween-80 (polyoxyethylene sorbitan monooleate) on a shaker for 2 hr (20 leaves per 500 ml). Plate counts were made from wash water and values that represent bacteria per gram fresh weight of leaf tissue were calculated. Termination of the sampling period in each of two seasons differed depending on maturity and disease incidence on leaves of cultivars. Pathogenicity tests were made by smearing a water suspension containing 10^6–10^8 cells per milliliter on leaves of greenhouse-grown plants injured by abrasion with 22-μm (600-mesh) Carborundum. Samples representative of the soybean pathogen (15–20 colonies from each leaf surface population assay) were used to inoculate garden bean, lima bean, and three cultivars of soybean (Acme, Chippewa-64, and Merit). In addition, isolates were grouped according to the five major characteristics selected by Lelliot et al (9): oxidase reaction, production of arginine dihydrolase, levan production, rotting of potato tuber tissue, and induction of a hypersensitive reaction (HR) in tobacco. This simplified system tends to place together many species formerly separated by differences in plant pathogenicity.

RESULTS

Total bacterial count. In the first year, populations of fluorescent pseudomonads of the three cultivars fluctuated similarly until the second week in August when the numbers increased on Acme (a highly susceptible, early maturing cultivar) and then dropped sharply (Fig. 1). In the second season, populations on all three cultivars were increasing rapidly by the first week in July and by mid-July, Acme was so severely infected that symptomless leaves were unavailable for assay. In both years, bacterial populations tended to decline as plants approached maturity. Also, rainfall consistently caused an immediate reduction in bacterial populations, but they were quickly reestablished.

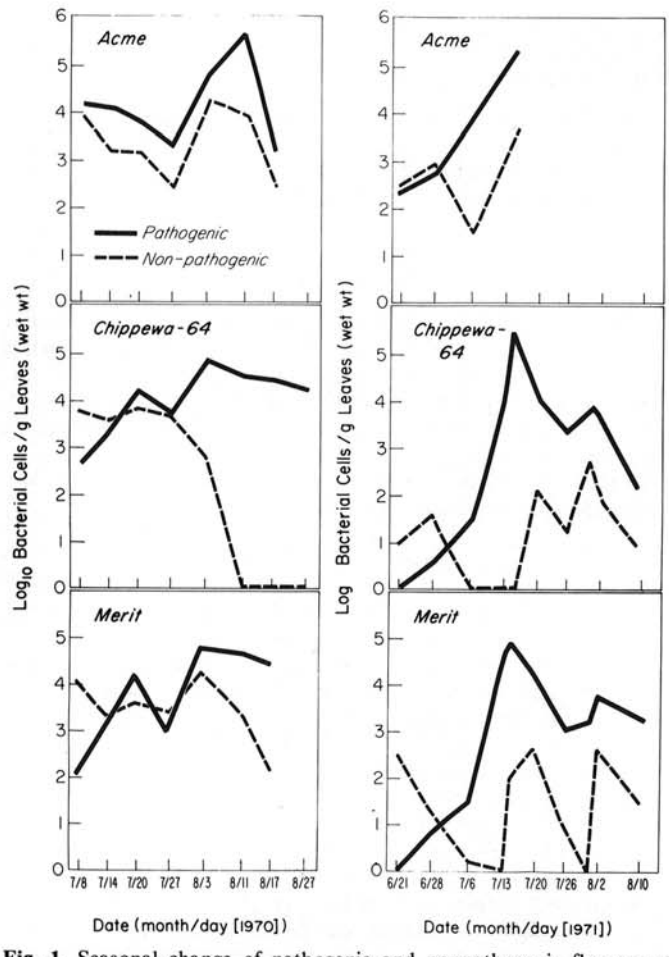

Fig. 1. Seasonal change of pathogenic and nonpathogenic fluorescent pseudomonads in the phyllosphere of three soybean cultivars during two seasons. Each point represents the mean of four replications, 20 leaves per replicate; points ~≤1 are arbitrary.

Saprophytic isolates. Two fast-growing, mucoid, fluorescent pseudomonads were isolated frequently throughout both seasons. Colonies of both were blue-transparent on the medium employed, and one was characterized by a yellowish center. Populations of both were consistently lower than were those of pathogenic forms on plants, and they showed no antagonism to pathogens in vitro.

Pathogenic isolates. On SPD agar plates, colonies of pathogenic fluorescent pseudomonads were large, dome-shaped, and slow growing; those of the saprophytes were flat and fast growing. Numbers of pathogenic isolates were often greater than nonpathogenic components of the fluorescent pseudomonad population (Fig. 1).

Pathogenic races. Isolates were initially designated as Acme, Chippewa-64, and Merit. Analysis of pathogenicity (ability to cause typical angular, water-soaked bacterial blight lesions on leaves, often with chlorotic halos beyond margins of lesions) indicated that the pathogenic component of the population differed in each of the two seasons (Table 1). These differences were likely due to presence of different pathogenic races and variation in seasonal weather patterns.

In the first year, all isolates from leaves of the susceptible cultivar Acme were pathogenic to it regardless of when they were isolated during the growing season. Early in the season, however, isolates from Acme leaves tended to be specific to that cultivar. In the more resistant Chippewa-64, more than half of the pathogenic isolates washed from leaves were pathogenic to both Chippewa-64 and Acme and 35% to Merit, a cultivar that was usually intermediate in resistance. Late-season isolates tended to have greater percentages capable of attacking not only the cultivar from which they were isolated, but the other two cultivars as well. In the second year, there was a tendency for each cultivar to harbor a single compatible predominant race early in the season and for it to persist throughout the season.

Two distinct races were recognized that persisted in the phyllosphere of field-grown soybeans during both years: Race 1 was pathogenic to cultivar Acme and it was most predominant on this cultivar early in the season. Chippewa-64 and Merit were resistant. Race 2 was pathogenic to all three cultivars in this study and became predominant late in the season. Although pseudomonad populations were detected in the phyllosphere of Chippewa-64 and Merit that were pathogenic to those cultivars only, these were encountered infrequently and were only a small part of the total population.

Reaction of nonhosts. Cross-inoculation tests with isolates of pathogenic fluorescent pseudomands collected from the three soybean cultivars indicated that none of 40 isolates tested were

TABLE 1. Pathogenicity of fluorescent pseudomonads isolated from the phyllosphere of three soybean cultivars during two seasons

Origin of phyllosphere isolates[a]		Pathogenicity of phyllosphere isolates (%)					
		Acme		Chippewa-64		Merit	
Cultivar	Time	1970	1971	1970	1971	1970	1971
Acme	Early[b]	100	75	2	28	8	46
	Late[b]	100	85	63	58	55	75
Chippewa-64	Early	57	22	57	68	35	56
	Late	87	90	98	74	83	60
Merit	Early	50	75	50	43	75	84
	Late	87	85	74	75	90	90

[a] Based upon a total of 207 isolates in 1970 and 186 isolates in 1971.
[b] In 1970, all samples collected in July were designated early and all those collected in August were designated late. In 1971, early samples were collected 23 June–7 July, late samples 15 July–1 August.

virulent to field-grown *P. vulgaris* (snap bean) cultivar Bush Blue Lake or to *P. limensis* (lima bean) cultivar Fordhook 242. However, pv. *glycinea* was detected in the phyllosphere of snap bean cultivar Bush Blue Lake. Lima bean was hypersensitive in the field, but developed water-soaked lesions (with no halo or systemic infection) on leaves of artificially inoculated greenhouse-grown plants.

Taxonomy. At approximately 2-wk intervals throughout the growing season of both years, five pathogenic and five nonpathogenic isolates of fluorescent pseudomonads were chosen at random from population analyses on the three soybean cultivars and evaluated according to the scheme of Lelliott et al (9). Taxonomic grouping of a final total of 80 isolates, on the basis of five determinative tests, indicated that the pathogens fit Lelliott's Group IA fluorescent pseudomonads, whereas the nonpathogenic forms apparently were members of either Group IB or V. Pathogenic isolates were positive for tobacco HR and levan production and negative for oxidase, arginine dihydrolase, and potato soft rot.

Natural occurrence of bacterial blight in the field. Lesion counts were made weekly on leaves comparable in age and number to those used for surface population analyses in 1971. Samples were taken at random from each cultivar (20 leaves from each of four replications). The object was to correlate the relationship between bacterial populations on symptomless leaves and the incidence of disease. Bacterial blight was most severe at about the sixth week after planting; this correlated closely with high populations of pathogens washed from symptomless leaves (Fig. 1).

DISCUSSION

Total populations of fluorescent pseudomonads changed with the season and growth stages of soybean plants, but were not notably different among three cultivars of soybean during two seasons. Leaves of cultivar Acme were more susceptible to pathogenic components of the bacterial population than was Chippewa-64 or Merit. Although pathogenic pseudomonads on Acme appeared earlier than on the other two cultivars, the population peak was reached at about the same time on all three. This seasonal variation in population of the pathogen on foliage differs from the results of Crosse (2), who compared the leaf surface population of *P. morsprunorum* in autumn on two cherry cultivars in England. He found consistently higher populations on late season leaves of a susceptible cultivar compared to a resistant one.

Early in the growing season, pathogenic populations tended to be specific to the cultivar from which they were isolated. Late in the season, the percentage of the isolates that were pathogenic to a number of cultivars increased and races specific to a single cultivar declined. Apparently, soybean cultivars support races of pv. *glycinea* that do not cause symptoms on them in the field. The observation that fewer cultivar-specific isolates occur late in the season suggests that several races can exist on old or senescing leaves.

Observation during 1970 and 1971 indicated that leaves of *P. vulgaris* cultivar Bush Blue Lake were not attacked by pathogenic fluorescent pseudomonads that were abundant on leaves of nearby soybeans growing in adjoining rows where there was frequent intermingling of leaves. However, fluorescent pseudomonads pathogenic to soybean were present in low numbers on snap bean leaves. It is significant that *P. limensis* cultivar Fordhook 242 inoculated in the greenhouse with pathogenic fluorescent pseudomonads isolated from soybean leaves developed water-soaked lesions, but was consistently hypersensitive to inoculations in the field. Our colleagues have noted wide susceptibility of this cultivar to pathogenic pseudomonads in the greenhouse (11). We conclude that the two groups of pathogens, although taxonomically similar, are pathogenically distinct; there is no evidence from our study that either pathogen will consistently infect its specific host in the field. The magnitude of this specificity, compared to that encountered in the greenhouse, is a point worthy of further study. A point needing further clarification deals with the extent of reproduction on leaf surfaces compared to mere survival after deposit. Since *P. vulgaris*, which is not a host, had pv. *glycinea* on the foliage, spread from lesions on nearby infected soybean foliage may be more significant in determining abundance of this pathogen than is growth on leaf surfaces.

Conclusions drawn from our results are consistent with and extend those of Daft and Leben (3), who found pv. *glycinea* in water from wet field plants following a rainstorm; apparently these bacteria subsequently can either increase or remain viable on healthy leaves for considerable periods of time. In view of the numbers of pathogenic fluorescent pseudomonads present on symptomless soybean leaves in the field by the sixth week after planting in Minnesota, we concluded that blight could, and probably does, develop on soybeans infected by inoculum from such a source.

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