Growth of Pseudomonas glycinea on the Surface of Soybean Leaves

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ABSTRACT

Each of three pathogenic races of *Pseudomonas glycinea* were sprayed on leaves of three soybean cultivars in the greenhouse and assays were made of bacterial populations on symptomless leaves at intervals up to 240 hr. On susceptible leaves, bacteria increased by about 1,000-fold within 1-2 weeks. Populations were unchanged or declined on leaf surfaces of resistant cultivars. On leaf surfaces of plants intermediate in susceptibility, population increased during the 1st week, but declined during the 2nd week. Phytopathology 61:715-716.

Additional key words: epiphytes.

Bacterial blight of soybean (*Glycine max* [L.] Merr.) caused by *Pseudomonas glycinea* Coerper is a common leaf disease and is especially prevalent in the upper midwestern areas of the United States. The pathogen apparently can live on the surface of buds (6). In view of the fact that the nomenspecies comprises a number of pathogenic races (1), our attention was drawn to the possibility that the resident phase may be influenced by host genotype. In order to improve our understanding of survival and multiplication of *P. glycinea* on foliage, we proceeded to determine population changes of three races on the leaf surface of three symptomless cultivars of soybean in the greenhouse.

MATERIALS AND METHODS.—Expected reaction of various host-parasite combinations used to study epiphytic populations is listed in Table 1. Each race of the pathogen was either virulent, intermediate, or avirulent on one of the three host differentials. Nine possible combinations were assayed, using four replications of each combination and repeating each treatment in a subsequent experiment.

Soybean seeds were surface-disinfected with 0.5% sodium hypochlorite for 10 min, planted in 5-inch pots of pasteurized soil, and grown in insect cages for protection from possible contamination between treatments. Cultures for inoculum grown on YDC (yeast-dextrose-calcium carbonate) agar for 48 hr were washed twice, and the resulting suspension was adjusted to about 1.7×10^8 viable cells/ml. Before use, suspensions were also adjusted to contain 0.003% Tween 80 (polyoxyethylene sorbitan monooleate). Bacteria were introduced by either soaking seeds in suspensions before planting or by spraying the suspensions on leaves. By the first method, spread of bacteria from seeds to leaves was detected by noting symptoms after gentle abrasion of leaf surfaces with Carborundum. By the latter method, bacteria were gently atomized on plants by use of an airbrush until plants were wet but without runoff. By this method, where extreme caution was used to avoid injury and presumably to place bacteria on rather than in leaves, only occasionally (on ca. 1% of the leaves) did a lesion form on susceptible plants. Each of 10 leaves selected for an assay was examined under magnification for presence of lesions, and all assays were made on symptomless leaves.

TABLE 1. Reaction of three soybean cultivars to three races of *Pseudomonas glycinea*

Race of pathogen	Natural reaction of host[a]		
	Acme	Chippewa	Merit
R1	S	R	R
R2	S	S	S
R5	R	I	I

[a] R = resistant; I = intermediate; S = susceptible.

At intervals of 4, 24, 48, 120, and 240 hr after spray inoculation, 10 leaves were sampled at random and washed for 1 hr by shaking in 100 ml of water containing 0.001% Tween 80; bacterial numbers were determined by dilution series on a modified Kado's Medium D, selective for *Pseudomonas* (5). For our assays, 3% sucrose and 0.0001% crystal violet were added; at least 80% of the colonies isolated with this medium were *P. glycinea* as indicated by pathogenicity tests. In every assay, 10 randomly selected colonies were evaluated on a differential host series to assure that the medium was in fact selective for races 1, 2, or 5 of *P. glycinea*.

RESULTS.—*Fate of bacteria placed on seeds.*—Blight lesions developed on leaves after seed inoculation with a race of *P. glycinea* to which plants were susceptible. This confirms the results of Leben et al. (7), who found that bacteria on buds can originate from infected seeds. No symptoms developed on comparable plants inoculated with a race to which plants were resistant.

Fate of bacteria placed on leaves.—Multiplication of bacteria on leaves was evident in every combination where susceptibility or an intermediate reaction was expected (Fig. 1). In contrast, bacteria did not multiply on uninjured leaf surfaces of cultivar-race combinations expected to result in host resistance by standard inoculation procedures.

DISCUSSION.—These data indicate that race specificity of *P. glycinea* correlates with the resident phase of the bacteria on leaf surfaces of soybeans. Population

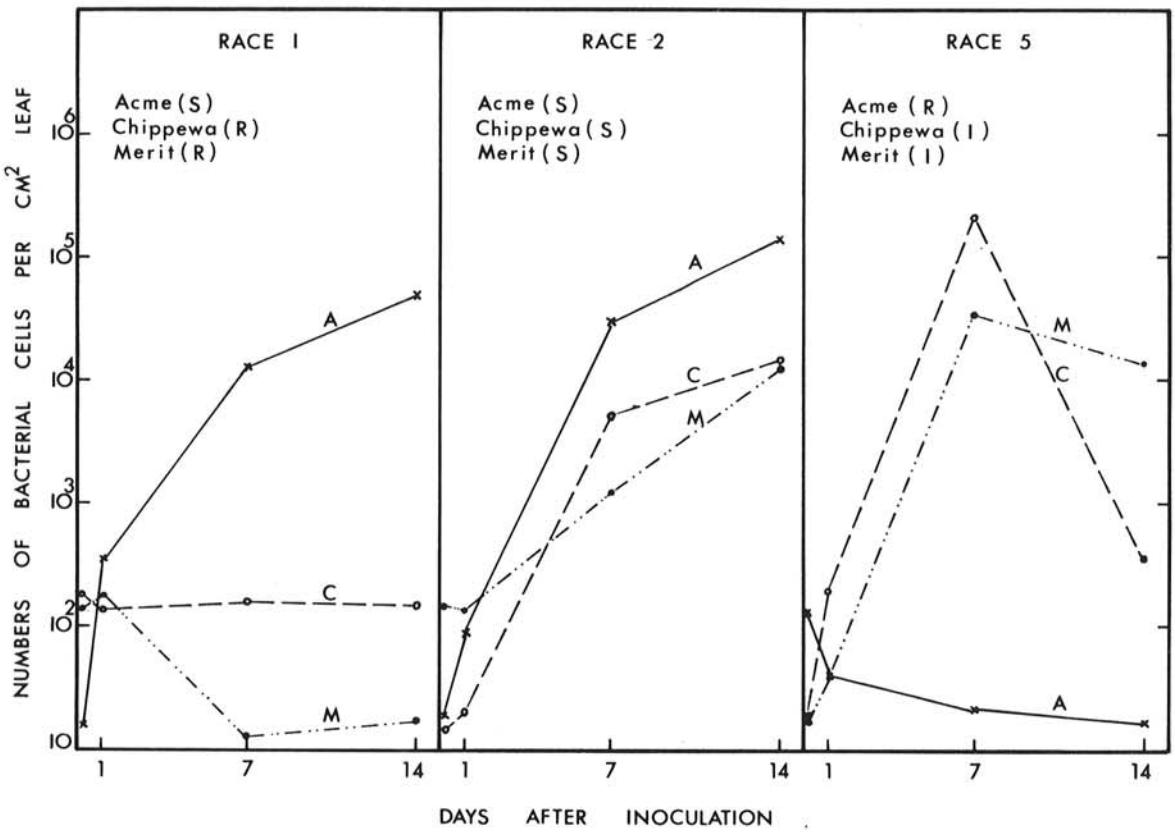

Fig. 1. Growth of *Pseudomonas glycinea* on susceptible (S), intermediate (I), and resistant (R) soybean leaf surfaces.

levels increased only on susceptible leaves. Bacteria tended to increase, then diminish more quickly, on surfaces of leaves possessing intermediate susceptibility compared to more susceptible leaves.

Our data imply that external factors apparently influence specificity of the resident phase. The data presented by Crosse (2, 3) indicate a similar effect of the host on populations of *P. morsprunorum* during preepidemic stages. Furthermore, the epiphytic microflora of cherry and pear plants examined by Ercolani (4) at intervals after the plants had been artificially infested with either a cherry strain of *P. morsprunorum* or a pear strain of *P. syringae* were both found to be established on leaves of their natural hosts. They invariably failed to colonize the leaf surface of the nonhost plant, and pathogenicity of bacteria reisolated from either plant was found to be unchanged.

Field studies are underway to evaluate the significance of this information as it may apply to natural conditions in view of the possibility that it may have important implications relative to survival and abundance of inoculum.

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