Dissemination and Survival of Pseudomonas alboprecipitans Ascertained by Disease Distribution

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ABSTRACT

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A field-scale spatial distribution of bacterial leaf blight of corn in central Florida was plotted to locate a source of initial inoculum of *Pseudomonas alboprecipitans*. Doublet analysis demonstrated a nonrandom aggregation of diseased plants. Graphs of log of infection vs. log of distance were used to compare plant-disease dispersal gradients within plots. Comparison of correlation coefficients implicated farm equipment as a means of dissemination and ditchbank weeds as a reservoir for initial inoculum. A diagnostic medium based primarily on the utilization of Lactalysate® was used for the isolation of *P. alboprecipitans* from ditchbank flora. It was found that long-term survival of the bacterium occurred in association with vaseygrass (*Paspalum urvillei*). The bacterium survived in association with vaseygrass seed as well as in leaf tissue.

Additional key words: *Zea mays*, epidemiology, disease distribution.

A bacterial disease of sweet corn [*Zea mays saccharata* (Sturtevant) Bailey] caused by *Pseudomonas alboprecipitans* Rosen caused significant economic losses in Florida and elsewhere during the past several years (5, 17). The most common symptoms on corn plants are long, narrow lesions on the foliage and basal rot of the ear. Other symptoms include stalk lesions, lodging, and a tassle rot.

Rosen (15) first observed *P. alboprecipitans* on foxtail *(Setaria lutescens)* and described the pathogen in detail in 1922 (16). In 1949, Johnson et al. (10) reported *P. alboprecipitans* as the causal organism of bacterial leaf blight and stalk rot of corn. Recently, it was proposed that *P. alboprecipitans* and *P. avenae* were synonymous (17). It was recommended that *P. avenae* be adopted as the current nomenspecies as it is the older of the two epithets. However, an authentic type-culture of *P. avenae* does not exist and its original description was inadequate. In the original description, Manns (14) described the apparent presence of endospores in old cultures of *P. avenae* and it is not clear that he worked with a pure culture. Elliott (3) speculated that Manns in reality had worked with several bacterial diseases, one of which was halo-blight of oats caused by *P. coronafaciens*. Dye et al. (2) proposed not to retain *P. avenae* as a legitimate name; we concur and choose to use *P. alboprecipitans* as the name of the bacterial leaf blight and stalk rot pathogen.

At present, there are no successful control measures for bacterial leaf blight and stalk rot of corn. To develop an effective disease control program for *P. alboprecipitans*, information directly relating to the overseasoning mechanisms of the pathogen is needed. Plant disease dispersal gradients can be used to locate the site of the overseasoning pathogen as well as a source of inoculum (9, 18). We plotted the spatial distribution of bacterial leaf blight of corn in an extensive field study to locate the source of inoculum through the interpretation of a disease gradient. Because of the observed spatial distribution of the disease and reports (16, 21) of *P. alboprecipitans* occurring on wild grasses, the possible role of vaseygrass *(Paspalum urvillei* Steud.) in the survival of the pathogen also was examined. An abstract of this research has been published (7).

MATERIALS AND METHODS

The spatial distribution of bacterial leaf blight of sweet corn was observed at 1-wk intervals during a natural epidemic on the cultivar Gold Cup in experimental plots. Plots consisted of 34 rows (60 plants per row) and four replicates. Rows were spaced 91 cm apart and plants were thinned to 30 cm spacing in-the-row. The locations by row number and plant position of diseased plants were recorded and plotted on scaled graph paper. Field plots were sprayed weekly for control of the corn earworm, *Helothis zea* Boddie, and northern corn leaf blight caused by *Helminthosporium turcicum* Pass. *(Trichometasphaeria turcica* Luttrell). A plant was scored for bacterial blight on a plus or minus basis for foliar symptoms. Data were analyzed graphically, by chi-square analysis, linear regression analysis, and the doublet

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analysis method described by van der Plank (20).

Overseasoning of *P. alboprecipitans* in association with vaseygrass (*Paspalum urvillei* Steud.) was determined over an 11-mo period. This encompassed both the overwintering and oversummering periods in central Florida. Leaf samples of vaseygrass were collected every 6 wk from a group of ditchbank weeds in the sweet corn-growing area of Zellwood and examined for the presence of *P. alboprecipitans*. Vaseygrass seeds were collected from symptomless plants prior to the spring planting of sweet corn and examined for the presence of external and internal bacteria. Isolations were made on a medium consisting of 2.0% Lactalysate, (lot no. J6DCHQ; Baltimore Biological Laboratory, Cockeysville, MD 21030). 1.8% agar, 0.5% meat extract, contamination with *P. alboprecipitans,* vaseygrass seeds, surface-sterilized by ethanol flaming or nonsterilized, were triturated in 0.5 ml of 0.75% saline solution. The resulting suspensions were streaked onto the diagnostic medium to test for the presence of *P. alboprecipitans* and incubated at 36 C for 72 hr. Bacteria isolated from vaseygrass were identified by the production of a precipitate on the diagnostic medium, growth rates at 36 C, and symptomatology on sweet corn when inoculated by the Carborundum leaf-rub method. In addition, bacteria suspected of being *P. alboprecipitans* were separated from similar nonfluorescent phytopathogenic pseudomonads by several key tests, as outlined by Goto and Starr (8). All isolates were tested for fluorescent pigment production in King's Medium B (11) and production of a hypersensitive response in tobacco (12).

RESULTS

Bacterial leaf blight developed in the field plots within 30 days after seedling emergence. An unequal distribution of diseased plants among the four plot areas was found (Fig. 1). The easternmost plot (plot A) contained the largest number of diseased plants (13.3%). In field plots B, C, and D, 2.2%, 1.5%, and 1.9% of the plants were diseased, respectively. The density of diseased plants on the eastern edge of plot A was highest at observed access points for farm machinery. Tractor-mounted spray equipment entered the plots at the northeast corner of plot A and covered eight rows per swath. Doublet analysis, which provides a test for aggregation, demonstrated a nonrandom distribution for diseased plants within these plots (Table 1). In the eight-row

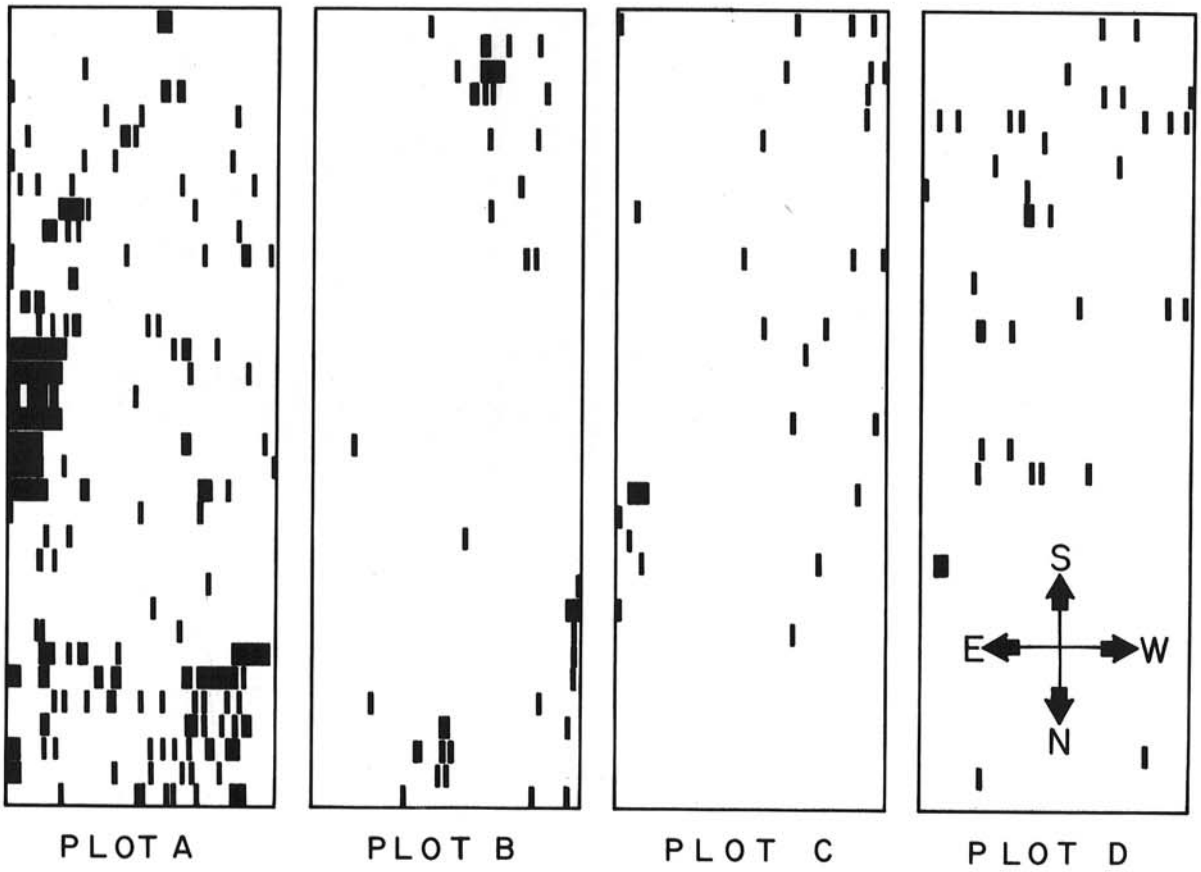

Fig. 1. Spatial distribution of bacterial leaf blight of corn (which is caused by *Pseudomonas alboprecipitans*) in the spring of 1975. Data represent primary spread of initial inoculum when corn plants were in the mid-whorl stage (collar of eighth leaf visible). Machinery entered plots at lower left corner of plot A and covered eight rows successively in plots A through D.

swaths where machinery moved east to west, there was a significantly greater number of diseased plants than in the eight-row swaths where machinery moved west to east (Table 2). Correlation coefficients of linearity for rows 1-8 ($r = -0.81$) and 17-24 ($r = -0.71$) were not significantly different at $P = 0.01$. They were significantly different from correlation coefficients for rows 9-16 ($r = -0.45$) and rows 25-32 ($r = -0.45$) at $P = 0.01$. Thus, eight-row swaths which had significantly more disease also had a gradient of diseased plants from east to west, whereas eight-row swaths with less disease had no demonstrable gradient.

We observed that ditchbank weeds grew within 14 m of the eastern edge of plot A, but no weeds were in close proximity to the western edge of plot D. Percentages of total diseased plants in plots A-D plotted against the distance from the assumed inoculation point (eastern edge of plot A) produced a negatively exponential curve similar to those associated with disease gradients (Fig. 2). When the log of percent plants diseased was plotted against the log of distance from the assumed inoculation point, a straight line with a negative slope was the result (Fig. 3). Linear regression analysis of the data had a correlation coefficient of −0.84. The data represent a primary gradient as there was no evidence of secondary spread.

In a survey of the ditchbank flora along the eastern edge of plot A, only vaseygrass frequently was found to be diseased with *P. alboprecipitans*. *Pseudomonas alboprecipitans* consistently was isolated from vaseygrass over an 11-mo period. This indicates that the bacterium can overseason in association with the foliage of this wild host. The bacterium was also isolated from crushed surface-sterilized and uncrushed nonsterilized vaseygrass seeds, but not from uncrushed, surface-sterilized seeds. Of 12 isolates of *P. alboprecipitans* from vaseygrass and vaseygrass seeds, all were pathogenic to sweet corn. The characteristics of isolates from vaseygrass in key tests agreed closely with isolate PA 117 used in the

TABLE 1. Doublet analysis of the distribution of corn plants infected with *Pseudomonas alboprecipitans* in four plots of sweet corn. Analysis is a test of aggregation of diseased plants and the measurement of disease spread from the source of inoculum

Plot	Expected doublets (no.)	Observed doublets (no.)
A	36.0	99.0[a]
B	1.0	9.0[a]
C	0.5	6.0[a]
D	0.7	4.0[a]
Total	38.2	118.0[a]

[a]Significantly different at $P = 0.01$ from the expected number of doublets as determined by chi-square analysis. This implies nonrandom aggregation.

TABLE 2. Number of corn plants with bacterial blight in eight-row swaths in relation to east/west movement of farm machinery

Row numbers	Direction of machinery	Number of diseased plants over total sampled
Plot A:		
1-8	West	116/480[a]
9-16	East	37/480[c]
17-24	West	76/480[b]
25-32	East	41/480[c]
All plots:		
1-8	West	136/1920[a]
9-16	East	66/1920[c]
17-24	West	91/1920[c]
25-32	East	86/1920[c]

[a]Significantly greater at $P = 0.01$.
[b]Significantly greater than 9-16 and 25-32 at $P = 0.01$.
[c]Not significantly different, $P = 0.05$.

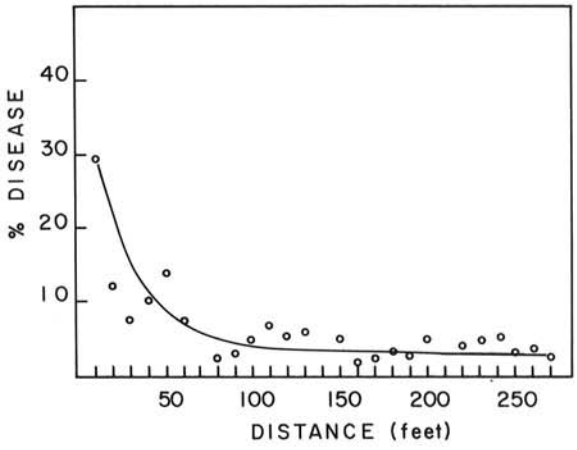

Fig. 2. Percentage of sweet corn plants in plots A through D scored positive for bacterial leaf blight of corn plotted against distance from the eastern edge of plot A.

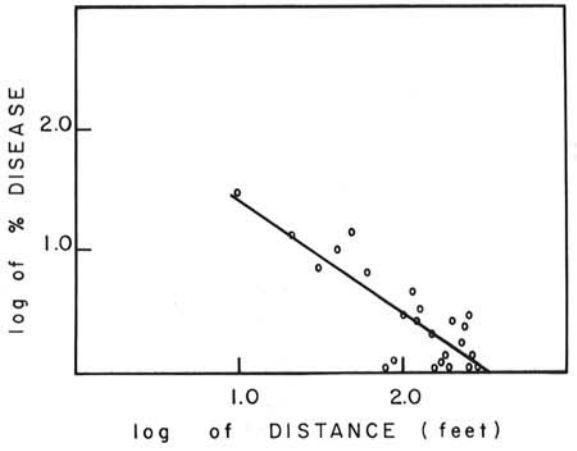

Fig. 3. The log of the percentage of sweet corn plants in plots A through D scored positive for bacterial leaf blight of corn plotted against the log of distance from the eastern edge of plot A. Correlation coefficient of linearity was −0.84.

characterization tests performed by Goto and Starr (8). Vaseygrass isolates were Gram-negative, produced a hypersensitive response in tobacco, did not produce fluorescent pigment in King's Medium B, were negative for arginine dihydrolase and starch hydrolysis but positive for Kovac's oxidase and nitrate reduction. All isolates, including PA 117, produced a clear halo within a white precipitate when grown on the diagnostic medium and all were capable of rapid growth at 36 C.

DISCUSSION

The importance of plant residue in the survival of phytopathogenic bacteria in soil is well recognized (1, 13). However, considering the rapid decomposition of plant materials in humid subtropical soils and the previous failure to recover a streptomycin-resistant mutant of *P. alboprecipitans* from soil (6), the possibility of soil survival of this bacterium is considered low.

We therefore looked for other sources of initial inoculum of *P. alboprecipitans*. The observed dispersal gradient in sweet corn that existed after primary spread of the bacteria was interpreted as evidence that there was a local source of inoculum. It was concluded that inoculum entered the plots from ditchbank weeds along the eastern edge of plot A. Farm machinery was incriminated as the disseminating agent of *P. alboprecipitans* from: (i) the high density of diseased plants at machinery access points, (ii) the pattern of distribution of diseased plants with significant differences among eight-row swaths, (iii) the development of a disease gradient from east to west, and (iv) the nonrandom aggregation of diseased plants. It was significant that increased dissemination occurred when machinery moved in an east-to-west direction. Strandberg (19) observed similar results with cabbage black rot and considered it evidence for spread by equipment. It would be desirable to ascertain how farm machinery disseminated the bacteria and how dissemination occurs in fields sprayed by aerial application.

The theory that a source of inoculum was centered in the eastern ditchbank weeds was supported by the discovery of diseased vaseygrass in that area. This is the first report of this perennial grass as a host of *P. alboprecipitans*. The symptoms are similar to the foliage symptoms observed on corn. It would be desirable to know the significance of resident and parasitic phases of the bacterium on vaseygrass. Ercolani et al. (4) found that epiphytic survival of *P. syringae* on hairy vetch was significant in the epidemiology of bacterial brown spot of bean. Since vaseygrass is a widespread weed in corn-growing areas of Florida, a resident phase could be important to the epidemiology of this disease.

The association of *P. alboprecipitans* with vaseygrass seed may be significant in long-term survival and dissemination. A grass seed that provides protection for the pathogen might be wind-blown or water-borne over greater distances than unprotected bacteria. It is conceivable that an infested grass seed in association with a corn plant could protect the bacterium until conditions were favorable for infection. The seed-borne phase in vaseygrass may also be important in spreading and increasing initial inoculum.

At present, vaseygrass is widespread along ditches and roadsides in the sweet-corn-growing areas of central Florida but rarely occurs within corn fields. Possible control measures for this disease would be the eradication of vaseygrass within a specified distance from corn fields or exclusion of farm machinery from contacting ditchbank weeds.

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