Spread of Bacterial Tan Spot of Soybean in the Field

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ABSTRACT

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The spatial distribution of bacterial tan spot of soybean caused by *Corynebacterium flaccumfaciens* was observed over time in four field plots in which two soybean plants were inoculated near the center of each plot. There was no evidence of spread by 2 wk after inoculation. By 3 wk after inoculation, secondary spread had occurred to plants neighboring the source, and by 4 wk after inoculation, disease was detected on plants in adjacent rows. Considerable disease was present at that time, and bacterial tan spot was more evident within rows than across rows. Mean size of the four areas of diseased plants 5 wk after inoculation was 19 × 5 m. There was no significant difference between mean disease levels within rows and across rows by 2 and 3 wk after inoculation, but there was a difference (significant at $P<0.05$) by 4 and 5 wk after inoculation. Bacterial tan spot was not observed in any of the four control plots.

Additional key word: dissemination

Bacterial tan spot of soybean (*Glycine max* (L.) Merr.) caused by a strain of *Corynebacterium flaccumfaciens* (Hedges) Dowson pathogenic to both bean (*Phaseolus vulgaris* L.) and soybean was found in all of the 95 counties sampled in Iowa in 1982. Tan spot was most prevalent in an 11-county area in the southwestern section of the state in which 84% of the fields were affected by the disease. Disease incidence was low in all fields. Of 407 infested fields observed (four 30-m rows examined per field), the mean number of diseased plants per row was 3.9 (4), equivalent to 0.004% incidence assuming a plant spacing of 3 cm.

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Symptoms of bacterial tan spot are dissimilar to those of bacterial blight (caused by *Pseudomonas syringae* pv. *glycinea* (Coerper) Young, Dye, & Wilkie) and bacterial pustule (caused by *Xanthomonas campestris* pv. *glycines* (Nakano) Dye), the two most commonly observed bacterial diseases of soybean in the northern United States. Lesion diameter in the latter two diseases is limited to a few millimeters unless lesions coalesce, whereas a single bacterial tan spot lesion may enlarge to cover an entire leaflet (2).

There is no epidemiological information available on bacterial tan spot of soybean. The objective of this study was to determine the extent of secondary dissemination of the pathogen under field conditions when two plants were artificially inoculated as the primary source of inoculum.

MATERIALS AND METHODS

Inoculum was prepared by centrifuging cells of *C. flaccumfaciens* from 24-hr cultures in nutrient broth/yeast-extract broth (7), resuspending the resulting pellets in sterile 0.6% saline, and adjusting the concentration to 10^8 colony-forming units per milliliter. Plants to be inoculated were dusted lightly with 400-mesh Carborundum before leaves were rubbed briskly with a cheesecloth pad saturated with inoculum. Susceptibility of Clark 63 to *C. flaccumfaciens* strain ATCC 33802 (American Type Culture Collection, Rockville, MD) was established previously (2). In four of the plots, a single plant near the middle of each of two rows passing through the central sampling area was inoculated with *C. flaccumfaciens* strain ATCC 33802 (2). Plants were inoculated in the seedling stage as the second trifoliolate leaves were unrolling. The remaining four plots served as uninoculated controls. Leaves of a single plant near the middle of each of two rows passing through the central sampling area of each of four control plots were dusted lightly with Carborundum before they were rubbed briskly with a cheesecloth pad saturated with 0.6% saline.

The test was conducted in central Iowa, near Ames, on fertile, well-managed soil with a nearly level topography. An area planted to Clark 63 soybeans was divided into eight plots 60 × 60 m, with rows spaced at 0.91 m. The pattern of distribution of sampling areas within the plots was that of Imhoff et al (5). To determine the extent of disease spread, 41 areas 1.83 × 1.83 m each, spanning two rows, were examined weekly in each plot after plants in the central area of four plots were inoculated. The sampled areas were located at the midpoints of the parallel sides and the corners of a series of five squares concentric around the initially inoculated center area (Fig. 1). To determine the rate of disease spread, distances were measured as the distance from the center of the initially inoculated central sampling area to the center of any other given sampling area. Thus, in each of eight compass directions, plants sampled at the corners and sides of five

concentric squares around the inoculated center yielded a total of 10 distances from the center (five distances in four directions to the corners of each concentric square and five distances in four directions to the midpoint of each side of each concentric square). Concentric squares within plots were numbered from 1 to 5 from the central sampling areas toward the margins of the plots. Sampling areas within plots were identified by one of eight compass directions (north, northeast, east, southeast, south, southwest, west, and northwest) from the central plot and by the number of the concentric square (Fig. 1).

Disease incidence, incidence of diseased leaves, and number of sampling areas in which bacterial tan spot occurred were obtained 5 wk after inoculation. Disease incidence was expressed as the percentage of diseased plants and was based on 50-plant samples (25 consecutive plants near the middle of each of two rows passing through the same sampling area). Incidence of diseased leaves was expressed as the number of diseased leaves per plant and was based on the same samples as those used to determine incidence of diseased plants.

The experimental design was a randomized complete block with four replicates. Means were tested for significance by computing the least significant difference (LSD). Control plots were observed each week as a check for possible bacterial contamination by movement of equipment and research workers. Plants were not cultivated after plants in the central areas were inoculated. The test was terminated 5 wk after inoculation when plant canopies began to close the rows, thus increasing the possibility of spurious disease spread.

RESULTS AND DISCUSSION

The first symptoms of bacterial tan spot were observed on inoculated plants 8–9 days after inoculation. No symptoms were detected on other plants in the field by 2 wk after inoculation. By 3 wk, the disease was evident on uninoculated plants in all central sampling areas as well as on plants in one or more adjacent sampling areas in all plots (Fig. 2). Bacterial tan spot was confined to the same rows as inoculated plants in all plots. Outside the central inoculated areas, the disease occurred in concentric square 1 (sampling areas E1 in plots 1, 3, and 4; and in W1 in plots 1, 2, and 4). From the second to the third week after inoculation, the disease advanced a mean distance of 1.4 m from the central sampling area to sampling areas E1 and W1, but the advance was not sufficiently large by 3 wk after inoculation to differ significantly ($P<0.05$) from other plots in concentric square 1 in which the disease did not occur (Table 1).

There was considerable disease by 4 wk after inoculation, and it was apparent that spread of the bacteria occurred more rapidly within rows than across rows (Fig. 2). The disease had spread to concentric square 2 in all four plots, and the mean distances that the disease had spread in areas E2 and W2 (the direction of rows) by 4 wk after inoculation were 2.8 and 4.1 m, respectively (Table 1).

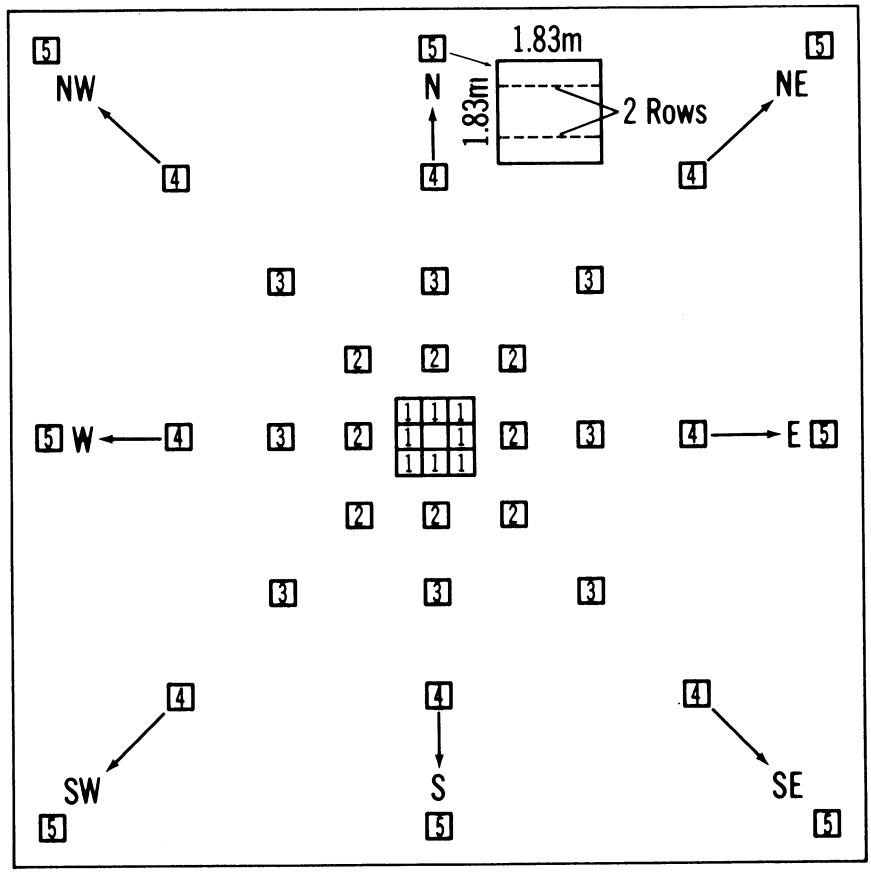

Fig. 1. Arrangement of 40 bacterial tan spot disease sampling areas in five concentric squares surrounding a central sampling area in which two soybean plants near the center of the area were inoculated with *Corynebacterium flaccumfaciens*. Individual sampling areas were identified by one of eight compass directions from the central disease sampling area and the number of the appropriate concentric square. Sampling areas were 1.83 × 1.83 m, and an entire plot was 60 × 60 m.

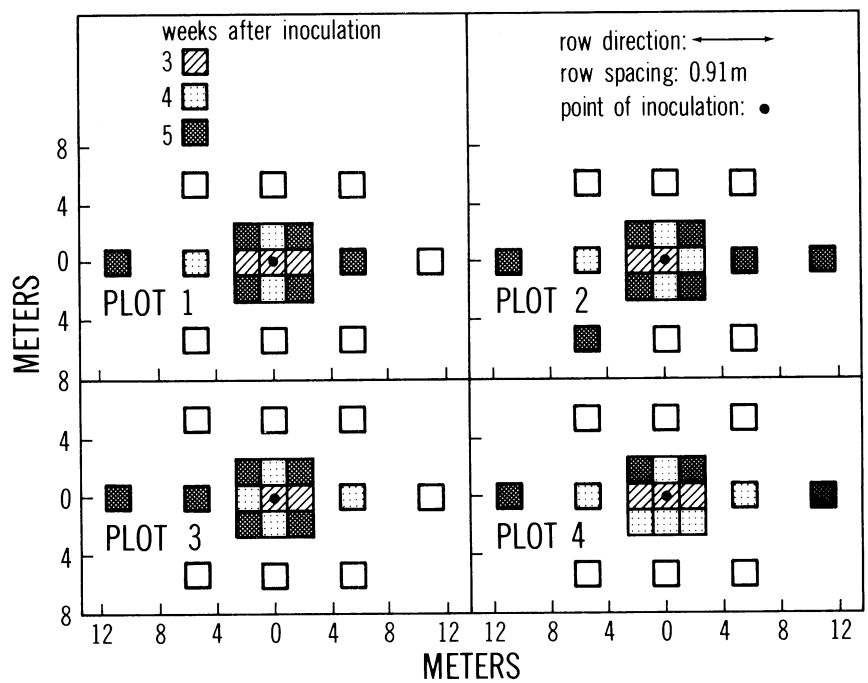

Fig. 2. Distribution patterns of bacterial tan spot in four plots of Clark 63 soybeans at weekly intervals after inoculation of two plants in each of the central sampling areas. No plants infected by *Corynebacterium flaccumfaciens* were observed in concentric square numbers greater than 3.

Table 1. Mean distances in eight compass directions from the centers of the central sampling areas of four soybean plots to the centers of sampling areas in concentric squares where infected plants were found during the current week[w]

	Mean[x] distance (m) to currently affected sampling area (wk after inoculation of plants in central sampling areas)								
	3			4			5		
Direction	CS 1[y]	CS 2	CS 3	CS 1	CS 2	CS 3	CS 1	CS 2	CS 3
North	0.0	0.0	0.0	1.8 a[z]	0.0 a	0.0	0.0 a	0.0 a	0.0 a
Northeast	0.0	0.0	0.0	0.0 b	0.0 a	0.0	1.8 b	0.0 a	0.0 a
East	1.4	0.0	0.0	0.5 ab	2.8 b	0.0	0.0 a	2.8 b	11.0 c
Southeast	0.0	0.0	0.0	0.7 ab	0.0 a	0.0	1.4 ab	0.0 a	0.0 a
South	0.0	0.0	0.0	1.8 a	0.0 a	0.0	0.0 a	0.0 a	0.0 a
Southwest	0.0	0.0	0.0	0.7 ab	0.0 a	0.0	1.4 ab	1.9 b	0.0 a
West	1.4	0.0	0.0	0.5 ab	4.1 b	0.0	0.0 a	1.4 ab	5.5 b
Northwest	0.0	0.0	0.0	0.0 b	0.0 a	0.0	1.8 b	0.0 a	0.0 a

[w] Two plants at the center of the central sampling area of each plot were inoculated with *Corynebacterium flaccumfaciens*, and tan spot was recorded weekly.
[x] Mean of the same sampling areas from four plots.
[y] CS = concentric square. No plants infected by *C. flaccumfaciens* were observed in concentric square numbers greater than three.
[z] Numbers within the same concentric square, in the same week, followed by a common letter are not significantly different at $P<0.05$ according to LSD. Absence of a letter indicates nonsignificance.

Table 2. Mean disease incidence, disease severity, and sampling areas affected 5 wk after two plants in the central sampling area of each of four plots were inoculated with *Corynebacterium flaccumfaciens*[v]

	Diseased plants (%)			Diseased leaves per plant			Sampling areas affected		
Direction	CS 1[w]	CS 2	CS 3	CS 1	CS 2	CS 3	CS 1	CS 2	CS 3
North	22.5 c[x]	0.0 a	0.0	2.0[y]	0.0	0.0	1.0[z]	0.0 a	0.0 a
Northeast	10.0 a	0.0 a	0.0	1.8	0.0	0.0	1.0	0.0 a	0.0 a
East	56.2 d	33.7 b	8.7	4.2	2.0	1.2	1.0	1.0 b	0.8 b
Southeast	12.5 abc	0.0 a	0.0	1.8	0.0	0.0	1.0	0.0 a	0.0 a
South	21.2 bc	0.0 a	0.0	1.8	0.0	0.0	1.0	0.0 a	0.0 a
Southwest	11.2 ab	0.0 a	0.0	1.5	0.5	0.0	1.0	0.3 a	0.0 a
West	57.5 d	38.7 b	0.1	4.7	2.5	0.5	1.0	1.0 b	0.8 b
Northwest	7.5 a	0.0 a	0.0	1.5	0.0	0.0	1.0	0.0 a	0.0 a

[v] Sampling areas were located in eight compass directions from the central sampling area and arranged around it in concentric squares.
[w] CS = concentric square. No plants infected by *C. flaccumfaciens* were observed in concentric square numbers greater than three.
[x] Represents mean of four 50-plant samples. Numbers within the same concentric square followed by a common letter are not significantly different at $P<0.05$ according to LSD. Absence of a letter indicates nonsignificance.
[y] Represents mean of four 50-plant samples.
[z] Represents mean of four replicates.

Bacterial tan spot had reached concentric square 3 by 5 wk after inoculation. The mean distances that the disease had spread in the direction of rows was 11.0 m (east) and 5.5 m (west). Also, all sampling areas in concentric square 1 had been affected by the disease at that time.

By 5 wk after inoculation, disease incidence was greatest in sampling areas in the direction of rows (east and west) of concentric square 1, where more than 50% of the plants were diseased (Table 2). Least disease incidence in concentric square 1 (<13%) occurred in areas diagonal to the direction of rows. The mean number of diseased leaves per plant was greater in the east and west sampling areas of concentric squares 1–3 than in other sampling areas of the same concentric squares, but these differences were not significant at $P<0.05$ (Table 2). Bacterial tan spot was never present in any of the control plots.

Disease movement from the central sampling area was invariably in the order of consecutive increasing numbers of concentric squares, i.e., no sampling areas were passed over (Fig. 2). The mean rate of disease movement was greatest to the east (2.2 m/wk) and second greatest to the west (1.1 m/wk). The mean rate of disease movement was identical for the north and south directions (0.5 m/wk). Thus, disease movement to the east (in the direction of the rows) was 4.8 times more rapid than in a north or south direction (across rows). Strandberg (6), in a study of the distribution of cabbage black rot caused by *X. campestris* (Pammel) Dowson, stated that spatial patterns of distribution of plant pathogens, like other natural populations of organisms, are seldom truly random or regular in nature. In soybean bacterial tan spot, such patterns are not random but are best described as a tendency for the disease to move at a more rapid rate within rows than across rows or diagonal to rows. Berger and Luke (1), in their study of the spatial and temporal spread of oat crown rust (*Puccinia coronata* Cda. f. sp. *avenae* Fraser & Led.), reported mean rates of disease spread as high as 1.2 m/day. Contrasted with oat crown rust, the maximum mean rate of disease spread of 2.2 m/week for bacterial tan spot was very slow.

Initial sightings of bacterial tan spot of soybean in Iowa fields after the disease was discovered in 1975 ranged from a few adjacent plants up to areas 6 m wide (2). In a later study involving 821 soybean fields (4), a few larger areas up to 30 × 10 m were observed. These elongate areas usually were oriented with the longest dimension in the same direction as the rows, as observed in this study. In a study of yield losses in soybean induced by bacterial tan spot, the greatest mean yield loss (16.9%) occurred at a location where hail caused some leaf damage (3). Although spread of bacterial tan spot from the central sampling areas was confined to relatively small areas of a susceptible cultivar (mean size was 19 × 5 m), the total crop damage resulting from the disease would be a function of the number of seedlings initially infected in a field, and this might be determined by the percentage of diseased seed sown.

ACKNOWLEDGMENT

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