Prevalence of *Corynebacterium flaccumfaciens* as Incitant of Bacterial Tan Spot of Soybean in Iowa

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ABSTRACT

Dunleavy, J. M., Keck, J. W., Gobelman, K. S., Reddy, S., and Thompson, M. M. 1983. Prevalence of *Corynebacterium flaccumfaciens* as incitant of bacterial tan spot of soybean in Iowa. Plant Disease 67:1277-1279.

Bacterial tan spot, caused by *Corynebacterium flaccumfaciens*, occurred in 51.1% of 818 Iowa soybean fields examined in 1982. The mean number of plants infected per 30 m of row was 3.9. The disease was found in all 95 counties sampled. Greatest prevalence of tan spot occurred in a nine-county area in the southwestern section of the state, where 84.3% of the fields were affected by the disease. The lowest disease prevalence occurred in the nine-county east central section, where 32.1% of the fields were affected. There was no correlation between the percentage of fields affected by tan spot in a particular section of the state and the mean number of infected plants in affected fields. We tested diseased leaves from one field in each county sampled to determine if *C. flaccumfaciens* produced the symptoms observed. All isolates of the bacterium recovered from these leaves were identified as *C. flaccumfaciens* on the basis of laboratory tests, were pathogenic to soybean, and produced symptoms of tan spot in a greenhouse test.

Additional key words: *Glycine max*

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, has been observed in 16 Iowa counties from 1975 through 1981 (3). Leaf chlorosis, the first observable symptom of bacterial tan spot, frequently begins at the leaflet edge and progresses toward the midrib. In later stages, the chlorotic tissue dries and turns tan. High winds may then cause the necrotic areas of leaflets to fall to the ground, giving a ragged appearance to diseased leaves (1,3).

C. flaccumfaciens also causes bean wilt, a systemic disease in bean (*P. vulgaris*), in which pods are infected and the causal bacterium is transmitted in seed (12). Isolates of the bacterium that are pathogenic to soybean infect only leaves. The disease is not systemic and is not transmitted in seed. Soybean cultivars vary in disease reaction from very resistant to very susceptible (3). Yield loss of 8% has been reported for tan spot (2).

Information on distribution of the disease in the state in previous years was obtained from observers familiar with the disease, but no systematic survey of bacterial tan spot has been conducted in the state. The objective of this study was to determine the prevalence of bacterial tan spot in Iowa by sampling a large number of soybean fields.

MATERIALS AND METHODS

Observations were made between 13 July and 13 August 1982. We sampled 818 randomly selected fields in 95 Iowa counties. The number of fields sampled per county was determined by the soybean acreage in the county in 1981 at the rate of one field per 4,050 ha of soybeans grown in each county. Allamakee, Clayton, Dubuque, and Jackson counties were not sampled because of their low soybean production (less than 4,050 ha in each county in 1981). Four rows selected at random, each 30 m long, were examined in each field. Disease incidence was recorded as the number of infected plants per 30 m of row.

Leaves with bacterial tan spot symptoms (Fig. 1) were collected from one field in each county in which soybean fields were sampled. Identity of the causal organism producing tan spot symptoms on leaf samples was established by crushing three diseased leaflets from each sample with a sterile pestle in a sterile mortar containing 5 ml of autoclaved, distilled water. The resulting sap was inoculated by wounding (3) to three Clark 63 soybean plants and to three Kentucky Wonder pole bean plants that were 3 wk old. Controls consisted of one set of six pots of plants (three pots of Clark 63 and three pots of Kentucky Wonder) that were inoculated similarly with sap from crushed, healthy leaves and another set of six pots of plants inoculated with sap from soybean leaves infected with an isolate of *C. flaccumfaciens* known to be pathogenic to soybean (ATCC 33802, American Type Culture Collection, Rockville, MD 20852). Greenhouse temperatures ranged from 20 to 33 C. Development of tan spot symptoms under greenhouse conditions were as described elsewhere (3).

After inoculated leaves showed disease symptoms, bacteria were isolated from lesion margins by crushing chlorotic tissue on a sterile glass slide with a sterile wire loop. A mixture of sap and crushed tissue was streaked on the surface of three nutrient broth-yeast extract agar plates

Fig. 1. A field-grown soybean leaflet naturally infected by *Corynebacterium flaccumfaciens*, the cause of bacterial tan spot.

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(NBY) (9), and isolated bacterial colonies were transferred to other NBY plates. Bacteria isolated from diseased soybean leaves were compared with ATCC 33802. Methods used for characterization of isolates were as described previously by Schuster et al (7).

Inoculum was prepared from 48-hr NBY agar plate cultures of the test bacteria by suspending bacteria in 10 ml of 0.85% saline solution added to each of the plates. For pathogenicity tests, young trifoliolate leaves of greenhouse-grown Clark 63 plants were dusted lightly with autoclaved 400-mesh Carborundum before leaves were rubbed briskly with a cheesecloth pad saturated with inoculum. Three plants were inoculated with each isolate.

Disease prevalence, as used in this study, is defined as the percentage of fields attacked by a disease (11), whereas disease incidence is defined as the number of plants infected (5).

The bacterial nomenclature used is that proposed by Rogosa et al (6). An alternative nomenclature for plant-pathogenic bacteria has been proposed by Young et al (10), and using this system, the name of the tan spot bacterium is *C. flaccumfaciens* pv. *flaccumfaciens* (Hedges) Dowson.

RESULTS AND DISCUSSION

Bacterial tan spot (Fig. 1) occurred in 51.1% of 818 fields examined, and the disease was found in all counties sampled (Table 1). The mean number of plants infected per 30 m of row was 3.9. Data were summarized by combining counties into nine geographical sections in Iowa (Fig. 2). The southwestern section was the only one where more than 76% of the fields were affected by bacterial tan spot. In two sections, central and the south central, between 51 and 75.9% of the fields were affected. In the remaining six sections, between 26 and 50.9% of the fields were affected.

Bacterial tan spot was usually localized in soybean fields, and infested portions varied from a single plant to areas as large as 10 × 30 m. These elongate diseased areas were usually oriented with the longest dimension in the same direction as the rows. The disease was not believed to be a significant problem in any of the fields observed.

In any particular section of the state, there was no correlation between percentage of fields affected by tan spot and mean number of infected plants in affected fields ($r = -0.08$) (Table 1). The southwestern section had the highest percentage of fields affected by the disease but had only a mean of 2.5 plants infected per row. The northeastern section had the greatest number of plants infected per row (10.1) but had 50% of the fields affected (fifth highest). Because the number of fields sampled in each section was weighted for soybean acreage (one field sampled for each 4,050 ha of soybeans produced), comparisons of the number of fields sampled with both percentage of fields affected and mean number of infected plants was of interest. Soybean acreage of a section (number of fields sampled) was not correlated with either presence of tan spot (percentage of fields affected) ($r = -0.23$) or incidence of the disease (mean number of plants infected) ($r = -0.36$).

Bacterial tan spot is distinguished easily from other bacterial diseases of soybean, but the disease produces symptoms similar to those of Phyllosticta leaf spot (8). We tested diseased leaves from one field in each county sampled to determine if *C. flaccumfaciens* produced the symptoms observed. Soybean and bean plants showed disease symptoms 4 to 6 days after inoculation with sap from crushed leaves from all counties sampled. Soybean plants showed symptoms of tan spot and bean plants showed symptoms of bean wilt. Bacterial isolates obtained from inoculated soybean plants (total of 95, one from each county sampled) were gram-positive, formed a medium-yellow endopigment on NBY medium, were motile, grew well at 37 C, did not produce urease, and were pathogenic to soybean. These characteristics were identical to those of *C. flaccumfaciens* ATCC 33802 (3) and, with the exception of being pathogenic to soybean, were the same as those reported for *C. flaccumfaciens* by Rogosa et al (6). We concluded that all bacterial isolates from soybean were *C. flaccumfaciens*.

We did not expect to find bacterial tan spot distributed so widely in Iowa. Hedges (4) first reported *C. flaccumfaciens* as a new pathogen of bean in the neighboring state of South Dakota in 1922; therefore, the bacterium may be indigenous in this area. With such wide distribution but low frequency of occurrence, release of new cultivars with high susceptibility to bacterial tan spot may result in the disease being more of a problem than it is at present.

Table 1. Prevalence and incidence of bacterial tan spot of soybeans in Iowa in 1982

Section of state	Counties sampled (no.)	Fields sampled (no.)	Fields affected (%)	Mean[a] plant infection in affected fields (plants/30 m of row)
Northwestern	12	136	50.7	1.5
North central	11	134	34.3	1.5
Northeastern	8	64	50.0	10.1
West central	12	116	45.7	3.4
Central	12	131	54.9	3.8
East central	9	56	32.1	1.9
Southwestern	9	70	84.3	2.5
South central	11	47	63.8	2.0
Southeastern	11	64	43.8	8.8
Entire state	95	818	51.1	3.9

[a] Mean of four rows sampled.

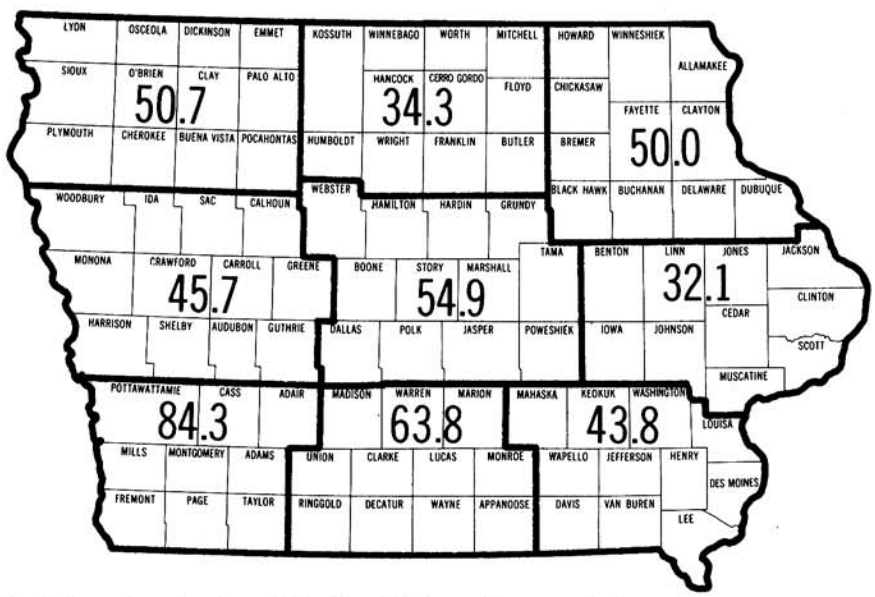

Fig. 2. Percentage of soybean fields affected by bacterial tan spot in nine sections of Iowa in 1982.

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