Virulence of Cephalosporium gregatum and Verticillium dahliae in Soybeans

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ABSTRACT

Verticillium dahliae was isolated from diseased soybean plants, and subsequently proved pathogenic to soybeans. *Verticillium dahliae* produced vascular discolorations similar to those caused by *Cephalosporium gregatum* but did not produce typical brown stem rot symptoms caused by *C. gregatum*. Leaf symptoms produced by the two fungal pathogens were distinct. When soybean cultivars resistant and susceptible to *C. gregatum* were inoculated with *V. dahliae*, they were similarly resistant and susceptible. Midwest and Ontario were the most resistant and susceptible cultivars, respectively. Average infection percentage for nine cultivars decreased from 82.9 at 18 C to 49.8 at 28 C when plants were inoculated with *C. gregatum*. Infection decreased only slightly, from 97.1% at 18 C to 94.4% at 28 C, when plants were inoculated with *V. dahliae*. Average length of vascular tissue discoloration was similar at 18 and 28 C for *C. gregatum*, while plants inoculated with *V. dahliae* had slightly more discoloration at the higher temperature. Plant height reduction was greater with *V. dahliae*- than with *C. gregatum*-inoculated plants. Phytopathology 61:565-568.

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Cephalosporium gregatum Allington & Chamberlain, the pathogen causing brown stem rot of soybeans (*Glycine max* [L.] Merr.) was first reported by Allington & Chamberlain (2) from Illinois, Indiana, Iowa, Missouri, and Ohio. Shortly thereafter, it was reported from Minnesota (8). Ross & Smith (14) identified the fungus in North Carolina and Virginia, and more recently, Morgan & Dunleavy (13) identified it from diseased specimens from Mexico.

Verticillium dahliae Kleb. is not known to be a disease problem in soybeans, but Wiles (17) artificially inoculated soybeans and cowpea (*Vigna sinensis*) with *Verticillium albo-atrum* from cotton (*Gossypium hirsutum*) and obtained some infection in both hosts. Soybeans were considered resistant because severe external symptoms appeared only in certain cowpea cultivars and not in soybeans. Soybean cultivars tested were Bragg, Hill, Lee, and Semmes. Because of Wiles' report (17) and the similarity of symptoms between brown stem rot vascular discoloration in soybeans and *Verticillium* vascular symptoms on many crops, I investigated *V. dahliae* as a possible pathogen of soybeans.

Specific objectives of the study were (i) to determine if *V. dahliae* occurs as a pathogen in Iowa soybean fields; (ii) to make comparative pathogenicity studies between *V. dahliae* and *C. gregatum* in soybeans; (iii) to study relative susceptibility and resistance of soybean cultivars to the two fungi; and (iv) to determine the effect of temperature on virulence.

MATERIALS AND METHODS.—Four soybean plants with vascular tissue discolorations in lower portion of stem suspected of *Verticillium* disease were obtained from Toddville, Iowa, in September 1968. Plants were about 10 days from maturity and had attached dead leaves. Similar plants occurred sparsely throughout the field. Typical pith-browning symptoms of brown stem rot were not evident. After 8-cm stem sections were washed in running tap water overnight, they were aseptically sliced longitudinally. Minute pieces of darkened tissue from freshly exposed vascular tissues were excised and plated onto potato-dextrose agar (PDA). An Iowa isolate of *C. gregatum* (10) was used for the comparative pathogenicity tests. Two- and 3-week-old cultures of *V. dahliae* and *C. gregatum* grown on PDA were used for inoculation. Plants were inoculated by the wound-inoculation method described for *Phytophthora megasperma* var. *sojae* (7). The only modification was that small (ca. 1 × 2 mm) pieces of the agar culture were inoculated directly into wounds rather than blending the culture before inoculation.

Pathogenicity tests were conducted in adjacent greenhouse units at 18 and 28 C, ±4 C. Natural daylight was supplemented with artificial VHO fluorescent lamps for a min of 1,000 ft-c and 16-hr days. Plants were grown in 4-inch clay pots, 6 plants/pot. Soil was a 5:2:1 (Clarion sandy loam, peat, and sand) mixture, steam autoclaved at 15 psi for 4 hr. Plants were grown at 28 C and inoculated 12 days after seeding. Four replicates were used, and data were recorded 6 weeks after inoculation. Data included external leaf symptoms, plant height, and length of vascular tissue discoloration. The discoloration was differentiated as to *Verticillium* type (restricted to vascular tissues) and brown stem rot (BSR) type (browning extended into pith).

The soybean cultivars Clark, Clark 63, Ford, Harosoy, Hawkeye, Lincoln, Midwest, Ontario, and Wayne were selected to represent a wide range of reaction to *C. gregatum*. Ford, Lincoln, and Ontario are susceptible, and Hawkeye, Harosoy, and Midwest are resistant to *C. gregatum* (2, 9, 16). Clark 63 and Wayne were resistant, and Clark and Ford seemed susceptible, to *V. dahliae* in preliminary tests.

RESULTS.—*Isolation.*—A *Verticillium* sp. was isolated from the diseased soybean specimens obtained from the field. Morphological characters, including microsclerotia, hyaline mycelium, conidiophores, and conidia, and growth rate on PDA and prune agar fitted descriptions of *V. dahliae* by Smith (15) and Devaux & Sackston (4). The soybean isolates were compared with known isolates of *V. dahliae* and *V. albo-atrum* obtained from C. A. Martinson, Iowa State Univ. They closely resembled the known isolate of *V. dahliae.* Koch's postulates were completed during preliminary pathogenicity tests with soybean cultivars Wayne, Clark, Clark 63, and Ford to prove pathogenicity of the new isolates.

Comparison of symptoms caused by V. dahliae and C. gregatum in soybeans.—Sudden leaf wilt symptoms were produced by *V. dahliae* in soybeans 7 to 10 days after inoculation. The wilt, however, did not generally encompass entire leaves. Early wilt symptoms were evident as apical, marginal, or unilateral wilting of primary leaves (Fig. 1-B). Young affected tissues remained green, and dried without initially turning chlorotic, but ultimately become blanched. Similar symptoms occurred on first and second trifoliate leaves but not, or only rarely, on younger leaves. At times, leaf symptoms were similar to those reported for *Phyllosticta* leaf spot (Fig. 1-B, Right). When wilted leaves were placed in a humidity chamber, typical mycelial growth of *V. dahliae* occurred. In contrast, leaves of plants infected with *C. gregatum* did not develop the sudden wilt nor did the fungus grow from leaves held in the humidity chamber. Typical leaves on plants infected with *C. gregatum* showed a gradual unilateral chlorosis with vein-reddening (Fig. 1-A, Right). Two to 3 weeks were required for symptom development. The symptoms invariably developed from the petiole end and spread toward the leaf tip. Field leaf symptoms as described by Allington & Chamberlain (2), and frequently illus-

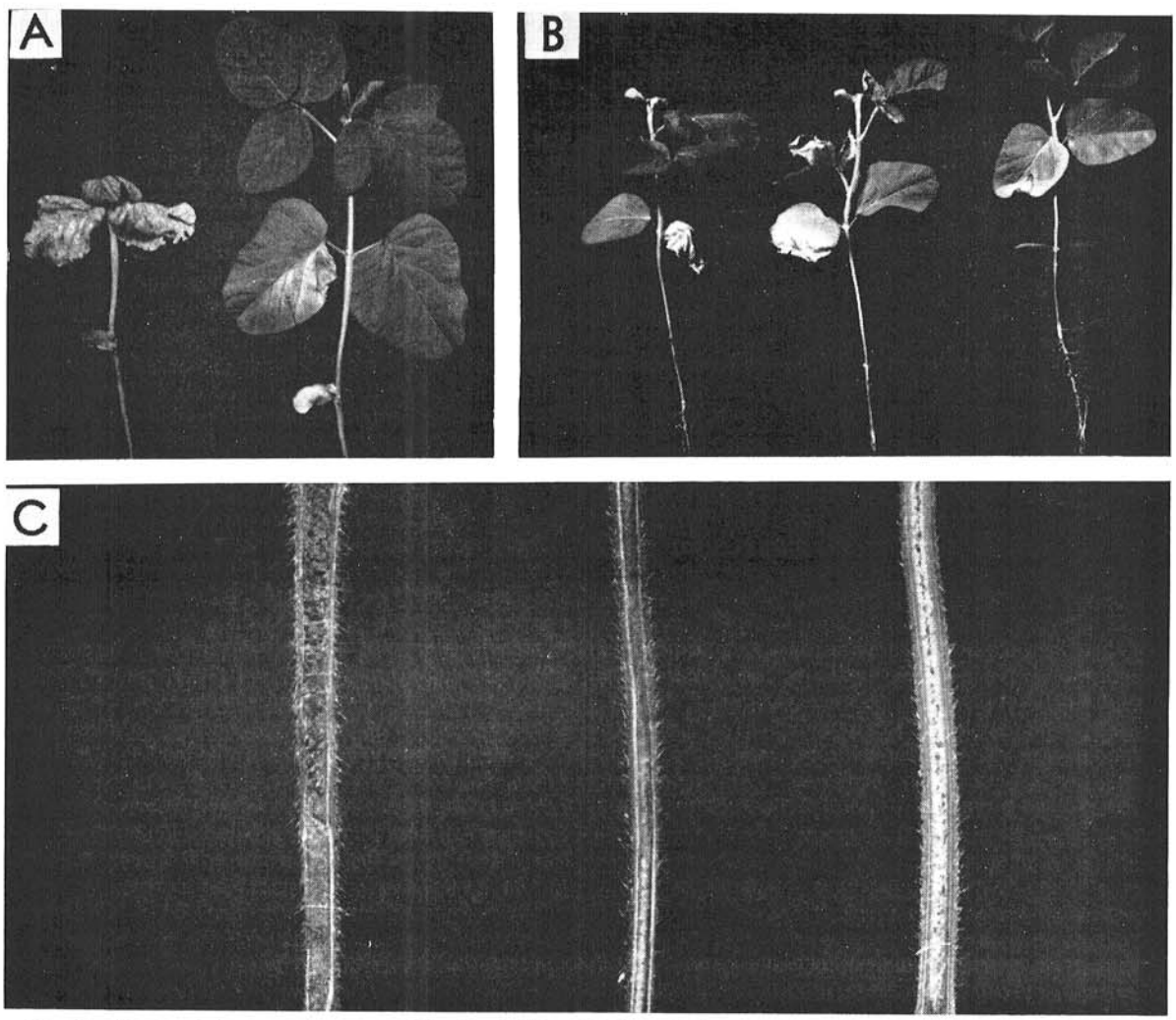

Fig. 1. Leaf and stem symptoms caused by *Verticillium dahliae* and *Cephalosporium gregatum* in artificially inoculated soybeans. **A)** Atypical leaf symptom (Left) and typical leaf symptom (Right) by *C. gregatum;* **B)** Leaf symptoms produced by *V. dahliae;* and **C)** stem symptoms by *C. gregatum* (Left), *V. dahliae* (Center), and noninoculated control (Right).

trated for BSR, were produced in only one of 1,350 inoculated plants (Fig. 1-A, Left). This plant was infected also with a *Fusarium* sp., which may have entered through the diseased cotyledon (Fig. 1-A, Left). Normal cotyledons without lesions were present on plants with typical BSR leaf symptoms observed in these studies (Fig. 1-A, Right). The combination vascular tissue and pith discoloration recognized as BSR were generally produced in plants inoculated with *C. gregatum* (Fig. 1-C, Left), and only rarely in plants inoculated with *V. dahliae*. The latter included two plants each of Ontario and Lincoln at 18 C. Discoloration produced by *V. dahliae* was restricted to vascular tissues and was reddish brown to black (Fig. 1-C, Center).

Relative susceptibility and resistance of cultivars to the two fungi.—No cultivar was immune from both fungi, and some infection occurred in all cultivars (Tables 1, 2). Total infection percentages for all cultivars at the two temperatures, 18 and 28 C, were 97.1 and 94.4, respectively, for *V. dahliae* and 82.9 and 49.8, respectively, for *C. gregatum*. Differences in resistance and susceptibility of cultivars to both pathogens also were evident in the extent of vascular tissue discoloration. Vascular discoloration was greater (7.1 and 7.2 cm) with *C. gregatum*-inoculated plants than with *V. dahliae* (5.9 and 6.6 cm) for both 18 and 28 C, respectively.

Cultivars susceptible and resistant to *C. gregatum* usually showed a corresponding pattern of reaction to *V. dahliae* (Tables 1, 2). Ontario was the most susceptible cultivar to both fungi, and Midwest, the most resistant. Clark was slightly more susceptible to *V. dahliae* than its isogenic counterpart, Clark 63, at both temperatures. When inoculated with *C. gregatum*, Clark was more susceptible than Clark 63 at 28 C, but equally susceptible at 18 C. Lincoln was more susceptible to *C. gregatum* than to *V. dahliae*. Although the differences were slight, Ford was consistently more susceptible than Wayne to both fungi at both temperatures. In general, except for the relatively greater resistance of Midwest and susceptibility of Ontario to both fungi, differences among other cultivars were small.

Effect of temperature on virulence.—The virulence of *V. dahliae* was affected only slightly by temperature, while the virulence of *C. gregatum* was affected significantly. Infection occurred in 94.4% of plants inoculated with *V. dahliae* at 28 C. At 18 C, infection was 97.1%. In contrast, infection by *C. gregatum* was 49.8% at 28 C and 82.9% at 18 C. Thus, infection increased by 33.1% when temperature was decreased 10 C. Although temperature affected the percentage of plants infected by *C. gregatum*, the amount of vascular discoloration did not change. Vascular discoloration for *V. dahliae*-inoculated plants decreased from 6.6 to 5.9 cm at 28 and 18 C, respectively. Plant height was reduced more at both temperatures by *V. dahliae* than by *C. gregatum*. *Verticillium dahliae* reduced the average height of the cultivars by 11.8 and 10.3 cm at 18 and 28 C, respectively; corresponding reductions for *C. gregatum* were 7.7 and 4.4 cm.

DISCUSSION.—*Verticillium dahliae* and *C. gregatum* are both virulent vascular pathogens of soybeans. It was not possible to distinguish between vascular discoloration caused by the two pathogens by visual examination, which may be a problem in disease identification if vascular tissue discoloration alone is used for diagnosis. Cultivar reactions are not helpful, because most cultivars, whether resistant or susceptible, react similarly to the two pathogens. Although leaf symptoms produced by the two pathogens in the present study are distinct, they are no longer evident or present late in the season when vascular discoloration is apparent. Pith browning when present is generally characteristic of BSR, and sometimes can be used to identify this disease, but isolation from vascular tissue remains the only sure method for accurate differentiation of the two diseases.

Although the economic importance of *V. dahliae* in soybeans remains unknown, several observations indicate a possible significance of the fungus in this crop. During seed assay studies for the USDA in 1965 and 1966 of soybean samples from northern and southern United States, *Verticillium* sp. were consistently isolated, but not *C. gregatum* (Lois Tiffany, *personal communication*). The geographic distribution of *Verticillium*-infected seeds included Florida, Georgia, Mississippi, and North Carolina. Seeds from Minnesota did not contain the fungus, and seeds from Iowa, Illinois, Indiana, and Delaware carried the fungus to a lesser extent than those from the south. Because *Verticillium* wilt of cotton has been a major problem in the south

TABLE 1. Infection, vascular tissue discoloration, and plant height reduction of nine soybean cultivars inoculated with *Verticillium dahliae* and *Cephalosporium gregatum* in the greenhouse at 18 C

Cultivar	*V. dahliae* Ratio infected: inoculated	*V. dahliae* Avg vascular discoloration in cm	*V. dahliae* Avg plant height reduction in cm	*C. gregatum* Ratio infected: inoculated	*C. gregatum* Avg vascular discoloration in cm	*C. gregatum* Avg plant height reduction in cm
Ontario	23:24	7.6	9	17:23	10.0	9
Harosoy	24:24	5.0	12	22:24	6.2	4
Hawkeye	22:23	5.1	16	21:24	7.1	8
Lincoln	22:23	7.8	12	14:21	9.3	12
Ford	23:24	7.1	8	21:24	7.7	8
Wayne	23:23	5.4	0	24:24	5.6	0
Clark	22:23	6.4	15	23:25	6.5	12
Clark 63	22:23	5.6	12	15:22	6.5	8
Midwest	23:23	3.2	23	17:23	5.2	9

TABLE 2. Infection, vascular tissue discoloration and plant height reduction of nine soybean cultivars inoculated with *Verticillium dahliae* and *Cephalosporium gregatum* in the greenhouse at 28 C

Cultivar	*V. dahliae* Ratio infected: inoculated	*V. dahliae* Avg vascular discoloration in cm	*V. dahliae* Avg plant height reduction in cm	*C. gregatum* Ratio infected: inoculated	*C. gregatum* Avg vascular discoloration in cm	*C. gregatum* Avg plant height reduction in cm
Ontario	22:23	10.1	9	14:24	10.0	2
Harosoy	21:23	6.9	2	13:24	5.6	0
Hawkeye	21:24	6.1	15	11:24	6.7	6
Lincoln	23:24	5.1	7	10:24	7.0	5
Ford	23:23	8.7	11	14:24	7.9	7
Wayne	24:24	5.5	16	14:24	7.0	9
Clark	23:24	7.2	12	8:24	9.6	0
Clark 63	22:24	6.0	12	16:24	6.1	9
Midwest	22:24	3.8	9	7:24	5.2	2

and because soybean production in recent years has been increasing rapidly in that region, the disease may be of potential importance with soybeans.

The high infection percentage by *C. gregatum* at cooler temperatures indicates that brown stem rot should be more common in the north. A recent Iowa BSR survey (5) indicated disease incidence to be greater in the southern sections of the state. Similar distribution patterns occurred in Indiana in 1965, 1966, 1967, and 1969 (1, 3, 11, 12). Furthermore, BSR occurs in North Carolina and Virginia (14) and as far south as Mexico (13). The field observations of greater BSR incidence in the warmer southern climate do not agree with greenhouse studies of temperature effects on pathogenicity (i.e., greater infection at lower temperatures observed in the present study as well as by Allington & Chamberlain (2). Factors other than temperature per se must be involved in BSR incidence and distribution in the field.

The observation of significant difference in infection percentages and extent of vascular discoloration remaining the same for *C. gregatum* at different temperatures confirms previous data by Kunkel (9), who found no definite relationship of temperature to rates of symptom development, even though infection percentages differed.

No information is available on the effect of stem browning on yield, although stem browning has been measured in studying incidence of BSR (5). Infection percentage, however, has been related to yield losses (6). Yield losses increased with increasing numbers of infected plants. Thus, infection percentage may be a more useful criterion than stem browning for evaluation of BSR resistance.

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