Role of Temperature, Plant Age, and Fungus Isolate in the Development of Brown Stem Rot in Soybeans

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ABSTRACT

Temp and plant age were not limiting factors preventing leaf symptoms in soybean plants inoculated with a defoliating isolate of *Cephalosporium gregatum*. Soybean plants inoculated with this isolate of the fungus were defoliated at 22 and 28 C. Only the defoliating isolate produced characteristic leaf symptoms on plants inoculated in the field.

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Although *Cephalosporium gregatum* Allington and Chamberlain, the cause of brown stem rot (BSR) of soybeans *Glycine max* (L.) Merr., has been reported in soybean-producing areas for more than 20 yr (1), the effect of factors such as temp and plant age on BSR development are not well understood. For example, Allington and Chamberlain (1), and Schneider et al. (9) reported that vascular browning in plants inoculated with *C. gregatum* was more extensive in plants kept at 20 C than at higher temp. Allington and Chamberlain (1) also reported that BSR symptoms were most prevalent in the field during periods of cool, dry weather in late August

and September. Phillips (7) reported that air temp were probably not a limiting factor in BSR development in Georgia, and Dunleavy (3) failed to find any correlation between air temp and BSR development in Iowa.

Plant age has been reported to influence development of BSR symptoms in soybeans. Allington and Chamberlain (1), Chamberlain (2), and Phillips (7, 8) reported that vascular browning developed to a greater extent in plants inoculated after the onset of flowering, than during the vegetative stage. In contrast, Schneider et al. (9) reported that BSR developed to a greater extent in plants inoculated in the field in the vegetative stage of growth than during pod-fill.

Leaf symptoms attributed to BSR in field-grown plants are well documented (1, 3). However, evaluation of the temp and plant age influence on BSR development in greenhouse-inoculated plants has been based primarily on the extent of vascular browning (3, 7, 8, 9). Only two reports have indicated leaf symptoms and defoliation associated with BSR in greenhouse-inoculated plants (1, 5). Recently it has been shown that defoliating and nondefoliating strains of the fungus exist, and that only the defoliating strains produced leaf symptoms on inoculated plants (5). Because there is some uncertainty whether defoliating or nondefoliating isolates of *C. gregatum* were used in earlier research, it was my aim to determine the influence of temp and plant age on BSR development (leaf symptoms as well as vascular browning) in plants inoculated in the greenhouse and field with a defoliating isolate of *C. gregatum*.

MATERIALS AND METHODS.—*Soybean cultivars.*—All soybean cultivars used in both greenhouse and field experiments were susceptible to *C. gregatum*. The cultivar 'Wayne' was used in the 1971 field experiment and, because of its smaller size, 'Chippewa 64' was used in greenhouse experiments. All greenhouse experiments were repeated three times.

Fungus culture and plant inoculation.—A defoliating strain of *C. gregatum*, isolated from a soybean plant and used in previous work (6), and a nondefoliating isolate (ATCC culture No. 11073) were grown on liquid soybean-seed broth medium (five soybean seeds in 50 ml water, autoclaved 20 min at 121 C) for 3 wk at 22 C. Greenhouse plants were inoculated by placing a small piece of the mycelial mat into a wound made in the stem 0.5 cm below the soil line. The site of inoculation was covered with pasteurized soil. Field plants were inoculated by injecting triturated mycelia of the fungus into soybean stems at the soil line with an 18-gauge hypodermic needle. Inoculum was prepared by triturating in a Waring Blendor one fungal mat from a 3-wk-old broth culture in 50 ml of sterile water.

Culture of greenhouse plants.—Chippewa 64 soybean plants were grown in 15-cm diam pots of soil-sand mixture (1:1, v/v), which had been pasteurized at 100 C for 2 hr on 2 consecutive days. A light intensity of 21,500 lux for a 15-hr photoperiod was used, and the temp varied as required for each experiment. Each pot received 100 ml weekly of a 4 g/liter nutrient solution made up from commercial Ra-Pid-Gro[3] (Ra-Pid-Gro Corp., Dansville, N.Y.).

Comparison of pathogenic isolates on field grown plants.—The effects of a defoliating and a nondefoliating isolate of *C. gregatum* were compared on the cultivar Wayne in field plots in 1971. In previous experiments the area was free of *C. gregatum* (6). Four (8-ft) rows, replicated four times, were used for each fungus isolate and for the control. The plots were planted on 16 May 1971. The plants were stem-inoculated 6.5 wk after planting. Plants were observed for leaf symptoms during the growing season, and were scored for BSR at maturity.

Influence of temperature and plant age on BSR development.—Chippewa 64 plants were seeded in two groups that were 3 wk and 6 wk old (initial flowering) at the time they were inoculated with the defoliating isolate of *C. gregatum*.

Fifteen inoculated and control plants were used for each plant age group and were placed at either 22 C or 28 C after inoculation. The 22 C corresponded to the reported near-optimum for BSR development (1, 2, 9), while 28 C was selected as a temp possibly limiting BSR development (1, 2, 9). The plants were observed daily after inoculation for leaf symptoms. Four weeks after inoculation, the plants were removed, the stems were split, and vascular browning was measured.

Effect of temperature and pathogenic isolate of C. gregatum on BSR development.—Chippewa 64 plants were used to determine how temp influences BSR development in plants inoculated with defoliating and nondefoliating isolates of *C. gregatum*. The defoliating isolate, identical to the one used in previous greenhouse and field experiments, and the ATCC culture (No. 11073), typical of the nondefoliating isolates (9), were used to inoculate separate groups of 20 plants 6 wk after seeding. Twenty plants for each isolate and 20 control plants were placed in a chamber at 22 C; a similar series was maintained in a greenhouse at 28 C. Three wk after inoculation the plants were observed for vascular browning.

RESULTS.—*Comparison of pathogenic isolates on field-grown plants.*—Only the defoliating isolate of *C. gregatum* produced leaf symptoms on inoculated Wayne plants in the field. A gradual yellowing of the lower leaves, which progressed to the upper leaves, appeared on plants inoculated with the defoliating isolate 4 wk before maturity. Some of the upper leaves on inoculated plants

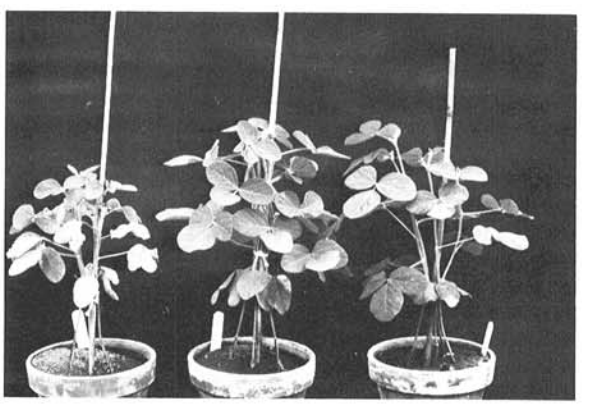

Fig. 1. 'Chippewa 64' plants inoculated with a defoliating isolate (left) and nondefoliating isolate (middle) of *Cephalosporium gregatum*, and control (right) held at 28 C for 3 wk after inoculation.

suddenly curled and became dry 3 wk before maturity, and interveinal necrosis on upper leaves was evident on inoculated plants 3 wk before maturity. The interveinal leaf necrosis was identical to that reported in earlier work (1, 3). Plants inoculated with the defoliating isolate matured 10 days earlier than control plants. At maturity, 100% of the plants inoculated with the defoliating isolate had vascular browning. Only 80% of the plants inoculated with the nondefoliating isolate had vascular browning. None of the control plants developed BSR. Plants inoculated with the nondefoliating isolate developed vascular browning but showed no leaf symptoms, and matured at the same time as noninoculated control plants.

Influence of temperature and plant age on BSR development.—Four wk after inoculation, plants inoculated with the defoliating isolate in the *vegetative stage* of growth (before initiation of flowering) had 421 mm of vascular browning at 22 C and 386 mm of vascular browning at 28 C. Plants inoculated in the reproductive stage of growth (during flowering and pod initiation) had 621 mm of vascular browning at 22 C and 534 mm at 28 C. After 4 wk, leaf necrosis, leaf yellowing, and defoliation were evident on inoculated plants of both age groups held at 22 C and 28 C. The higher ambient temp (28 C) did not prevent leaf symptoms although vascular browning was reduced in both age groups of plants maintained at 28 C.

Effect of temperature and pathogenic isolate of C. gregatum on BSR development.—Three wk after inoculation, plants inoculated with the defoliating isolate of *C. gregatum* showed severe leaf necrosis at 22 and 28 C. The average extent of vascular browning above the point of inoculation was 350 mm in plants held at 22 C and 285 mm in plants held at 28 C. The ATCC culture (No. 11073) did not produce leaf symptoms (Fig. 1). On inoculated plants held at 22 C, it produced 132 mm of vascular browning and on those held at 28 C, 114 mm of vascular browning (Fig. 1).

DISCUSSION.—Recently it was shown that only defoliating isolates of *C. gregatum* significantly reduce soybean yield compared to control plants (6). This raises an important question: what is the best measure of BSR severity, extent of vascular browning or defoliation?

Results from the present investigation demonstrated that neither temp nor plant age prevented leaf symptom development on plants inoculated with a defoliating isolate of the fungus. However the extent of stem vascular browning was reduced in the younger plants compared to the older plants.

Under field conditions, only the Wayne plants inoculated with the defoliating isolate of the fungus developed typical leaf symptoms 3 wk before maturity. Wayne plants inoculated with the nondefoliating isolate of the fungus did not develop leaf symptoms; although vascular browning was evident, they matured at the same time as the control plants.

My studies show that only the defoliating isolate of *C. gregatum* produced symptoms on inoculated plants under both greenhouse and field conditions that are identical to those reported in earlier observations (1, 3); i.e., stem vascular browning as well as leaf necrosis and defoliation. My results suggest that the defoliating strain of *C. gregatum* should be used in studies of host resistance, and the effects of environmental factors on disease development.

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