Evidence for Toxin Production by a Strain of Cephalosporium gregatum

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ABSTRACT

Extracts made from soybean stems infected with the Type I (defoliating) isolate of *Cephalosporium gregatum* induced wilting and death of detached trifoliolate soybean leaves, but extracts made from stems infected with the Type II (non-defoliating) (ATCC) culture did not. Both extracts caused vascular browning in the petiole. Leaves of cultivars Amsoy 71, Beeson, Calland, and Wayne wilted after three days in the Type I extract; P.I.'s 84,946-2 and 86,150 did not. Wilting was not entirely attributable to vascular plugging. It is suggested that a toxin produced by Type I isolates of *C. gregatum* is responsible for the difference in symptomatology induced by the two isolates.

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Additional key words: brown stem rot, defoliating strain, wilt.

Variation in pathogenicity in isolates of *Cephalosporium gregatum* Allington and Chamberlain within a given geographical area has been demonstrated. Gray (3) found two types of isolates in central Illinois: Type I caused wilting and defoliation in addition to vascular browning; Type II, corresponding to the ATCC (No. 11073) culture of *C. gregatum,* induced only vascular browning. The wilt symptoms induced by the Type I isolate suggested that a toxin might be involved.

To explore this possibility, a series of soybean plants [*Glycine max* (L.) Merr. 'Chippewa 64'] was inoculated (1, 3) with a culture of *C. gregatum* Type I. A second series was inoculated with the ATCC culture, and a third series was left for an uninoculated control. Each series was harvested 6 weeks after inoculation, and an extract was prepared as follows: 15 g of stem sections from each series was homogenized in a blender in 200 ml distilled water. The homogenate was strained through a clarifying filter, autoclaved for 20 minutes, and cleared by centrifugation. The extract was then sterilized by filtration and stored at 5 C until used.

Trifoliolate leaves of uniform size from Chippewa 64 soybean plants were used to assay the extracts. A 1:4 (v/v) dilution of the extract with sterile distilled water was placed in each of four 10-ml vials for each extract. Leaves were severed from the plant near the pulvinus and held under water while about one cm was sliced from the proximal end of the petiole with a razor blade to eliminate air bubbles in the vessels. A foam rubber plug, split to enclose the petiole, held the leaf upright in the vial with

the petiole immersed in the extract. The leaves were observed daily, and sterile distilled water was added to the vials as needed.

Leaves in the Type I extract became flaccid within two days. Interveinal necrosis was noted in three days, followed by complete drying of the leaves. Leaves in the other extracts had no symptoms.

Another series of four extracts was made from stems and leaves of plants infected with a Type I culture, and from stems and leaves of plants infected with a Type II culture. The original extract was diluted 1:1 (v/v) with sterile distilled water. Yellowing, wilting, and necrosis of the leaves in extracts from both stems and leaves of Type I-infected plants were noted in three days. Leaves in the other extracts showed no symptoms. A repetition of this experiment, including a nonautoclaved extract for comparison, indicated that autoclaving did not influence the symptoms produced on the leaves.

To determine the effect of dilution, the basic extract from Type I-infected plants was diluted 1:1, 1:3, 1:9, and 1:49 (v/v) with sterile distilled water. Triplicate vials were used for each dilution with the leaf petioles inserted through the stoppers as previously described. One series of vials of the undiluted extract was used for comparison. Comparable dilutions of healthy plant extract were maintained for controls. Wilting of the leaves occurred within 24 hours in the basic extract and in the 1:1 dilution, and in 2-3 days in the 1:3 dilution. Leaves in the higher dilutions showed no effect. In the healthy-plant-extract controls, wilting occurred in the basic extract in which bacterial growth was evident. The test was repeated three times with the 1:1 and 1:3 dilutions. To inhibit bacterial growth 0.125 mg of tetracycline hydrochloride and 0.1 mg of streptomycin were added to each vial in this and all subsequent experiments. Both dilutions of the diseased-plant extract induced wilting in all trials, whereas healthy-plant extracts induced no symptoms.

Gray (3) reported that *C. gregatum* Type I did not cause wilt or defoliation on P.I. 84,946-2. To determine whether there might be differential host reaction to the Type I extract, leaves from cultivars Wayne and Calland were compared with leaves of two resistant introductions, P.I. 84,946-2 and P.I. 86,150, in extracts made from plants infected with Type I and Type II isolates. Leaves of bush beans (*Phaseolus vulgaris* L. var. *humilis* Alef.) along with two additional soybean cultivars, Amsoy 71 and Beeson, were also included in the experiment. As controls, leaves of the same strains were maintained in healthy-plant extract and in sterile distilled water. Within three days, the leaves of Wayne, Amsoy 71, Beeson, and Calland in the Type I extract wilted and dried, but those of the two introductions and the bush bean were not affected. None of the leaves in the healthy plant extract or in the water controls wilted.

The possibility of toxin production in culture was then explored. Isolates of Type I and ATCC cultures were grown in soybean stem broth (1) for three weeks, a period considered minimal for the extremely slow-growing *C. gregatum*. The broth was strained through glass wool to remove mycelium, autoclaved, flash-evaporated from 50 to 10 ml, and finally sterilized by filtration. The extract was diluted 1:4 (v/v) with sterile distilled water, and assayed as before with excised Chippewa 64 trifoliolate leaves. Controls consisting of soybean stem broth in which no organism had grown were maintained. The leaves in the Type I culture extract wilted within three days; those in the ATCC culture extract showed no symptoms. The controls likewise remained symptomless.

To determine whether wilting might have been induced by plugging of the vessels, leaves that had been held in the Type I extract until the onset of wilting were transferred to vials of fast-green stain. At the same time, leaves from the water controls were also transferred to the stain solution. The leaves were observed at hourly intervals. After 2 hours, the leaves from the water controls showed a green color throughout the vascular elements of the petiole, midrib, and lamina. Leaves from the infected-plant extract had vascular staining in the petiole only. After five hours, however, the green stain was evident in the vessels throughout the lamina of the leaf. A similar reduction in progress of the dye was also noted in leaves that had been held in Type II extract. Although partial reduction in vascular transport accounted for the extra time required for the dye to permeate the leaves from both the Type I and Type II extracts, it was evident that wilting could not be attributed to plugging of the vessels.

The material extracted from plants infected with the Type I defoliating strain of *Cephalosporium gregatum* induced symptoms on detached leaves essentially like those on soybean plants infected with this strain of the organism (3). In vitro production of the wilt-inducing material was indicated by the effect of culture filtrate on detached soybean leaves. The material is heat-stable, since the effect was unchanged after autoclaving at 1.0 atmosphere for 20 minutes.

The results of this investigation suggest that a toxin is involved in pathogenesis. The results of the uptake of fast green stain show that plugging of the vessels could not account entirely for the wilting of the leaves. Partial reduction of water flow in the vessels has been demonstrated previously by diseased stem extract (2), which would account for the slower movement of the dye in the leaves previously held in the Type I extract. The extract (Type II) used by Chamberlain (2), however, did not cause wilting of the leaves when tested by the same methods used in this investigation. Therefore, we suggest that the difference in symptoms induced by isolates of the Type I and Type II cultures is attributable to a toxin produced by Type I isolates.

The original descriptions of brown stem rot symptomatology included leaf symptoms like those found on plants infected with Type I isolates. Subsequent observations have shown that these symptoms are extremely erratic in frequency of appearance. We suggest that this erratic behavior may be explained by the relative prevalence of Type I and Type II strains of *Cephalosporium gregatum*.

LITERATURE CITED

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