

Twin-Stem Abnormality Disease of Soybean Seedlings Caused by *Sclerotium* Sp.

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ABSTRACT

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A growth abnormality disease incited in soybean seedlings by a *Sclerotium* sp. has three distinct severity forms. The internally seedborne pathogen cannot be isolated from seed placed on potato-dextrose agar or moist blotting paper. Infected seeds in a seed lot can be detected by planting the seeds in sterilized sand and observing the seedlings for symptoms, which appear only after the cotyledons have opened. Seed treatment with fungicides is ineffective.

A growth abnormality of soybean (*Glycine max*) seedlings was observed in the field during the 1976-1978 growing seasons in the state of Minas Gerais, Brazil. An identical abnormality was observed in routine emergence tests of seeds planted in methyl bromide-treated sand. Seeds from lots producing such seedlings yielded no microorganisms when planted on potato-dextrose agar (PDA) or blotting paper. The soybean breeder and the agronomist at the Universidade Federal de Viçosa had been observing the abnormality for years and believed an abiotic factor was the cause. Doubts about this theory arose when two seed lots of the soybean cultivar UFV-1 grown at different locations were planted in sterile sand in a greenhouse. The abnormality appeared in 73% of the seedlings of one lot but in only a few of the other lot (Fig. 1), suggesting the inciting factor might be seedborne. This article describes this previously unreported (1) disease of soybean seedlings.

Symptoms appear only after the cotyledons have opened and have three distinct severity forms. In the severe form, designated Type I, elongation of the first internode that bears the primary leaves is completely inhibited. The apical meristem looks like a convex knob between the cotyledons on the cotyledonary node (Fig. 2A). The cotyledons are large and spongy, and the hypocotyl is thick. The seedling remains at this stage for 7-10 days, then two pairs of primary leaves, with rudimentary petioles and internodes, form (Fig. 2B). At maturity, the seedling is stunted and bushy and may have two main stems originating from the cotyledonary node; the name "twin-stem abnormality disease" is derived from this symptom.

In the moderately severe form, designated Type II, the first internode with underdeveloped primary leaves elongates to a limited extent. The original primary leaf primordials are necrotic. Two secondary pairs of primary leaves develop from the cotyledonary node, one on each side (Fig. 3). One or both pairs continue to grow. Adult plants are stunted and bushy and may have two main stems originating from the cotyledonary node.

In the less severe form, designated Type III, the first internode with underdeveloped primary leaves elongates

normally or excessively. The leaves remain wrapped around each other (Fig. 4A) and are bleached at the apex. When the leaves open in 3-5 days, the bleaching extends to the margin and inward, the apex is curved inward, and the entire leaf is wrinkled. The first trifoliolate leaf is formed with little or no elongation of the second internode (Fig. 4B). One or two pairs of leaves may form at the cotyledonary node but generally remain rudimentary (Fig. 4B).

MATERIALS AND METHODS

Surface-disinfected hypocotyls, primary apical meristems, and primary leaves with bleaching and/or curling and wrinkling were collected from naturally diseased seedlings and cultured on PDA. In all cases, a nonsporulating, sclerotium-forming fungus was isolated. Several attempts by us and the Commonwealth Mycological Institute to induce sporulation failed. The fungus was identified as a *Sclerotium* sp. on the basis of well-differentiated sclerotia and the presence of clamp connections. The fungus grew

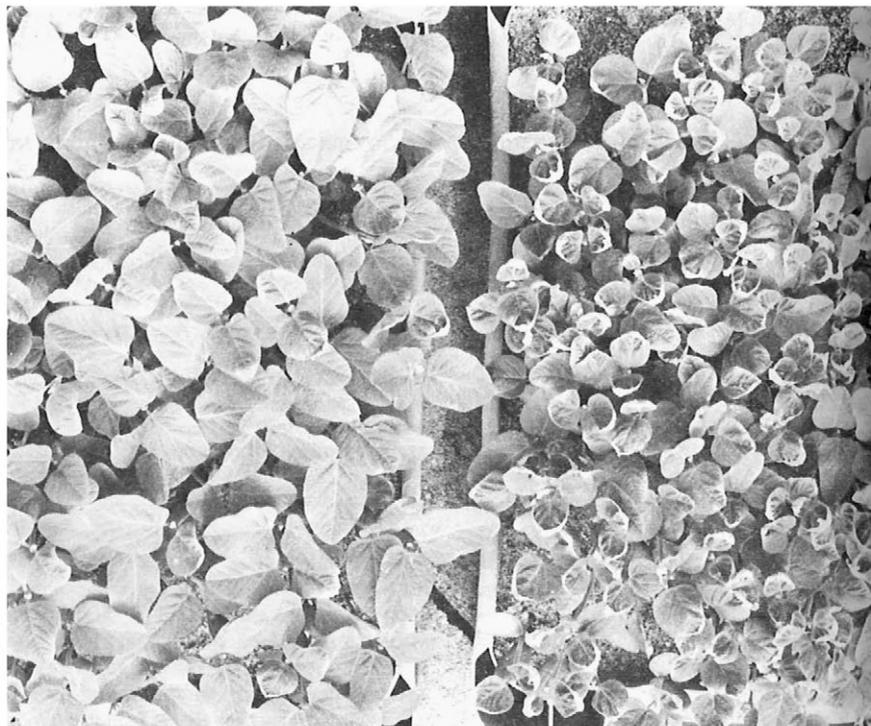


Fig. 1. Seedlings from two seed lots of the soybean cultivar UFV-1. About three-fourths of the seedlings on the left but only a few of those on the right show symptoms of growth abnormality disease.

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rapidly on PDA, with appressed mycelium. Sclerotia began to form after 10–15 days, first at the edge of the colony and then elsewhere. Sclerotia were round, immersed in the medium, 0.5–1.8 mm in diameter, and light red to dark brown-orange in color and germinated by multiple germ tubes.

Pathogenicity of the fungus was tested by inoculating seeds of the soybean cultivar UFV-1 from a lot known to have no natural infection. A mycelial mat grown on soybean seed extract broth for 7 days was minced in 100 ml of sterile water, and seeds were soaked in the mycelial suspension for 5 min. Seeds soaked in sterile water only served as controls. Inoculated and noninoculated seeds were planted in the greenhouse in methyl bromide-treated sand in four batches of 200 seeds each. Emergence counts and observations for symptoms were made 15 days after planting.

RESULTS AND DISCUSSION

The emergence percentage of seedlings was similar in inoculated (89%) and noninoculated seeds (87%). All seedlings from inoculated seeds showed growth abnormality symptoms identical to those observed in the field. In the field, Type III symptoms were more common than Type I or Type II. In the greenhouse, 40% of the seedlings showed Type I symptoms, 38% Type II, and 22% Type III; these results were expected because of the high inoculum level in artificially inoculated seeds. A small superficial canker observed at the base of the hypocotyl of many seedlings

emerging from inoculated seeds (Fig. 5) usually was not seen on seedlings from naturally infected seeds.

Reisolations were made from 30

artificially inoculated seedlings with symptoms. The *Sclerotium* sp. was recovered from cankers, apical meristems, primary leaves, and hypocotyls, suggest-

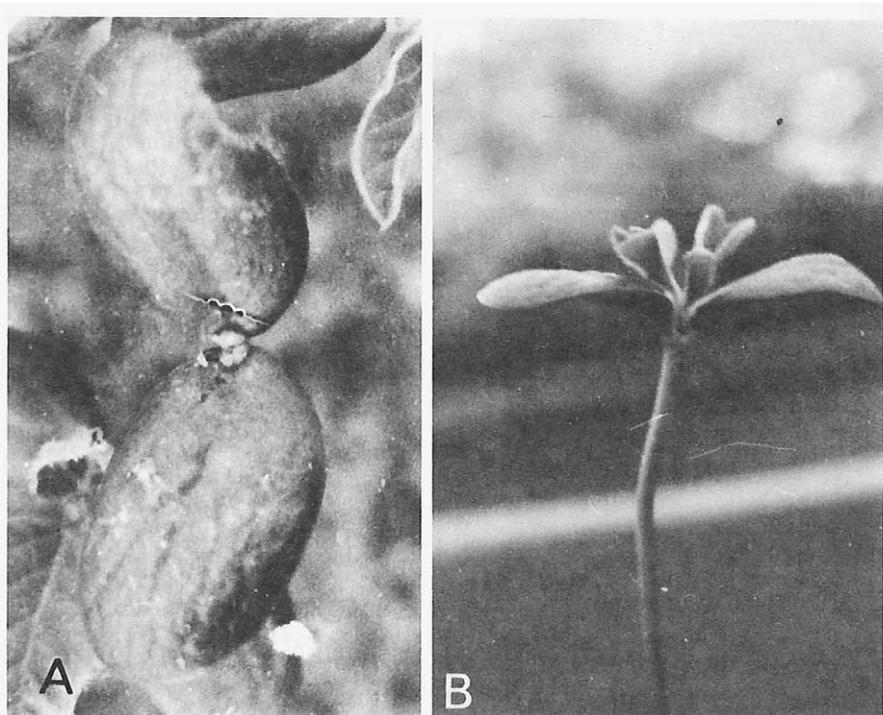


Fig. 2. Severe symptoms (Type I) on seedlings emerging from soybean seed artificially inoculated with *Sclerotium* sp. include (A) complete inhibition of elongation of the first internode and (B) formation of two pairs of primary leaves on the cotyledonary node 7–10 days later.

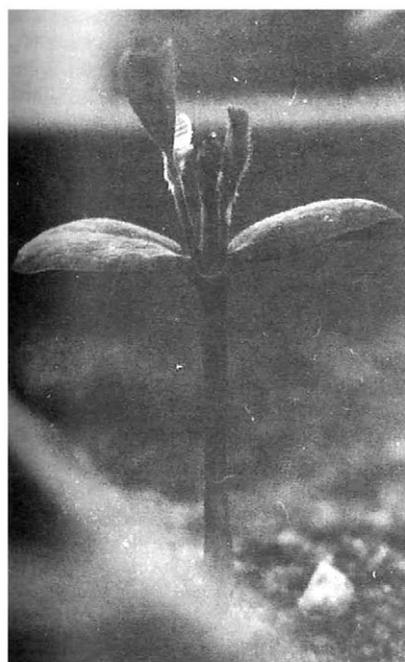


Fig. 3. Moderately severe symptoms (Type II) on seedling emerging from soybean seed artificially inoculated with *Sclerotium* sp. include a stunted first internode and two pairs of primary leaves at the cotyledonary node.

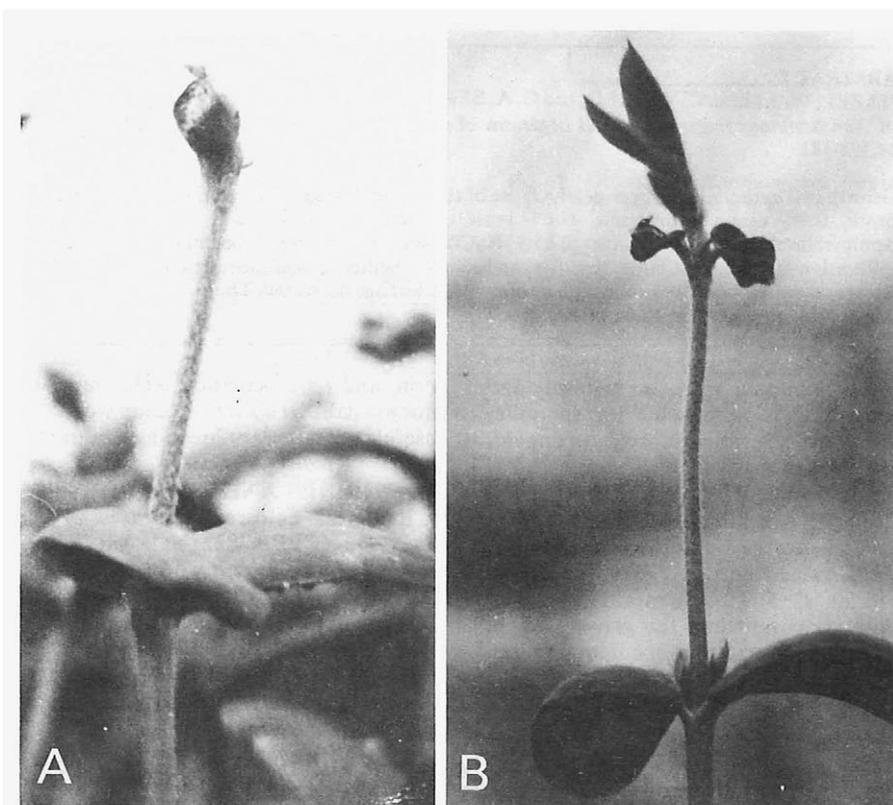


Fig. 4. Less severe symptoms (Type III) on seedlings emerging from soybean seed artificially inoculated with *Sclerotium* sp. include (A) excessive elongation of the first internode and unopened primary leaves and (B) formation of the first trifoliate leaf with little elongation of the second internode.

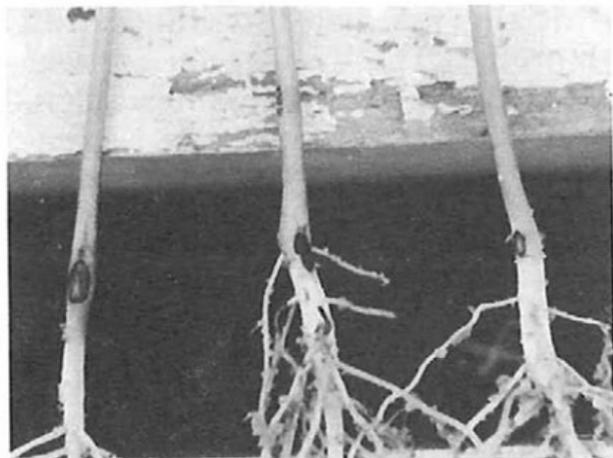


Fig. 5. Canker at or near the crown of seedlings emerging from soybean seed artificially inoculated with *Sclerotium* sp.

ing a systemic infection. The fungus could not be isolated from roots or from seed placed on PDA or moist blotting paper.

At present, the only method of detecting the pathogen in seed lots is to plant the seeds in sterilized sand and observe for symptoms.

Because the pathogen is seedborne, controlling the disease by fungicide treatment was attempted. Seeds from lots with 82% infected seeds and 98% emergence were treated with one of the following fungicides at a rate of 1% (w/w) active ingredient: benomyl (Benlate 50W), thiophanate methyl (Cycosin 70W), thiabendazole (Tecto 60W), fenopronil (RH 2161-25L), pentachloronitrobenzene (PCNB, Brassicol 75W), captan (Captan 75W), chlorothalonil (Daconil 2787-75W), and thiram (Arasan 75W). Untreated seeds served as controls. Treated and untreated seeds were planted in sterilized sand in four replicates of 200 seeds each. Emergence and seedlings with

growth abnormality were counted 15 days after planting.

Emergence varied between 96 and 98% in both treated and untreated seeds. The percentage of seedlings with growth abnormality varied between 76 and 84 in treated and control seeds, with no significant difference among treatments. Thus, seed treatment with the fungicides tested was ineffective.

Twin-stem abnormality disease of soybean seedlings appears to be common in Brazil. Over 30% of the seedlings from 58% of the seed lots tested between 1976 and 1978 showed symptoms of the disease.

LITERATURE CITED

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