Effects of Plant Age Upon Development of Necrosis and Occurrence of Intraxylem Sclerotia in Soybean Infected with Macrophomina phaseolina

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ABSTRACT

Soybean (*Glycine max*) plants up to 60 days of age were wound-inoculated at 10-day intervals with mycelium of *Macrophomina phaseolina (Rhizoctonia bataticola).* Pith necrosis increased significantly with each increase in plant age. Intraxylem sclerotia were produced in stems of 30-, 40-, 50-, and 60-day-old plants. Symptoms of charcoal rot in soybean have been attributed to enzymes and toxin production. Data reported here indicate that sclerotia in xylem vessels also contribute to wilting and death of infected plants.

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Additional key words: charcoal rot, vascular plugging.

Macrophomina phaseolina (Tassi) Goid. [*Rhizoctonia bataticola* (Taub.) Butler] causal agent of charcoal rot of soybean, is found in the warmer growing areas of the U S and India (2, 4, 7). This soilborne fungus causes a root and stem disease on a variety of crops and weeds (2, 7). On soybean, *M. phaseolina* causes a progressive wilting, flagging, defoliation, loss of vigor, and yield reductions (1, 5, 8, 10). We show that intraxylem sclerotia may be involved in symptom production.

Soybean [*Glycine max* (L.) Merr.], cultivar 'Amsoy,' seedlings were planted in clay pots containing autoclaved, sandy loam soil and placed in an Isco growth chamber programmed for 32 C, 50% relative humidity (RH), and a 14-hr day of 86,080 lx (8,000 ft-c).

M. phaseolina was isolated from an Illinois field cropped for 6 yr in soybeans and was maintained on Difco potato-dextrose agar (PDA) (5, 7, 9). Plants 10, 20, 30, 40, 50, and 60 days old were wounded 4 cm above the soil line with a sterile scalpel. There were six pots of three seedlings per pot for each plant age. A small portion of mycelium from a 5-day-old soybean broth culture grown at 25 ± 3 C was placed into the wound cut of half of the plants (5, 7). Then all wounds were covered with petroleum jelly (Vaseline) to prevent drying (7). After 14 days, lesion-size determinations were made then all plants were cut off at the soil line, split longitudinally, and observations on pith necrosis were made. The length of necrotic pith was measured (in cm) from the crown upward.

The cut stems and tap roots were examined under a dissecting microscope, and the average percentage of stem tissue with intraxylem sclerotia was determined.

A second group of plants were grown in the growth chamber for 50 days. Three plants in each of two pots (total, six plants) were wound-inoculated with *M. phaseolina* as described above. Three wounded, but noninoculated, plants served as controls. When individual plants first expressed wilt symptoms (5-14 days after inoculation) they were cut off at the soil line. Stem pieces (5-mm long) were cut from diseased and control plants, fixed in FAA, and embedded in paraffin (3). These were sectioned with a rotary microtome and stained with safranin + fast green (3). Sections were examined under a light microscope for the presence of *M. phaseolina* mycelium and sclerotia.

All wound-inoculated plants developed dark brown lesions about the point of inoculation. No discoloration developed on controls. All inoculated plants wilted and died within 14 days as lesions enlarged and girdled the stems. The youngest plants (10 to 20 days old) wilted and died within 5 to 8 days and the oldest plants (50 to 60 days old) died within 6 to 14 days after inoculation. The average lesion size at the time of wilting increased with increase in plant age and was related to stem size. The average length of stem lesions in cm for each plant age was: 1.4, 10; 4.0, 20; 4.9, 30; 5.4, 40; 5.7, 50; and 7.8, 60. Histological studies of stems showed that necrosis developed in the cortex, phloem, and cambium tissues, as well as in the pith.

The extent of pith necrosis increased significantly for each increase in plant age. The average length of necrotic pith for each plant age group was: 4.8 cm at 10 days; 10.6 cm at 20 days; 16.6 cm at 30 days; 23.5 cm at 40 days; 27.1 cm at 50 days; and 30.6 cm at 60 days. The LSD at 0.05 level of confidence was 2.2 and at 0.01 level, 2.9. The histopathology and isolation of the fungus from stem portions suggested that the fungus mycelium grows rapidly in xylem vessels and is associated with stem discoloration. Sclerotia and mycelium of *M. phaseolina* were found in the xylem and pith of all plants inoculated at 30, 40, 50, and 60 days of age (Fig. 1) and mycelium, but no sclerotia were found in xylem and pith of 10- and 20-day-old plants. The percentage of stem length in which sclerotia formed did not vary with plant age.

The production of intraxylem sclerotia by *M. phaseolina* is reported for the first time. Production of sclerotia by the same fungus was reported in corn and bean stems (6), in woody stem of *Grevillea robusta* (8), and in roots of tea and *Hevea brasiliensis* (8). In plants inoculated at 50 days after planting, sclerotia were produced by the fungus in cortex and pith tissues as well as xylem vessels. Mycelium was located intracellularly in xylem vessels and intercellularly in xylem parenchyma. Sclerotia were formed after the invasion of the xylem by the fungus. Intraxylem sclerotia developed above and below the point of inoculation. The number of xylem vessels with sclerotia in 30 randomly selected, transverse histopathological sections varied from 10 to 21 and in longitudinal sections, the average number in 70, 5-mm sections ranged from 2 to 10. The length of 200 sclerotia varied from 80 to 360 μm, with an average of 148 um. The width of sclerotia varied and was determined by the diam of individual xylem vessels.

Charcoal rot development in soybean plants 10 and 20 days old is probably due to tissue disintegration, caused by enzymes (1) and toxin activity (O.D.Dhingra, *personal communication*) since infected seedlings may die before

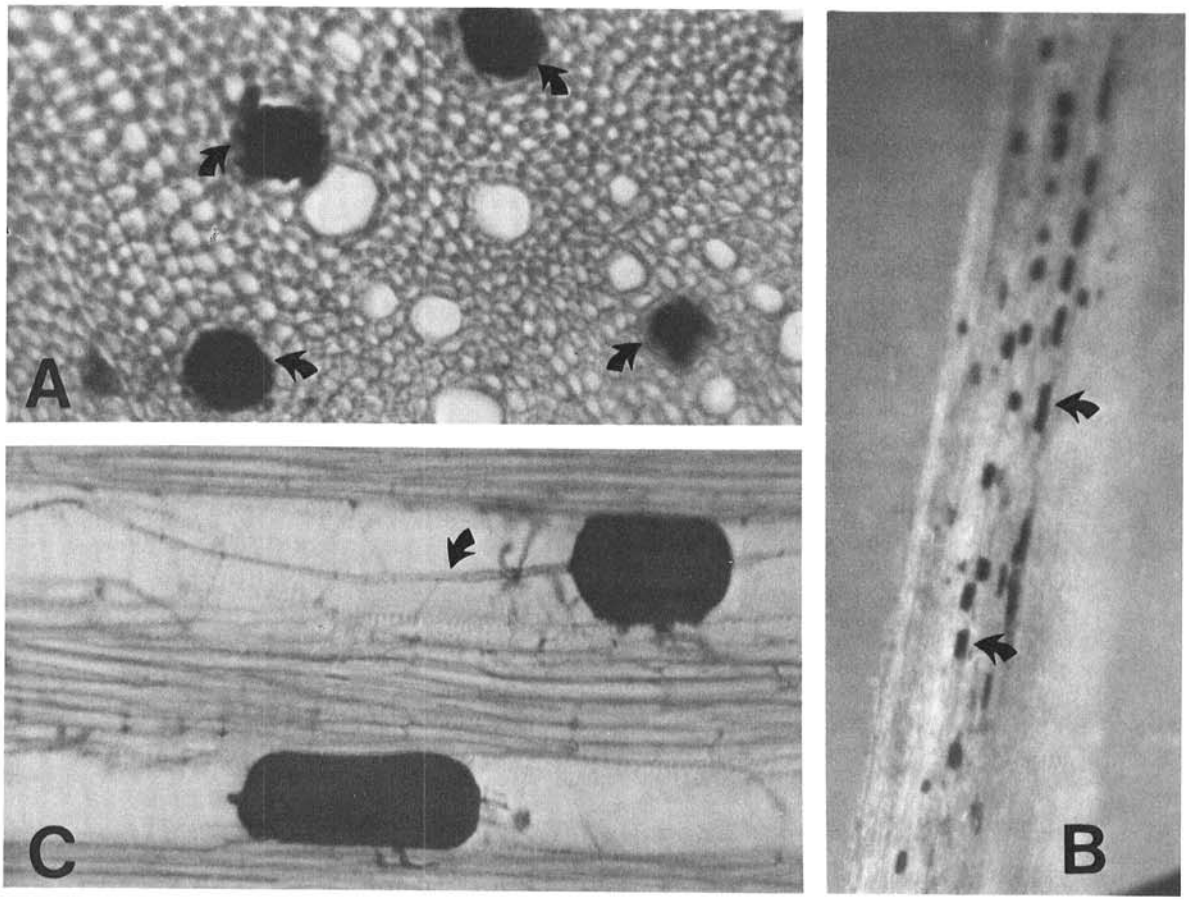

Fig. 1-A, B, C. Photomicrographs of soybean (*Glycine max*) stem sections inoculated with *Macrophomina phaseolina*. **A)** Cross section with sclerotia (arrows) in xylem vessels (×320); **B)** Longitudinal section showing intraxylem sclerotia (arrows) (×30); and **C)** Longitudinal section with sclerotia and mycelium (arrow) in xylem vessels (×320).

sclerotia are formed. However, in plants 30 to 60 days old, plugging of xylem vessels by intraxylem sclerotia may contribute to symptom development (Fig. 1), assuming that they occur in naturally infected plants. Also, viable sclerotia of the fungus in soybean plant debris may be a source of inoculum for stored seed or for introducing the pathogen into noninfested fields.

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