Mycelial Development and Control of *Phomopsis sojae* in Artificially Inoculated Soybean Stems

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ABSTRACT

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The direction and rate of mycelial growth of *Phomopsis sojae* were traced from a single inoculation site in greenhouse-grown soybean (*Glycine max* cv. Davis) plants. Serial stem sections from inoculated plants were plated on potato-dextrose agar (PDA) and observed with a scanning electron microscope. Mycelium remained within 2 cm of the point of inoculation until senescence began. The pathogen grew terminally approximately 5.5 cm by the late R8 stage of growth. In another greenhouse test, soybean plants in the V3 growth stage were inoculated with a conidial suspension of *P. sojae* 24 hr before, at the time of, and 7, 10, 14, 17, and 21 days after a foliar application of benomyl at 0.3 g/L. Growth of *P. sojae* was detected on PDA only from stem sections inoculated 21 days after the fungicide treatment.

The sudden appearance of *Phomopsis sojae* Leh. pycnidia on the entire surface of field-grown soybean stems at maturity indicated a systemic infection. Kilpatrick (4) suggested that the disease became systemic after its establishment in soybean plants. Gerdemann (3) believed that *P. sojae* remained semidormant throughout the growing season and became systemic as the plants matured.

We attempted to trace the movement of *P. sojae* in inoculated stems and to gain some insight into the fungicidal action of benomyl.

MATERIALS AND METHODS

Davis soybean seeds were surface-sterilized for 10 min in Rada's solution (5), rinsed for 1 min in a 10% sodium hypochlorite solution, and soaked for 10 min in sterile, distilled water. Seeds were then placed one per plate on sterile potato-dextrose agar (PDA) and incubated for 7 days at room temperature. Only seeds that germinated with no visible signs of contamination and were considered to be disease-free were used.

Roots of seedlings were treated with *Rhizobium japonicum* (Kirchner) Buchanan, planted five per pot in steam-sterilized soil in 10-in. clay pots, and watered with deionized water. Plants were grown in the greenhouse during the winter months without supplementary light and reached a height of 30–35 cm.

Plants in the VI stage of growth (2) were inoculated either by injection with a hypodermic syringe at the cotyledonary scar or by placing a drop of inoculum on the cut surface of the stem after excising the cotyledon. Four plants in each pot were inoculated; the fifth plant, the control, was injected with sterile, distilled water. Conidial suspensions of 1.8×10^5 cells per milliliter were used as inoculum.

Stems assayed for *P. sojae* were excised 3 cm below the point of inoculation, cut serially into 2-cm lengths, sterilized for 5 min with each of 10% sodium hypochlorite solution and Rada's solution, and plated on PDA. For convenience, we refer to the 2-cm stem section 1 cm above and below the point of inoculation as A, the section 1–3 cm above the point of inoculation as

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B, and the section 3–5 cm above the point of inoculation as C. The section 1–3 cm below the point of inoculation was designated as the basal stem section (BSS).

Flowers, buds, pods, and seeds from all test plants were surface-sterilized as described, incubated at 24 C, and observed for the presence of *P. sojae*.

A scanning electron microscope was used to determine the tissue through which the pathogen moved. Twenty-one inoculated and 14 control plants at the R4 growth stage were used in each test. A 4-cm section that included the cotyledonary scar was taken from the stems 1 cm above the basal end, surface-sterilized, and plated on PDA, using three inoculated and two control stem sections per plate.

For 5 days after plating the stem sections, we removed two inoculated and one control stem section from the plates every 24 hr. The remaining sections were used to confirm the presence of *P. sojae* and to check for contamination to insure that the organism we observed with the scanning electron microscope was the pathogen.

The sections removed from PDA were cut into 5-mm sections and placed in a fixative solution (75% Formalin, 20% ethanol, and 5% glacial acetic acid) for 24 hr. They were then rinsed in several changes of deionized water, dehydrated in 2,2-dimethoxypropane, transferred to acetone, and dried by the critical point method (1). The stem sections were then mounted on aluminum stubs, sputter-coated with palladium, and viewed in a Hitachi S-500 scanning electron microscope for the presence of hyphae. In addition, fresh sections were collected from stems at various growth stages and prepared as above for examination in the scanning electron microscope.

A second study was undertaken to determine the effectiveness and duration of control of pod and stem blight with benomyl. Plants in the V5 stage were divided into two groups. The first group of 20 plants was injected with a conidial suspension of *P. sojae* (1.8×10^5 cells per milliliter). Fifteen of these plants received a foliar application of benomyl at 0.3 g/L 24 hr later. The five remaining plants served as controls. Two inoculated and one control plant were removed at 7-day intervals for 5 wk. The stems were excised, sectioned, sterilized, and plated as described above to test for the presence of *P. sojae*.

A second group of 15 plants received a foliar application of benomyl (0.3 g/L). This group was then divided into five control plants and 10 plants injected with freshly prepared conidial suspensions at the time of and 7, 10, 14, 17, and 21 days after fungicide was applied. These plants were removed 30 days after treatment and tested as described above for the presence of *P. sojae*.

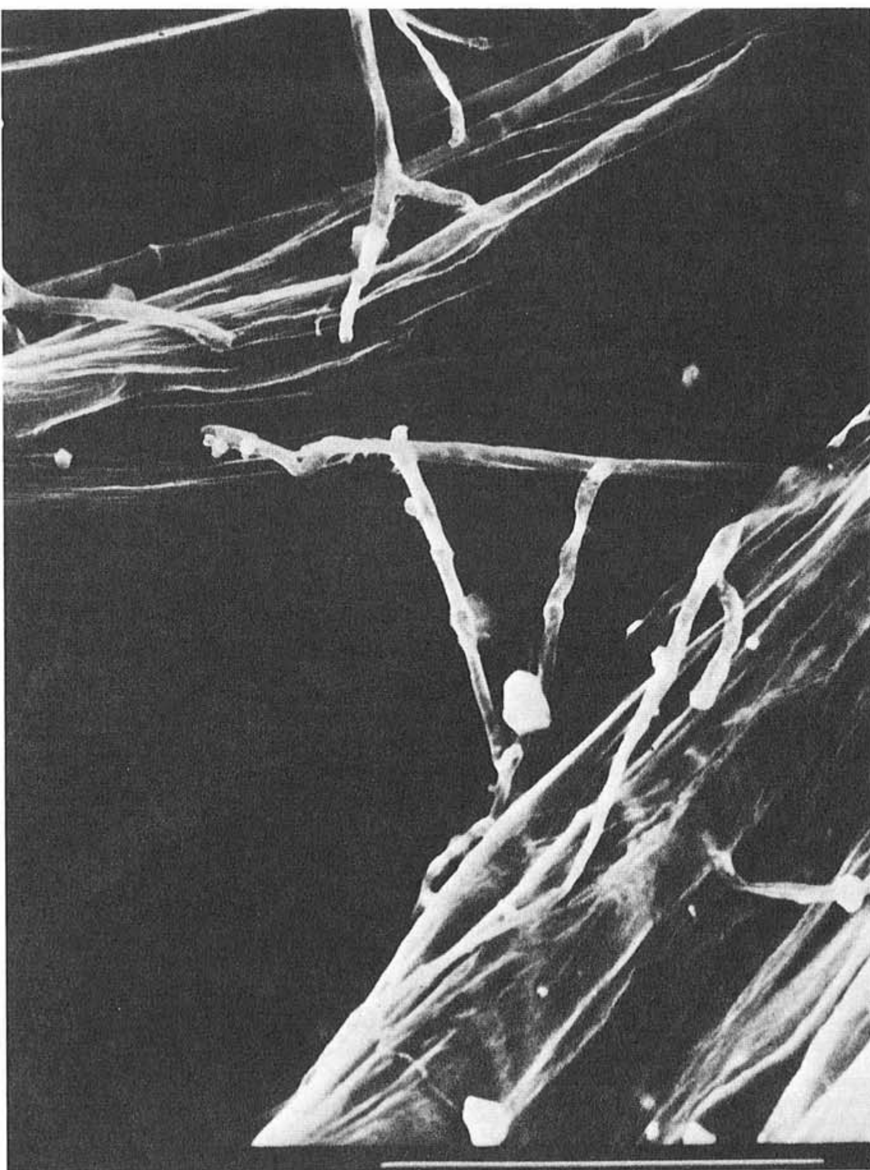

Fig. 1. Soybean stem pith tissue with *Phomopsis sojae* hyphae beginning to appear 48 hr after plating on potato-dextrose agar. Bar represents 50 μm.

RESULTS

Inoculation tests. *P. sojae* was isolated from stem sections A in the first (V2) through the ninth (R5) week after inoculation and from stem sections B and BSS once in the V3 and again in the R2 stage during this period. These results indicated the inactivity of the pathogen.

At R6, the fungus became active and grew terminally within the stems. At this stage, the stems began to lose their green color rapidly and became tan. A dark necrotic area developed near the point of inoculation and enlarged through the R6–R7 stages; by late R7, it had completely encircled the stem and was 4.0–5.5 cm long.

The characteristic linear row of pycnidia formed proximally from the point of inoculation. Weekly isolations from stem sections taken during the R6–R7 stages confirmed the presence of *P. sojae* in stem sections A and B by mid-R6 and in section C by late R7. *P. sojae* was never isolated from the stem sections of control plants.

Scanning electron microscopy. Scanning electron microscope observations of inoculated stems did not indicate the pathogen's presence until the R6 and R7 stages, when hyphae began to appear and colonize the pith tissue and epidermis of stem sections A, B, and C. No mycelial development of *P. sojae* was evident during the first 24 hr after inoculation. At 48 hr, small hyphal strands were seen in the pith area (Fig. 1), but none were observed on the epidermis. By day 3, the hyphae had ramified throughout the pith and had begun to appear on the outside or within the cells of the epidermis, where they produced appressoria (Fig. 2). At day 4, the hyphae had permeated the pith and covered the epidermis.

Very limited mycelial growth occurred on the sieve elements. No mycelial growth was noted on the exposed cross sections of the stem until day 5, when mycelium completely covered the section. No

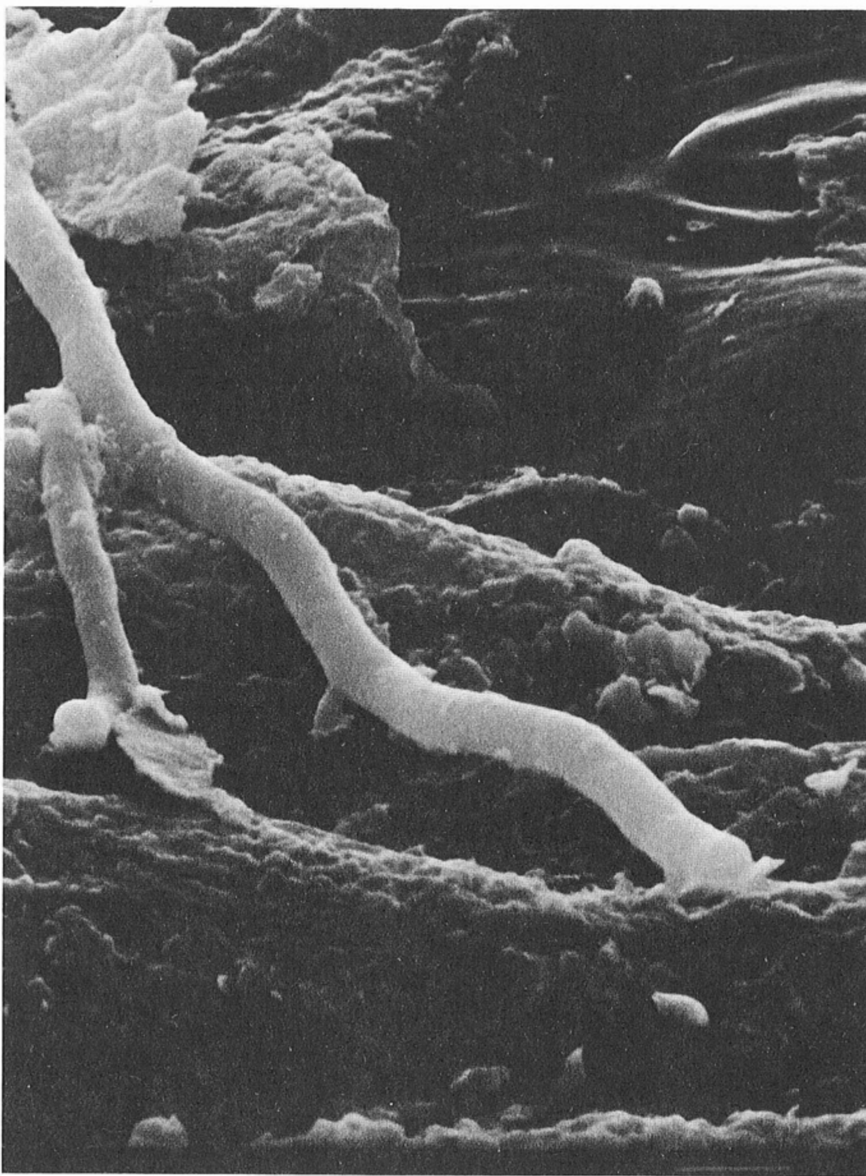

Fig. 2. Appressoria of *Phomopsis sojae* on soybean stem epidermis 72 hr after plating on potato-dextrose agar. Bar represents 5 μm.

fungal growth was observed in the vascular tissue.

Benomyl tests. Plants sprayed with benomyl remained free of *P. sojae* for 17 days, regardless of whether the inoculum was already present, was introduced just before spraying, or was injected up to 17 days after spraying. Plants inoculated 24 hr before being sprayed or inoculated and sprayed immediately after injection remained free of *P. sojae* after the 30-day test period. Plants inoculated 7, 10, 14, and 17 days after being sprayed were also free of the pathogen, while plants inoculated 21 days after being sprayed were infected.

DISCUSSION

Researchers have suggested that after becoming established in soybean plants, *P. sojae* either becomes systemic (4) or remains semidormant through the growing season and then becomes systemic as the plant matures (3). The argument for the systemic activity of *P. sojae* was based on observations of pycnidia that appeared suddenly on stems and petioles at maturity and/or on isolations of the pathogen from seeds within healthy pods.

The research presented here indicates that the pathogen is not systemic. The sudden appearance of pycnidia late in the season under field conditions probably results from multiple localized infections. Inoculation tests and electron microscopy showed that *P. sojae* remained semidormant and close to the point of inoculation until the plant began to mature.

Benomyl applications appeared to provide protection against pod and stem blight for at least 17 days. Benomyl's protective ability deteriorated rapidly after this time and disappeared 21 days after application.

The recommended fungicidal spray program for control of pod and stem blight of soybeans in Louisiana is correctly scheduled. Spraying at late flower–early pod set followed by another application 14 days later protects plants during the crucial periods of seed development and maturity.

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