

Development of *Phytophthora megasperma* var. *sojae* in Soybean Roots

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ABSTRACT

A histological stain was developed which is differentially absorbed by oospores of *Phytophthora megasperma* var. *sojae* (PMS) in soybean root tissues. Intracellular hyphae and haustorium-like bodies were detected in PMS-infected root tissues by light-microscope studies. A sand medium favored production of oospores in root tissues of cultivars

susceptible ('Amsoy'), field tolerant ('Wayne') and resistant ('Amsoy 71') to PMS. Wayne roots had as many oospores and greater dry weight when compared with Amsoy roots. Amsoy 71 roots contained few oospores. Oospores were found in Amsoy 71 roots that overwintered in field soil.

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Germ tubes of zoospores of *Phytophthora megasperma* var. *sojae* (PMS) penetrate soybean roots (7), and after colonization, oospores form within the tissues. These germinate to form exogenous sporangia. Electron microscope studies (8) showed the hyphal penetration of soybean hypocotyl tissues by PMS and the formation of haustorium-like bodies. Since there are no detailed light-microscope studies on the penetration and development of PMS in soybean roots, we report here the development of PMS hyphae and oospores in root tissues of soybean cultivars that are resistant, susceptible, and field tolerant to PMS.

MATERIALS AND METHODS.—An isolate of *Phytophthora megasperma* Drech. var. *sojae* Hild. race 1 (12) isolated from diseased soybean [*Glycine max* (L.) Merr.] plants collected in Champaign County, Illinois, was used in this study. Cultures were maintained on V8-CaCO₃ agar (V8A) (11). Inoculum was prepared by cutting 1-cm disks with a sterile cork borer from the edge of an actively growing PMS colony on V8A. Each plug was placed on a 1.5-cm cork disk floating in 150 ml of autoclaved (15 min at 110 C) commercial V-8 juice diluted 1:4 with distilled water. The medium was adjusted to pH 6.5 with 1 N NaOH before it was autoclaved. The mycelial mat from a 10-day-old culture (23–25 C) was removed, separated from the cork and inoculum plug, and the excess medium removed by blotting on sterile filter paper. Mats were blended twice in a blender (Virtis 45 Omnimix) at low speed for 15 sec with two changes of sterile 0.05 M phosphate buffer (pH 7). The excess liquid was removed by placing the mycelial mass on sterile, 7-cm diam filter paper disks which were folded and placed inside a centrifuge tube previously filled with absorbent tissue (Kimwipe), and centrifuged for a few min at 6,000 g.

Soybean seed of the cultivars 'Amsoy' (AS), 'Wayne' (WS), and 'Amsoy 71' (AR), susceptible, field tolerant, and resistant, respectively, to PMS (2, 9, 13) were hand-selected for uniformity and surface-sterilized by immersion in a 2.75% sodium hypochlorite solution for 3 min, then in 70% ethanol for 1 min, followed by a sterile distilled water rinse and allowed to air dry.

All roots were washed in tap water, and 2-cm sections from 2,645 root tips were examined microscopically for presence of oospores prior to staining. A histological stain (100 mg bromphenol blue + 3 g AgNO₃ in 50 ml of 95% ethanol) (BPBA) was used to stain oospores within root sections. Root samples with oospores were placed in Karpechenko's fixative (1) for 72 hr, rinsed in tap water, dehydrated in an alcohol series, embedded in paraffin (Paraplast), and sectioned on a Spencer rotary microtome at 10 μ . Paraffin was removed with xylene, tertiary butanol, and 95% ethanol before the sections were stained with BPBA for 30 min. After a tap water rinse, stained sections were immersed for 5 sec in 95% ethanol, dipped for 1 sec in a saturated solution of methylene blue in ethanol, and immediately rinsed in running tap water. No disruption of tissues took place during this process. The surrounding tissues were stained a light blue. The sections were dehydrated in absolute ethanol, rinsed in tertiary butanol and xylene, and a cover slip affixed with mounting medium (Permount).

Infected roots came from seedlings grown either in a PMS-infected medium or in overwintered soil collected in a field in which AR soybeans were grown the previous

season. The infesting medium was prepared by blending 1.7 g of PMS inoculum with 175 ml of the phosphate buffer. The percent transmission of the inoculum suspension diluted 1:4 was 18 on a Beckman DB-G spectrophotometer set at 550 nm. This suspension was mixed immediately with 1,000 g of dry, sterile quartz sand (15).

In one experiment, 25 seedlings of each variety were grown in 450-cc plastic (Styrofoam) cups filled with either nonautoclaved or autoclaved (15 min at 212 C) field soil or autoclaved quartz sand. Four seedlings were planted in each cup. The growth media were either noninfested (controls) or infested by mixing (2:1) with the infesting medium.

In a second experiment, only sterile quartz sand was used because oospore formation was greater in roots of seedlings grown in this medium than in field soil. The infesting medium prepared in a similar manner (1:1) had a percent transmission of 10 at 55 nm. This was blended with 300 ml of water and mixed with sterile, dry sand. There were 10 plastic cups filled with infested sand, each with four seeds of each cultivar. Cups with noninfested sand served as controls.

RESULTS AND DISCUSSION.—The BPBA stain aided in studying the life cycle of PMS in soybean root tissues. Oospores were stained black to light blue (Fig. 1-A) and mycelia stained a light blue (Fig. 1-B). Alcoholic nitrate stains were used for neurotoxicological impregnations (4), but this is the first use of a silver nitrate-bromphenol blue stain for differentiating fungal structures in higher plant tissues (1). The differences in uptake of the stain by oospores may be due to physiological differences among them.

PMS became established and developed with the root tissues of WS and AS (Fig. 1-B, D). We showed for the first time in light-microscope studies the intercellular growth of PMS and globular (Fig. 1-E) and digitate (Fig. 1-F) haustorium-like structures that invaginated or penetrated soybean root cells. Klarman & Corbett (8) claimed to have electron-microscope photographs (*unpublished*) of intracellular hyphae and haustorium-like structures of PMS in soybean hypocotyls. Other studies on PMS were from *in vitro* cultures (5, 16).

Roots of WS seedlings contained as many oospores as those of AS seedlings. Oospores were observed in 40, 30, and 4% of WS roots and 43, 18, and 0% of AS roots from seedlings grown in sand, autoclaved and nonautoclaved soil, respectively. One percent of roots from AR seedlings grown in sand contained oospores. No oospores were found in control plants. This is the first report of oospores being formed in AR seedling roots. Root diam measured at 0.75 cm above the root tip ranged from 200 to 700 μ . More oospores were observed in roots 500 μ in diam than in smaller ones.

In the second experiment after 5 days, dead, dying, or nonemerged seedlings were removed, and their roots were examined for oospores. Oospores were found in the discolored roots of 10 out of 17 AS plants, and in roots of 5 out of 19 WS seedlings. The roots of three of these latter five plants were slightly discolored. AR and control seedling roots were not discolored, and did not contain oospores. All remaining seedlings were removed at 7 days and their roots examined for oospores. The root tips from 18 of 20 AS and 16 or 19 WS plants contained oospores.

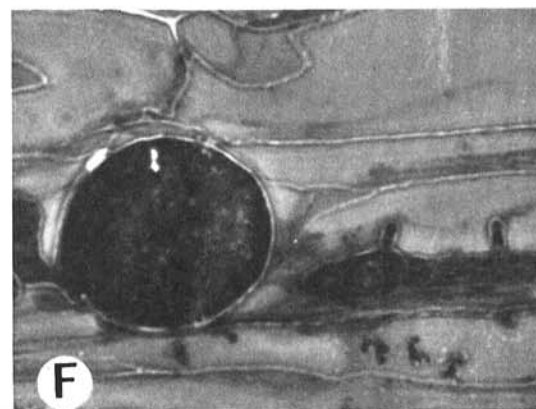
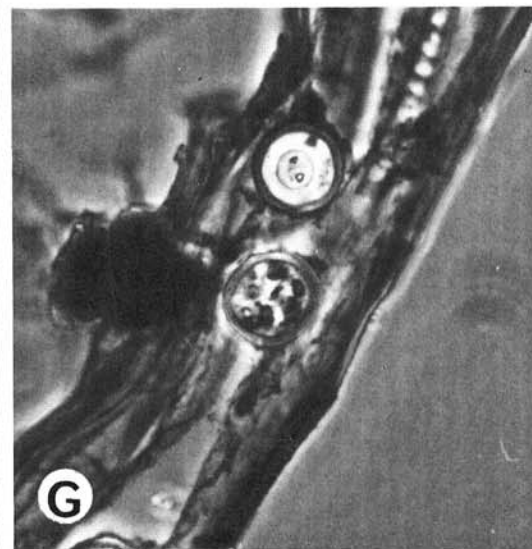
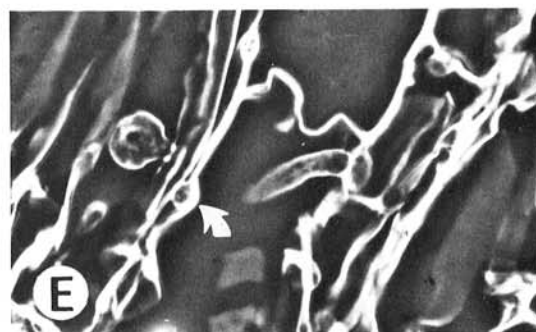
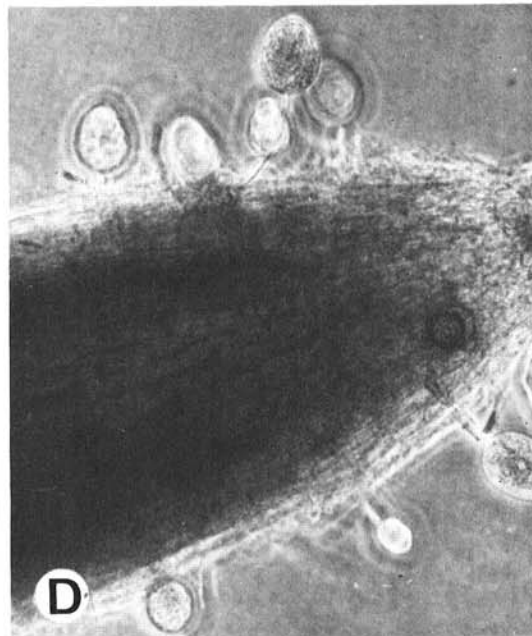
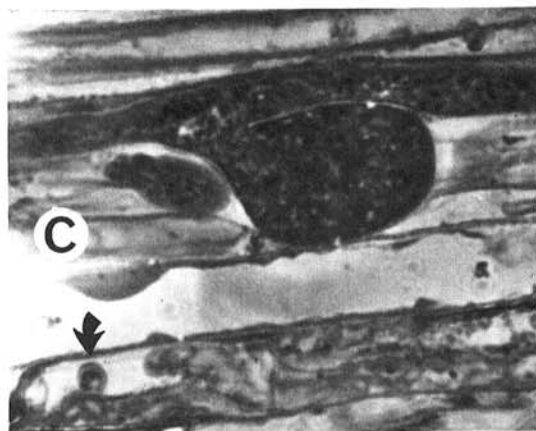
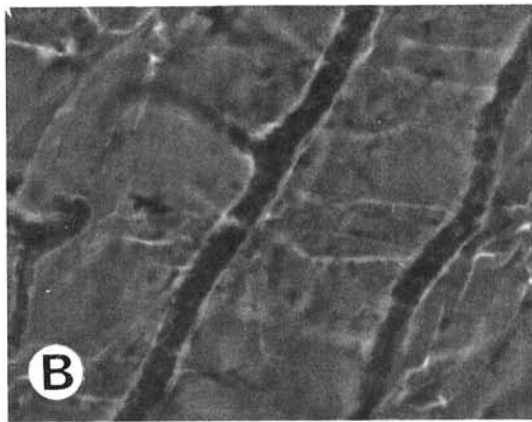
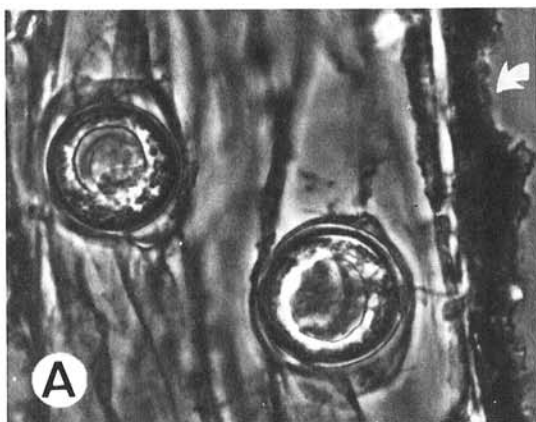


Fig. 1. Developmental stages of *Phytophthora megasperma* var. *sojae* (PMS) in soybean seedling roots susceptible ['Amsoy' (AS)], field-tolerant ['Wayne' (WS)], or resistant ['Amsoy 71' (AR)] to PMS. A) Stained oospores within WS root tissue; arrow indicates root epidermis ($\times 800$). B) AS root tissue with hyphae stained in granular pattern ($\times 600$). C) Formation of oogonial initial in AS root tissue; note cross section view of hypha penetrating host cell (arrow) ($\times 800$). D) Immature sporangia forming outside of AS root ($\times 150$). E) Hyphae penetrating two cells, forming globular, haustorium-like bodies; hyphae also growing intercellularly (arrow) ($\times 400$). F) Hyphae penetrating or invaginating cells, forming digitate, haustorium-like bodies; note immature oogonium ($\times 800$). G) Oospores in AR root from sand-grown plant ($\times 300$).

Roots of 26 AR seedlings and controls of the three cultivars contained no oospores.

The dry weights of the root system and top portion of 20 seedlings were recorded separately from each cultivar grown in sand. There was no relationship between the presence of oospores in roots and the dry weights of roots and tops. There were no measurable differences in top dry weights among varieties. The dry weights of roots were higher for WS and AR than for AS. WS had 14% lower root dry weight than AR. The mean top weights (g) were 1.11, 1.11, and 1.13 for AS, AR, and WS, respectively. The mean dry weights of roots were 0.38, 0.56, and 0.65 g, respectively.

Root discoloration has been used as an index of PMS infection (10); we found no correlation between presence of oospores in root tissue and discoloration, however. Our observations showed that AS roots of infected seedlings were generally discolored but those of WS either were not or were only slightly discolored. Oospores and chlamydospores of *Phytophthora* spp. are considered to be primary survival units in soil (16). Since WS roots contained oogonia and mature oospores, it is suggested that this variety produces overwintering inoculum equal to that of the susceptible AS. Roots from AR seedlings occasionally contained oospores. Schmitthenner & Williams (14) reported that PMS-resistant 'Blackhawk' stands and yields were less in plots planted annually with soybean than in those rotated with corn and soybeans. PMS inoculum buildup in soil may arise from the small percentage of infected susceptible plants found among those of a resistant variety (3, 6).

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