Histopathology of Soybean Seed and Seedling Infection by *Macrophomina phaseolina*

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ABSTRACT

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Soybean seeds infected by *Macrophomina phaseolina*, cause of charcoal rot of soybean (*Glycine max*), were used. Histological examination of symptomatic seeds showed that hyphae and sclerotia were ecto- and endophytic; hyphae were inter- and intracellular in tissues of the seed coat, endosperm, and embryo. Seed coats of severely infected seeds were fragmented. The fungus produced sclerotia in 4% of asymptomatic seeds after 2 days of incubation at 25 ± 2 C on acidified (pH 4.5) potato-dextrose agar. These sclerotia developed near or adjacent to the seed coat endosperm and hypodermis. The fungus apparently can penetrate and colonize soybean seeds without producing symptoms, but subsequently forms sclerotia in asymptomatic seeds when conditions are favorable for seed germination. After 3–4 days of incubation, sclerotia were formed in the cotyledons of asymptomatic seeds and after 4–5 days, in the hypocotyl-radicle axis. This is the first report of *M. phaseolina* being seedborne in field-grown soybeans in Illinois.

Additional key words: *Rhizoctonia bataticola*.

Macrophomina phaseolina (Tassi) Goid. [synonym: *Rhizoctonia bataticola* (Taub.) Butler] is found worldwide and causes charcoal rot on more than 500 hosts (12). In tropical climates, the fungus causes a postemergence damping-off of soybean (*Glycine max* (L.) Merr.) seedlings with plant losses up to 77% (6). On mature soybean plants *M. phaseolina* causes a progressive wilt, flagging, yellowing, premature drying, loss of vigor, reduced emergence and yield, and a grayish black discoloration of stem tissues due to abundant production of sclerotia (1,3,6,10,12,16,17).

The fungus is seed transmitted and seedborne in soybeans and can survive for 2–3 yr in seeds (5,14). It was found more frequently in soybean seeds grown in tropical than in temperate climates (5). Symptoms on infected seeds appear as indefinite black spots or blemishes on the seed coat. There is no published information on the histopathology of soybean seeds colonized by *M. phaseolina*.

We report herein the growth and development of *M. phaseolina* in naturally infected asymptomatic and symptomatic seeds and the tissue changes associated with this fungus. A portion of this study was published as an abstract (9).

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MATERIALS AND METHODS

In 1983, soybean seeds were harvested from naturally infected plants (cultivar Amsoy) showing symptoms of charcoal rot in an experimental field plot at the University of Illinois Agronomy-Plant Pathology South Farm at Urbana. Approximately 1,000 seeds were examined under a stereoscopic microscope for symptoms of *M. phaseolina*, but none were found.

In 1984, soybean (cultivar Corsoy 79) seeds were harvested from naturally infected plants that had been killed by *M. phaseolina* in an experimental field plot at the UIUC Cruse Farm at Urbana, IL. Eight of these seeds showed typical symptoms of infection by *M. phaseolina*.

The asymptomatic seeds of 1983 and the symptomatic seeds of 1984 were surface sterilized for 5 min with 0.5% sodium hypochlorite and then washed in two changes of sterilized deionized water.

Approximately 1,000 asymptomatic seeds were plated on 9-cm-diameter culture plates containing potato-dextrose agar (Difco) acidified with lactic acid (pH 4.5) at five seeds per plate and incubated in the dark for 5 days at 25 ± 2 C. All fungi growing from the seeds were isolated and identified. One hundred germinated asymptomatic seeds with or without colonies of *M. phaseolina* from the 1983 seed lot were collected every 24 hr for the 5 days and fixed in formalin-acetic acid-alcohol for 48 hr (8). In addition, ungerminated asymptomatic and symptomatic seeds were soaked

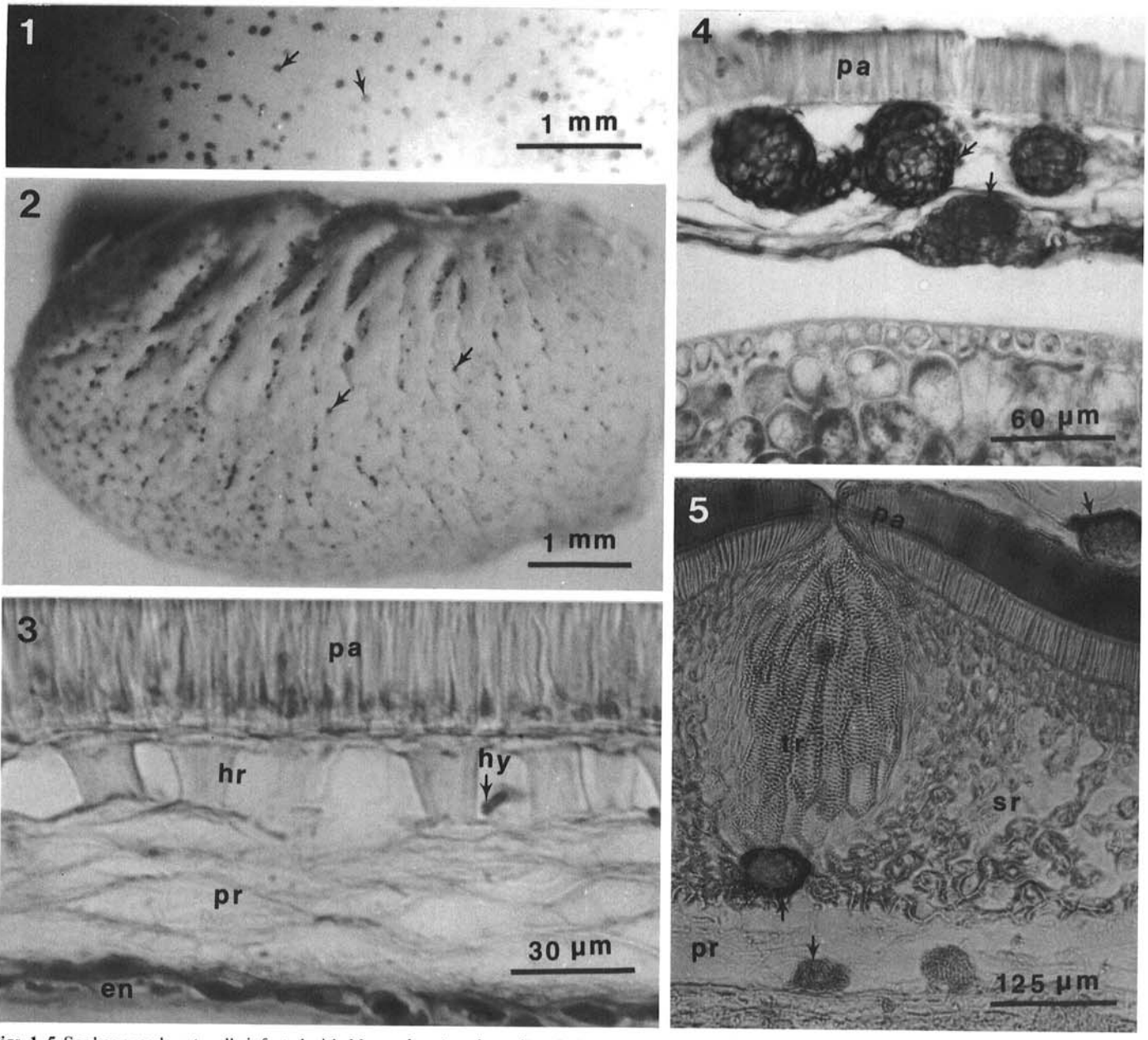
in deionized water for 2 hr at 60 C and then fixed in formalin-acetic acid-alcohol for 48 hr. Germinated and ungerminated seeds were dehydrated in a tertiary butyl alcohol series, embedded in Paraplast (Sherwood Medical Industries, Inc., St. Louis, MO), and sectioned on a rotary microtome at 10–20 μm (8). Sections were stained either with safranin followed by light green (8), or with toluidine blue O (11). All sections were mounted in Canada balsam and examined under a bright-field compound microscope.

RESULTS

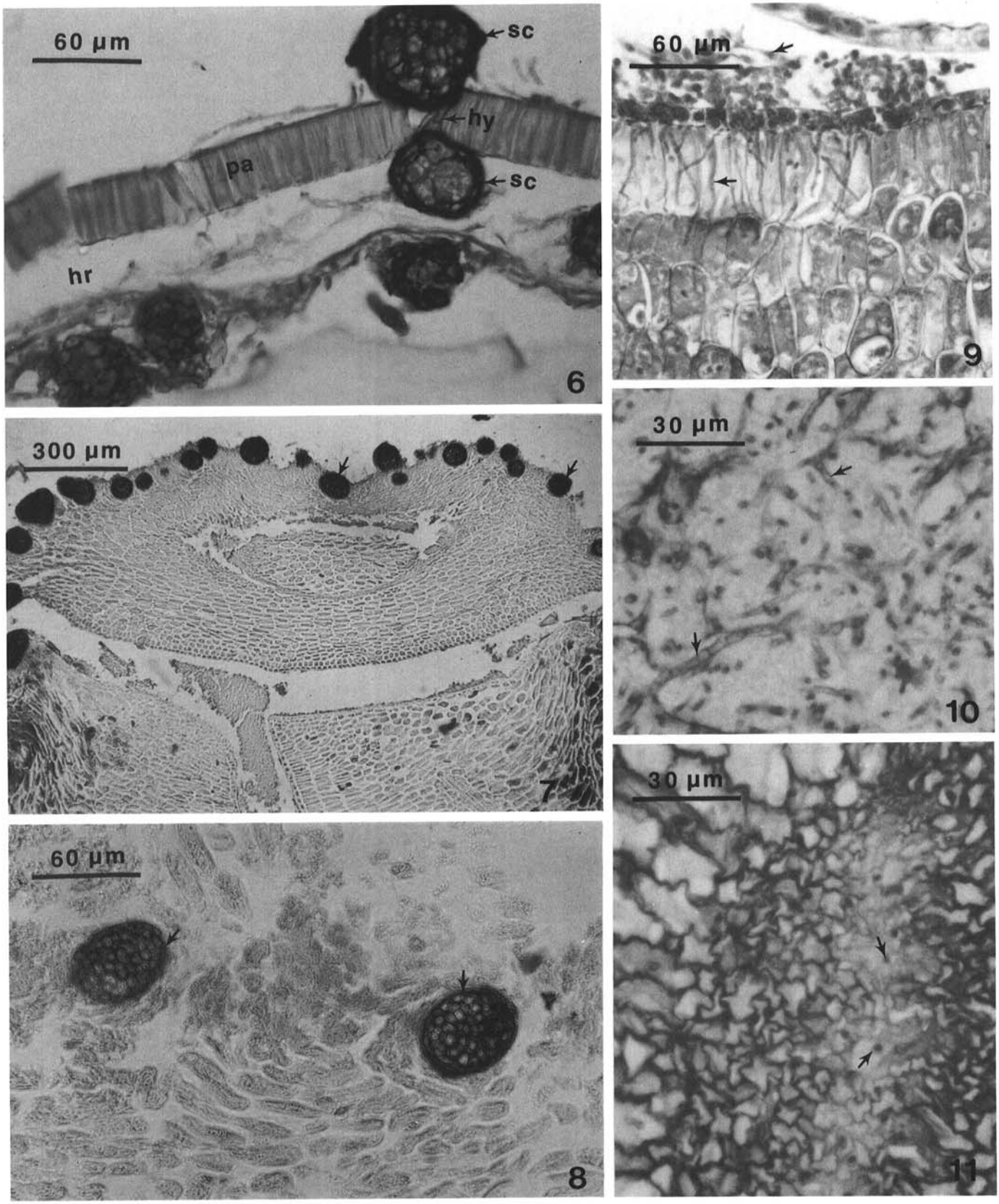
Four percent of the asymptomatic seeds from 1983 and all of the symptomatic seeds from 1984 yielded *M. phaseolina* on acidified (pH 4.5) potato-dextrose agar. Asymptomatic seeds infected with *M. phaseolina* had translucent, immature, brown sclerotia below the seed surface after day 2 of incubation (Fig. 1). By day 3 of incubation, the sclerotia had increased in number and size, turned black, and occasionally could be seen on the seed coat surface. Infected seedlings from such seeds were covered with sclerotia by day 5 and were dead. Seed coat tissues in symptomatic seeds were fragmented and sclerotia, mostly within the seed coat, could be seen easily due to its fragmentation (Fig. 2). Heavily infected seeds appeared black because of abundant sclerotial production.

Soybean seeds consist of a seed coat, endosperm (aleurone layer and parenchymatous cells), and an embryo made up of two large fleshy cotyledons, a plumule, and hypocotyl-radicle axis. The seed coat covered with cuticle consists of three distinct layers, epidermis (one-cell-thick palisade layer), hypodermis (one-cell-thick hourglass layer), and endodermis (six- to eight-cell-thick parenchymatous layer) (4).

Sections of infected asymptomatic seeds showed the presence of sparse intercellular hyphae only in the hourglass cell layer (Fig. 3). No tissue damage was observed. Sections of seeds colonized by *M. phaseolina* and incubated for 2 days showed inter- and intracellular hyphae in the three seed coat layers with sclerotia in the hourglass cell layer, endodermis, and endosperm which were indistinguishable



Figs. 1–5. Soybean seeds naturally infected with *Macrophomina phaseolina*. **1,** Asymptomatic seed after 2 days of incubation, showing subepidermal brown sclerotia (arrows). **2,** Symptomatic seed with fragmented seed coat and black sclerotia (arrows) within the seed coat. **3–5,** Photomicrographs of transverse sections of soybean seeds. **3,** Asymptomatic seed with sparse hyphae (hy) in hourglass layer (hr). The palisade (pa), hourglass layer (hr), parenchyma (pr), and endosperm (en) of the seed coat are undamaged. **4,** Asymptomatic seed after 2 days of incubation showing sclerotia (arrows) in all the layers of the seed coat except the palisade (pa). **5,** Sclerotia (arrows) in the hilum region above the palisade layer (pa), below the tracheid (tr), and in the sclerenchyma (sr) and parenchyma (pr) layers.



Figs. 6–11. Photomicrographs of transverse sections of symptomatic soybean seeds naturally infected with *Macrophomina phaseolina*. **6–9,** Seeds incubated for 3 days. **6,** Hyphal connection (hy) between sclerotia (sc) above the palisade (pa) and below in the disintegrated hourglass layer (hr). Sclerotia also are in the indistinguishable parenchyma and endosperm layers. **7,** Sclerotia (arrows) in the outer cell layers of hypocotyl-radicle axis. **8,** Sclerotia (arrows) deep within cotyledonary tissues. **9,** Hyphae (arrows) penetrating from the adaxial side of a cotyledon. **10–11,** Symptomatic nonincubated seeds. **10,** Cotyledonary cells lysed, with their contents depleted and digested, and filled with hyphae (arrows) in symptomatic seeds. **11,** Inter- and intracellular hyphae (arrows) in the vascular bundle of a cotyledon showing the adjacent cells shrunken and devoid of cell contents.

(Fig. 4). Cells of the hourglass and endosperm layers in the proximity of the sclerotia were collapsed and disintegrated. Hyphae were observed at the tip of the hilar tracheid and in the stellate parenchyma of the hilar region, but not in the hilar tracheid. Sclerotia were present outside and within the hilar region, below and on the sides of the hilar tracheid, in the stellate parenchyma, and in the parenchymatous cells below the hilar region (Fig. 5). Hourglass cells in the hilar region remained intact despite the presence of sclerotia.

In 3-day-old seedlings from asymptomatic seeds, sclerotia were infrequent on the seed coat surface. Hyphal connections were observed between sclerotia on the seed coat surface and below the palisade layer (Fig. 6). Hyphae were inter- and intracellular in embryo tissues. Sclerotia were formed in the outer cell layers of the hypocotyl-radicle axis (Fig. 7) and abaxial side of the cotyledons. Sclerotia were observed deep within cotyledonary tissues in only two seeds (Fig. 8). Hyphae were observed between the cotyledons, penetrated the adaxial epidermis, and colonized the palisade cell layer (Fig. 9) and deeper tissues of the cotyledons. Hyphae also were observed in the plumule and vascular elements of the cotyledons. There was no apparent difference between cells of infected and uninfected cotyledons.

In 4- and 5-day-old seedlings, hyphae and sclerotia were observed in all the tissues of the radicle. Sclerotia were never formed on the adaxial side of the cotyledon.

Sections of symptomatic seeds had fragmented seed coats. Hyphae and sclerotia were present mostly within the seed coat. Hyphae were inter- and intracellular in all the layers of the seed coat and embryo. In heavily infected seeds, the seed coat was broken and disintegrated, and numerous sclerotia were present below the palisade layer. The hypodermis, endodermis, and endosperm layers could not be distinguished and the space between the palisade cell layer and cotyledon was filled with hyphae and sclerotia. Hyphae and sclerotia also were abundant outside and inside the hilar region. The hourglass cells of the hilum region in moderately to heavily infected seeds were broken and disintegrated. The hilar tracheid had abundant hyphae. Mature hyphae were observed frequently outside the seed coat and within the tissues. Sclerotia were abundant in the outer abaxial layers of cotyledons and the hypocotyl-radicle axes. In some seeds, sclerotia were observed in the adaxial side of the cotyledon. The cytoplasm in the cotyledon and the hypocotyl-radicle usually was vacuolated, coagulated, and fragmented. In heavily infected seeds, individual cells of cotyledons or hypocotyl-radicle axes could not be distinguished. In less heavily infected seeds the cells appeared to be lysed, their contents to be digested and depleted, and the resulting space to be filled with hyphae (Fig. 10). The vascular bundles in cotyledons also had inter- and intracellular hyphae (Fig. 11) and the cells were shrunken and devoid of cell contents.

DISCUSSION

M. phaseolina is reported for the first time to be seedborne in field-grown soybeans in Illinois. Symptoms may or may not be evident on infected seeds. The environmental conditions that influence symptom production is not known. In sections of asymptomatic seeds, sparse hyphae were present in the hourglass cell layer; sclerotia formed inside seed coat tissues within 2 days of incubation but were rarely formed on the seed coat surface. With increasing incubation period, the color of sclerotia changed from brown to black. Thus, *M. phaseolina* may penetrate and colonize soybean seed tissues without symptom production, and sclerotial formation may not take place until seeds germinate. Singh and Singh (13) reported that sclerotia of *M. phaseolina* formed freely on and within the seed coat and in the embryo in sesame (*Sesamum indicum* L.) seeds. Their observations differed from ours; they found that infection induced division in palisade cells of the embryo.

Gangopadhyay et al (5) excised embryonic axes, cotyledons, and seed coats from artificially infected soybean seeds and plated them separately on PDA following surface sterilization; they found evidence of invasion by *M. phaseolina* only in seed coats. However, we observed hyphae and sclerotia in all parts of naturally infected, germinated asymptomatic, and ungerminated symptomatic soybean seeds.

Singh et al (13) reported failure of seed germination and the presence of browning and rotting of seedlings in sesame. Sclerotia developed on roots, hypocotyls, and cotyledons during incubation of seeds infected with *M. phaseolina*.

Basu Chaudhary and Pal (2) studied sann hemp (*Crotolaria juncea* L.) seeds infected with *M. phaseolina* and found that the pathogen was present in all parts of the seeds and that infected seeds had either sclerotia, pycnidia, or mycelia. Seeds with either pycnidia or sclerotia failed to germinate.

Cells of cotyledons in soybean seeds incubated for 2 days appeared normal, but in heavily infected symptomatic seeds the cell walls were indistinguishable and cell contents were depleted. Seed coat tissues in symptomatic seeds were disintegrated. This may be due to the activity of cellulolytic and pectinolytic enzymes produced by the fungus as suggested by Goel and Mehrotra (7), or it may be the result of mechanical pressure exerted by the developing sclerotia.

Sinha and Khare (15) studied cowpea (*Vigna unguiculata* Walp.) seeds naturally infected with *M. phaseolina* and found that upon incubation, pycnidia were formed instead of sclerotia and that these emerged through the seed coat.

M. phaseolina can be seedborne in symptomless soybean seeds; thus, the pathogen can be carried over long distances by human activities. These facts are important in studying the epidemiology of charcoal rot. Since the pathogen can colonize the seed coat and embryo of soybean seeds, conventional fungicide seed treatments probably would not be effective in control.

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