Colletotrichum truncatum Borne Within the Seedcoat of Soybean

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ABSTRACT

Colletotrichum truncatum was isolated from, and observed within, the seedcoats of surface-sterilized soybean seeds, but not from embryos or cotyledons of the same seeds. The mycelium was confined to the middle "hourglass" layer of the seedcoat and to naturally occurring wounds. *C. truncatum* also was obtained from water washings of infected soybean seeds. The seedcoat symptoms of soybean seed infected with *C. truncatum* are similar to those described for *Macrophomina phaseolina (Rhizoctonia bataticola).* Histochemical tests showed the hourglass layer to consist of, in part, an amorphous starch-rich matrix.

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Colletotrichum truncatum (Schw.) Andrus and Moore causes anthracnose of soybean [*Glycine max* (L.) Merr.] in the U.S. and other countries (5, 7, 9, 10). Lin and Wu (5) considered it the most destructive soybean disease in Taiwan. It has been reported to be seedborne (1, 5, 7) and Tiffany (10) observed *C. truncatum* mycelium in embryos and cotyledons of germinating seeds within 3 or 4 days of seed inoculation. This paper reports additional information of the location of *C. truncatum* in soybean seed and histochemical tests of infected tissue.

Initially we wished to study the seedborne nature of *Macrophomina phaseolina* (Tassi) Goid. [*Rhizoctonia bataticola* (Taub.) Butler]. For this purpose 'Bragg' soybean seeds grown in India were selected based on symptoms described by Gangopadhyay et al. (2) for infection by *M.* phaseolina. These seeds came from a seedlot known to contain a high percentage of infection by *C. truncatum* (7). The symptoms consisted of irregular, gray discolorations (1 to 4 mm in diam) composed of minute black specks. Ten such seeds were selected and washed separately in 2 ml sterile, distilled water by vigorous shaking. The washings from each seed were plated separately on potato-dextrose agar containing 30 μg/ml streptomycin sulfate (SPDA). The washed seeds were then surface-sterilized by immersion in 0.25% sodium hypochlorite for 4 min, then in 70% ethanol for 2 min, and rinsed in sterile, distilled water (8). Each seed was aseptically dissected into seedcoat, cotyledons, and embryo, and each part plated on SPDA.

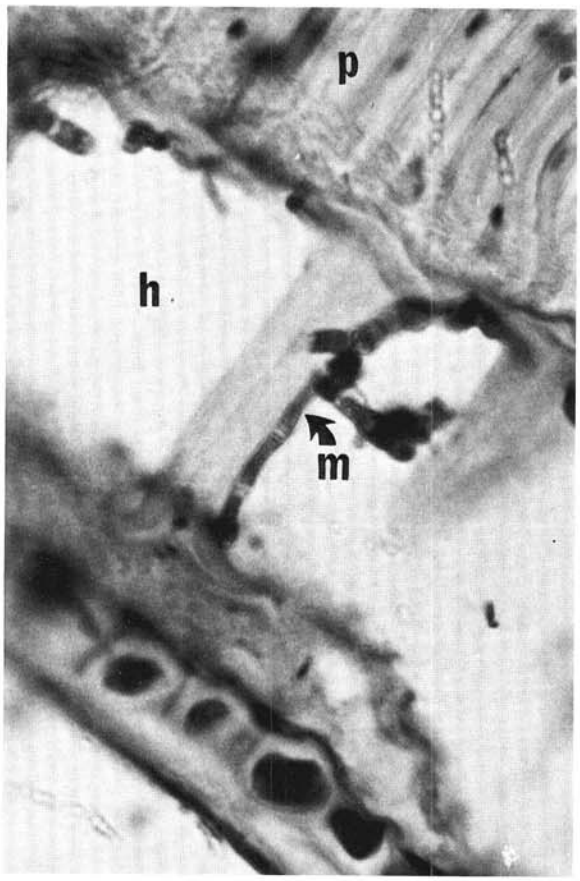

Fig. 1. Location of *Colletotrichum truncatum* mycelium (m) in the hourglass cell layer (h) in a cross section of an undamaged seedcoat (× 300). p = palisade cell layer.

Each part then was washed separately in sterile water before being plated on SPDA. The washings from this final step were separately plated on SPDA as described above.

Five other similarly infected seeds were dehydrated in tertiary butyl alcohol, embedded in Paraplast (Sherwood Medical Ind., Inc., St. Louis), sectioned (10-μm), and stained in a safranin-fast green series (4). Seedcoats were cleared by boiling in chloral hydrate and stained with lactopheno-cotton blue. Schultze's and Gram's iodine reagents were used to test for starch, cellulose, and lignin in seedcoat thin sections (3).

The symptoms of seed infected with *C. truncatum* are similar to those infected with *M. phaseolina* (2), but we found only *C. truncatum* in the seeds examined. *C. truncatum* grew from water washings of six nonsurface-sterilized seeds; five surface-sterilized seedcoats, and one embryo, but not from cotyledons. Isolation of the pathogen from the embryo probably resulted from contamination during dissection. For identification, *C. truncatum* was grown on SPDA at 25 C; dimensions and morphology of conidia and setae agreed with those given by Lin and Wu (5). No other fungi, including *M. phaseolina,* were detected.

The seedcoat of a healthy soybean seed consists of a cuticle, and three distinct cell layers: the palisade, "hourglass", and parenchyma layers (11). When the seedcoat cuticle is damaged, the hourglass cell layer is exposed and *C. truncatum* mycelium can be seen in the wound. Light microscope examination showed the mycelium of *C. truncatum* to be in the hourglass cell layer but not in the other two cell layers (Fig. 1). *C. truncatum* was identified in vivo by its thick, dark, irregularly-shaped cells. The pathogen was never observed in the cotyledons or embryo tissues.

The palisade layer stained deep yellow with Schultze's reagent, indicating heavy lignification, while the hourglass layer appeared to be an amorphous, starch-rich matrix which stained dark blue with Schultze's and Gram's reagents (3). This layer may provide a rich nutrient source for *C. truncatum*.

Wolf and Baker (11) postulated that breaks in soybean seedcoats, which appear to be genetically controlled (6), could provide potential sites for entrance and colonization by fungi. We demonstrated that *C. truncatum* is localized in wounds of the soybean seedcoat which expose the hourglass cell layer. Other seedborne fungi also may enter through wounds and into the seedcoat, particularly under conditions favorable for dissemination of the pathogens. Nicholson (7) showed that the incidence of *C. truncatum* and other seedborne pathogens in soybean seeds increased when seeds were harvested during rainy weather conditions.

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