SOIL TEMPERATURES AND INOCULATION TECHNIQUES AFFECT EMERGENCE AND REISOLATION OF SCLEROTINIA SCLEROTIORUM FROM SOYBEAN

by

J. F. NICHOLSON, O. D. DHINGRA & J. B. SINCLAIR*

ABSTRACT

Emergence of 'Amsoy' soybean (Glycine max) seed inoculated with Sclerotinia sclerotiorum was significantly reduced below noninoculated seed at soil temperatures of 25°, 30°, and 35 °C, but not at 20 °C. S. sclerotiorum was readily reisolated from wound-inoculated stems of seedlings and nearly mature plants above the point of inoculation and below to the crown area, but not from roots. The fungus was recovered from stems but not roots of 15-day seedlings grown in sterile soil before infestation of the soil surface with a suspension of mycelium and sclerotia and assayed at 15 days after soil infestation. When compared to healthy, seeds, infected seeds with S. sclerotiorum were characterized by appearing flattened.

Sclerotinia sclerotiorum (Lib.) de Bary, the incitant of stem rot of soybean (Glycine max (L.) Merr.), has been considered of little importance except for sporadic outbreaks (1,2,3). The fungus has been shown to be internally seed-borne in three soybean varieties grown in six states and to reduce in vitro germination and field emergence (4). This paper reports: (i) the effect of soil temperature on emergence of seed either noninoculated or inoculated with S. sclerotiorum; (ii) the reisolation of the fungus from stems of inoculated seedlings and nearly mature plants; and (iii) the tissues colonized by fungus in emerging seedlings from infested soil.

MATERIALS AND METHODS

Isolates of S. sclerotiorum from soybean seed were maintained on either Difco potato-dextrose agar (PDA) or soybean seed broth (100 g seed/liter) (SSA) in 250 ml flasks. One fungal mat containing mycelia and sclerotia from a 7-day-old SSA culture had a fresh weight of approximately 7 g. Registered 'Amsoy' seed was sterilized in a 1.7 % sodium hypochlorite solution for 5 min then in ethanol.

* Graduate Research Assistants and Professor, respectively, Department of Plant Pathology, University of Illinois, Urbana 61801.

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for 2 min, finally rinsed in sterile, distilled water and dried with forced warm air.

For soil temperature studies, 1000 sterilized seed were mixed with three macerated fungal mats from SSA. Fifty noninoculated (control) or inoculated seed were planted in each of eight glazed crocks (7.5 l capacity) with 5 cm depth of a 1 : 1 sterilized sand-soil (volume basis) mixture for each of four temperatures (20°, 25°, 30° and 35°C). The crocks were then placed in controlled, water temperature tanks 24 h before planting. Soil temperatures and moisture were checked daily. Emergence of seedlings was recorded 7 days after planting. At the end of the experiment, seedlings from inoculated and noninoculated seed were selected at random from each temperature and plated on PDA and incubated at 22° ± 2°C for 10 days.

Stem (hypocotyl) inoculations at 2 cm above the soil line of 15-day-old seedlings and nearly mature plants were made to determine if mature stems escape infection (1). Fifteen seedlings were inoculated and at 15 days after inoculation, the roots, tissues below the point of inoculation, cotyledons, and epicotyl were assayed on PDA for presence of the fungus.

Eight soybean plants were grown to maturity in a growth chamber (70 F ± 1°C, 60% relative humidity, 14 h light at 6000 ft-candles). About 2 weeks before pods were mature, stems of four plants were wound inoculated and the wound immediately covered with petroleum jelly. Four wounded, but noninoculated plants served as controls. At 21 days after inoculation, roots and stem tissue above and below the point of inoculation were assayed on PDA.

Seedling infection from infested seed and soil was studied. Eighty sterilized seed were rolled in crushed mycelium from a SSA culture of the test fungus. Twenty noninoculated (control) or inoculated seed were planted in each of six clay pots containing a 1 : 1 autoclaved, soil-sand mixture. Soil was infested with a mycelia-sclerotia suspension of the test fungus either by mixing with soil and then planting sterilized seed 15 days after infestation, or by sprinkling the suspension on soil at the base of 15-day-old seedlings growing in autoclaved soil. Seedlings from the first trial were assayed for the presence of the fungus 30 days after planting and for the second at 15 days after soil infestation.

A 'Lee 68' soybean seed lot known to have 87% infection with S. sclerotiorum (4) was used to confirm the observation that soybean seed infested with the fungus appear flattened (1). One hundred flattened seed were selected and plated on PDA and the percent recovery of S. sclerotiorum and other fungi recorded.

RESULTS AND DISCUSSION

There was no significant difference between the emergence of noninoculated and inoculated seed at 20°C, however, at 25°, 30° and 35°C, the difference was significant.
The mean percent emergence for inoculated seed at 20°, 25°, 30° and 35 °C was 62, 56, 65, and 50 respectively, and for noninoculated seed: 74, 84, 82, and 80 respectively. The LSD01 was 16.

*S. sclerotiorum* was not recovered on PDA plates from nongerminated seed, or emerged plants from noninoculated seed, but spp. of *Rhizopus, Penicillium, Alternaria, Fusarium* and unidentified bacteria colonies were isolated. Seedlings emerging from inoculated seed appeared to be either healthy or dwarfed and the cotyledons were reddish at all temperatures. *S. sclerotiorum* was recovered from the cotyledons of both types at all temperatures; from the stems of the unhealthy plants; but not from the roots of plants from any of the treatments. Seed that did not germinate was assayed, and those seed covered with a white mycelial growth yielded *S. sclerotiorum*.

The assay of seedlings grown in soil infested 15 days after planting, showed that the test fungus grew from tissue near the soil line of six plants; from the cotyledonary node of three and from the crown area of two; but not from roots. The assay of wound-inoculated seedlings showed *S. sclerotiorum* to be throughout the hypocotyl of 11 seedlings in tissues at 2 cm above the inoculation point in two; and in all tissues of two dead plants. The fungus grew from the tissue 5 cm above the point of inoculation from all four of nearly mature plants; at the soil line; and 30 cm above the point inoculation, but not from the roots nor from noninoculated plants. Split stems of inoculated plants showed discolored (tan) vascular elements at approximately 5 cm above the point of inoculation. *S. sclerotiorum* was isolated from these tissues.

Of the fungi isolated from flattened seed, 75 % showed presence of *S. sclerotiorum*, 16 %, *Fusarium* sp., and 10 % bacteria.

*S. sclerotiorum* is not considered a major disease of field grown plants (1). It is seed-borne in soybean and can reduce in vitro germination and field emergence. The reduction in emergence was not significant at 20 °C, but it was at 25 ° (1) and our data show that the reduction is greater with increase in soil temperature from 25 ° to 35 °C. Seed-borne *S. sclerotiorum* may effect seed quality and stands in the field.

Regardless of the method of inoculation of seedlings or mature plants, the fungus invades only above ground tissues and not the roots. CHAMBERLAIN (1) was not successful in inoculating the stems of nearly maturing plants, but our results show that these tissues to be as susceptible as hypocotyls of seedlings. The fungus produces a discoloration on cotyledons and in mature stems, which may be confused with similar symptoms produced by other pathogens. *S. sclerotiorum* did not infect roots of any plants and would appear that on soybean the fungus is strictly a stem disease organism.

Seed infected under field conditions are flattened (1) and this appears to be a good means to visually separate infected from noninfected seed.
Summary

*Sclerotinia sclerotiorum* isolated from soybean seed, significantly reduced germination at soil temperatures of 25°C, 30°C, and 35°C, but not at 20°C. Seedlings grown in infested soil and nearly mature plants wound-inoculated with the fungus developed stem decay, but the fungus was not isolated from roots. The fungus appears to be strictly a hypocotyl and stem pathogen on soybean. Harvested seed from infected field plants are flattened, when compared to noninfected seed. Visual separation of infected seed from healthy seed is possible. The fungus can affect the seed quality of soybean.

References