A Stem Rot of Bean Seedlings Caused by a Sterile Fungus in Florida

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ABSTRACT

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A stem rot disease of bean seedlings, which first was observed in central Florida in 1968, resembled southern blight which is caused by *Sclerotium rolfsii*. However, lesions on infected stems never extended above the soil line and there were no sclerotia on the stems or on the surface of surrounding soil. Isolations from the stems consistently yielded a sterile fungus which failed to produce sclerotia after prolonged growth in culture or on infected plants. Pathogenicity of this fungus was proved on beans grown in artifically infested soil. The identity of the fungus was not determined because of the lack of reproductive structures. Hyphal clamp connections indicate that it is a Basidiomycete, and its cultural and other characteristics are similar to those of *Athelia* spp. that have been classified on the basis of the perfect state.

Additional key words: *Phaseolus vulgaris*.

In 1968, a severe wilt of bush bean (*Phaseolus vulgaris* L. 'Harvester') seedlings in the one- to four-leaf stage of growth was observed causing a 30% to 40% stand reduction in two fields in central Florida. The disease has recurred every fall in bean crops that have been grown in these two fields since 1968, and has been found on the fall crop in two of seven additional fields that have been surveyed. The disease apparently is favored by high temperatures; it has not been found in any spring crops even in fields in which it was severe the previous fall. The average daily mean temperatures during the seedling stages of the spring and fall crops are ~ 16 C and 27 C, respectively. In some respects, the disease resembles southern blight which is caused by *Sclerotium rolfsii* Sacc. When wilting plants are pulled, a collar of mycelium and soil adheres to the stem at the soil line. Usually there is a somewhat watery soft rot of the underground portions of infected stems during periods of wet weather. These lesions sometimes are a light salmon color, but usually are not discolored. When plants are infected in the field during periods of dry weather, the lesions are dry and light tan. Regardless of the soil moisture level, the entire underground portion of the stem usually is affected by the time plants begin to wilt. Loose rope-like strands of mycelium usually extend along the infected part of the stem (Fig. 1-A) and tap root and sometimes along some of the larger lateral roots.

This disease differs from southern blight in that the stem lesions do not extend above the soil surface, sclerotia are not produced in or on the infected plant tissue or in the surrounding soil, and strands rather than compact layers of mycelium grow on the infected tissues below ground, but not on the stem aboveground or on the soil surface.

Isolations from wilting plants consistently yielded a sterile fungus which did not produce sclerotia on potato-dextrose agar (PDA) or water agar. In this paper, we report results of experiments that were carried out to determine the pathogenicity and other characteristics of this fungus.

MATERIALS AND METHODS

Pathogenicity experiments.—The causal fungus was isolated from infected bean hypocotyls and maintained in pure culture on PDA. Ten-cm diameter clay pots filled with field soil were placed in paper bags and autoclaved for 2 hr. The soil from groups of six pots then was placed in a container and 3.0 g of $Ca(OH)_2$ was incorporated to adjust the pH to $\cong$ 6.0. Three cultures of the pathogen grown for 2 or 3 wk on PDA in petri dishes were triturated in 375 ml of sterilized distilled water for 30 sec in a Waring Blendor and mixed into the soil which then was replaced into the pots. Sterilized PDA was used instead of fungus cultures for soil of control pots. Seeds of Harvester and Tendergreen bush beans were surface-sterilized by immersion in 0.5% sodium hypochlorite solution for 15 min and rinsed four times with sterilized distilled water. Ten seeds were planted in each pot. Three pots of each cultivar in infested soil and three in control soil were placed under constant fluorescent illumination of ~ 1,883 lux intensity in the laboratory at 30 ± 3 C and three pots of each were placed in a greenhouse with fluctuating temperatures. The average daily mean temperatures in the greenhouse were ~ 27 C and 19 C in experiments 1 and 2, respectively. In experiment 1, cultivar Harvester was

grown under the same conditions in soil infested with *S. rolfsii* in the same manner and rate as described for the sterile fungus. In experiment 2, both cultivars were grown in soil infested with *S. rolfsii*. All pots were watered daily to maintain a high level of soil moisture. The plants were observed for 8 wk after emergence; plants that developed definite wilt were pulled and examined for hypocotyl lesions and mycelial strands. All plants, including the controls, remaining in the pots after 8 wk were pulled and examined for disease signs and symptoms.

Characterization of the pathogen.—For purposes of comparison, the sterile fungus and *S. rolfsii* were grown on PDA in petri dishes under constant fluorescent illumination (~ 1,883 lux) at 30 ± 2 C. Samples of mycelium for microscopic comparisons were taken from 1-wk-old cultures. The following methods were utilized in attempts to induce the sterile fungus to produce a perfect stage: (i) cultures were grown on PDA, cornmeal agar (CM), cornmeal agar plus malt- and yeast-extracts (CMMY), potato-dextrose plus yeast-extract agar (PDAY), oatmeal agar (OMA), and water agar (WA); (ii) the sterile fungus was plated adjacent to *S. rolfsii* in the same petri dish; (iii) cultures on PDA and OMA were grown under near-ultraviolet light (360 nm and 753 lux) (2); (iv) blocks of PDAY were excised from actively-growing cultures and placed on WA (3); and (v) the soil-over-culture method was used (3).

RESULTS

Pathogenicity experiments.—Within a few days after bean plants emerged in soil infested with *S. rolfsii*, the mycelium had grown on the soil surface around some of the plants. A watery soft rot and brown discoloration which extended 2-7 cm above the soil line usually had developed on stems of severely-attacked plants. Mycelium and scleroita were common on the lesions (Fig. 1-B) and the plants died within a few days. Usually there were compact layers of mycelium on the underground portions of these stems. Most of the surviving plants which were pulled at the end of the experimental period had stem lesions (Table 1) which were dry, firm, light-tan, and usually located below the soil surface. Compact layers of mycelium usually were present on these lesions. Roots of all infected plants were rotted. Isolations from infected plants yielded only *S. rolfsii*.

In soil infested with the sterile fungus, mycelium never was seen on the soil surface, and stem lesions were dry, firm, and tan, and never extended above the soil surface (Fig. 1-B). Affected plants usually were wilted completely

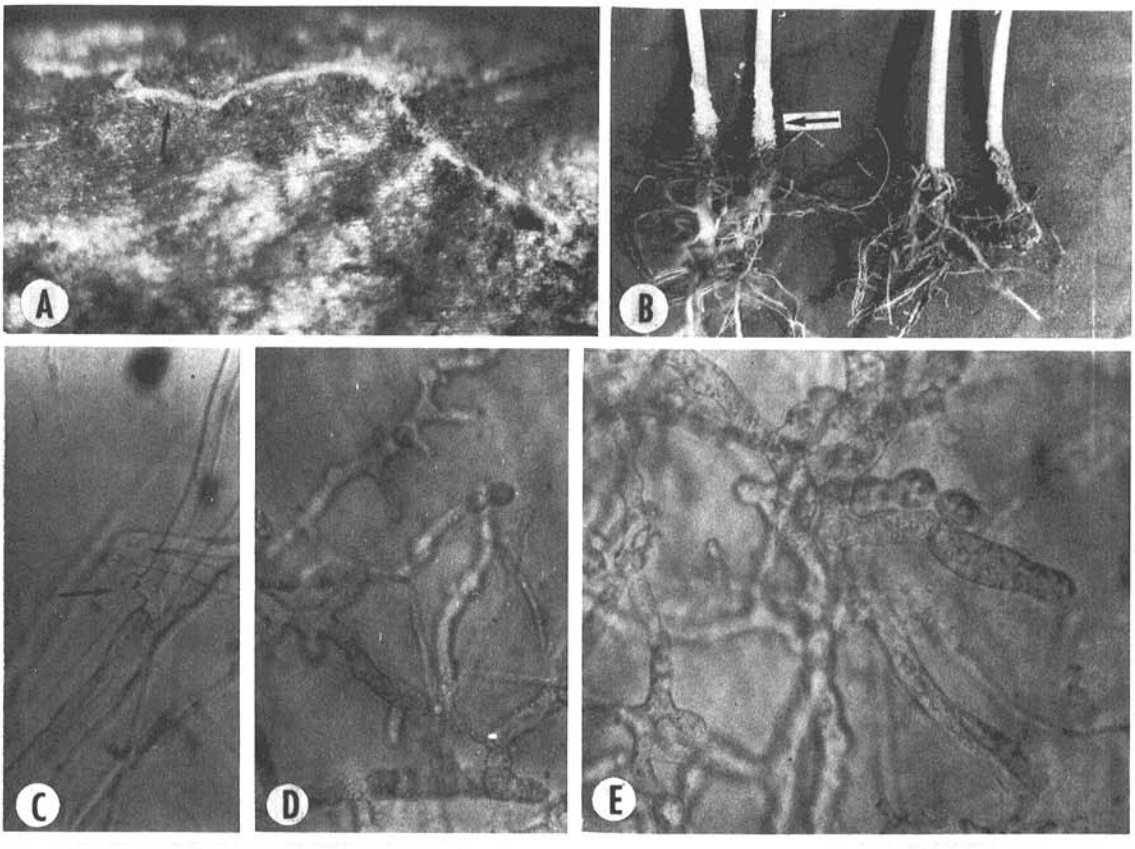

Fig. 1-(A to E). A) Mycelial strand (arrow) of the sterile fungus on a bean stem. **B)** Mycelium and sclerotia (arrow) on bean stems infected by *Sclerotium rolfsii* (left) and by the sterile fungus (right) showing symptoms only on underground portions. **C)** Clamp connection and hyphal branching (arrow) of the sterile fungus. **D, E)** Submerged mycelium of the sterile fungus.

within 3 days after the first signs of wilt and died soon thereafter. Dry rot was present on the entire underground portions of these stems. Dry rot also was present on the entire underground portions of the stems of many of the plants that survived for 8 wk, but only one small lesion (~4 mm in diameter) had developed on some of them. A few plants had only root rot and no stem lesions. Mycelial strands had formed on the roots or underground portions of the stems of most of the infected plants (Table 1). Compact layers of mycelium never were formed on plants infected by the sterile fungus. Isolations from infected plants yielded only the sterile fungus.

Both the sterile fungus and *S. rolfsii* killed a high percentage of the plants of both cultivars under the low light intensity and higher temperature conditions of the laboratory. In the greenhouse with lower temperatures and higher light intensity, *S. rolfsii* killed 23% of Harvester plants in experiment 1, and 43% of Harvester and 41% of Tendergreen plants in experiment 2. The sterile fungus killed only 5% of Harvester plants in the greenhouse in experiment 1, none in experiment 2, and no Tendergreen plants in either experiment. No control plants in either experiment were killed, and the roots and underground portions of the stems appeared to be white and healthy when the experiments were terminated after 8 wk.

Characterization of the sterile fungus.—During the first 5-7 days the sterile fungus and *S. rolfsii* cultures on PDA were very similar macroscopically. *Sclerotium rolfsii* then began to form sclerotia, whereas the sterile fungus did not form sclerotia regardless of how long the cultures were kept. Within 2 wk, strands of mycelium sometimes were formed in cultures of the sterile fungus, but never were formed in the cultures of *S. rolfsii*.

The aerial mycelium of both fungi consisted of coarse and slender hyphae. Clamp connections (usually two at each septum where they were present) were produced on both sizes of hyphae of both fungi, but were much more prevalent in cultures of the sterile fungus. The coarse hyphae of both fungi often produced a branch in place of one of the clamps at a septum as described by Higgins (1) for *S. rolfsii* (Fig. 1-C). Cells of the aerial mycelium of both fungi were binucleate, and the nuclei were stained by a slightly acidified solution of aniline-blue in 50% glycerin (4). The dolipore septa were not stained by this solution.

Diameters of 25 of the coarse- and 25 of the slender hyphae of each fungus were determined. The coarse hyphae of the sterile fungus measured 3.5 - 7.3 μm (avg. 5.3) in diameter and the slender hyphae measured 1.2 - 2.9 μm (avg. 2.3). These measurements corresponded closely to those of the *S. rolfsii* hyphae. The submerged hyphae of the two fungi were very different. Submerged hyphae of *S. rolfsii* had slender, mostly straight cells, whereas submerged hyphae of the sterile fungus had mostly monilioid cells (Fig. 1-D, E).

Colonies of the sterile fungus on PDA, PDAY, and CMMY were fast-growing and had thick, white, floccose mycelium; on CMA and WA, growth was submerged and scanty. Blocks of mycelium on PDAY that were plated on WA produced scanty, submerged growth and submerged clumps of monilioid cells. When cultured on the same plate, *S. rolfsii* overgrew the sterile fungus with no evidence of anastomoses. None of the methods described induced production of sclerotia or a basidial stage.

DISCUSSION

The experimental data and field observations reported here show that the sterile fungus is about equal to *S. rolfsii* in virulence on bean seedlings under conditions of high temperatures, but is considerably less virulent than *S. rolfsii* at lower temperatures. The disease that it causes may have been overlooked heretofore because of its close similarity to southern blight.

TABLE 1. Percentages of potted plants of two bean cultivars infected or killed following inoculation with *Sclerotium rolfsii* or a sterile fungus and incubation in laboratory or greenhouse environments, and percentages of infected plants on which rope-like strands of mycelium developed

Exp. no.	Location[a]	Bean cultivars	Treatment[b]	Plants infected[c] (%)	Infected plants with mycelial strands (%)	Plants killed (%)
1	Lab	Harvester	Sterile fungus	100	100	92
			S. rolfsii	. . .	0	65
		Tendergreen	Sterile fungus	100	100	83
	GH	Harvester	Sterile fungus	100	91	5
			S. rolfsii	. . .	0	23
		Tendergreen	Sterile fungus	100	71	0
2	Lab	Harvester	Sterile fungus	96	100	83
			S. rolfsii	100	0	100
		Tendergreen	Sterile fungus	86	92	59
			S. rolfsii	83	0	56
	GH	Harvester	Sterile fungus	100	88	0
			S.rolfsii	100	0	43
		Tendergreen	Sterile fungus	87	23	0
			S. rolfsii	100	0	41

[a]Experiment locations: Lab = laboratory bench, 30 ± 3 C and constant light [1,883 lux (= 175 ft-c)]; and GH = greenhouse bench, avg. daily mean temperatures ~ 27 C (Exp. 1) and 19 C(Exp. 2).

[b]Noninoculated plants of both cultivars grown in noninfested soil were not infected.

[c]Includes plants killed.

This fungus possesses some characteristics of both *Rhizoctonia* and *Sclerotium* spp. The formation of clamps with lateral outgrowths and wide-angled branching of the hyphae are characteristic of the genus *Sclerotium*. However, the lack of sclerotia production excludes it from this genus. The formation of monilioid cells by the sterile fungus is characteristic of the first stage of sclerotium development by *Rhizoctonia* spp. Tu (3) has characterized the genus *Athelia* as follows: binucleate, wide-angled branching hyphae with dolipore septa that do not stain in aniline-blue 50% glycerin, the presence of clamps, and sometimes the forming of sclerotia. The similar characteristics of the sterile fungus lead us to believe that if the perfect state is produced, the basidia probably will be characteristic of the genus *Athelia*.

Cultures of the sterile fungus are on deposit with the American Type Culture Collection as ATCC 28344 and with the Commonwealth Mycological Institute as IMI 163081. Cultures have been sent to mycologists in the United States, England, Australia, and Canada. None of them has been able to induce the formation of fructifications or sclerotia and, therefore, none could identify it. All agree that it is not typical of *S. rolfsii*. Until more information on the sterile fungus is obtained, it cannot be properly classified.

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