Crown Canker of Pigeon Pea (*Cajanus cajan*) Caused by a Sterile White Basidiomycete in Puerto Rico

WALTER J. KAISER, Research Plant Pathologist, USDA, ARS, Western Regional Plant Introduction Station, Washington State University, Pullman 99164-6402; PEDRO L. MELÉNDEZ, Professor, Crop Protection Department, University of Puerto Rico, Mayagüez 00708; RICHARD M. HANNAN, Horticulturist, USDA, ARS, Western Regional Plant Introduction Station, Washington State University, Pullman; and MILDRED ZAPATA, Graduate Student, Crop Protection Department, University of Puerto Rico, Mayagüez

ABSTRACT

Kaiser, W. J., Meléndez, P. L., Hannan, R. M., and Zapata, M. 1987. Crown canker of pigeon pea (*Cajanus cajan*) caused by a sterile white basidiomycete in Puerto Rico. Plant Disease 71:1006-1009.

A sterile white basidiomycete (SWB) was isolated from crown cankers on naturally infected pigeon pea (*Cajanus cajan*) plants in western Puerto Rico. Fungal mycelium resembled that of *Sclerotium rolfsii* but did not produce sclerotia in culture or on infected plant tissues. Hyphal cells of the SWB were binucleate, and clamp connections were present on the hyphae. At times, fleshy, bractlike structures reminiscent of fruiting bodies developed beneath the soil surface in cankers of inoculated plants. No basidia were observed on these fleshy structures. In greenhouse inoculation tests, the pathogen caused preemergence and postemergence damping-off of pigeon pea. Sunken, reddish brown lesions often developed in the crown area of older, inoculated plants. Wounding of pigeon pea hypocotyls increased the size of cankers but not the number of symptomatic or dead plants. Infection of pigeon pea seedlings was greater at 30 C than at 15–25 C. The SWB was pathogenic to food crops from 11 genera and four families of plants.

Pigeon pea (*Cajanus cajan* (L.) Huth) is the most important food legume cultivated in Puerto Rico. A number of plant pathogens infect the stems, foliage, pods, and seeds of this high-protein crop (5); however, little information is available on soilborne diseases that affect the root and crown tissues of seedlings and larger plants under field conditions. Cook (2) observed a disease of the basal regions of pigeon pea plants caused by a *Sclerotium* sp. Kaiser and Meléndez (*unpublished*) isolated *Sclerotium rolfsii* Sacc. from diseased pigeon pea seedlings in western Puerto Rico and demonstrated its pathogenicity to pigeon pea. This fungus also incited a disease of pigeon pea seedlings in Trinidad, West Indies (8). Kaiser et al (6) demonstrated that a crown canker disease of this crop in Puerto Rico was caused by a sterile white basidiomycete. In 1977, Howard et al (3) described a new stem rot disease of bean (*Phaseolus vulgaris* L.) seedlings in Florida that appears very similar to the sterile fungus from pigeon pea in Puerto Rico.

The objectives of this study were to examine the etiology of the crown canker disease of pigeon pea in Puerto Rico and to investigate the pathogenicity and host range of the pathogen.

Accepted for publication 18 March 1987.

MATERIALS AND METHODS

Isolation studies. Isolations were made from crown cankers on pigeon pea plants (cultivar Kaki) beginning in September 1973. Crown tissues from healthy and diseased plants were rinsed in running tap water for 15–30 min and surface-sterilized in 0.25% NaOCl for 5 min. Small pieces of tissue were plated on 2% water agar (WA) and potato-dextrose agar (PDA). Studies were discontinued from April 1974 until March 1979 because of transfer of the first author. In April 1979, permits were obtained to import cultures of the sterile white basidiomycete in Puerto Rico (SWB-PR) and the sterile white basidiomycete described by Howard et al (3) in Florida (SWB-FL).

Pathogenicity tests. Pathogenicity tests were conducted in greenhouses at Mayagüez, PR, with cultures of the SWB-PR and *S. rolfsii* (from pigeon pea seedlings). Similar tests were also performed at Pullman, WA, with the SWB-PR and SWB-FL. Fungal isolates were grown on PDA plates for 5–7 days at 22–25 C. Cultures were cut into small pieces (about 1 cm^2), and one plate was incorporated into the top 3–4 cm of greenhouse potting medium (55% peat moss, 35% pumice, and 10% sand) in 15-cm-diameter plastic pots. Uninfested controls containing sterile PDA were included in all tests. Untreated seeds of pigeon pea, cultivar T-21, were planted 2–3 cm deep in all pots. Crop species in the Cucurbitaceae, Fabaceae, Poaceae, and Solanaceae that were commonly grown in association and/or rotation with pigeon pea in Puerto Rico were also tested by planting seeds or seedlings in potting medium infested with the SWB-PR.

Five-month-old pigeon pea plants (with stem diameters of 0.3–0.5 cm) were inoculated with the SWB-PR. One PDA plate seeded with the SWB-PR was incorporated into the soil 2–3 cm deep around the base of each wounded or unwounded plant in 15-cm-diameter plastic pots. Wounds were made by jabbing the hypocotyl (below the soil surface) once with a sterile scalpel. In the control treatment, sterile PDA was mixed around the bases of plants. Plants were placed in a complete random design in a growth cabinet with three replicates of five plants per treatment.

Inoculation experiments were carried out in the greenhouse where temperatures ranged from 15 to 25 C or in growth cabinets at selected constant temperatures (15–30 C) with a 12-hr photoperiod (11,500 lux) and >75% relative humidity.

Characterization of pathogen. Laboratory and greenhouse tests were conducted to compare the SWB-PR and SWB-FL and to induce formation of basidiocarps. The fungi were cultured on the following Difco-Bacto agar media, which were prepared according to the directions on the label: malt agar, malt extract agar, oatmeal agar, cornmeal agar, and PDA. Both fungi also were cultured on the following natural media that were prepared in 2% WA: pigeon pea seed, cowpea pods, bean pods, and barley straw. The center of each 9-cm-diameter petri dish was seeded with a 3-mm-diameter plug from the edge of an actively growing colony on PDA. Seeded plates were incubated at different constant and/or fluctuating temperatures and in constant light (two fluorescent lights at 4,500 lux), 12 hr of light and 12 hr of darkness, or total darkness.

Sterile and nonsterile field soil was placed on the surface of PDA previously colonized by the SWB-PR and SWB-FL. Plates were incubated at 23–25 C in 12 hr of light and 12 hr of darkness or in total darkness. Infected pigeon pea hypocotyl tissue was incubated in moist chambers at various temperature and light regimes.

The nuclear condition of the SWB-PR and SWB-FL was observed following the

Figs. 1–3. (1) (Left) Girdled stem of T-21 pigeon pea inoculated with the sterile white basidiomycete from Puerto Rico. **(Right)** Healthy plant. Arrow indicates soil surface. **(2) (Right)** Wilting of T-21 pigeon pea inoculated with the sterile white basidiomycete from Puerto Rico. **(Left)** Healthy plant. **(3)** Naturally infected Kaki pigeon pea with white mycelial strands of the sterile white basidiomycete from Puerto Rico.

procedure of Jensen (4). The fungi were grown on dialyzing membrane on malt agar at 22–25 C. The mycelium-covered membranes were fixed in chromic acid-acetic acid-formalin (CRAF III) and stained with Heidenhain's iron-alum hematoxylin. After dehydration, small pieces of membrane were mounted in Harleco synthetic resin.

Anastomosis of the hyphae of the SWB-PR and/or SWB-FL was observed on thin strips of Czapek solution agar placed in the center of sterile glass microscope slides and incubated at 25 C in sterile petri dish moist chambers for 7 days. The agar strip was then peeled off, the glass slide was stained with cotton blue-lactophenol, and the mycelium was viewed under the compound microscope.

Pieces of SWB-PR fleshy structures that developed on inoculated pigeon pea hypocotyls were stained in cotton blue-lactophenol before examination for basidia and clamp connections.

PDA slants of the SWB-PR and SWB-FL were sealed with Parafilm and incubated at 4 or 23–25 C for 4 yr to test survival.

RESULTS

Symptoms and signs of disease. A sterile white basidiomycete was consistently isolated from reddish brown to dark brown cankers in the crown region and just below the soil surface of naturally infected pigeon pea plants at Isabela, PR. Cankers, which ranged in length from 0.5 to 10 cm, frequently girdled the stems (Fig. 1), causing plants to wilt and/or lodge (Fig. 2). White mycelial strands of the fungus often extended above and below the cankers (Fig. 3). Sclerotia or fruiting bodies were never observed on pigeon pea plants naturally infected by the sterile white basidiomycete in Puerto Rico.

In greenhouse inoculation studies, symptoms and signs of disease on inoculated pigeon pea plants were identical to those observed on naturally infected plants. One or more cankers often developed in the hypocotyl region of bean, cowpea, and pigeon pea plants inoculated with the SWB-PR and SWB-FL. White mycelial strands were observed within and around the cankers.

The pathogen. In western Puerto Rico between October and November 1973, crown canker of pigeon pea was observed primarily on the cultivar Kaki in the Isabela area. Disease incidence rarely exceeded 1%. A sterile white basidiomycete was isolated from >85% of the crown cankers assayed. Mycelium of the fungus resembled *S. rolfsii*, but sclerotia were absent on naturally infected pigeon pea and did not develop on different culture media (Fig. 4). Although *S. rolfsii*

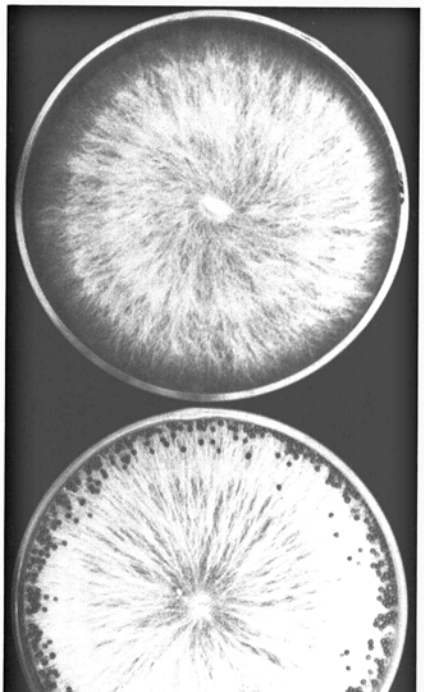

Fig. 4. (Top) Sterile white basidiomycete from Puerto Rico showing resemblance to **(bottom)** *Sclerotium rolfsii* on potato-dextrose agar except for sclerotial development.

can cause a disease of pigeon pea seedlings in Puerto Rico, it was rarely isolated from cankers on older plants. Brown sclerotia were observed on stem tissues of pigeon pea seedlings infected by *S. rolfsii*. A culture of the SWB-PR was sent to the Commonwealth Mycological Institute, Kew, Surrey, UK, for identification. The fungus (IMI 180605) was recognized as a basidiomycete with clamp connections, but it could not be identified. A culture of the fungus was also deposited at the American Type Culture Collection, Rockville, MD (ATCC 46988).

Fig. 5. Fleshy bractlike structure attached to a crown canker on T-21 pigeon pea inoculated with the sterile white basidiomycete from Puerto Rico. No hymenium was observed on the fleshy structure.

On different culture media, the SWB-PR and SWB-FL had morphological characteristics similar to those of *S. rolfsii* but differed from *S. rolfsii* in that small, round sclerotia were absent (Fig. 4). No sclerotia or fruiting bodies developed when the SWB-PR and SWB-FL were cultured on various Difco and natural media at different temperature and light regimes. Basidiocarps did not form on naturally infected hypocotyl tissues of pigeon pea in moist chambers exposed to different temperature and light regimes. Reproductive structures did not develop on PDA when the mycelium of both fungal isolates was covered with sterile or nonsterile field soil.

Hyphal cells of the SWB-PR and SWB-FL were binucleate, and clamp connections were produced by the hyphae of both isolates. Anastomosis of the hyphae was observed between the two isolates.

At times, fleshy bractlike structures reminiscent of fruiting bodies formed beneath the soil surface on hypocotyls of wounded and unwounded, inoculated plants (Fig. 5). The fleshy bodies (3–9 mm broad × 10–20 mm wide × 2–5 mm thick), which were cream-colored to tan, developed within 21 days in cankers of inoculated pigeon pea plants that were 0.5–1.2 cm in diameter. Hymenia were not observed on the upper or lower surfaces of these fleshy structures, but clamp connections were present on the hyphae. No basidia formed on the fleshy structures when they were placed in moist chambers and incubated at 25–30 C in alternating light and darkness or constant darkness. A fungus identical to the SWB-PR was isolated from the fleshy structures, and it was pathogenic to pigeon peas, producing symptoms indistinguishable from the SWB-PR.

On PDA, both fungal isolates lost viability within 3–5 mo at 4 C but remained viable after 4 yr at 23–25 C.

Pathogenicity studies and host range. The SWB-PR and SWB-FL were highly pathogenic to pigeon pea plants of varying size and age. Both fungal isolates caused a seed rot and postemergence damping-off of pigeon pea. Emergence of T-21 pigeon pea seedlings in infested soil was reduced by > 30%, and frequently > 20% of the emerged seedlings damped-off. Sclerotia did not develop on or around rotted seeds or infected plants inoculated with either fungus. The SWB-PR and SWB-FL produced identical symptoms on inoculated T-21 pigeon pea, Bountiful bean, and California Blackeye cowpea.

Inoculated pigeon pea plants, wounded and unwounded, developed cankers at or below the soil surface (Table 1). Wounding did not affect the number of diseased plants. The length of cankers on the wound-inoculated plants averaged twice that of cankers on the unwounded plants. Twenty-seven percent of the wounded and 7% of the unwounded, inoculated plants died. Although there was no statistical difference in the ultimate number of infected plants that died, plant mortality in the wounded treatment was 20% higher than in the unwounded treatment.

In inoculation tests at different temperatures, infection and mortality of T-21 pigeon pea was significantly higher at 30 C than at the lower temperatures tested (Table 2).

S. rolfsii also caused a seed rot and postemergence damping-off of pigeon pea in greenhouse inoculation studies but rarely induced disease or death in larger, established plants. Brown sclerotia developed around the bases of inoculated seedlings.

Emergence of different plant species seeded in potting medium infested with the SWB-PR ranged from 30 to 97%, whereas infection of emerged seedlings ranged from 20 to 100% (Table 3, Fig. 6).

Table 1. Effects of hypocotyl wounding of T-21 pigeon pea plants on development of crown cankers caused by a sterile white basidiomycete[a]

Treatment	Plants infected[b,c] (%)	Mean length of cankers[c] (cm)	Plants killed[c] (%)
Uninoculated,			
Unwounded	0	...	0
Wounded[d]	0	...	0
Inoculated,			
Unwounded[e]	87	1.4*	7
Wounded	100	3.0*	27

[a] Plants 0.3–0.5 cm in diameter were grown singly in 15-cm-diameter plastic pots with three replicates of five plants per treatment. Plants were incubated in a growth cabinet at a fluctuating temperature of 30/25 C (12 hr each) with a 12-hr photoperiod.
[b] Includes both plants with cankers and those killed.
[c] There was no significant difference in the number of diseased or killed plants, but there was a significant difference in the size of cankers when analyzed using linear contrast (* = $P = 0.05$).
[d] The hypocotyl region below the soil surface was jabbed once with a sterile scalpel.
[e] Potato-dextrose agar inoculum was incorporated around the base of the plant to a depth of 2–3 cm.

Table 2. Effects of temperature on crown canker disease of T-21 pigeon pea[a]

Temperature (C)	Plants infected[b] (%)	Plants killed (%)
15	30[c]	15[c]
20	60	50
25	65	50
30	75	75

[a] Twenty-day-old seedlings were transplanted to soil infested with potato-dextrose agar inoculum of the sterile white basidiomycete and evaluated for disease after 12 days of incubation.
[b] Includes both plants with cankers and those killed.
[c] Numbers of plants infected or killed within the temperature range are significantly different in the first degree (linear) of an orthogonal polynomial contrast ($P = 0.05$).

Table 3. Pathogenicity of the sterile white basidiomycete from pigeon pea to different plant species[a]

Family, species, and cultivar[b]	Emergence (%)	Plants infected[c] (%)
Cucurbitaceae		
Citrullus lanatus var. *lanatus* 'Sugar Baby'	50	100
Cucumis melo 'Hale's Best'	30	100
Cucumis sativus 'Ohio MR-17'	80	25
Cucurbita pepo 'Early White Bush'	30	65
Fabaceae		
Cajanus cajan 'T-21'	67	50
Glycine max 'Bragg'	79	47
Phaseolus vulgaris 'Bountiful'	84	63
Vigna radiata var. *radiata* 'Berken'	90	22
Vigna unguiculata subsp. *unguiculata* 'California Blackeye'	97	73
Poaceae		
Zea mays subsp. *mays* 'Golden Bantam'	67	60
Solanaceae		
Lycopersicon lycopersicum[a] 'Rutgers'	100	20

[a]Ten seeds of all species except tomato, were planted in soil infested with potato-dextrose agar inoculum of the fungus in 15-cm-diameter plastic pots, replicated two or three times. Thirteen-day-old tomato seedlings were transplanted to infested soil.
[b]Tests were carried out in a growth cabinet at 30 C with a 12-hr photoperiod (11,500 lux).
[c]Number of emerged plants with lesions.

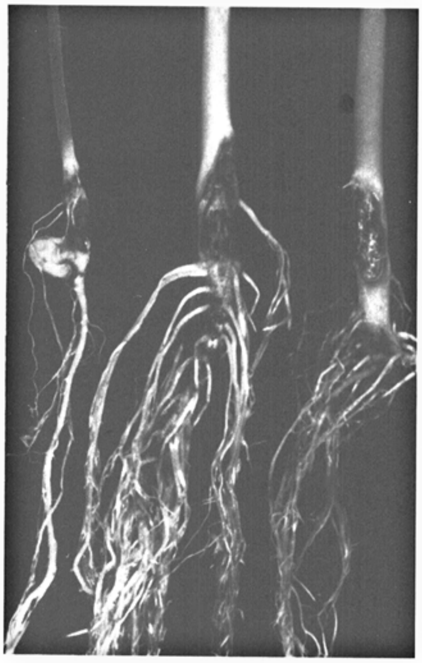

Fig. 6. Hypocotyl lesions on **(left)** T-21 pigeon pea, **(middle)** California Blackeye cowpea, and **(right)** Bountiful bean inoculated with the sterile pigeon pea fungus from Puerto Rico.

The poorest emergence (30%) occurred with Hale's Best cantaloupe (*Cucumis melo* L.) and Early White Bush squash (*Cucurbita pepo* L.), whereas the highest incidence of emerged, infected plants (100%) was in Hale's Best cantaloupe and Sugar Baby watermelon (*Citrullus lanatus* var. *lanatus* (Thunb.) Matsum. & Nakai). Within 3–4 days of emergence in infested soil, seedlings of these three cucurbits began to wilt and die. The incidence of disease was lowest in Rutgers tomato (*Lycopersicon lycopersicum* (L.) Karsten) (20%), Berken mung bean (*Vigna radiata* (L.) Wilczek var. *radiata*) (22%), and Ohio MR-17 cucumber (*Cucumis sativus* L.) (25%).

DISCUSSION

This appears to be the first report of a crown canker disease of pigeon pea caused by a sterile white basidiomycete. Cook (2) reported a foot rot disease of pigeon pea caused by *Sclerotium* sp. that was adversely affecting cultivation of the crop in different areas of Puerto Rico. It is possible that the pigeon pea pathogen identified by Cook (2) is similar or identical to the fungus we describe in this paper. Cook, however, did not give a detailed description of the *Sclerotium* sp. or disease symptoms, so it is only possible to hypothesize on the relationships of the diseases and pathogens.

In 1977, Howard et al (3) reported a stem rot disease of bean seedlings in Florida caused by a sterile white basidiomycete. Subsequently, diseases of bean, corn, cowpea, peanut, soybean, and squash in Georgia (1,10) and of pigeon pea (6) and yams (*Dioscorea* spp.) (7) in Puerto Rico were attributed to a similar, if not identical, soilborne fungal pathogen. It is highly probable that the sterile white basidiomycete will be identified as a pathogen of food crops in other tropical and subtropical countries of the Americas. The experimental host range of the pigeon pea pathogen is extensive, infecting crops in 11 genera and four families. In Georgia, Sumner et al (9,10) investigated the host range of a sterile white basidiomycete from corn and found that it infected 28 crops in 12 genera.

From morphological characteristics and pathogenicity, the sterile basidiomycete described by Howard et al (3) in Florida on bean seedlings is identical to the sterile pigeon pea fungus. Sumner et al (10) compared their corn isolates of a sterile basidiomycete that caused gray to black lesions on most hosts to Howard's bean isolate from Florida and considered them to be identical based on disease symptoms, host range, cultural morphology, and aminopeptidase profiles. The *Sclerotium*-like mycelial basidiomycete identified by Mignucci et al (7) in Puerto Rico on yam tubers probably belongs to this group of sterile white basidiomycetes.

The taxonomy of these sterile basidiomycetes that cause rot of the hypocotyl, crown, and/or roots of a wide range of crop plants would be greatly simplified if the basidial stage could be found in nature or induced to develop under laboratory or greenhouse conditions. Howard et al (3) stated that if a perfect state were found, it would probably belong to the genus *Athelia*. Development, characterization, and identification of the basidial state of this group of sterile basidiomycetes would be a subject worthy of future investigation.

ACKNOWLEDGMENT

The late J. D. Jensen gave us valuable assistance and technical advice in our studies with the sterile white basidiomycete.

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