Formation and Dispersal of Cylindrocladium crotalariae Microsclerotia in Infected Peanut Roots

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ABSTRACT

Survival of *Cylindrocladium crotalariae* in air-dried, naturally-infected peanut roots was demonstrated. Peanut plants grown in fumigated and nonfumigated soil in which *C. crotalariae*-infected roots had been incorporated in September and exposed to field conditions until April became uniformly infected. *C. crotalariae* microsclerotia grown in culture incited root rot of peanuts 4 wk after inoculation. Microsclerotia of *C. crotalariae,* found in the root cortex of naturally and artificially-infected peanut plants, were highly variable in number and size, and averaged 52.7 μm by 88.4 μm. They first appeared in infected peanut roots 55 days after inoculation in the field and numbers increased rapidly after 90 days. Root fragments containing microsclerotia were found in debris expelled from combines operating in infested fields. Fragments large enough to carry microsclerotia were trapped 235 m downwind.

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Cylindrocladium black rot (CBR) of peanut (*Arachis hypogaea* L.) caused by *Cylindrocladium crotalariae* (Loos) Bell & Sobers was described from Georgia in 1966 (1). Since its appearance in North Carolina in 1970 (4), the fungus has become established in virtually all areas of the state where peanuts are grown (10). Although *C. crotalariae* appears to move readily over long distances, its dispersal mechanisms have not been reported.

Two species closely related to *C. crotalariae* (*C. scoparium* Morgan and *C. floridanum* Sobers & Seymour) have been studied extensively as pathogens of conifers and woody ornamentals. Viable microsclerotia of *C. floridanum* have been isolated from naturally infested soil and are thought to be the primary survival and dispersal propagules of that fungus (8, 9, 12). Microsclerotia were found in the root cortex of spruce seedlings (*Picea* sp.) 2-3 wk after inoculation with *C. scoparium* (3, 11). Microsclerotia of both *C. scoparium* and *C. floridanum* were found in the cortex of infected roots of *Azalea* sp. after the plants had been dead for at least 2 mo (6). Elongate microsclerotia of *C. floridanum* were observed in root xylem and phloem elements of infected yellow poplar (*Liriodendron tulipifera* L.) seedlings (5).

The purpose of this study was to investigate: (i) the possible formation of *C. crotalariae* microsclerotia in infected peanut roots and (ii) the potential role of these propagules in spread of the disease.

MATERIALS AND METHODS.—*Survival in naturally-infected roots.*—Air-dried, naturally CBR-infected peanut roots were collected just prior to harvest in late September. Plants had been dug, air-dried for 10 days in inverted windrows, and were ready for combining. Secondary roots were excised from plants selected at random, chopped, thoroughly mixed, and 1.0 g samples were placed ca. 2-3 cm beneath the soil surface adjacent to the taproots of 3-mo-old peanut plants (cultivar Florigiant). The plants were evaluated for CBR infection 10 wk later by isolation from necrotic roots.

Overwintering.—Complete root systems of naturally infected plants were chopped into lengths 1-3 cm long and distributed on the surface of fumigated and nonfumigated field soil in six flats (45 × 60 × 12 cm). The infested soil was placed outside until mid-December when the roots in four of the six flats were incorporated into the soil. Rye (*Secale cereale* L.) was then sown as a cover crop. After 3 mo, the soil in each flat was mixed and placed in the greenhouse 2 wk later. Twenty peanut plants were sown per flat, grown in the greenhouse for 6 mo, and then evaluated for CBR infection.

Pathogenicity of microsclerotia.—Microsclerotia were produced in culture by growing the fungus in 250-ml flasks containing 40 ml of 2% malt extract broth. After 6-8 wk, the mycelial mats were comminuted in a Waring Blendor and washed in running water through a series of nested, 15-cm diam sieves with 246-, 149-, 104-, and 74-μm openings (60-, 100-, 150-, and 200-mesh). Most microsclerotia were collected on the 149- and 104-μm sieves, washed into a beaker, and rinsed in distilled water to remove any remaining mycelial fragments. Two-wk-old peanut plants (cultivar Florigiant) were inoculated with a syringe by injecting a 1-ml suspension of ca. 200-300 culturally-grown microsclerotia along the taproot 2-3 cm below the soil line. Inoculated plants were sampled at 2-wk intervals and evaluated for CBR-infection by isolation.

Detection of microsclerotia within infected roots.—CBR-infected roots were collected from greenhouse-grown peanut plants 6-10 wk after inoculation with a fungal suspension (10) and from naturally-infected, field-grown plants at harvest. A modification of a technique described by Bevege (2) was used to clear small, blackened, lateral roots excised from sampled plants. Roots were inserted in vials of 1.0 N KOH and placed in boiling water for 10-12 min. The KOH solution was changed twice as it darkened. Roots were then rinsed twice in distilled water and placed in 3% sodium hypochlorite for 30-60 s (until the roots were a pale straw color). They were then rinsed again in distilled water and mounted in lactophenol.

Rate of microsclerotia development.—A 3-m row of peanuts (cultivar Florigiant) was inoculated in the field every 2 wk from June through September. A 1-cm-diam plug taken from a 1- to 2-wk-old potato-dextrose agar plate of *C. crotalariae* was placed next to the taproot of each plant ca. 3-4 cm below the soil surface. Six plants were dug at random from each inoculated area in mid-October. All secondary roots from each treatment were excised, mixed, and chemically cleared. Fifteen to twenty cleared roots were selected at random from each treatment for microscopic observation. After microsclerotial counts were made, the material was oven dried for 24 h at 40 C and weighed.

Collection of plant debris expelled from a combine.—Plant debris was collected as it was expelled from a peanut combine operating in a CBR-infested field. Large fragments were removed and the remaining debris was passed through a series of nested, 15-cm-diam sieves with 2.14-, 0.97-, 0.70-, and 0.50-mm openings (10-, 20-, 30-, and 40-mesh). A portion of the material collected on the 0.50-mm sieve was chemically cleared and examined for the presence of *C. crotalariae* microsclerotia in root tissue fragments. Peanut plants were inoculated in the greenhouse with 0.1-g samples of the remaining 0.50-mm fragments in the manner previously described in survival tests.

Dispersal of debris by combines.—Greased microscope slides were suspended 1 and 2 m above the ground at intervals from 7 to 234 m downwind from a combine operating in winds of < 4 m/s. As the combine passed the trap slides, airborne dust and plant fragments expelled from the machine were sampled. Similar slides were exposed to prevailing winds for an equal time, as controls. Trapped plant fragments sufficiently large to carry microsclerotia ($>$ 300-μm diam) were counted as a function of distance.

RESULTS.—After 10 wk, perithecia of *Calonectria crotalariae,* the perfect stage of *Cylindrocladium crotalariae,* had formed at the bases of ca. 30% of the peanut plants inoculated with root fragments collected in

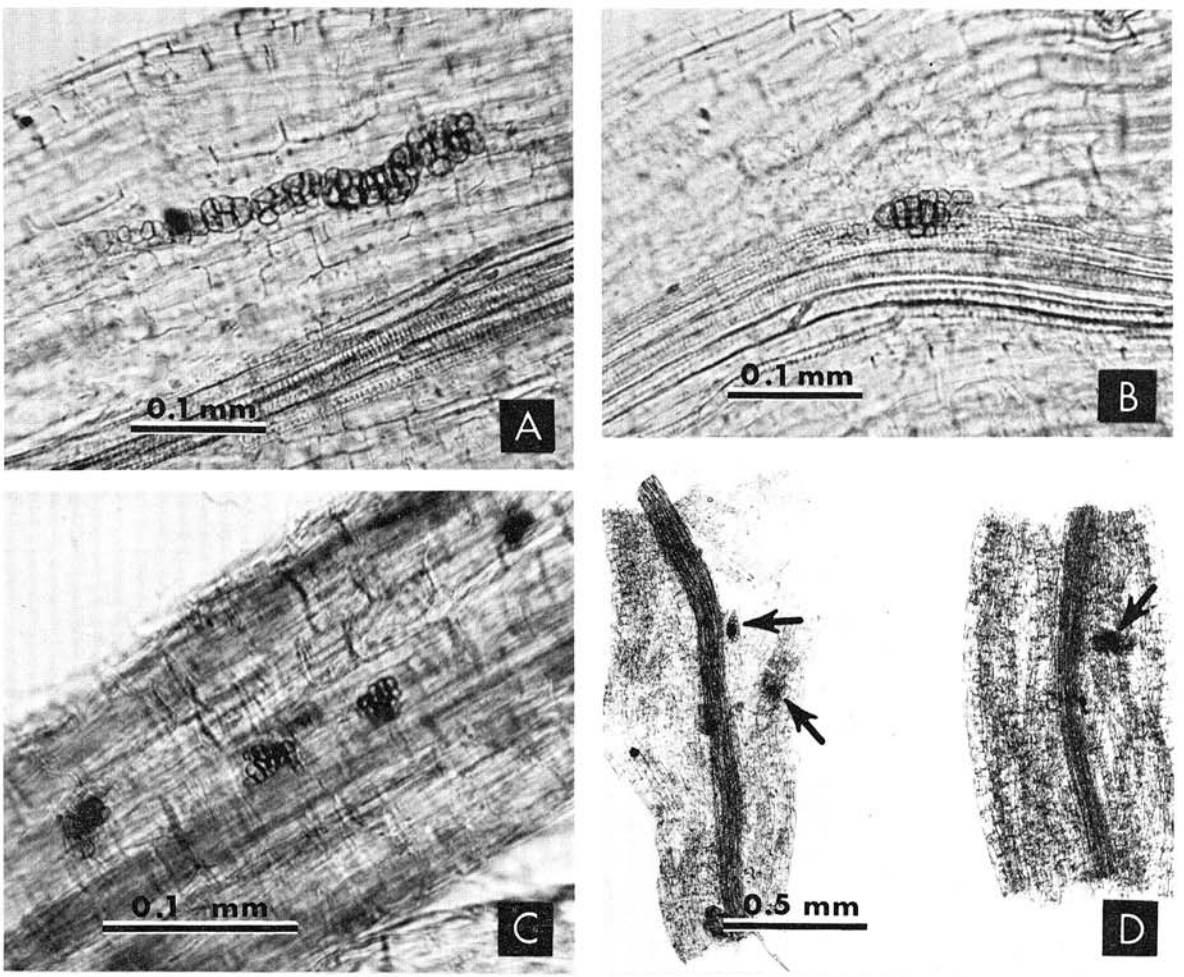

Fig. 1-(A to D). *Cylindrocladium crotalariae* microsclerotia in the cortex of cleared, infected peanut roots. **A,B)** Greenhouse-inoculated plants; **C)** Naturally-infected, field-grown plants; **D)** Root fragments collected from debris expelled from peanut combines.

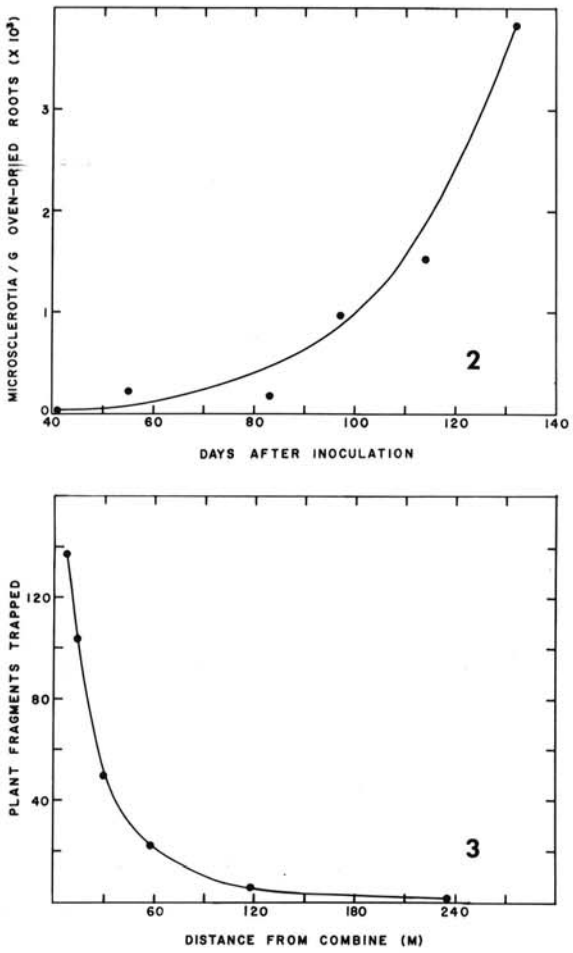

Fig. 2-3. 2) Numbers of *Cylindrocladium crotalariae* microsclerotia per gram of oven-dried lateral roots excised from field-grown, inoculated peanut plants. 3) Plant fragments > 300-μm diam trapped in 4 min on a 19 cm^2 greased surface downwind from operating peanut combines.

the field from air-dried, naturally infected plants. Isolation of the fungus from necrotic roots confirmed infection of all remaining plants.

Peanuts planted in early April in field soil infested with CBR-infected roots in September and left outside throughout the winter, became uniformly infected after 6 mo. The fungus can thus survive the mild winters of eastern North Carolina (average minimum temp during January and February, 1973 = −1 C) within infected roots. CBR was more severe on peanuts growing in infested, fumigated soil than in infested, nonfumigated soil. Moreover, disease severity did not differ when infected roots were incorporated 8 wk after harvest, or when rye was sown as a cover crop 3 mo prior to planting.

Inoculation of greenhouse-grown peanut plants with suspensions of *C. crotalariae* microsclerotia grown in culture caused lateral root necrosis and some taproot necrosis after 4 wk. *C. crotalariae* was consistently reisolated from these necrotic areas.

At ×10 magnification, microsclerotia were readily visible without staining in the cleared root cortex of greenhouse-grown plants 6-10 wk after inoculation (Fig. 1-A). Numbers of microsclerotia were highly variable within infected roots, averaging ca. 14.6 per cm of root length. Length and width of microsclerotia, based on over 100 measurements, ranged from 33.3 μm to 311.1 μm (average 88.4 μm) and from 22.2 μm to 133.3 μm (average 52.7 μm), respectively (Fig. 1-A, -B). In all cases, microsclerotia were found in the root cortex–never in the vascular tissues.

Microsclerotia identical with the above description were found in roots of naturally-CBR-infected, field-grown peanuts collected just prior to harvest in October (Fig. 1-C). In field inoculation studies, very few microsclerotia had formed in peanut roots after 55 days. After 90 days, microsclerotial numbers increased rapidly to a high, at harvest (130 days), of 3,800/ġ oven-dried roots (Fig. 2).

Root fragments containing microsclerotia were found in plant debris expelled from a combine operating in a CBR-infested peanut field (Fig. 1-D). Less than 10% of the debris collected on the 0.50 mm screen was composed of root fragments, and less than 1% of the root fragments observed contained microsclerotia. These were identifiable only after chemical clearing of the debris. Although the capability of this debris to transmit CBR was demonstrated in the greenhouse by inoculation, perithecia of *Calonectria crotalariae* developed at the base of only one of five peanut plants inoculated 10 wk earlier. Attempts to isolate the fungus from the other plants were unsuccessful. Plant fragments large enough to carry microsclerotia (> 300-μm diam) were trapped as far as 235 m downwind from operating combines in wind that did not exceed 4 m/s (Fig. 3).

DISCUSSION.—Microsclerotia formed within CBR-infected peanut roots are effective survival propagules of *Cylindrocladium crotalariae*. As disintegration of root tissues progresses, these propagules may be released into the soil, and then spread locally during tillage and aqueous runoff.

Microsclerotia-containing roots that remain attached to infected plants throughout the season are unearthed when the plants are mechanically dug and inverted prior to harvest. During this process, soil in the root zone is also disturbed, bringing free microsclerotia to the surface. The 3- to 7-day drying period that follows digging allows the surface layer of sandy peanut soils to become a loose dust in which the microsclerotia can easily become separated from soil particles. During combining, entire plants, along with large quantities of this surface dust, are taken into the machine and pulverized. The peanut fruits are separated by density and the plant material, including small fragments containing microsclerotia, are forcibly expelled from the machine by jets of air.

Ludlam (7) reported that particles of 60- to 200-μm diam injected into the atmosphere at a height of 10 cm in a wind of ca. 8 m/ s would all fall within ca. 100 m. We were able to detect small numbers of plant fragments > 300-μm diam 235 m downwind from an operating combine. These particles, however, were forcibly ejected from the machine at a high rate of speed ca. 2-3 m above the ground and are much lighter than similar-sized particles of soil or sand.

Because of the unique harvest requirements for peanuts where plants are both dug and combined, microsclerotia formed in peanut roots can become airborne. Due to their large mass relative to fungal spores and pollen grains, microsclerotia, especially those within small plant fragments, would have little chance of entering the upper atmosphere and remaining suspended over long distances (7). Strong, turbulent winds that often occur during harvest, however, may transport some particles of this size for several kilometers before they settle out.

It seems unlikely that airborne microsclerotia can explain the apparent rapid spread of CBR across the southeastern United States. Once the fungus has been established in a locality, however, these propagules can be effective in regional dispersal.

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