Occurrence of *Cylindrocladium crotalariae* on Peanut (*Arachis hypogaea*) Seed

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ABSTRACT

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Propagules of *Cylindrocladium crotalariae*, cause of Cylindrocladium black rot (CBR) of peanuts, were detected in samples of sound peanut kernels from two sites at Tolga, Queensland. Samples were taken from mechanically harvested pods in storage as well as from gleanings collected from the field up to 8 wk after harvest. Propagules were detected on sucrose-QT agar after sieving (mesh sizes 36 and 63 μm) aqueous washings from kernel samples. Low numbers of propagules (1–80/kg of kernels) were recorded on seed-quality kernels from storage bins, whereas much higher counts were obtained from field gleanings. Seed transport is implicated in the spread of CBR to unaffected areas and should be considered during selection of peanut seed crops and postentry quarantine treatment of imported peanut lines.

Cylindrocladium black rot (CBR) of peanuts (*Arachis hypogaea* L.) caused by *Cylindrocladium crotalariae* (Loos) Bell & Sobers (perfect state *Calonectria crotalariae* (Loos) Bell & Sobers) was first recorded in 1965 in Georgia (1,4). The disease has since spread to other American peanut areas and to Japan (4). In Australia, *C. crotalariae* was first recorded on peanuts at Kingaroy in south Queensland in 1976 (BRIP 12048) and in north Queensland in 1978 (BRIP 12756).

C. crotalariae can produce conidia, ascospores, and microsclerotia in culture and in infected plants (7). Conidia have rarely been observed under field conditions but are capable of causing necrosis of roots and pods (3). Perithecia producing ascospores have been observed on infected plants at the soil line when sufficient moisture has been present (9). Ascospores and conidia have been reported to be sensitive to desiccation, so their role in CBR epidemiology has been reported as short-distance within-field spread of the disease (2).

Microsclerotia have been found in abundance in decaying peanut tissue and have been regarded as effective long-term survival propagules. Spread in contaminated soil (8) and aerial dissemination in windborne plant parts have been observed (2); however, worldwide spread of airborne and soilborne microsclerotia has been discounted.

Peanuts differ from most agricultural crops by producing true seed below the ground, where they are especially prone to contamination by soilborne pathogens. This paper reports work that demonstrates that CBR propagules can be transmitted on peanut seed.

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MATERIALS AND METHODS

Samples. During 1982, peanut pods were collected from three sites at Tolga, north Queensland. In the first field (site 1), CBR had been severe for three seasons, whereas in the second field (site 2), a moderate incidence had been observed. CBR had not been observed in the third field (site 3).

The sample consisted of gleanings (pods left in the field after harvest) and harvested pods from each site obtained from bulk storage bins. From site 1, gleanings were collected immediately after harvest and again 8 wk later. From site 2, gleanings were collected 5 and 8 wk after harvest. No field collections were made from site 3. Samples of pods (dried by forced air) from storage bins were collected 8 and 5 wk after harvest at the three sites, respectively.

Immediately before assay for *C. crotalariae*, gleanings collected 8 wk after harvest from site 2 were sorted into three categories: 1) shells and kernels undamaged and unblemished; 2) shells undamaged, kernels unsound and blemished; and 3) shells and kernels damaged.

All samples collected at harvest were stored unshelled in the laboratory (25 C) and assayed after 5 wk when gleaning samples were collected. The 8-wk samples were assayed within 24 hr of collection.

Assay method. Immediately before assay, samples were shelled either with an experimental shelling machine or by hand. At each shelling, samples were processed in order of increasing likelihood of *C. crotalariae* contamination. After shelling, kernels were separated into edible and oil grades. In commercial practice, seed kernels are selected from edible grades after removal of discolored kernels, splits, and loose dirt. Our samples were sorted to remove these fractions, which were bulked and assayed separately as "processing grade."

Sucrose-QT agar, a selective medium used to detect microsclerotia of *C. crotalariae* in soil sievings (6), was used to assay sample extracts. Within 2 hr of shelling, each assay sample was mixed with tap water and manually swirled for 180 sec. The washings were then passed through a nest of sieves (mesh sizes 250, 63, and 36 μm), using running tap water to ensure that each sieve retained only particles of a larger size. The residues on the 63- and 36-μm mesh sieves were removed with 5–10 ml of distilled water, and 1-ml aliquots were dispensed onto plates of sucrose-QT agar. The samples were sieved in order of increasing likelihood of propagule recovery. Sieves were rinsed thoroughly between each assay. The sieves were sterilized between the 5- and 8-wk sampling. Plates were incubated on a laboratory bench, and after 5 days, counts were made of *C. crotalariae* colonies. Three selected colonies from each positive sampling site were transferred to potato-dextrose agar (PDA) and later inoculated onto peanut seedlings (root dip using blended PDA colonies) to confirm pathogenicity.

Table 1. Incidence of *Cylindrocladium crotalariae* detected in peanut kernel assays

Sample details	Seed sample weight (g)	Number of colonies	
		36-μm mesh	63-μm mesh
Kernels from farm storage bins			
Site 1, severe CBR[a] incidence	1,000	45	80
Site 2, moderate CBR incidence	1,000	1	36
Site 3, CBR absent	1,000	0	0
Kernels from fields			
Site 1, at harvest	250	150	291
Site 1, 8 wk after harvest	250	900	900
Site 2, 5 wk after harvest	250	8	10
Site 2, 8 wk after harvest			
Category 1[b]	170	80	287
Category 2	80	900	900
Category 3	100	900	900
Processing grade[c]			
Site 1, from storage bin	100	3	9
Site 2, from field 5 wk after harvest	100	1	0

[a] Cylindrocladium black rot.
[b] Category 1 = seed from undamaged shells, category 2 = discolored kernels from undamaged shells, and category 3 = kernels from damaged shells.
[c] Processing grade = splits, oil-grade kernels, discolored kernels, and soil particles in sample after shelling.

RESULTS AND DISCUSSION

Colonies identified as *C. crotalariae* were produced from residues retained by both the 63- and 36-μm mesh sieves, particularly the former (Table 1). Propagules were detected in all samples from fields where CBR had previously occurred. They were not found in the samples from the unaffected field (site 3).

Incidence of propagules was much lower in samples from storage bins than from gleanings. Incidence was highest in samples from site 1, where CBR had been most severe, and increased in the later gleaning samples (5 and 8 wk after harvest). Counts were also higher in washings from blemished than from unblemished kernels.

The contamination of seed-quality kernels was of the same magnitude as the processing grade fraction in the same sample (after correction for sample weight).

Selected isolates of *C. crotalariae* from assay plates reproduced CBR symptoms and formed perithecia and microsclerotia when inoculated onto young peanut seedlings.

This study has demonstrated that *C. crotalariae* can be carried as surface contaminants on peanut kernels. Contamination is much higher on poor-quality kernels. The sample assayed as "seed," having been sorted and discolored kernels removed, were of high quality. Thus, *C. crotalariae* could be spread with apparently clean seed to unaffected areas in a region or another country. Although Garren and Porter (5) found *Cylindrocladium* spp. in cured, matured peanuts, Garren et al (4) were unable to isolate any *Cylindrocladium* spp. from cured seed, even seed hand-harvested from rows in which all plants were severely affected by CBR. They (4) conceded, however, that on standard media, *C. crotalariae* might be masked by faster-growing fungi. Since the development of selective media for detection of *C. crotalariae* propagules, no attempts to reexamine whether they could be seedborne appear to have been reported.

Because peanut kernels are produced below the ground, CBR might become seedborne by infection of seed before harvest or by contamination of kernels by infected trash/shell fragments during shelling. Microsclerotia produced by *C. crotalariae* can be seen in necrotic root tissue using a dissection microscope and have been observed abundantly in affected plant material in Queensland. Perithecia have only been observed in north Queensland during cool, moist weather. No sign of *C. crotalariae* propagules was detected when healthy seed kernels were examined under a stereo dissecting microscope or when sections of seed testa were examined under a binocular compound microscope.

Because microsclerotia have been observed in abundance in infected tissue, the seed examined in our study was probably contaminated by microsclerotia or else by fragments of CBR-infected peanut tissue within the particle size range retained by the sieves.

Colonization of the kernel testa by *C. crotalariae* has also been observed on low-grade peanuts in north Queensland and confirmed by isolation on PDA from freshly harvested pods. Possibly, minor colonization of some high-grade kernels could also occur in sites affected by CBR. The small number of propagules present on healthy seed would be very difficult to detect visually.

Although Garren et al (4) were unable to find *Cylindrocladium* spp. in cured peanut fruit or seed microflora, curing may have induced dormancy rather than death of *C. crotalariae* microsclerotia, which presumably were evolved for long-term survival. This would complicate its detection.

Tomimatsu and Griffin (10) found that although most germinating microsclerotia infected roots, only a low percentage (0.28%) of infections developed necrosis. In a field planted to seed contaminated with fewer than 100 microsclerotia per 1,000 seeds (one seed weighs about 1 g) visible disease symptoms might not occur until one or two seasons after introduction of inoculum. Because peanut pods develop below the ground, despite a low incidence of necrotic plants produced by seedborne inoculum, contamination of progeny kernels could be quite high. In this way, imported peanut seed contaminated by *C. crotalariae* and multiplied in postentry quarantine could escape detection.

The rapid spread of CBR within Queensland's two peanut-growing districts could be accounted for by airborne and soilborne inoculum. However, the introduction of CBR into Australia and its spread from one district to another (over 2,000 km) is not as easily explained. In the past, Queensland peanut seed stocks have been chosen from first-quality kernels with no consideration given to the disease history of the crop. Seed stocks move freely between the two districts, whereas imported lines are multiplied in postentry glasshouse quarantine before release. Seed transmission would provide a plausible explanation for the introduction and spread of CBR in these cases.

Peanut seed is normally treated with fungicide dressing before planting and this may reduce inoculum potential in addition to any effect curing may have

had. Examination of the effects of seed dressing fungicides and seed curing methods on the viability of *C. crotalariae* may offer alternatives to prevent seed transmission of CBR.

The present contention that CBR is not seedborne has led to a lack of caution in dealing with movement of seeds of this crop. Until satisfactory methods are demonstrated to preclude CBR seed transmission, this disease should be regarded as seedborne and treated accordingly.

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