Importance Of Seed Transmission In The Spread Of *Cylindrocladium Crotalariae*[1]

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ABSTRACT

Cylindrocladium crotalariae, which causes Cylindrocladium black rot (CBR) of peanut (*Arachis hypogaea*), can be isolated at a low frequency from nondamaged peanut seed (seed size ≥6.4 x 25.4 mm slotted screen) not treated with a seed protectant fungicide. Peanut seed obtained from peanut fields in Suffolk, VA, and Martin County, NC, where CBR was severe in 1985 were infected at a frequency of 1.5% and 1.4%, respectively. *Cylindrocladium crotalariae* was isolated at twice the frequency from pieces of seed than from whole seed. However, *C. crotalariae* was not isolated from seed treated for 2 weeks with a seed protectant (DCNA + captan). The fungus was not isolated from seed devoid of testae or seed embryos, but was isolated from seed testae at a frequency of 0.4%. Discolored seed (≥6.4 x 25.4 mm) were infected at a frequency of 7.9%, but following seed treatment, the isolation frequency dropped to zero. Discolored seed measuring < 6.4 x 25.4 mm were infected with *C. crotalariae* at a frequency often exceeding 10%.

Key Words: Peanut, seed contaminant, *Arachis hypogaea*, seed protectant.

Cylindrocladium crotalariae (Loos) Bell and Sobers was first described as a pathogen of peanuts in Georgia in 1965 (1). Their description of this destructive pathogen included symptoms of the pegs, pods and roots. All underground plant parts are susceptible to infection by *C. crotalariae* (8). Infected plant parts turn black; hence, the name Cylindrocladium black rot (CBR). This disease was first observed in Virginia in 1970 (3). The disease has since spread to most peanut producing areas of Virginia and North Carolina. Based on aerial infrared photography, CBR was observed in 19% of the 338 fields assessed and was noted throughout the production area of Virginia in 1979 (10). The mechanism responsible for the spread of the disease from one locality to another is not entirely known. However, the movement of *C. crotalariae* contaminated farm equipment can transport the pathogen from one location to another (7). This does not account for the long distance spread of the pathogen into areas where the disease is not present. Neither does it account for the introduction of this pathogen into other countries such as Australia (6), Japan (K. H. Garren, personal communication) and China (personal observation, senior author). Even today CBR continues to be observed in Virginia-North Carolina fields without prior disease histories. Movement of the pathogen into disease free areas only by mechanical means is unlikely. Indirect evidence such as pattern of distribution of the disease within a field suggests the possibility of the pathogen by birds (5). At the present time, to minimize production costs, Virginia and North Carolina growers produce ca 1/3 to 1/2 of their own seed peanuts. It is a normal production practice in Virginia and North Carolina to store unshelled seed peanuts in trailers under shelters or in bulk inside nonheated buildings. Pods are shelled in the spring (March and April), treated with a seed protectant fungicide and planted during the latter weeks of April or the first weeks of May. Growers usually identify peanut fields without obvious disease symptoms as potential

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"seed" fields. However, growers often select diseased fields for seed sources since disease-free fields are not available. Thus a study was initiated to elucidate the relationship of peanut seed infestation with the continued spread of the CBR fungus.

Materials and Methods

Peanuts utilized in this study were grown on a farm in Suffolk, Virginia, and one in Hamilton, North Carolina. Fields at both locations exhibited severe symptoms of CBR. Following standard harvesting and curing recommendations, pods at both locations were dug in mid-October, dried for 3-6 days in the field, mechanically picked and artificially dried to 10% moisture content. Pods from the Suffolk farm were stored in open drying trailers throughout the winter months. Pods from the Hamilton farm were stored in an unheated building.

In January 1986 pods were collected from three drying trailers located on the Suffolk farm. A 22.7 kg lot of pods was gathered from each trailer and placed in an unheated building. Pod subsamples were collected at random from throughout each bag for specific studies.

Three different 2.27 kg pod samples, collected from the Hamilton farm were pooled and placed in mesh bags. Pod subsamples were collected from this pooled sample.

Pod subsamples, collected during January through April 1986, were sized and shelled with a small sample pod sizer and peanut sheller. Foreign material and loose shelled seed were removed from each sample before shelling. Only seed riding a 6.4 x 25.4 mm screen were used in this study. Seed were divided into two groups: one with normal colored testae and one with discolored testae.

Seed, plated (5/plate) on the surface of sucrose QT agar (4) without surface disinfestation, were incubated in the laboratory for 12 days. All experiments were repeated at least twice. The data presented are averages of these experiments. Some seed used in this study were treated with recommended rates of DCNA-captan (60-20 dust). Two weeks following treatment, seed were washed gently in water to remove traces of the fungicide. In some experiments seed were cut into two equal apical and basal halves and during plating the apical half was placed with the cut end in contact with the agar surface. The testa was removed from the seed by soaking in sterile, distilled water for 2 hours. The seed embryo was removed with forceps and plated (5/plate) on the agar surface. Fungal colonies with growth characteristics typical of *C. crotalariae* were transferred to plastic test tubes (20 x 80 mm) containing potato dextrose agar. Pathogenicity tests were determined using peanut seedlings. Seedlings (cv. Florigiant) grown in a sterile potting mix were inoculated by inserting disks of agar containing actively growing mycelium of *C. crotalariae* into the moistened potting mix. Roots were observed for evidence of infection within 14 days.

Results and Discussion

Using a nonselective medium, Garren *et al.* (3) were not able to isolate *C. crotalariae* from peanut seed obtained from field sites severely infected with the fungus. However, isolates of *Cylindrocladium* spp. were obtained from seed with discolored peanut pods grown in apparently CBR-free fields in Puerto Rico (2). Propagules, presumably microsclerotia of *C. crotalariae* were detected in Australia (6) in washings from pod samples in mechanically picked pods and pod gleanings from CBR-infected fields in Australia. In our study using a selective medium (4) involving peanut seed obtained from two field locations when CBR was severe in 1985, *C. crotalariae* was isolated from seed at a frequency of about 1.5% (Table 1).

In the Australian study (6) the incidence of seed infestation by *C. crotalariae* was several times greater in seed obtained from field gleanings than in seed obtained from plants growing in areas where CBR symptoms were severe. In our study only mechanically harvested seed riding a 6.4 x 25.4 mm screen were utilized. These seed

Table 1. The isolation frequency (IF) of *Cylindrocladium crotalariae* in stored peanut seed.

Suffolk, VA	No. Seed[1]	% IF
Field site		
Seed from Trailer #1	600[2]	1.8
Seed from Trailer #2	600	1.4
Seed from Trailer #3	600	1.4
Seed treatment (DCNA-captan)		
Untreated seed with normal testae	830	1.6
Treated seed with normal testae	1800	0.0
Seed testae		
Whole seed with normal testae	2300	0.9
Whole seed with discolored testae	165	7.9
One-half seed[3], pieces with normal testae	550	3.1
One-half seed pieces with discolored testae	120	9.8
Hamilton, NC		
Seed treatment (DCNA-captan)		
Nontreated seed with normal testae	500	1.4
Nontreated seed with discolored testae	100	8.1
Treated seed with normal testae	500	0.0
Treated seed with discolored testae	100	0.0
Seed parts		
Whole seed with normal testae	500	1.5
Whole seed with normal testae removed	500	0.1
Testae only	500	0.4
Embryo only	500	0.0

1/ Only seeds riding a 6.4 x 25.4 mm screen were used.

2/ Total number of seed plated in two experiments.

3/ Seed were cut into two equal sized pieces, the apical end containing the germ was plated with exposed cotyledonons tissues in contact with the agar.

would be similar to the seed size utilized by the grower and included both seed exhibiting normal testae color as well as those exhibiting discolored testae. *Cylindrocladium crotalariae* was isolated more frequently from seed with discolored testae (7.9% at the Suffolk farm and 8.0% at the Hamilton farm) than from seed with normal testae (Table 1).

Cylindrocladium crotalariae was not isolated from peanut seed, regardless of testae color, provided seed had been treated with DCNA + captan, a recommended seed treatment (Table 1). This is an important consideration since all seed approved by the Virginia and North Carolina seed certification agencies have been treated with an approved fungicide protectant. Based on the results presented in our study, the probability of transmitting *C. crotalariae* via seed approved by the certification agencies is negligible. This finding reaffirms the importance of seed treatment, not only to protect seed from decay pathogens but to aid in minimizing the spread of undesirable fungi.

An effort was made to determine what part of the seed was colonized by *C. crotalariae*. The fungus was isolated more frequently from whole peanut seed than from any of its component parts and from whole seed with testae than from whole seed with testae removed (Table 1). Isolations from the testae were less than the frequency associated with seed having testae. This could be due to the fact that pieces of testae were difficult to plate since the pieces would not adhere flat to the agar surface but tended to roll up as they dried out. Since washing in water (for 2 hours) did not remove the infective propagule (microsclerotia or thallus) from the testae and seeds without the testae rarely supported growth of *C. crotalariae*, it appears that the mycelia of the pathogen mainly colonize the testae and rarely penetrate into the cotyledons. This hypothesis is further supported by the fact that a

seed protectant applied directly to the seed surface eradicates the fungus.

The plating procedure utilized influenced the frequency of isolation of *C. crotalariae* from peanut seed (Table 1). Isolates of *C. crotalariae* were obtained more frequently from cut seed with normal testae than from whole seed with normal testae (Table 1). Similar trends were observed when cut seed with discolored testae were plated. This method of plating will probably give higher isolation frequency of the fungus from the infected seed even though it is more time consuming.

The effects of low ambient temperatures on the survival of microsclerotia of *C. crotalariae* has been demonstrated (9,11). Microsclerotial populations in the soil and those embedded in infected plant tissues sharply declined under severe winter conditions where temperatures often dropped to ≤ 0 C for prolonged periods. In field studies, microsclerotial populations declined sharply in frozen soil (9), but enough survived to initiate disease in subsequent years. Although the initial at-harvest infestation frequency of *C. crotalariae* is not known, in our study the fungus survived the winter in storage trailers parked under open-sided buildings even though ambient wintertime temperatures often dropped below 0 C. Freezing and below freezing temperatures did not eradicate *C. crotalariae* from peanut seed.

This study demonstrates that proper fungicide protectant eliminates the probability of seed transmission of *C. crotalariae.* Since seed peanuts planted in the United States are normally treated with a seed protectant, other avenues must be explored to determine the factors responsible for the spread of this serious pathogen.

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