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NATIONAL PEANUT BOARD/SOUTHEAST PEANUT RESEARCH INITIATIVE
QUARTERLY PROGRESS REPORT FOR WORK Dec 31, 2003
DONE UNDER RESEARCH AGREEMENT _____
INSTITUTION: University of Georgia

PROJECT TITLE: Epidemiological significance of seedborne *Cylindrocladium* black rot of peanut

RES. AGR. NO.: 25-21-RC299-293 PROJECT LEADER: Dr. Ron Walcott

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FINAL REPORT:

Cylindrocladium black rot has become a serious problem for Georgia peanut growers over the past 5 years. This sudden upsurge has prompted investigation into the possible sources of inoculum. Recently, it has been confirmed that CBR can be seed borne and seed transmitted; however, no research has been conducted in Georgia to determine the epidemiological significance of infested peanut seed. The objectives of this research project were to 1) determine the prevalence of CBR in peanut seedlots in Georgia; 2) determine the effects of CBR on peanut seed quality and CBR transmission; and 3) determine the epidemiological significance of seedborne CBR.

1) Determine the prevalence of CBR in peanut seedlots

To determine the prevalence of CBR in Georgia peanut seed, 145 lots were collected from anonymous peanut seed producers throughout the southeast. Samples (n=400 peanut seed) from each seedlot were visually examined for *C. parasiticum* infestation (speckled seed). Additionally, 100 seed from each lot were tested for the pathogen by an agar plate assay. Based on visual inspection 24 % (35/145) of the peanut seedlots collected from commercial sources were infested with *C. parasiticum*. The proportion of speckled (infested) seeds ranged from 0.25 to 1% with 13% of the lots containing one infested seed, 6% of the seedlots containing two infested seeds and 3% containing three infested seeds. Two percent of the lots had four infested seeds. Attempts to isolate *C. parasiticum* from naturally infested seedlots were unsuccessful. One possible explanation for this may be that the fungus becomes dormant during post-harvest processing.

2) Determine the effects of CBR on peanut seed quality and CBR transmission

With the assistance of Dr. Tim Brenneman, peanut seedlots (cv Georgia Green) were harvested in 2001 and 2002 from research plots with natural CBR incidence. Visual field estimates of CBR ranged from 5.2 to 54.1% in 2001, and from 25 to 39% in 2002. In both years, there were strong correlations between CBR field estimates and the level of seedlot infestation (by visual examination) ($R^2 = 0.85$ and 0.99 in 2001 and 2002, respectively) (Figure 2). There was also a strong relationship between CBR field estimate and seed infestation as determined by the agar plate assay for seeds harvested in 2001 (Fig 2A). Similar correlations between the field estimates of CBR and seed infestation have been reported previously. Hence, it is likely that seeds produced in *C. parasiticum* -infested fields will become infested. Avoiding such fields by restricting seed production to pathogen-free fields should reduce the risk of seed infestation.

Throughout the experiment we observed significant discrepancies in seed infestation determined by visual examination as compared to the agar plate assay. In general, seed infestation determined by the agar plate assay was significantly lower than that observed by visual examination (Fig 2A). We failed to isolate *C. parasiticum* from seed produced in 2002 because the seedlots were heavily infested with *Aspergillus niger* and other saprophytic fungi that rapidly colonized the semi-selective agar. This contributed to the failure to detect *C. parasiticum* in the seed. The discrepancy in the two seed detection assays may be due to the fungus becoming dormant and difficult to revive on semi-selective agar.

Overall, for the naturally occurring range of seed infestation, *C. parasiticum* had no effect on seed quality. In both seasons, as seed infestation increased, there was no significant change in warm ($R^2 = 0.11$ and 0.02 in 2001 and 2002, respectively) or cold germination (indication of seedling vigor) ($R^2 = 1.5$ and 1.55 , respectively) (Fig 3.). Seedling emergence was higher for seeds harvested in 2001 than in 2002, possibly because of the high levels of *Aspergillus niger* in the 2002 seedlots. Nevertheless, it was clear that in the range of seed infestation studied, *C. parasiticum* had little or no effect on emergence (Figure 4) or seed weight ($R^2 = 0.33$).

Seed transmission of CBR. For seeds harvested in 2001 and 2002, the percentage of seedlings displaying CBR symptoms ranged from 12 to 20% and 4 to 8%. Symptoms included blackened crowns but no wilting or perithecial development was observed. Additionally, none of the seedlings died prematurely due to CBR. Despite these observations we were unable to recover

viable *C. parasiticum* strains from the suspected seedlings. Since we could not confirm that these seedlings were infected we conclude that no CBR transmission occurred during this experiment.

Seed transmission has been reported for CBR by researchers in North Carolina and Virginia, however, we did not observe similar results in this study. Explanations for this discrepancy include different host responses to CBR (e.g Georgia Green vs. cultivars used in other studies). It is also possible that the natural levels of seed infestation that we studied (0-7%) were too low to induce significant levels of CBR transmission. Higher levels of seed infestation could possibly lead to disease transmission and significant reductions in seed quality.

Epidemiological significance of CBR.

The soil to which naturally infested seed were planted was repotted (soil from 98 containers reconstituted into one pot) and clean seed (n= 10-15 seed) (cv. Georgia Green) were replanted into each pot. During plant development typical CBR symptoms were not observed. Pods from each pot were harvested 90 days after planting and after manual shelling, they were assayed for *C. parasiticum* infestation as described above. From soil in which seed harvested in 2001 were planted, seedlots were produced with speckled testae. The levels of speckled seed ranged from 2.5 to 10% by visual examination; however, we were unable to recover the fungus using semi-selective agar. To confirm that the harvested seed were infested with *C. parasiticum*, seed coats were removed from 4 to 5 speckled seed from selected lots and DNA was extracted and subjected to polymerase chain reaction (PCR) using *C. parasiticum*-specific primers, CBR1x/2x. Using PCR, we did not detect *C. parasiticum* in the peanut seed tissue nor in samples (n=5g) of soil in which the seed were produced. Based on these observations, we conclude that naturally infested peanut seedlots with low (1 - 7% infestation) may not be important in the epidemiology of CBR.

Summary

Approximately 24% of the commercial seedlots surveyed were contaminated with *C. parasiticum* at infestation levels that ranged from 0.25 - 1%. At low levels of infestation (1 - 7%) *C. parasiticum* had a negligible effect on seed quality; however, higher levels of seed infestation may negatively affect seed quality and increase the chances of seed transmission. In the future, we will explore the relationship between seed quality and seed infestation using

artificially generated seedlots with higher infestation levels. Additionally, we will explore the ability of heavily infested seed to transmit *C. parasiticum* to soil. While such experiments may yield a different result, it may not be relevant since the natural levels of infestation range from 0 - 1%. At these low levels of seed infestation, we conclude that seedborne CBR has little epidemiological significance.

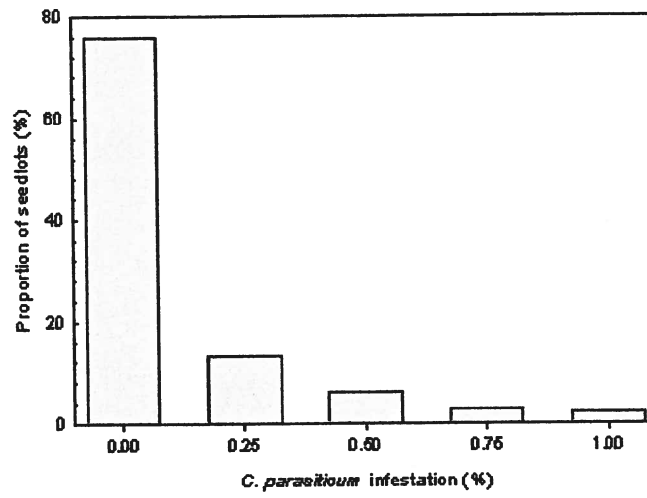


Figure 1. Results of the survey of commercial peanut seedlots for *C. parasiticum*.

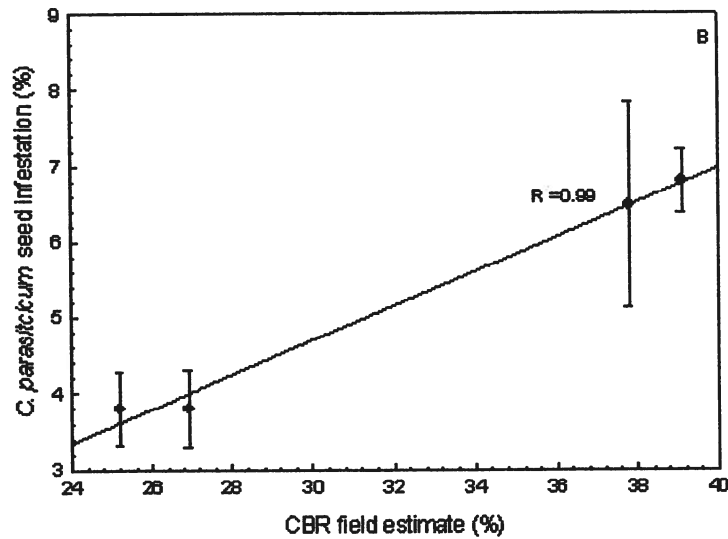
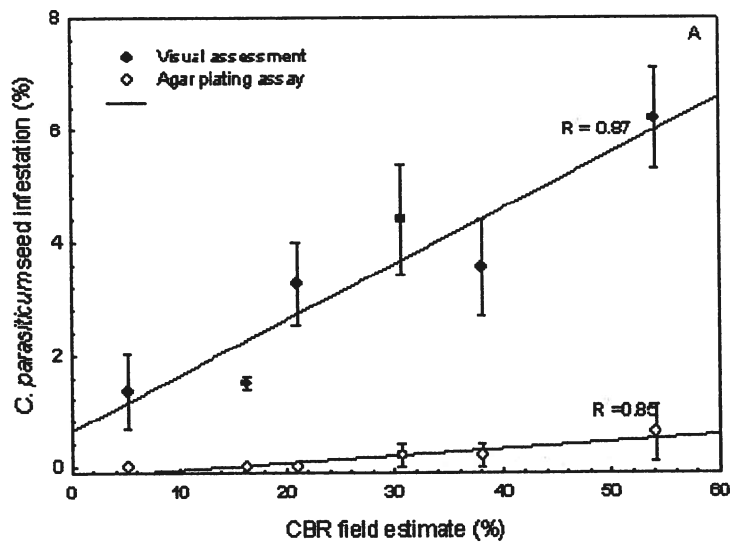


Figure 2. The relationships between field estimates of CBR and levels of *C. parasiticum* infested seed in the lots harvested from the plots. Seed infestation was assessed by visually examination (n=400 seeds) or by agar plate assay (n=100 seed). Graphs 2A and 2B indicate the data collected for seed harvested in 2001 and 2002 respectively. Vertical lines indicate the standard error of the means.

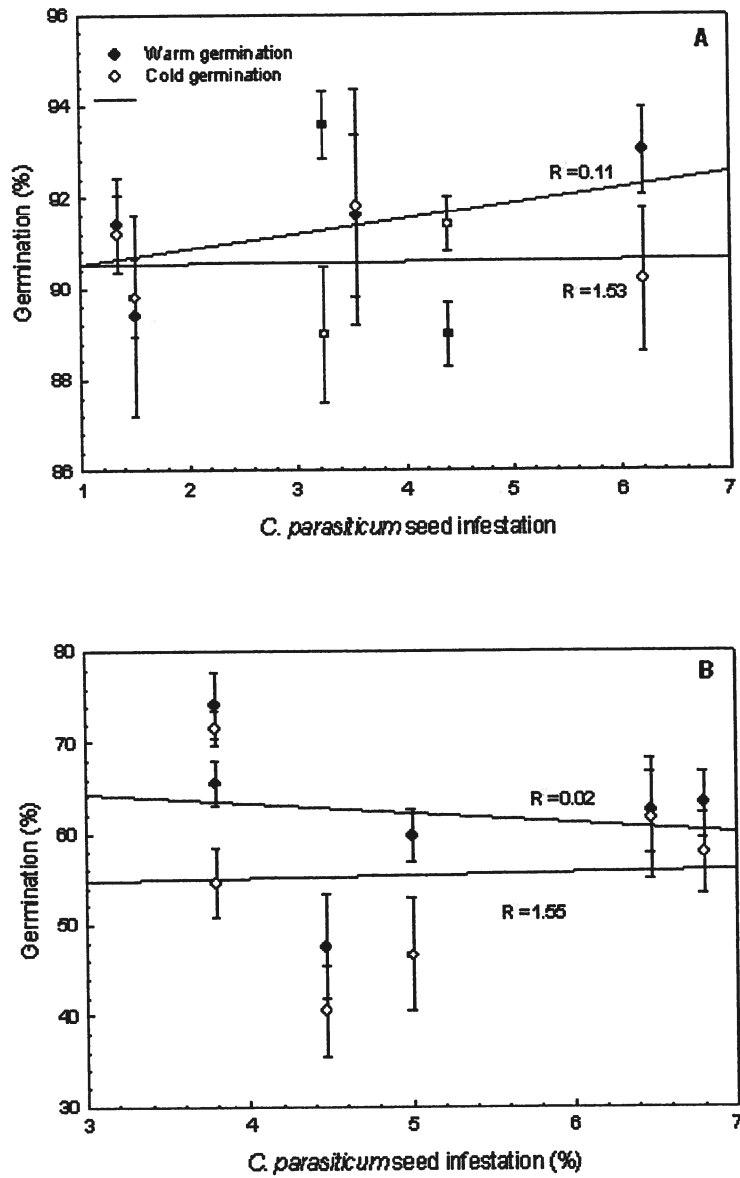


Figure 3. The relationships between peanut seed infestation with *C. parasiticum* and seed quality as determined by warm and cold germination. Graphs 3A and 3B indicate data collected for seedlots harvested in 2001 and 2002, respectively. Vertical lines indicate the standard error

of the means.

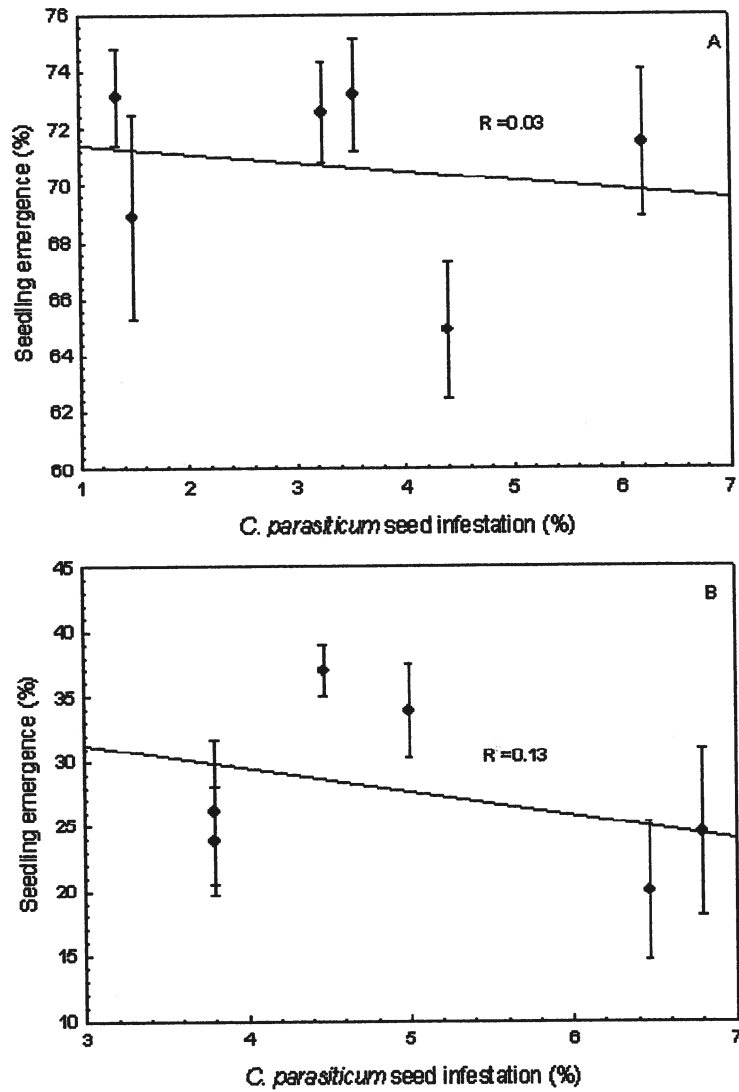


Figure 4. The relationship between *C. parasiticum* seed infestation and seedling emergence under greenhouse conditions for naturally infested peanut seed harvested in A) 2001 and B) 2002. Vertical lines indicate the standard error of the means.

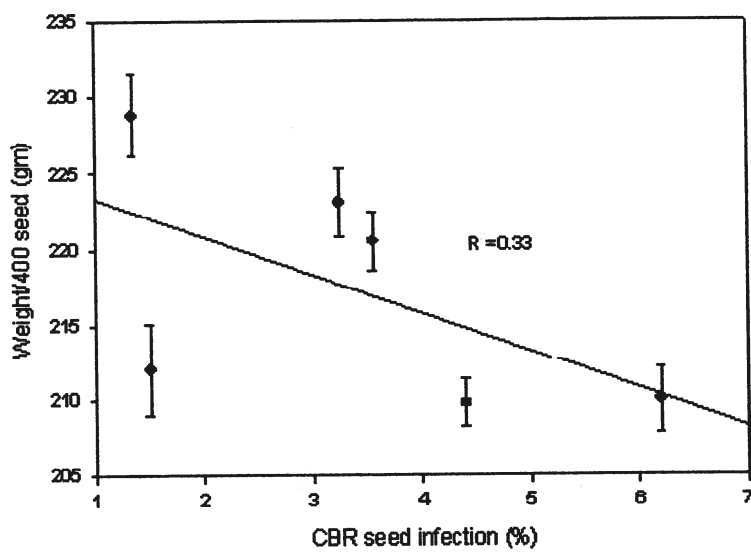


Figure 5. Figure 5. The relationship between *C. parasiticum* seed infestation and kernel weight for seed produced in 2001. Peanut seed weight is for 400 kernels. Vertical lines indicate the standard error of the means.

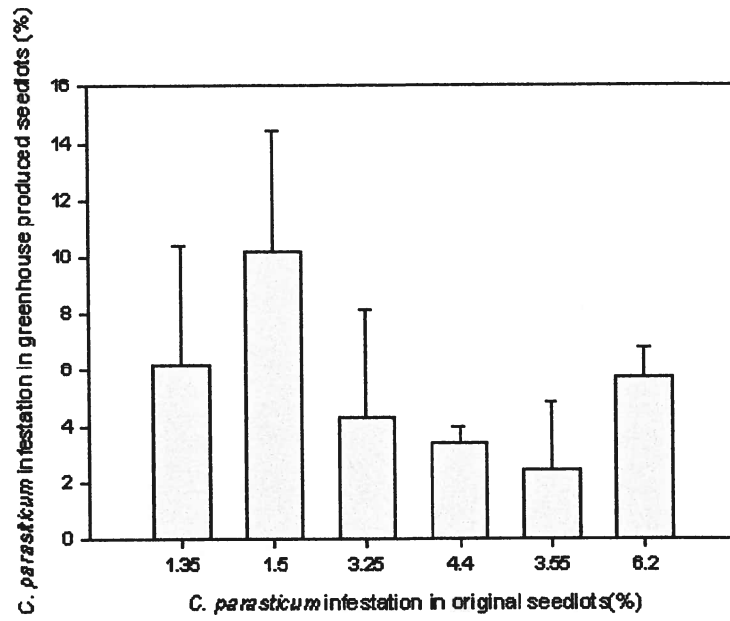


Figure 6. Bar chart indicating the percentages of speckled seed produced from soil that was previously planted to naturally infested peanut seed harvested in 2001.