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NATIONAL PEANUT BOARD/SOUTHEAST PEANUT RESEARCH INITIATIVE
QUARTERLY PROGRESS REPORT FOR WORK June 30, 2003
DONE UNDER RESEARCH AGREEMENT_____
INSTITUTION: University of Georgia

PROJECT TITLE: Genetic Diversity of *Cylindrocladium parasiticum*, causal agent of Cylindrocladium black rot of peanut

RES. AGR. NO.: 25-21-RC299-306 PROJECT LEADER: Dr. Ron Walcott

EXPIRATION DATE: December 31, 2003
SPRI CONTACT: Emory Murphy NPB CONTACT: Stephen O'Brien

FINAL REPORT:

Funds were requested to support a graduate research assistant to work on this project. Unfortunately, it took longer than expected to find a graduate student to fill the position and the start of the project was delayed. As a result, while considerable progress has been made, some aspects of the research are ongoing, and will be completed as part of the graduate research requirements for the student.

Between 2002 and 2003, 263 strains of *C. parasiticum* were collected from peanut, soybean sicklepod and partridge pea in multiple locations in Georgia, Florida, Alabama and Virginia. Before the genetic diversity of this population could be assessed, it was necessary to develop a system to confirm the identity of the strains. To accomplish this, we designed a set of oligonucleotide primers CBR 1X (5'-CTG GGG ATT CAC TAA CAT TG-3') and CBR 2X (5'-TCG AGG GAC ATA CTT GTT GC-3') based on the conserved beta tubulin gene of a known *C. parasiticum* isolate. Using the polymerase chain reaction (PCR), CBR1x/2x directed the amplification of a 560-bp fragment from *C. parasiticum* DNA. On the other hand, the primers did not amplify DNA from other fungi including *Fusarium sp.*, *Bipolaris maydis*, *Colletotrichum sp.*, *Alternaria sp.*, *Sordaria sp.*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *C. angustatum*, *C. rumohrae*, *C. avesiculatum*, *C. spathiphylli*, *C. floridanum* and *C. gordoniae* (Fig. 2). While the PCR protocol still need to be optimized, these primers will be useful to rapidly identify *C. parasiticum*. Currently, to confirm fungal identification, it is necessary to examine morphology and in some cases, strains must be sent to experts in other states. With CBR1x/2x, *C. parasiticum* can be objectively confirmed in less than a day, which may increase the speed by

which management decisions can be made. Additionally, this assay can be used to rapidly detect *C. parasiticum* in soil and seed tissues. This may be useful for research or for routine seed or soil testing in the future.

Genetic diversity of *C. parasiticum*

DNA was extracted from the single-conidium cultures and used for random amplified polymorphic DNA (RAPD) analysis. To determine the best RAPD primer to use 11 ten-base pair random primers (or primer combinations) were evaluated using *C. parasiticum* isolates from different locations. Primers OPE 14/OPE 20 were selected because they generated the largest number of markers per strain. Based on RAPD analysis, no differences were observed in the DNA fingerprints of the of *C. parasiticum* strains collected from Georgia, Virginia, Florida and Alabama (Figure 3). This was a surprising observation since *C. parasiticum* is homothallic and readily undergoes sexual reproduction to produce abundant perithecia. With sexual reproduction, there is genetic recombination of DNA so we expected a high degree of genetic diversity. One possible explanation for this observation is that sexual reproduction plays an insignificant role in the survival of *C. parasiticum*. Alternatively, the fungus may survive mainly via vegetative microsclerotia and mycelia in the soil. It is also possible that RAPD analysis may lack the resolving power to discriminate among the strains of *C. parasiticum*. To address the latter concern, a powerful DNA fingerprinting technique called amplified fragment length polymorphism (AFLP) was used to compare representative *C. parasiticum* isolates. AFLP generated more than 100 markers for *C. parasiticum* DNA; however, even with this technique, there was little genetic variability within the population.

The second part of this study was contingent upon discovering genetic diversity among *C. parasiticum* populations. Since we found that there was no genetic diversity using RAPD DNA fingerprinting, we were unable to complete this aspect of the project. However, we compared the aggressiveness of strains from different geographical locations. For this study we included CBR 01-06 recovered from soybean in Georgia; CBR 84 recovered from peanut in Suwanee Co. Florida; 202-13P recovered from peanut in Plains GA; 201-21 recovered from Headland AL; CBR 783 recovered from peanuts Levy Co. FL (Dr. Kucharek) and CBR97-11 recovered from partridge pea in McDaniel Co. GA. Despite the apparent genetic uniformity of *C. parasiticum* population, there were striking differences in the severity of CBR induced by the

strains. CBR783 and CBR9711 were the most aggressive strains with average estimated disease indices of 3.85 and 3.5 (on a scale of 1 - 5 with 1 being a healthy root system and 5 being a completely dead plant with a rotten root system). These were followed by CBR202-13P (2.14) and CBR201-21 (2.21). The least aggressive strain was CBR 84(1.29). The differences in relative aggressiveness do not reflect divisions of populations as CBR 783 and CBR 84 differed significantly in aggressiveness but both originated in Florida.

Summary

At present we have no evidence that *C. parasiticum* populations in Florida, Virginia, Georgia and Alabama are genetically distinct. If population of isolates studied represents the entire population, it would be impossible to distinguish between indigenous and exotic strains that may have be introduced by peanut seed. We plant to continue AFLP analysis using a different set of primers and run conditions to further compare strains in our collection.

If *C. parasiticum* populations in the southeast are genetically homogenous it is likely that resistance breeding programs will be successful in the long-term. Additionally, chemical-based management strategies should be effective regardless of the region in which they are applied. Work on this project will continue over the next two years as part of the graduate requirements of Ms. Anita Castro.

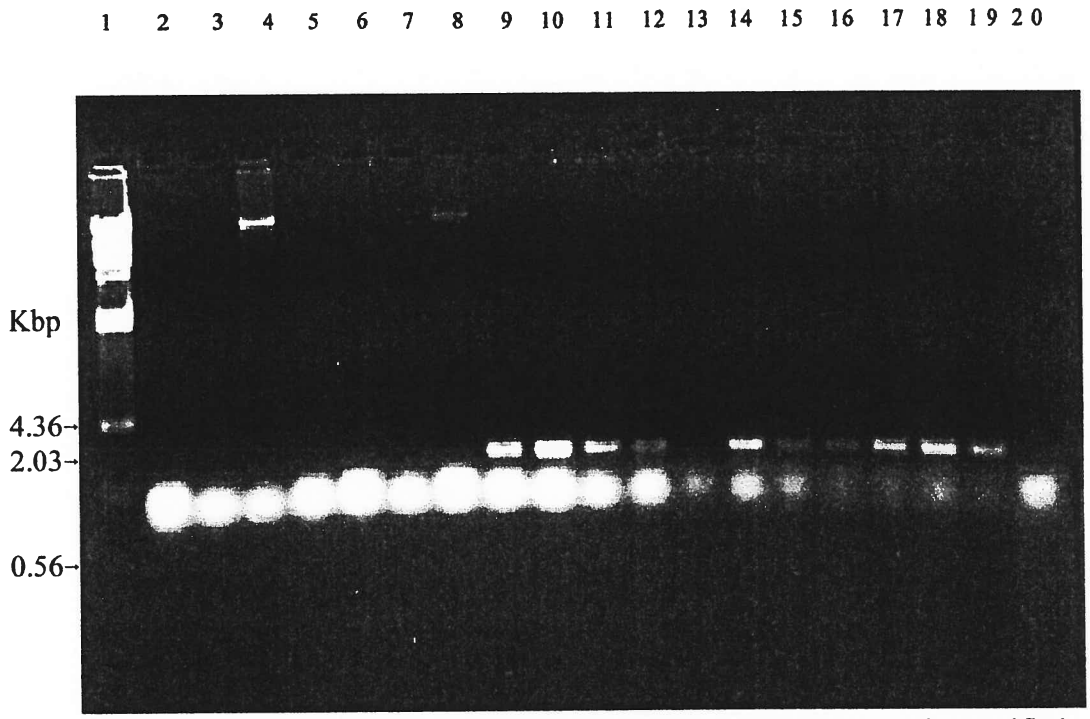

Figure 1. CBR1X/2X primer specificity on different fungal genera using purified DNA from each sample. Lane 1 contains *Hind*III marker. Lanes 2-20 contain: *Alternaria* sp., *Colletotrichum* sp., *Fusarium* sp., *B. maydis*, *Rhizoctonia solani.*, *Sclerotium rolfsii*, *Sordaria* sp., CBR 202-1G, CBR 202-2G, CBR 202-3G, CBR 202-4G, CBR 202-5G, CBR 202-6G, CBR 202-7G, CBR 202-8G, CBR 202-9G, CBR 202-10G, CBR 94-8, and water

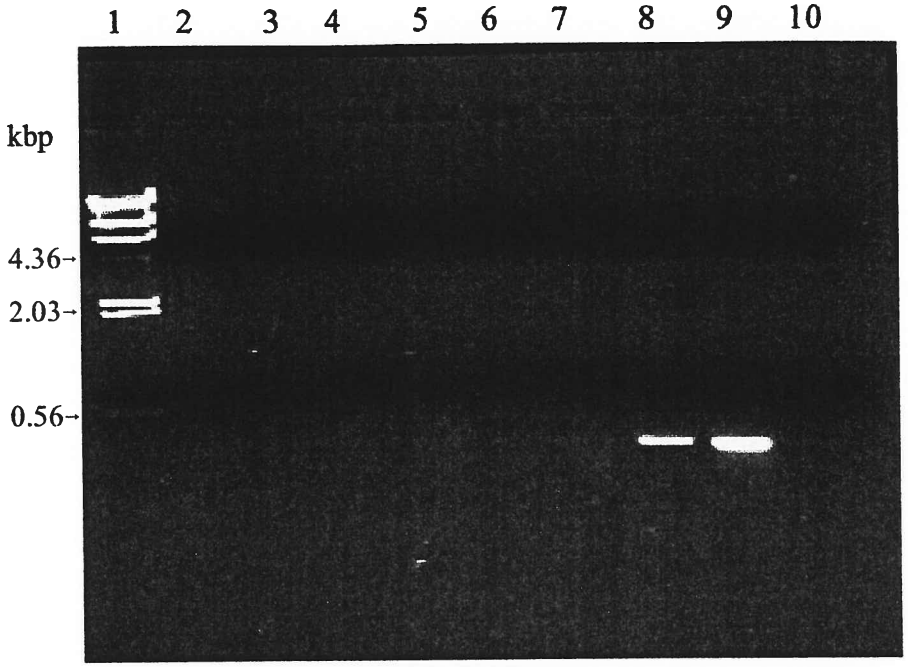

Figure 2. PCR to verify CBR1X/2X specificity using different *Cylindrocladium* sp. Lane 1 contains λ phage DNA digested with *Hind*III. Lanes 2-10 contain PCR products from *C. angustatum* P99 0454, *C. angustatum* P99 1321, *C. avesiculatum* 6825, *C. gordoniae* P02 6389, *C. rumohrae* p94 3976, *C. spathiphylli* 0576, CBR 95-4, CBR 96-1, and water as a negative control, respectively.

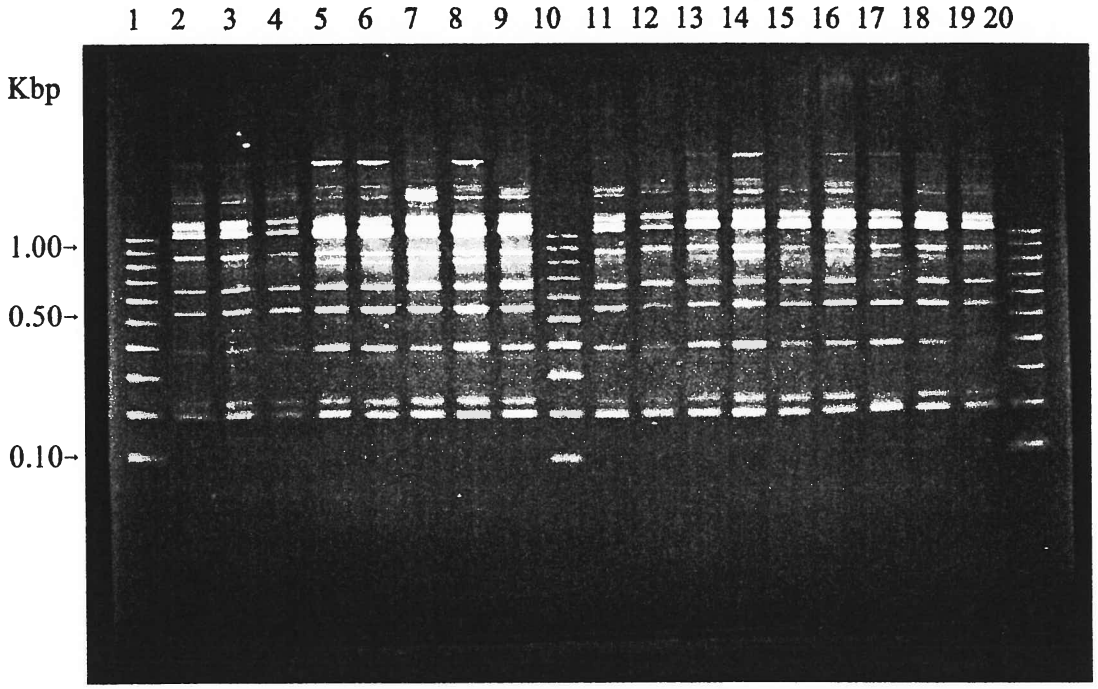

Figure 3. RAPD on *C. parasiticum* isolates from different geographical locations using OPE14 and OPE20 primers. Lanes 1,10 and 20 contain 100bp low ladder marker. Lanes 2-9 and 11-19 contain randomly amplified PCR products from DNA from CBR 84 Suwanee Co., FL, CBR 85 Suwanee Co., FL, CBR 91 Suwanee Co., FL, CBR 782 Levy Co., FL, CBR 780 Decatur Co., GA, CBR 781 Decatur Co., GA, CBR 984 Decatur Co., GA, CBR 93-4 Screven Co., GA, CBR 98-7 Screven Co., GA, CBRVA, VA, CBRVA3, VA, CBRVA4, VA, CBRVA5, VA, CBR 97-11 McDaniel Co., GA, CBR 98-11 Benson Farm, GA, and CBR 95-1 Mitchell Co., GA