Chapter 1.

Introduction and Objectives

Cylindrocladium black rot (CBR) was first reported on peanut in Georgia in 1965 (2). Five years later, the disease was observed in a peanut field in Nansemond County, Virginia (6). Today, CBR is present in approximately 75 % of the peanut acreage in Virginia (P.M. Phipps, pers. commun.). The disease caused an estimated yield loss of 2.2, 3.2 , and 6.7 million dollars for the state in 1998, 1999, and 2000, respectively. CBR and Sclerotinia blight are the two most economically important diseases of peanut in Virginia (20).

CBR is caused by the fungus *Cylindrocladium parasiticum* Crous, Wingfield and Alfenas (teleomorph=*Calonectria ilicicola* Boedijn and Reitsma) (2, 4). In literature prior to 1993, the pathogen is listed as *Cylindrocladium crotalariae* (Loos) Bell and Sobers and *Calonectria crotalariae* (Loos) Bell and Sobers as the imperfect and perfect stages, respectively.

C. parasiticum overwinters in soil and plant debris as pigmented, thick-walled cells known as microsclerotia. Microsclerotia are 33.3 to 311.1 μm in length and 22.2 to 133.3 μm in width (31). These propagules can survive in soil during mild winters, but survival decreases when temperatures fall below 5°C for 4 to 5 weeks or when soil water in the plow layer freezes (24). Saturated soil conditions plus chilling temperatures can also reduce overwintering populations of microsclerotia in soil during winter months (7). Populations are greatest at 7.6 to 15.2 cm depths (3 to 6 in.) (32). Root exudates stimulate germination of microsclerotia, and subsequent taproot infection is favored when soil temperatures are 20 to 25°C and soil moisture is near field capacity (13, 23). Fungal mycelia proliferate both inter- and intracellularly within the cortex of infected taproots and form additional microsclerotia (11, 31). Other underground plant parts including secondary roots, hypocotyls, pegs, pods and seed are also susceptible to infection (2).

Additional infective propagules produced by the pathogen include ascospores and conidia. Under moist conditions, orange-red perithecia can be found near the soil surface on stems of infected plants. These fruiting bodies are about 320 to 465 x 290 to 370 μm in size (2). Two-celled, hyaline ascospores (34 to 58 x 6.3 to 7.8 μm) are produced within the asci and may be passively discharged through the ostiole in a viscous ooze. Forcible discharge is triggered when humidity levels drop such as in early morning hours (30). *C. parasiticum* produces

hyaline, cylindrical, and usually 3-septate conidia (58 to 107 x 4.8 to 7.1 μm) (2). Leaves of infected plants appear chlorotic and wilted, plant growth may be stunted, and plant death usually follows (2, 6, 22). Aboveground symptoms are more pronounced when high levels of soil moisture are present early in the season followed by moisture stress in later months (1). Infected taproots, secondary roots, hypocotyls, pegs, and pods are blackened and necrotic (2). The testae of infected seed have a cinnamon-brown speckled appearance (28). In early reports, infected seed was described as "blemished," "discolored," or "exhibiting lesions" (10, 25, 26).

The dispersal of ascospores, conidia, and microsclerotia leads to spread of CBR. Ascospores can be dispersed by forcible discharge from perithecia, rain splash, or insects (30). Less than 0.1 % of ascospores were viable after 30 min of exposure to conditions typical of the Virginia/North Carolina growing season (33°C and 73 % RH) (30). When soil was infested with plant material containing all three propagule types, only microsclerotia remained viable after eight months (9). Neither ascospores nor conidia are capable of long-term survival in soil (9). Due to their sensitivity to desiccation, ascospores and conidia are limited to short-distance dispersal such as within a field during periods of moisture (9, 30). CBR incidence has often been noted to follow drainage patterns in fields; runoff waters during heavy rains may carry ascospores, conidia, or microslerotia to previously uninfested areas (12, 31). Microsclerotia can also move within and among fields by farm equipment such as cultivators, plows, and tillers carrying infested soil (12, 31). Infested soil and plant debris can also be transported by winds during combining. Winds have been shown to carry plant debris large enough to contain microsclerotia up to 235 m (771 ft) downwind of operating combines (31). Birds may also disseminate microsclerotia (8).

Early research suggested that seed transmission was not a major contributor in the spread of CBR (25, 27). However, the issue has resurfaced within the last 15 years due to unexpected disease incidence in certain areas. In 1985, Johnson reported that *C. parasiticum* could be carried as a surface contaminant on seed, especially those with blemished testae (10). He also reported that the pathogen had been isolated from freshly harvested seed in North Queensland, Australia. Since then, additional isolations of *C. parasiticum* from seed have been reported as well as observations of microsclerotia within the testae of speckled seed (25, 26, 27, 28). Field trials have provided additional evidence that *C. parasiticum* is indeed seedborne. The number of speckled seeds produced in a field has been directly correlated to CBR incidence (26, 28).

Low levels of speckled seed can escape current handling procedures in commercial shelling plants and are subsequently planted by growers. In a recent survey in North Carolina, the average percentage of speckled seed in 62 commercial seed lots was 1.3 % with a maximum of 5.6 % (28). Field trials in North Carolina indicate that planting speckled seed can result in seed transmission of *C. parasiticum* under field conditions. In 1993, Randall-Schadel et al. reported CBR incidence in a field with no history of peanut cropping after planting speckled seed either untreated or treated with fungicide (29). In another field test, disease incidence increased as the number of speckled seeds planted increased (28). The extent to which seed transmission and potential yield loss occurs in the commercial setting has not been determined.

Several strategies have been developed to manage CBR in problem fields. Delaying planting until after May 10 when temperatures are often less favorable for taproot infection is recommended in Virginia (22). Moderately resistant cultivars (NC 12C and Perry) are available; however, planting these cultivars should be combined with other disease management strategies. Crop rotation with non-leguminous crops such as tobacco, cotton, or corn reduces populations of microsclerotia in soil (24). Equipment used in infested fields should be cleaned before use in additional fields (22).

Resistant cultivars and cultural practices provide only limited disease control. Presently, the primary method for CBR control in Virginia is soil treatment with metam sodium at 36 kg a.i./ha (Metam 42 %, UCB Chemical Corp.) (18). The fumigant is applied as a row treatment 20.3 cm below the soil surface by one injector shank in the center of each row, and then rows are bedded to mark the treated zone (22). High soil temperatures and moderate soil moisture favor the conversion of metam sodium to methyl isothiocyanate (MIT) (33). MIT is a biocide and destroys the soilborne microsclerotia in the area of taproot growth (3, 18). MIT also reduces nematode populations which may predispose roots to fungal invasion (5). Soil fumigation is recommended in fields heavily infested with *C. parasiticum.* Its use has greatly reduced yield loss due to CBR for Virginia growers. More than 75 % of the total acreage planted to peanut in Virginia is currently fumigated with metam sodium for control of CBR and nematodes (P.M. Phipps, pers. commun.).

Several fungicides applied as foliar sprays have been reported to suppress CBR. The mechanism by which the fungicides move from the foliage to the soil is not completely understood. Foliar applications of diniconazole, tebuconazole, benomyl, fluazinam, or iprodione

have been suggested as offering partial control of the pod rot phase of CBR (14, 15, 19, 21). However, no foliar fungicide has proven superior to soil fumigants containing metam sodium in the ability to reduce taproot colonization by *C. parasiticum*.

After lowering soilborne inoculum levels by soil fumigation, additional inoculum may be added to treated soils when infected seed is planted. Seed treatment fungicides are applied routinely to all peanut seed in the U.S. for control of several soilborne and/or seedborne pathogens such as *Rhizoctonia solani*, *Aspergillus niger*, *Sclerotium rolfsii*, *Sclerotinia minor*, *Rhizopus* sp., *Verticillium dahliae*, *Pythium myriotylum*, and *Thielaviopsis basicola* (16, 17). The efficacy of seed treatment fungicides at eliminating *C. parasiticum* from peanut seed is debatable. In early laboratory studies, *C. parasiticum* could not be isolated from seed treated with the following fungicides: captan + dicloran, captan + dicloran + carboxin, captan + maneb + pentachloronitrobenzene (pcnb) + etridiazole, pcnb + thiram, or carboxin + thiram + dicloran (27). However, CBR incidence was documented in a field after planting speckled seed treated with either captan + pcnb + carboxin or captan + carboxin + dicloran (28). More research is needed to determine the ability of currently available seed treatment fungicides to prevent transmission of *C. parasiticum* in peanut seed.

In addition to fungicide treatments, temperature treatments are another possibility for eliminating *C. parasiticum* from seed. Porter et al. found the ability to isolate the pathogen from seed during winter seed storage declined over time when seed was stored under ambient conditions in an unheated building or under refrigerated conditions at 5°C (27). Initial isolation frequencies were 6.8 % (NC 8C) and 37.2 % (NC 6) during the first month of storage. Thereafter, frequencies dropped to an average of 2.8 % after seven months of storage at either location. This study was conducted before the speckled testae symptom of infected seed had been described. Randall-Schadel documented a decline in recovery percentage of *C. parasiticum* from speckled seed over time when seed was stored unshelled in a warehouse (28). Initial isolation frequencies averaged 95 % immediately after digging and dropped to 7 and 22 % in VA-C 92R and NC 7, respectively, after seven months of storage. In addition to seed storage conditions, high temperatures encountered during pod drying procedures may adversely affect survival of *C. parasiticum* in speckled seed. The use of supplemental heat in outside drying trailers is often necessary to dry seed to moisture levels recommended for winter seed storage. The effect of high temperatures on pathogen survival in speckled seed needs to be determined.

Since the first report of CBR in Virginia over 30 years ago, the disease is still a problem in some fumigated fields. Seed transmission of *C. parasiticum* may explain some of this occurrence. Although recent research in North Carolina indicates that seed transmission can occur, the issue remains controversial. Additional research is needed to determine the extent to which seed transmission of *C. parasiticum* occurs under commercial growing conditions in Virginia as well as the economic impact that planting speckled seed has on growers and the industry. The objectives of the present study were to determine the incidence of *C. parasiticum* in commercial seed lots in Virginia and to examine the effects of pod drying and winter seed storage temperatures on pathogen survival in speckled seed (Chapter 2), to assess the role of speckled seed in transmission of *C. parasiticum* and to quantify potential yield losses due to the planting of speckled seed (Chapter 3), and to evaluate the efficacy of seed treatment fungicides in preventing seed transmission of *C. parasiticum* (Chapter 4).

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