IDENTIFYING RESISTANCE TO, AND INTERACTIONS OF, ROOT-KNOT NEMATODES AND CYLINDROCLADIUM BLACK ROT IN PEANUT

by

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(Under the Direction of Timothy B. Brenneman)

ABSTRACT

Root-knot nematodes (RKN, *Meloidogyne* spp.) and Cylindrocladium black rot (CBR, *Cylindrocladium parasiticum*) are important soilborne diseases on peanut. Utilization of peanut cultivars with resistance to both diseases is a desirable management approach. Greenhouse, microplot, and field studies were conducted to improve screening techniques and identify new resistance sources and to enhance RKN and CBR resistance breeding in peanut. Greenhouse and microplot studies were also conducted to evaluate the interactions between *M. arenaria* and *C. parasiticum* in peanut.

A gall index based on percentage of galled root was a reliable indicator of resistance to *M. arenaria* at early harvest dates after inoculation with 8000 or more eggs/plant from 10 to 30 days after planting. The selected genotypes could then be assessed for eggs/g root at 8 weeks after inoculation to verify the resistance level based on egg production. Twenty-six peanut genotypes with some resistance to *M. arenaria*, *M. javanica*, or *M. hapla* were identified from 60 accessions based on eggs/g root and gall index in the greenhouse. Results with molecular

markers indicate that different resistance genes exist in the selected genotypes. The resistant selections should be valuable sources for pyramiding resistance genes to develop new cultivars with broad and durable resistance to *Meloidogyne* spp. Differentials of resistance to CBR exist in runner type peanuts. Peanut genotypes are most reliably screened in inoculated or uniformly infested natural fields, but greenhouse evaluations may be useful to identify and characterize components of resistance.

In the greenhouse experiments, root rot ratings were increased by addition of 500-3000 eggs/plant of *M. arenaria* with low inoculum level of *C. parasiticum*, but not at high levels. Gall indices were not affected by *C. parasiticum* inoculations in the greenhouse or microplots. In microplot experiments, synergistic interactions between *M. arenaria* and *C. parasiticum* were observed on plant mortality in both 2006 and 2007. The root rot ratings from nematode-susceptible genotypes were higher in plots infested with *M. arenaria* and *C. parasiticum* than those with *C. parasiticum* alone. Simultaneous inoculation with *M. arenaria* decreased yield incrementally on RNK-susceptible C724-19-25 and Georgia-02C as *C. parasiticum* inoculum levels increased, but not on RNK-resistant C724-19-15.

INDEX WORDS: Root-knot nematode, *Meloidogyne arenaria, Meloidogyne javanica, Meloidogyne hapla,* Cylindrocladium black rot, *Cylindrocladium parasiticum*, Host resistance, Resistance breeding, Screening method, Interaction

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Peanut or groundnut is one of the principal oilseeds and protein meals in the world. According to USDA estimates for the crop year 2006/2007 (FAO Food Outlook, 2007), peanut's share was approximately 8% and 2.6% from world total oilseeds production of 404.33 million metric tons and protein meals production of 225.00 million metric tons, respectively. The cultivated peanut (*Arachis hypogaea* L.) originated in South America (Bolivia and adjoining countries) (Stalker and Simpson, 1995). This crop was grown widely by native peoples of the New World at the time of European expansion in the sixteenth century and was subsequently taken to Europe, Africa, Asia, and the Pacific Islands. Peanut was introduced to the present southeastern United States during colonial times. It is currently grown throughout the tropical and warm temperate regions of the world, with 35.7 million tons of world-wide production in 2006 (FAO Food Outlook, 2007). India, China, and the United States have been the leading producers from 1960s and grow about 70% of the world crop. Peanut was ranked ninth in acreage among major row crops in the United States during 2005, and second in dollar value per acre. Production of peanut in the U.S.A. during 2005-2006 was about at 1.85 million tons or about 5.3% of the world production of 34.9 million tons (FAO Food Outlook, 2007). In 2006, Georgia, Texas, Alabama, and Florida grew 82.6% of the 1,233,000 acres of peanut in the United States. North Carolina, South Carolina, Oklahoma, Virginia, New Mexico, and Mississippi were the other states with more than 10,000 acres of peanut (Anonymous NASS, 2006).

Peanut is an extremely important crop in Georgia, with around 45% of domestic production occurring in the state. The industry contributes an estimated $1 billion to Georgia's economy each year, and supports over 250 companies dedicated to the industry, and an excess of 150,000 Georgians (Perdue, 2003). In 2006, peanut was planted to more than 575,000 acres across the state, with production exceeding 1.44 billion pounds of in-shell peanuts (Anonymous USDA FAS, 2007).

Peanut is a self-pollinating, indeterminate, annual, herbaceous legume. Peanuts are most productive if conditions are warm and moist during the growing season, and dry during harvest (Beasley, 1997). Fertilized flowers form gynophores that grow toward and into the soil where the fruit develop below the soil surface (Beasley, 1997). The fruit is a pod with one to five seeds that develops underground attached to a needlelike structure called a peg, an elongated ovarian structure. This results in not only the roots, but also the pods being subject to damage by many soilborne pathogens.

Root-knot caused by the root-knot nematodes (RKN, *Meloidogyne* spp.) and Cylindrocladium black rot (CBR, *Cylindrocladium parasiticum*) are two soilborne diseases of economical importance in peanut production. In Georgia alone, RKN and CBR cost farmers an average of $13.6 and 6.2 million in damages and control costs for each of the last 7 years (Williams-Woodward, 2000, 2001, 2002, 2003; Pearce, 2004; Martinez, 2005, 2006). Root-knot nematodes and *C. parasiticum* are frequently found together in peanut fields, and previous reports showed that *Meloidogyne* sp. increased CBR severity on some virginia type peanut, with or without resistance to CBR (Culbreath et al., 1992; Diomande and Beute 1981a & b; and Diomande et al., 1981).

Literature Review

Root-Knot Nematodes

Species and Distribution. Root-knot nematodes (*Meloidogyne* spp.) are the most important plant-parasitic nematodes in agriculture. They are distributed worldwide and cause severe loss of crop yield (Sasser and Freckman, 1987). The major species of root-knot nematodes known to damage peanut are *Meloidogyne arenaria* (peanut root-knot nematode) race 1 (Neal) Chitwood, *M. hapla* (northern root-knot nematode) Chitwood, and *M. javanica* (Javanese root-knot nematode) race 3 (Treub) Chitwood. Neal (1889) reported a root-knot nematode that produced severe galling on peanuts in Florida which he described as *Anguillua arenaria*, and which Chitwood (1949) renamed *Meloidogyne arenaria*. Sasser (1954) reported that peanuts were host for *M. arenaria* and *M. hapla* and non-host for *M. incognita*, and *M. javanica*. However, Martain (1958) indicated that peanuts in South Rhodesia were infected by *M. javanica*. Additionally, a new species of root-knot nematode causing disease on peanut in Texas, USA was recently described as *M. haplanaria* (Eisenback et al., 2003).

Meloidogyne arenaria, *M. hapla*, and *M. javanica* are known to occur in peanut-producing regions all over the world, including North, Central and South America, Africa, Asia, Europe, and Australia (Sasser, 1977; Song et al., 1992). *Meloidogyne arenaria* and *M. javanica* are common in warm peanut-growing regions, whereas *M. hapla* occurs mainly in cool regions. In the United States, *M. arenaria* and *M. hapla* exist throughout the peanut-producing areas. *Meloidogyne arenaria* is the predominant species parasitizing peanut in the southern regions, especially in Alabama, Florida, Georgia, Texas, and South Carolina, where up to 40% of the fields are infested, and yield losses in heavily infested fields can exceed 30% (Dickson, 1998; Ingram and Rodrigue-Kabana, 1980; Koenning et al., 1999; Minton and Baujard, 1990; Wheeler

and Starr, 1987). *Meloidogyne hapla* is the most damaging species in more northerly states, including North Carolina, Virginia, and Oklahoma (Anon., 1987; Schmitt and Barker, 1988; Koening and Barker, 1992). Populations of *M. javanica* parasitic on peanut are common in Egypt (Tomaszewski et al., 1994) and India (Sharma et al., 1995), but they are rare in the United States, having been described only from a few fields in Florida, Georgia, and Texas (Lima et al., 2002; Minton et al., 1969; Tomaszewski et al., 1994).

Life Cycle and Damage. The life cycle of the root-knot nematode consists of the egg, four juvenile stages, and the adult. Second-stage juveniles (J2) hatch from the egg, which migrate in soil and invade plant roots, gynophores (pegs), and pods (Minton, 1963). After entering roots, the J2 migrate intercellularly through the cortical tissue and establish feeding sites composed of three to five altered host cells, called giant cells, in the vascular tissue. Giant cells are multinucleate, have dense cytoplasm, elevated rates of metabolism, and altered cell wall structure. These cells are the permanent feeding site for the nematode. The J2 become sedentary, molt to the third stage after feeding on a susceptible host, and enlarge as they go through the forth stage, and then become adults. These mature adults lay eggs on the gall surface. Eggs are able to survive in unfavorable conditions in the soil for many months. It takes 20 to 60 days to complete the life cycle of the root-knot nematode (Hussey and Janssen et al., 2001; Song and Dong, 1992). The damage caused by root-knot nematodes leads to serious losses in yield and quality of peanut (Abdel-Momen and Starr, 1997; Song et al., 1994). Symptoms of nematode infection are retarded growth; galling of root, pegs and pods; and wilting (Dickson, 1998). Galls formed on peanut roots are of two general types depending on the nematode species. Sasser (1954) found that peanut roots infected with *M. hapla* developed small galls and extensive root proliferation, and the roots often formed a dense mat when infection was severe. Galls caused by

M. arenaira and *M. javanica* on peanut roots are larger than those caused by *M. hapla*. Infected plants tend to have fewer small rootlets than normal, and symptoms might be characterized as a combination of galls and coarse roots. The galls tend to be large and involve the main roots. Above-ground symptoms for root-knot nematodes include stunting and, in extreme cases, death of the plant. The foliage may appear light green to yellow, indicative of nutrient deficiencies. Infected plants are more susceptible to drought damage than noninfected plants.

Management. Many tactics are available for managing root-knot nematodes on peanut, including crop rotation, biological control, chemical control, and use of nematode-resistant peanut cultivars. However, each tactic has limitations and none is sufficient to provide complete control.

Rotation of peanut with nonhost or poor host crops can be effective in decreasing the potential for substantial yield losses (Luc et al., 1990; Whitehead, 1998) and reducing nematode populations (Dunn, 1988). Rotational crops recommended for *Meloidogyne* management on peanut in the United States vary with the nematode species present, cultivar of rotational crop, etc. Cotton, maize, small grains, bahiagrass, bermudagrass, millet, sorghum, and pasture grasses (Koenning et al., 1998; Dunn, 1988; Hagan, 1988; Kinloch, 2001) have been utilized as rotational crops. Maize and watermelon are also recommended rotational crops for managing *M. hapla* on peanut in Queensland Australia and China (Broadley, 1981; Vance, 1981; Song et al., 1992). However, rotations should not be expected to abruptly reduce root-knot nematode populations since 1) profitable rotational crops are not always available for the nematode-infested fields; 2) the most “resistant” crop plant may support at least a low nematode population, and 3) every cultivated fields have at least a few weeds that are good hosts for nematode reproduction.

Several biological control agents effective against root-knot nematode have been reported. *Pasteuria* spp. is one of the most effective biological agents that parasitize *Meloidogyne* spp. (Dickson et al., 1991; Sayre and Starr, 1985). Unfortunately, effective use and application of biological agents such as *Pasteria* spp. have not been developed, mainly due to the difficulties in reproduction of the agent in industrialization.

In some instances chemicals are the only reliable means to control root-knot nematodes of peanuts. However, use of nematicides is problematic because of the short-term efficacy (Dickson and Hewlett, 1989; Culbreath et al., 1992b) and the cost to growers. Public concerns for nematicide residues in the environment also have resulted in increased restrictions on their use.

Host–Plant Resistance. Host resistance to nematodes, which can be defined as the suppression of nematode reproduction by the resistant plant relative to reproduction on a susceptible genotype of the same plant species (Williamson and Hussey, 1996), is a desirable approach to manage nematodes. Use of resistant cultivars to manage root-knot nematodes is advantageous in that 1) it does not require advanced technology, 2) it leaves no toxic residues in the environment, and 3) it is usually cost effective (Trudgill, 1991).

Prior to the mid-1980's, no confirmed resistance to root-knot nematode species in peanut or related *Arachis* species was known, despite the screening of several thousand genotypes of *A. hypogaea* (Miller, 1972; Minton and Hammons, 1975; Holbrook et al., 1983; Baltensperger et al., 1986). In the past 30 years, great advances have been made in identifying and utilizing sources of resistance to *Meloidogyne* spp. in peanuts. A total of 25 accessions of *A. hypogaea* were selected from 2321 plant introductions for resistance to *M. arenaria* (Holbrook and Noe, 1992; Holbrook et al., 1996). The selections supported less egg production per gram fresh root compared with the susceptible cultivar, Florunner. Holbrook et al. (2000a) reported 36 out of 741 accessions in the

U.S. peanut core collection showed resistance to *M. arenaria*, with a reduction in root galling, egg-mass ratings, egg count per root system, and egg count per gram of root in comparison to the susceptible control, Florunner. Holbrook et al. (2000b) also evaluated accessions from 30 clusters having resistant indicator accessions. This second stage screening identified 259 accessions that had reduced egg-mass production and 28 that had greatly reduced numbers of egg masses. Holbrook et al. (1998) identified several peanut breeding lines with good resistance to *M. arenaria*. Those lines produce greater yield than susceptible cultivars when grown in soil heavily infested with *M. arenaria* (Holbrook et al. 1998). However, no high levels of resistance have been observed originating from *A. hypogaea*. If different mechanisms were involved in different genotypes, then it should be possible to combine the mechanisms to improve the level and durability of the resistance. Unfortunately, different mechanisms of resistance in six moderately resistant genotypes have not been comfirmed (Timper et al., 2000).

High levels of resistance to *M. arenaria* exist in other *Arachis* species (Baltensperger et al., 1986; Nelson et al., 1989; Holbrook and Noe, 1990), and the different mechanisms of resistance in these wild species suggest they have unique genes for resistance (Nelson et al., 1990; Starr et al., 1990). This resistance has been introgressed into *A. hypogaea* though two different pathways. Stalker et al. (2002) made an interspecific cross between *A. hypogaea* (PI 261942) and *A. cardenasii* Krapov. and W.C. Gregory (GKP 10017, PI 262141). The *A. hypogaea* parent ($2n = 4x = 40$) is a subspecies *fastigiata* var. *fastigiata* with high susceptibility to *M. arenaria* (Neal) Chitwood (Guok et al., 1986; Stalker, 1984). This line was introduced from the Guarani, Paraguay region of South America. *Arachis cardenasii* is a diploid ($2n = 2x = 20$) wild species from Robore, Bolivia. Several studies confirmed that *A. cardenasii* had high resistance to *M. arenaria* (Holbrook and Noe, 1990; Nelson et al., 1989). The hybrids of the first generation were

treated with colchicine to restore fertility at the hexaploid (2n = 6x = 60) level. A fertile plant was self-pollinated, and the chromosome number of numerous progenies from a heterogeneous population were found at the tetraploid ($2n = 4x = 40$) chromosome level. This resulted in the release of GP-NC WS 5 and GP-NC WS 6 as highly resistant germplasm lines to *M. arenaria* by the North Carolina Agricultural Research Service in 1997 (Stalker et al., 2002). Fewer galls and eggs per gram root ($P \leq 0.05$) were produced on the two interspecific hybrids than those produced on *A. hypogaea* with moderate resistance to *M. arenaria* (Holbrook and Noe, 1990; Stalker et al., 2002). Suppression of galling is conditioned by a single dominant gene (*Mag*), and a second dominant gene (*Mae*), conditions decreased reproduction as expressed by reduced egg production (Garcia et al., 1996). The two genes are linked at 16 ± 2.5 cM.

Two germplasm lines NR 0812 and NR 0817 were jointly released with resistance to *M. arenaria* by the USDA-ARS and the University of Georgia, College of Agricultural and Environmental Sciences (Anderson et al., 2006). The two lines were derived from a cross of AgraTech 108 (PVP no. 9600322) and GP-NC WS 5 (Stalker et al., 2002) made in 1995. Resistance to *M. arenaria* in NR 0812 was confirmed in the greenhouse in 2001 (Timper et al., 2003) and in two *M. arenaria*–infested fields in Decatur County, GA, and Headland, AL, in 2001 and 2002. In the greenhouse evaluation, NR 0812 also showed resistance to *M. javanica* race 3 and susceptibility to *M. hapla* Chitwood (Timper et al., 2003). NR 0817 was included in the 2002 field tests and expressed root-knot nematode resistance similar to NR 0812.

Resistance to *M. arenaria* also has been introgressed into *A. hypogaea* by using a complex interspecific hybrid pathway [released as TxAG-6 by Simpson et al. (1993)] from the three nematode resistant species, *A. batizocoi, A. cardenasii,* and *A. diogoi* Hoehne (Simpson, 1991). TxAG-7 was derived from the first backcross generation of *A. hypogaea* cv. Florunner ×

TxAG-6 (Simpson et al., 1993). Both TxAG-6 and TxAG-7 show extensive cross-compatibility with a wide range of *A. hypogaea* genotypes and are particularly noted for their high resistance to *M. arenaria*. A backcrossing program was used to introgress the root-knot nematode resistance from TxAG-6 into peanut breeding populations (Starr et al., 1995). In the backcross introgression pathway, TxAG-6 was the resistant parent and Florunner (*A. hypogaea* subsp. *hypogaea* var. *hypogaea*) was the recurrent parent. The fifth backcross produced the designated breeding line TP262-3-5, which was released as COAN, the first peanut cultivar with a high level of resistance to *M. arenaria,* in 1999 (Simpson and Starr, 2001). The seventh backcross produced the breeding line TP301-1-8, which was registered as a new cultivar NemaTAM with high resistance to *M. arenaria* and *M. javanica* and better yield potential than COAN (Simpson et al., 2003). The resistance in COAN and NemaTAM was derived from *Arachis cardenasii* and segregates as a single dominant gene (Choi et al., 1999; Church et al., 2000). However, the resistance in COAN does not appear to involve a necrotic, hypersensitive response as that reported for *A. cardenasii* (Bendezu and Starr, 2003). In addition, a recessive gene for resistance to *M. arenaria* has been identified in TxAG-6 (Church et al., 2005). However, it is not known whether the recessive gene exists in COAN and NemaTAM.

Castillo et al. (1973) reported resistance to *M. hapla* in four introductions of unidentified wild *Arachis spp*. and only moderate susceptibility in eight *A. hypogaea* entries. Also, Subrahmanyam et al. (1983) reported a wild *Arachis spp*. resistant to *M. hapla*. In Florida, resistance to *M. hapla* was reported from *A. glabrata*, a wild species that is incompatible with *A. hypogaea* (Baltensperger et al., 1986). In Texas, resistance to *M. hapla* was observed in two *Arachis* species and one interspecific hybrid (Nelson et al., 1990). In China, moderate resistance and tolerance to *M. hapla* were identified from over 5000 accessions of peanut germplasm in

naturally infested fields and artificially infested nursery (Song et al., 1995; Dong et al., 2001). However, no peanut cultivars or interspecific germplasm have resistance to *M. hapla* (Timper et al., 2003). Additionally, Sakhuja and Sethi (1985) reported resistance to *M. javanica* in four cultivars.

Resistance Screening Methods. The development of new peanut cultivars with resistance to nematodes will require reliable and efficient resistance screening techniques for identifying resistant progeny within segregating breeding populations. Evaluation of breeding lines can be done in naturally infested fields and artificially infested nursery gardens; however, the non-uniformity of root-knot nematode infestations, seasonal restrictions, seed limitation, and polyspecific nematode communities are disadvantages to field and nursery screening. The screening method used to identify root-knot nematode resistant breeding lines should be capable of readily and reliably evaluating the thousands of genotypes encountered in a breeding program (Boerma and Hussy, 1992). This requirement is best fulfilled in a greenhouse environment that permits tests to be conducted throughout the year. Additional benefits of using greenhouse cultures as the source of inocula include standardization of inoculum levels, uniform distribution of inoculum, evaluation of resistance in localities where a specific root-knot nematode species or host race are not indigenous, and the elimination of seasonal restrictions when evaluating genotypes (Hussey and Boerma, 1981).

Several greenhouse screening methods to identify resistance to *M. arenaria* in peanuts are available. As early as the 1970s, Minton and Hammons (1975) evaluated 512 peanut accessions for resistance to *M. arenaria* in the greenhouse. In their study, 1500 juveniles were used to inoculate each pot at planting, and resistance was evaluated based on galling at 6 weeks after planting. After a new egg collecting technique was developed by Hussey and Barker (1973), eggs

became the most widely used type of inoculum for screening in peanuts (Holbrook et al., 1983, 2000a, 2000b; Choi et al., 1999; Star et al., 1995; Burow et al., 1996; Garcia et al., 1996; Church et al., 2000 and 2005; Abdel-Momen et al., 1998; Timper et al., 2003). The inoculum levels used in these studies were 3500 eggs/plant (Holbrook et al., 2000a) to 10000 eggs/plant (Choi et al., 1999; Burow et al., 1996; Church et al., 2005; Star et al., 1995). Plant ages at inoculation were 5 (Choi et al., 1999) to 21 days after planting (Timper et al., 2003; Garcia et al., 1996; Church et al., 2005). The duration of the evaluation could be from 40 (Holbrook et al., 1983) to 90 (Holbrook et al., 2000a & b) days after inoculation in the greenhouse. According to the definition of plant resistance to nematodes (Williamson and Hussey, 1996), many researchers evaluated the resistance to root-knot nematodes in peanuts based on eggs per gram fresh root (Abdel-Momen et al., 1998; Choi et al., 1999; Burow et al., 1996; Church et al., 2005; Timper et al., 2003; Star et al., 1995). Holbrook et al. (1983) developed a method to use Phloxine B to stain the eggs masses in the root, which greatly expedited the egg mass screening process. From then on, an egg-mass index based on egg-mass number and a gall index based on gall number were commonly used to evaluate resistance of breeding lines to *Meloidogyne* spp. (Holbrook et al., 1983, 2000a & b; Chu et al., 2007). A gall index based on percentage of galled roots (Zhou and Star, 2003; Zhang et al., 2006) and gall numbers (Harris et al., 2003) have also been used during greenhouse screening to assess resistance to root-knot nematodes.

All the methods mentioned above have been successfully used in screening resistance to *Meloidogyne* spp. in peanut. However, they are all time-consuming, requiring 60 to over 100 days before results are available.

Marker-assisted Selection. Classical breeding techniques have contributed to the development of resistant varieties, but these techniques are laborious, expensive, and take a long

time to develop a new resistant cultivar. Using DNA marker technologies in breeding programs can improve these weak points of classical backcross programs (Hussey and Janssen, 2001). DNA marker technologies for marker-assisted selection can improve efficiency methods for selection of resistant individuals in segregating populations (Stalker and Mozingo, 2001). The recurrent genome can be recovered faster than by classical backcross programs using phenotypic selection procedures.

Breeding for nematode resistance represents the first practical use of marker-assisted selection (MAS) in peanut. Several molecular markers have been developed to expedite breeding for nematode resistance. Burow et al. (1996) identified three RAPD markers (RKN 229, RKN 410, and RKN 440) linked to *M. arenaria* resistance in several breeding populations derived from TxAG-7 in the fifth backcross generation. The resistance in each of the populations appeared to have been derived from *A. cardenasii* and was most likely due to a single gene. Subsequent studies (Choi et al., 1999) confirmed that the resistance in some of these populations was conferred by a single dominant gene from *A. cardenasii*. However, data from other populations indicated the possibility of a second gene for resistance. A genetic map of cultivated peanut (*Arachis hypogaea* L.) was achieved by RFLP analysis (Burow et al., 2001). A synthetic amphidiploid, TxAG-6 (Simpson, 1991), which was developed through the cross [*A. batizocoi* x (*A. cardenasii* x *A. diogoi*)] 4X was used as a donor to introduce the root-knot nematode resistance gene into cultivated peanut. Three hundred seventy RFLP loci were mapped (Burow et al., 2001). Based on this study, Choi et al. (1999) identified two useful RFLP markers, R2430E and R2545E. RFLP loci R2430E and R2545E showed distinct bands on resistant and susceptible phenotypes and were easy to score for genetic condition, homozygosity vs. heterozygosity, in individual plants. RFLP locus R2430E was 4.2 centiMorgans (cM) from the resistance locus (Choi et al., 1999). RFLP locus R2430E was derived from *A. cardenasii* and maps to linkage

group 1 (Burow et al., 2001). Church et al. (2000) reported the efficiency of marker-assisted selection using two RFLP markers, R2430E and R2545 to identify individuals homozygous for resistance to *M. arenaria* in segregating populations of peanut.

Although molecular markers are utilized to select resistance in individual plants, RFLP or RAPD analysis is impractical for routine identification in large populations. RFLP analysis requires several time-consuming steps, including DNA extraction from plant tissue, digestion, electrophoresis, and Southern blot hybridization. RAPD analysis often lacks reproducibility and thus is not sufficiently robust for routine marker-assisted selection programs. Therefore, there is a need to develop a reliable, robust PCR-based marker upon which a more efficient, reliable, and relatively simple marker-assisted selection procedure can be based (Paran et al., 1993). Sequence characterized amplified regions (SCARs) can overcome the problem of irreproducibility (Paran and Michelmore, 1993). Garcia et al. (1996) developed a RAPD Marker Z3/265 from an F2 population of GA6 (*A. hypogaea* (PI261942) x *A. cardenasii*) backcrossed with PI261942. A 265bp fragment derived from *A. cardenasii* was linked at 10±2.5 cM and 14±2.9 cM from the putative nematode resistance genes *Mag* and *Mae*, respectively. It was successfully converted into a SCAR (sequence characterized amplified region) marker. Recently, Chu et al. (2007) modified the marker RKN440 based on more complete sequencing data and established a new nematode resistance dominant marker 197/909. This SCAR marker amplifies fragments from both susceptible and resistant plants, but of different molecular weights, thus avoiding false negative classifications caused by failed reactions with dominant markers. This marker is reproducible and shows a high correlation with the phenotype data. When this marker was applied using a cost-effective, high-throughput DNA extraction method, it remained a robust assay.

Cylindrocladium Black Rot (CBR)

Distribution and Damage. Cylindrocladium black rot of peanut, caused by *Cylindrocladium parasiticum* Crous, Wingfield & Alfenas, was first found in southwest Georgia in 1965 (Bell and Sobers, 1966). Subsequently, it has been reported in all peanut-producing areas of the United States (Garren et al., 1972; Rodriquez-Kabana and Backman, 1975) and in Japan, India and Australia (Hammons et al., 1981). The disease threatens peanut production throughout the southeastern United States since the 1980s (Harris and Beute, 1982). On peanut, the pathogen can infect any below-ground tissue, but the region immediately behind the root tips is the primary infection court (Phipps and Beute, 1997). Taproots and hypocotyls become blackened and necrotic, with necrosis typically terminating at ground line. Root tips are sloughed off, leaving stubs. Sunken, blackish lesions appear on roots, pegs, and pods. Leaf tips and margins become chlorotic, wilted and blighted. Reddish-orange perithecia appear at, and just above, the soil line from mid-June through the end of the season. Ascospores may be exuded from perithecia in a visible thick, yellow liquid. Pod development is greatly reduced, and existing pods may be severely rotted (Bell and Sobers, 1966; Johnston and Beute, 1975.). In North Carolina and Virginia, where CBR has been a major yield limiting factor, some counties have as much as 75% of the peanut acreage infested (Black et al., 1984; Pataky et al., 1983a & b). Yield loses have been estimated to be approximately 250 to 450 kg/ha for each 10% increase in CBR incidence (Pataky and Beute, 1983a). Yield losses from CBR in some infested fields of peanut in Florida have exceeded 50% (Kucharek, 2000).

Pathogen. The fungus was first noticed in the US in southwest Georgia in 1965 in peanut fields (Bell and Sobers, 1966). It is thought that the pathogen was introduced from Asia during the establishment of a tea plantation in coastal Georgia in the 1950s. It was originally identified

as the anamorph of *Cercosporella theae* var. *crotalariae*, a common pathogen of tea (*Theae sinensis* L.) and crotalarias (*Crotalaria* spp.) in Asia. Bell and Sobers (1966) proposed raising this variety to the species level, and gave the name *Calonectria crotalariae* (Loos) Bell & Sobers to its perfect form and *Cylindrocladium crotalariae* to the imperfect form. It was subsequently decided that these names had not been validly published, and thus Crous et al. (1993) established the current name *Cylindrocladium parasiticum* Crous, Wingfield and Alfenas for the imperfect form. Crous et al. (1993) also determined that *Calonectria ilicicola* Boedign & Reitsma (1950) and *Calonectria crotalariae* Bell and Sobers (1966) were the same species, and thus the former name has priority for the teleomorph.

Cylindrocladium parasiticum Crous, M.J. Wingfield, & Alfenas

Cercosporella theae var. *crotalariae* Loos, nom. inval.

Cylindrocladium crotalariae (C.A. Loos) D.K. Bell & Sobers, nom. illeg.

Calonectria ilicicola Boedijn & Reitsma

Calonectria crotalariae (Loos) D.K. Bell & Sobres

Calonectria theae var. *crotalariae* C.A. Loos

Cylindrocladium parasiticum has a relatively wide host range (Crous, 2002). It can infect many kinds of legumes, especially alfalfa, clovers, and soybean, as well as blueberry (*Vaccinium ashei* and *V. corymbosum*), tea (*Camellia sinensis*), yellow poplar (*Liriodendron tulipifera*), sweetgum (*Liquidambar styraciflua*) and other hardwood seedlings. It has been reported on the commonly found legume weeds patridgepea, sicklepod, coffeeweed, and Florida beggarweed (Brenneman et al., 1998; Padgett et al., 1995; Kucharek, 2000). Cylindrocladium black rot is a serious problem on eucalyptus (*Eucalyptus* spp.), crotalarias and tea in China, Japan, India and Australia (Porter et al., 1991).

Calonectria ilicicola is an ascomycete in the Pyrenomycete group. The fungus is homothallic, and produces orange-red perithecia, 300-500µm high and 280-400 µm wide, oval to round or obovate with large, irregular and thin-walled cells. Asci are clavate and have eight falcate spores with 1-3 septae. In contrast, microsclerotia have dark brown, thick-walled cells (Hwang and Ko, 1976). Conidia are cylindrical, hyaline, have 1-3 septae, and are produced by apical budding; they have been measured between 38-68 × 4-5µm. Vesicles are clavate and 5-10 µm wide. Conidiophore-bearing stripes appear at right angles from the host (Crous et al., 1993; Bell and Sobers, 1966). The fungus can be grown on potato dextrose agar (PDA) medium, with optimum mycelial growth occurred at 26-28^{o}C, and perithecial development was enhanced by exposure to fluorescent light (Bell and Sobers, 1966).

Ecology and Epidemiology. The fungus, *C. parasiticum,* produces microsclerotia (small aggregates of hyphae with a hardened exterior) within infected tissue, particularly in roots (Rowe et al., 1974b). Microsclerotia serve as inoculum for CBR by germinating to form fungal strands (hyphae) in the soil. Hyphae penetrate the root cortex and *Rhizobium* nodules though intercellular within 24 hours of germination, and microsclerotia begin to be formed within several days. Peanut can produce protective periderms (dermal tissues typical of secondary growth) to wall off invaded and injured areas, and differences between susceptible and resistant varieties of peanut may be mainly due to the speed with which these periderms can be produced (Harris and Beute, 1982). Injury or the emergence of secondary roots may weaken epidermal to provide the pathogen additional entrance points. The decay of dead tissue releases microsclerotia into the soil. These propagules are not effective saprophytic competitors. A perithecium is a small flask-shaped fruiting body in ascomycetous fungi that contains the ascospores. If adequate moisture is available, perithecial initials can be found in large quantities on peanut stems within

few weeks after inoculation. In North Carolina, perithecia have been observed as early as mid-June. Mature ascospores can be present within 2 to 3 weeks after inoculation (Rowe and Beute, 1975). Ascospores are discharged both by ejection and in viscous droplets. Ascospore formation and discharge appear to be controlled by day-night relative humidity fluctuations, and they mature under 100% night-time humidity conditions. The drop in humidity that occurs at dawn triggers a widespread ascospore discharge coinciding with dew precipitation. Both ascospores and conidia are extremely sensitive to desiccation, and survival of either under normal day-time temperatures and humidity is under 10% after two minutes. Ascospore ejection occurs between 20-30^{o}C, and maximally at 25^{o}C, more or less coinciding with vegetative growth temperature optima.

A cool, damp spring can cause serious losses to CBR. The disease slows at temperature over 25^{o}C and ceases at 35^{o}C (Phipps and Beute, 1977). Microsclerotia are clumped rather than randomly distributed and environmental factors more than crop sequence affected survival (Griffin and Tomimatsu, 1983; Pataky and Beute, 1983b). Summer droughts and excessively cold winters destroyed microsclerotia and after such weather events CBR was less severe (Phipps and Beute, 1977). Griffin et al. (1978) evaluated the effects of various factors on viability of microsclerotia in samples, and concluded that maintaining samples at field moisture and temperature levels is critical. Germinability of microsclerotia was depressed when infested soil was incubated at 5^{o}C, -3^{o}C, or -10^{o}C for 4 weeks. Microsclerotia did not appear to survive the -10^{o}C regime. Air drying to -2000 bars also resulted in apparent loss of germinability. However, restoring moisture and incubation at 26^{o}C (not a critical temperature) for 2 to 4 weeks resulted in partial recovery of germinability (Griffin et al., 1978; Roth et al. 1979). In addition, disease severity may increase if roots are injured by preplant herbicides (Barron, 1981). Disease

development is also more likely in soils high in organic matter or otherwise more likely to retain moisture (Black and Beute, 1985).

Ascospores, conidia, and microsclerotia all may contribute to the spread of CBR. Ascospores are released from perithecia and may be dispersed within a field by forcible discharge, rain splash, or insects (Rowe and Beute, 1975). Conidia may also be produced on infected plants during the season (Bell and Sobers, 1966). Runoff water during heavy rains may carry both propagule types along drainage slopes within a field (Johnson, 1985). Ascospores and conidia lose their germinability rapidly after discharge into reduced humidities, which limits their role to short-distance dispersal such as within a field under favorable conditions (Hwang and Ko, 1976; Rowe and Beute, 1975). Unlike ascospores and conidia, microsclerotia are capable of long-term survival in soil and plant debris (Phipps and Beute, 1979). These multi-cellular structures have thick-walled cells and are produced in high numbers within the cortex of infected roots (Rowe et al., 1974b). Contaminated farm equipment, water runoff, and winds during harvesting operations can distribute microsclerotia within and among fields (Krigsvold et al., 1977, Rowe et al., 1974b). Birds have also been shown capable of moving microsclerotia (Hiller, 1975). Additional spread of the fungus within a field occurs with tillage of soil and harvest operations.

The dispersal mechanisms for movement of ascospores, conidia, and microsclerotia can account for local and regional disease spread, but fail to explain the movement of *C. parasiticum* over greater distances such as among states and continents. The dispersal of *C. parasiticum* through movement of infected seed has been hypothesized repeatedly (Garren et al., 1972; Johnson, 1985; Porter and Mozingo, 1991; Porter et al., 1991), but confirmation of seed transmission was only described recently (Randall-Schadel, 1999). Microsclerotia of *C.*

parasiticum were found within the multi-cellular layers of the testae of speckled seed and several studies have reported that the fungus was isolated from speckled seed at high rates (Glenn et al., 1999; Johnson, 1985; Porter and Mozingo, 1986; Porter and Mozingo, 1991; Porter et al., 1991; Randall-Schadel, 1999). On occasion, *C. parasiticum* has been isolated from normal seed at very low frequencies (Glenn et al., 1999; Randall-Schadel, 1999). This occurrence was rare and the contribution of normal seed in spread of CBR was thought to be insignificant. Field trials by Randall-Schadel (1999) showed that speckled seed can transmit *C. parasiticum* and cause CBR in emerging plants. CBR was observed in a field with no history of peanut cropping after speckled seed treated with fungicide was planted. In a separate study, CBR incidence increased as the percentage of speckled seed planted increased (Randall-Schadel, 1999). These results indicated that speckled seed can serve as a source of inoculum and contribute to disease spread. The rate at which seed transmission occurs when seed is planted under commercial growing conditions as well as the impact of seed transmission on yield have not been investigated.

Management. At this time, tactics that are likely to elicit total control for CBR on peanut are not available. The best control available is to utilize as many of the control tactics as possible for each field.

Crop Rotation. Higher levels of CBR are directly related to higher levels of microsclerotia in soil. Crop rotation with non-susceptible crops is useful to reduce the density of microsclerotia (Sidebottom and Beute, 1989). Susceptible weeds such as hairy indigo, beggarweed, and coffeeweed should be reduced or eliminated. However, crop rotations of peanut with four to five years of bahiagrass have not been adequate for suppression of CBR in some situations (Black and Beute, 1984b; Kucharek, 2000).

Cultural Practices. CBR is also a seedborne disease (Randall-Schadel, 1999). Removing all dark seeds during normal sorting and screening is important to protect other fields from this disease. Delaying tillage until spring keeps propagules away from insulating deep soil, and sometimes can reduce disease. Delaying planting to avoid cool, damp spring weather has been successful at reducing disease, but few yield increases have been realized due to the offsetting effects of later harvests (Rowe et al., 1974a). Applications of nitrogen to peanut fields also can reduce severity of CBR, presumably because fertilization reduces formation of *Rhizobium* nodules, thus providing fewer infection courts (Pateky et al., 1984). Fertilization of peanut, however, may result in lower yields due to delayed flowering.

Chemical Control. The soil fumigant, metam sodium, was first recognized in 1981 to have commercial value for control of CBR on peanut (Turner and Corden, 1963; Gerstl et al., 1977). Following chisel applications into soil, metam sodium converts rapidly to methyl isothiocyanate (MIT) which is the active ingredient. MIT is a highly toxic, broad spectrum biocide (Phipps, 1990). Depending upon the application rate and method, soil type, and environmental conditions, MIT can be highly effective in control of soil inhabitors such as weeds, nematodes, fungi and insects. Because of its highly toxic and nonspecific nature, MIT should be applied at least 2 weeks prior to planting to avoid crop injury. Metam-sodium at 36 and 72 kg/ha suppressed disease incidence in peanut cv. Florigiant (CBR-susceptible) by 39 and 85% and increased yields by 536 and 545 kg/ha, respectively (Phipps, 1982). In Florida, CBR has been suppressed in peanut by means of post-plant sprays of select sprayable fungicides (e.g. Folicur) at mid-season (Kucharek, 2000). Control from sprays has been slightly erratic, but usually wilt and black pods have been reduced and higher yields occurred. Provost, a combination of the triazole funigicides prothioconazole and tebuconazole, was recently registered for the suppression of CBR, as well as

for control of leaf spots, peanut rust, web blotch, leaf scorch, stem rot, and Rhizoctonia limb rot (Hagan, 2007). The primary activity on CBR comes from prothioconazole which will also labeled as Proline for use as an in furrow treatment (Brenneman and Young, 2007). The target fungi that have displayed increased tolerance or decline in sensitivity to tebuconazole tend to be sensitive to the prothioconazole componenet in Provost.

Resistance screening and resistance breeding. An important component in CBR management is the use of disease resistance. Although only partially resistant cultivars are available, they will have less disease and higher yield than susceptible cultivars when planted in fields with a significant amount of CBR. As early as 1973, results of a 1-year naturally infested field test showed that the spanish type cultivar, Argentine, was more resistant to CBR than the virginia type, Early Runner (Bell et al., 1973). Subsequently, several screening tests for resistance to CBR have been conducted (Wynne et al., 1975; Coffelt, 1980; Coffelt and Garren, 1982; Pataky et al., 1982, 1983c; Green et al., 1983). Results from these tests have shown that spanish type peanut are the least susceptible, valencia type peanuts the most susceptible, and virginia type peanuts are intermediate. However, large differences were observed in the susceptibility of cultivars within each group (Wynne et al., 1975). NC 3033, a virginia type whose pedigree includes spanish types, was considered one of the best resistance sources to CBR (Beute et al., 1976; Phipps and Beute, 1977). It was released in 1976 as the first CBR-resistant peanut germplasm (Beute et al., 1976). Although NC 3033 has a high level of resistance to CBR, it has small seeds and is low yielding. The use of NC 3033 in breeding programs resulted in the release of partially CBR-resistant virginia-type cultivars, 'NC 12C' (Isleib et al., 1997), and 'Perry' (Isleib et al., 2003). The CBR-resistant cultivars 'NC 8C' (Wynne and Beute, 1983) and 'NC 10C' (Wynne et al., 1991) were not developed from NC 3033, but the resistant parent of NC

3033 was contained in their pedigree (Isleib et al., 2001). CBR resistance breeding in runner peanut is behind the progress achieved in virginia peanut, although runner peanut is the major commercial type in the USA. In 2002, Georgia-02C was released as the first runner peanut cultivar with moderate resistance to CBR (Branch, 2003).

Most of the screening tests for resistance to CBR were conducted in naturally infested fields (Wynne et al., 1975; Coffelt, 1980; Coffelt and Garren, 1982; Pataky et al., 1983c; Green et al., 1983). However, microsclerotia are found in nonrandom or clustered pattern in field (Hau et al., 1982; Culbreath et al., 1990), which may affect the evaluation of genotypes because the resistance to *C. parasiticum* depends on inoculum density (Diomand and Beute, 1981a). Hammons et al., (1981) developed two screening methods, employing sterile and nonsterile media, for large-scale systematic screening of peanut genotypes for resistance to CBR in the laboratory. Pataky et al. (1983c) compared greenhouse, microplot, and field methods of evaluating CBR resistance while quantitatively characterizing CBR resistance for peanut breeding lines and commercial cultivars. In greenhouse and microplot evaluations, the substantial differences in CBR resistance among cultivars Florigiant and NC 8C could be observed, but the more subtle difference between two breeding lines, NC 18016 and NC 18229, was not detected. In field evaluations, NC 18016 was observed to be more resistant than NC 18229 when the data were categorized by inoculum density. Black and Beute (1984a) reported that in greenhouse tests, not only inoculum density, but also the size of microsclerotia were related to root rot severity. At the same inoculum density, root rot was more severe for large microsclerotia.

It is necessary in the development of resistant lines to have an accurate but rapid method of rating disease severity in order to screen large numbers of lines. Resistance can be considered to be the ability of the host to inhibit the growth and/or reproduction of the pathogen. For a

monocyclic disease, such as CBR, in which a soilborne fungus survives as microsclerotia, disease resistance can be evaluated from two practical aspects: differences in disease incidence and severity (ie, pathogen growth) and differences in microsclerotial production (ie, pathogen reproduction). Several evaluations of CBR resistance based on disease incidence or severity have been conducted in naturally infested fields (Coffelt, 1980; Coffelt and Garren, 1982; Garren and Coffelt, 1976; Green et al., 1983; Phipps and Beute, 1977; Wynne et al. 1975) and in the greenhouses (Harris and Beute, 1982; Phipps and Beute, 1977; Black and Beute, 1984a), but only three evaluations have considered microsclerotial production (Green et al., 1983; Taylor et al., 1981; Pataky et al., 1983c). The variables of root rot index, number of microsclerotia/g of root, and percentage of aboveground diseased and dead plants, were compared for evaluating CBR severity (Green et al., 1983). Results showed that error components exceeded the mean square value for differences among segregates for the root rot index and microsclerotia/g of root resulting in no significant differences among entries for these traits and low to no correlations among traits. Percent of dead and diseased plants was found to be the best of the three methods for screening peanut lines in the field for CBR resistance.

Generally, field plot data is the most meaningful; however, field evaluations of CBR resistance are not only time consuming, but also generally have large error components in the analysis of variance, due to the spatial pattern of microsclerotia. In greenhouse screening, the inoculum density is more uniform, but results reflect only root and not pod infection. The results of greenhouse trials have not always been highly correlated with field results, so the relationship between greenhouse and field results needs to be further quantified to determine the best method for further evaluation of germplasm for resistance to CBR. The development of more reliable and effective screening methods for evaluations CBR resistance in peanut is needed.

Interactions between C*ylindrocladium parasiticum* and Root-Knot Nematodes on Peanut

It has long been understood that the development of disease symptoms is not solely determined by the pathogen responsible, but is dependent on the complex interrelationship between host, pathogen and prevailing environmental conditions. In the case of soilborne pathogens, further opportunities exist for interactions with other microorganisms occupying the same ecological niche. The significant role of nematodes in the development of diseases caused by soilborne pathogens has been demonstrated in many crops throughout the world (Abdel-Momen and Starr, 1998; De Vay et al., 2001; Rupe et al., 1999; Walker et al., 2000; Wheeler et al., 2000). In many cases, such nematode–fungus disease complexes involve root-knot nematodes (*Meloidogyne* spp.), although several other nematodes (Castillo et al., 1998; Rupe et al., 1999; Vats and Datal, 1997) have been associated with diseases caused by soilborne fungal pathogens.

Several fungus-nematode interaction studies have included peanut or C*ylindrocladium parasiticum*. In a greenhouse study, a disease enhancement interaction was observed between *M. hapla* and *C. parasiticum* on CBR-resistant NC 3033 and CBR-susceptible Florigiant (Diomande and Beute, 1981a). An interaction was also observed between *Criconemella ornata* and *C. parasiticum* but only on Florigiant. Positions, but not the slopes of inoculum density-disease curves were changed by *M. hapla* on both NC 3033 and Florigiant, suggesting that physiological changes and root wounding caused by the nematodes may be important in these interactions (Diomande and Beute, 1981a). This was supported by a subsequent study (Diomande et al., 1981). Diomande et al. (1981) also found that the root rot index of NC 3033 increased in the presence of *M. arenaria* race 2, which is not a pathogen of peanut. In field experiments, positive correlations of *M. hapla* and *C. parasiticum* populations with CBR severity were observed on

both NC 3033 and Florigiant (Diomande and Beute, 1981b). Culbreath et al. (1992a) found that severity of black rot was increased in the CBR-susceptible genotype, Florigiant, by either *M. hapla* or *M. arenaria* with fungal inoculum densities of 0.05 and 0.5 microsclerotia 1 g soil. However, severity of black rot was not affected on genotypes moderately resistant to CBR (NC 10C or NC Ac 18016). In microplots, disease ratings of roots from NC 10C, NC Ac 18416, and NC Ac 18016 were higher in plots infested with either *M. arenaria* or *M. hapla* and *C. parasiticum* than in plots with *C. parasiticum* alone.

Of the nematodes associated with peanuts, *M. arenaria* is the most frequent and most damaging in the southeastern peanut-producing regions of the USA (Dickson, 1998; Ingham and Rodrigue-Kabana, 1980; Koenning et al., 1999; Minton and Baujard, 1990; Wheeler and Starr, 1987). Runner type peanuts have become the dominant peanut type grown in the USA due to the introduction in the early 1970's of the cultivar Florunner. Runners, grown mainly in Georgia, Alabama, Florida, Texas, and Oklahoma, account for 80% of total U.S. production (American Peanut Council, 2007). However, interactions between *M. arenaria* and *C. parasiticum* on runner type peanut have not been documented. In addition, runner peanut cultivars with high resistance to *M. arenaria* are now available. A better understanding of the effects of nematodes on CBR severity for nematode-resistant peanut may help to avoid unexpected losses.

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CHAPTER 2

COMPARISON OF METHODS FOR ASSESSING RESISTANCE TO *MELOIDOGYNE ARENARIA* IN PEANUT[1]

[1]Weibo Dong, C. Corley Holbrook, Patricia Timper, Timothy B. Brenneman, and Benjamin G. Mullinix. 2007. Journal of Nematology 39: 169-175. Reprinted here with permission of publisher, 11/13/2007.

ABSTRACT

Use of resistant cultivars is a desirable approach to manage the peanut root-knot nematode (*Meloidogyne arenaria*). To incorporate resistance into commercially acceptable cultivars requires reliable, efficient screening methods. To optimize the resistance screening protocol, a series of greenhouse tests were done using seven genotypes with three levels of resistance to *M. arenaria*. The three resistance levels could be separated based on gall indices as early as two weeks after inoculation (WAI) using 8,000 eggs of *M. arenaria* per plant, while four or more weeks were needed when 1,000 - 6,000 eggs/plant were used. High inoculum densities (over 8,000 eggs/plant) were needed to separate the three resistance levels based on eggs per gram of root within eight WAI. A gall index based on percentage of galled roots could separate the three resistance levels at lower inoculum levels and earlier harvest dates than other assessment methods. The use of eggs vs. second-stage juveniles (J2) as inoculum provided similar results; however, it took three to five more days to collect J2 than to collect eggs from roots. Plant age affected gall index and nematode reproduction on peanut, especially on the susceptible genotypes AT201 and D098. The genotypes were separated into their correct resistance classes when inoculated 10 to 30 days after planting, but were not separated correctly when inoculated on day 40.

Key words: *Arachis hypogaea,* assessment date, host-plant resistance, inoculation date, inoculum level, inoculum type, *Meloidogyne arenaria*, method, peanut, resistance evaluation, root-knot nematode.

The root-knot nematode *Meloidogyne arenaria* race 1 causes significant economic losses throughout the peanut (*Arachis hypogaea*) production regions of the world. In the southern U.S.

peanut-producing areas (AL, FL, GA, SC and TX), this nematode decreases peanut yield by 3 to 15% annually (Koenning et al., 1999). Management of root-knot nematode can include crop rotation, application of nematicides and use of resistant cultivars. Few profitable rotation crops are available because of the wide host range of *M. arenaria*. Use of nematicides is problematic because of the short-term efficacy (Dickson and Hewlett, 1989; Culbreath et al., 1992) and the cost to growers. There is a need for improved root-knot nematode management strategies, and the use of nematode-resistant peanut cultivars would be an effective and inexpensive approach to prevent yield and quality losses to *M. arenaria*.

Over the past two decades, progress has been made in identification and breeding for nematode resistance in peanut. Several sources of moderate and high resistance have been identified from germplasm of *A. hypogaea* and wild species of peanut (Holbrook and Stalker, 2003). High levels of resistance in wild species have been introgressed into *A. hypogaea*, which led to registration of interspecific germplasm TxAG-6 and TxAG-7 (Simpson et al., 1993), GP-NC WS 5 and GP-NC WS 6 (Stalker et al., 2002) and NR 0812 and NR 0817 (Anderson et al., 2006). A backcrossing program was used to introgress the root-knot nematode resistance from TxAG-7 into peanut breeding populations (Starr et al., 1995) and resulted in the release of COAN and NemaTAM, which are highly resistant to *M. arenaria* and *M. javanica* (Simpson and Starr, 2001; Simpson et al., 2003). However, neither COAN nor NemaTAM has been widely grown by farmers due to their susceptibility to tomato spotted wilt virus (TSWV) and low oleic acid content (Simpson and Starr, 2001; Simpson et al., 2003).

The development of new peanut cultivars with resistance to root-knot nematodes will require reliable and efficient resistance screening techniques for identifying resistant progeny within segregating breeding populations. Greenhouse screening techniques to identify peanut

germplasm with resistance to *M. arenaria* are available (Hussey and Boerma, 1981; Holbrook et al., 1983); however, the current evaluation methods can take up to 100 days before results are available (Holbrook et al., 2000a, 2000b) and are subject to high experimental error (Choi et al., 1999; Zhang et al., 2006). Thus, the objectives of this study were to: (i) determine the effects of inoculum type, inoculum level, inoculation date and assessment date on evaluating *M. arenaria* resistance in peanut, and (ii) optimize the resistance screening protocol used to identify root-knot nematode resistant peanut genotypes in the greenhouse.

MATERIALS AND METHODS

Peanut genotypes: Seven peanut genotypes with different levels of resistance to *M. arenaria* were used in all experiments. The genotypes included two highly resistant cultivars, COAN and NemaTAM; three moderately resistant breeding lines, C209-6-37, C209-6-60 and D099; one susceptible cultivar, AT201; and one susceptible breeding line, D098.

Nematode inocula: Meloidogyne arenaria race 1, originating from a peanut field in Tifton, GA, was cultured alternately on tomato (*Lycopersicon esculentum* cv. Rutgers) or eggplant (*Solanum melongena* cv. Blackbeauty) and peanut (*Arachis hypogaea* cv. Georgia Green). Eggs for inoculum were extracted from tomato or eggplant roots by agitating in 0.05% NaOCl for 2 to 3 min (Hussey and Barker, 1973). The eggs were then collected and rinsed with tap water on nested 150- and 25-µm-pore sieves. To collect the second-stage juveniles (J2) for use as inoculum, infected tomato or eggplant roots were placed in hatching dishes and incubated in a mist chamber. The J2 were then collected using 150- and 25-µm-pore sieves once a day for 3 to 5 d. During the collection period, J2 were stored in a 1-cm aqueous suspension at 5°C prior to inoculation of peanut plants.

Resistance assessment: For all assessment methods, peanut plants were uprooted and washed clean of soil 2 to 10 wk after inoculation (WAI). Criteria used to evaluate resistance levels in peanut were: gall number, gall index 1, gall index 2, egg mass number, egg mass index and egg number per gram root. Gall index 1 was on a scale of 0 to 5 (Taylor and Sasser, 1978), where 0 = no galls; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; and 5 = more than 100 galls. Gall index 2 was also on a scale of 0 to 5, but it was based on the percentage of the root system with galls (Hussey and Janssen, 2002), where 0 = no galling; 1 = trace infection with a few small galls; 2 ≤ 25% roots galled; 3 = 26 to 50%; 4 = 51 to 75%; and 5 > 75% roots galled. For the assessments based on root galling, the numbers of galls were counted, and the root systems were rated using the two indices. The roots were then placed in beakers containing approximately 300 ml of 0.05% phloxine B solution for 3 to 5 min to stain egg masses a bright red color so the number of egg masses per root system could be determined visually (Holbrook et al., 1983). Egg mass index was on a scale of 0 to 5 as described for gall index 1. Fresh root systems were weighed and then agitated in 1% NaOCl solution for 5 min to extract eggs. Eggs were collected on nested 150- and 25-µm-pore sieves and counted. Egg number per gram root was then calculated.

Inoculum level and harvest date: The experiment was a 4 × 7 × 4 factorial arrangement of treatments. There were four peanut genotypes in these experiments, including NemaTAM, C209-6-37, C209-6-60 and AT201. The seven inoculum levels were 0, 1,000, 2,000, 4,000, 6,000, 8,000 and 16,000 eggs/pot. The four assessment dates were 2, 4, 6 and 8 WAI. A split-plot treatment design was used with assessment dates as main plots. Subplots of inoculum level × genotype were randomized within six replicate main plots. Two seeds were planted in each 10 × 10-cm^2 plastic pot filled with 800 cm^3 steam-pasteurized (132°C for 6 hr) loamy sand (texture:

85% sand, 11% silt, 4% clay). After emergence, plants were thinned to 1 seedling/pot. Two holes about 5-cm deep and 1-cm wide were made in the soil around each 2-wk-old peanut seedling. A 2.5 ml aliquot of inoculum suspension was applied to each hole with a pipette. Unless otherwise noted, the plants were maintained in a greenhouse at 20 to 35°C and watered as needed. At harvest dates 1 (2 WAI) and 2 (4 WAI), resistance was assessed by gall number, gall index 1 and gall index 2. At harvest date 3 (6 WAI), gall number, gall index 1, gall index 2 and egg number per gram root were evaluated. At harvest date 4 (8 WAI), two additional variables, egg mass number and egg mass index, were also assessed. The entire experiment was repeated.

Inoculum type: The experiment was a 4 × 2 × 2 factorial arrangement of treatments. Four peanut genotypes, COAN, C209-6-37, C209-6-60 and AT201, were evaluated at two inoculum levels and two harvest dates. The peanut genotypes were grown and inoculated 2 wk after planting with either 2,000 J2 or 8,000 eggs of *M. arenaria* as previously described. A randomized complete block design with six replications was used. Gall index 2 was used to evaluate the resistance level in the selected peanut genotypes 2 WAI, whereas gall index 2 and egg number per gram were used to evaluate resistance 10 WAI. The entire experiment was repeated one time under similar conditions.

Plant age effect: Six peanut genotypes were evaluated: COAN, C209-6-37, C209-6-60, D099, AT201 and D098. The genotypes were planted in 10 × 10-cm^2 plastic pots filled with 800 cm^3 loamy sand/pot (texture: 85% sand, 11% silt, 4% clay) on five dates with 10-d intervals between dates. All plants were inoculated at the same date with 8,000 eggs/pot. The ages of the peanut plants at the time of inoculation were 0 to 40 d after planting (DAP). The experimental design was a split plot, with genotypes randomized within six replicate main plots (planting date). Plants were harvested at 8 WAI. Gall index 2 and egg number per gram root were used to assess resistance. The experiment was repeated one time.

Statistical analysis: Data from the two trials of each experiment were combined for analysis. Data were analyzed using Proc MIXED with ddfm = satterth option (a general Satterthwaite approximation for the denominator degrees of freedom) on the model statement (SAS v.9.1) (SAS Institute, Cary, NC), unless otherwise stated. Any interaction effects that were not significant were removed, and the reduced model evaluated again. Main effects were considered significant when $P \leq 0.05$ and adjusted with any significant interactions. Fisher's least significant difference (LSD) values at $\alpha = 0.05$ were computed using standard error and t values of adjusted degrees of freedom from the LSMEAN statement in Proc MIXED.

RESULTS

Inoculum level and harvest date: Galls on peanut roots were observed at 2 WAI at inoculation levels 1,000 to 16,000 eggs/plant. Eggs were extracted from infected roots at 6 WAI, although egg masses were not obvious until 8 WAI.

Initial inoculum level of *M. arenaria* affected gall index 2 in peanut (Table 2.1). For all four selected genotypes, gall index 2 increased as the inoculum level increased. However, the magnitude of the increase was not the same for all the genotypes (inoculum × genotype interaction, $P < 0.0001$). From 1,000 to 16,000 eggs/plant, the rate of increase in gall index 2 was greater for the susceptible genotype AT201 than for the moderately and highly resistant genotypes. On AT201, 4,000 eggs/plant caused greater ($P \leq 0.05$) gall index 2 than 2,000 eggs/plant did, while 8,000 eggs/plant were needed to cause gall index 2 to be greater than that for 2,000 eggs/plant on NemaTAM ($P \leq 0.05$). Across the harvest dates, the four genotypes could be separated into the appropriate resistance categories using 2,000, 6,000, 8,000 and 16,000 eggs/plant based on gall index 2. Low inoculum level (1,000 eggs/plant) could separate the

susceptible genotype AT201 from others, but it could not separate the highly resistant genotype NemaTAM from the moderately resistant genotypes.

Harvest date affected galling and egg production in peanut roots ($P < 0.0001$); however, there was a significant interaction of harvest date × genotype ($P \leq 0.01$) (Figure 2.1A, B). From 2 to 8 WAI, the increase of gall index 2 was greater for AT201 than for the other three genotypes. The gall indices did not differ between 4 and 6 WAI for AT201, C209-6-37 and C209-6-60, whereas they did for the highly resistant genotype NemaTAM. Eggs were obtained from all four genotypes by 6 WAI, but egg numbers increased dramatically by 8 WAI. The increase of egg number for NemaTAM was much lower than for the moderately resistant and susceptible genotypes.

Among all the combinations of seven inoculum levels × four harvest dates, use of gall index 2 could separate the three resistance levels correctly in 14 combinations (Table 2.2). Based on gall index 2, different resistance levels could be separated successfully as early as 2 WAI at high inoculation levels (8,000 to 16,000 eggs/plant) and could be separated at low inoculation level (1,000 eggs/plant) at the final harvest date (8 WAI). Based on eggs per gram root, the four peanut genotypes with three levels of resistance to *M. arenaria* were separated at the inoculation rate of 16,000 eggs/plant by 6 WAI and at 8,000 and 16,000 eggs/plant by 8 WAI (Table 2.2). At low inoculation levels (1,000 to 6,000 eggs/plant) and an early harvest date (6 WAI), the four genotypes were not separated into their appropriate resistance classification due to the high variability of eggs per gram root.

In addition to gall index 2 and eggs per gram root, gall number, gall index 1, egg mass number and egg mass index were also used to assess the resistance levels in the peanut genotypes. The ability of these assessment methods to accurately separate the different levels of resistance is

summarized in Table 2.3. Gall number could only separate the four genotypes correctly by 4 and 6 WAI at the highest inoculum level. Egg mass number was not adequate to separate the four genotypes in this study, whereas egg mass index was a good measure to discriminate between resistance levels with inoculum levels of 6,000 to 8,000 eggs/plant at 8 WAI. Generally, the higher the inoculum level used, the less time was needed to separate the genotypes correctly based on gall index 1 or 2. Both gall index 1 and 2 were positively correlated ($P < 0.0001$) with eggs per gram root ($r = 0.6047$ and 0.6773, respectively); however, gall index 2 was the most sensitive method of all measures used for assessing resistance. It provided more choices on combinations of inoculum level × harvest date to separate the four genotypes successfully than gall index 1.

Inoculum type: The hatch rate of the eggs used in this test was 24.7% after 6 d (144 hr, data not shown), thus inoculum levels of 8,000 eggs and 2,000 J2 were approximately equivalent. Eight thousand eggs and 2,000 J2 did not result in significant differences in gall index 2 and egg number at the two harvest dates (Table 2.4). The resistance classification was also similar between the two inoculum types. The three resistance levels in the four genotypes were distinguished from each other by 2,000 J2 and 8,000 eggs at 2 and 10 WAI, based on gall index 2 or eggs per gram root, except C209-6-37 was not separated from AT201 by 2,000 J2 at 2 WAI.

The coefficients of variation (CV) of gall index 2 for 2,000 J2 and 8,000 eggs were similar at both 2 and 10 WAI. The CV for eggs per gram root for 2,000 J2 (49.2%) was lower than that for 8,000 eggs (76.2%) at 10 WAI, which suggested that the inoculum of 8,000 eggs showed higher variability than 2,000 juveniles.

Plant age at inoculation: Among the six genotypes tested, D098 and AT201 were susceptible, C209-6-37, C209-6-60 and D099 were moderately resistant and COAN was highly resistant.

Plant age at time of inoculation affected gall development on the six genotypes (Figure 2.2); however, the effects on C209-6-60, D099 and COAN were not as great as on D098 and AT201. Inoculation on d 10 resulted in the highest gall index on all the genotypes except for C209-6-37, which had greatest galling when inoculated on d 20. Inoculation on d 40 could not separate the six genotypes in their correct resistance classifications due to the reduced gall indices of the susceptible genotypes D098 and AT201.

In this experiment, the genotype, DAP and genotype × DAP effects on nematode reproduction (Table 2.5) were significant ($P < 0.05$). The eggs per gram root for all the genotypes decreased on inoculation d 40 from the peak. In contrast, inoculation at 10, 20 and 30 DAP resulted in lower ($P \leq 0.05$) reproduction on the highly resistant genotype COAN than on the susceptible genotypes AT201 and D098. However, such differences between moderately resistant and highly resistant or between moderately resistant and susceptible genotypes were not always apparent (Table 2.5). The six peanut genotypes could not be separated into their appropriate resistance categories with inoculation at 0 and 40 DAP. Nematode reproduction was not different on the susceptible genotype D098 and the highly resistant genotype COAN at these two inoculation dates.

DISCUSSION

In plant nematology, resistance is used to describe the ability of a plant to suppress development or reproduction of the nematode (Roberts, 2002). For root-knot nematodes, the symptoms can be evaluated with as sufficient ease, accuracy and precision as for some fungal diseases, such as leaf spot and rust. Therefore, the term resistance is also used to describe the capacity of a host to suppress the disease (Sasser et al., 1984; Roberts, 2002) as in general plant

pathology. Peanut breeders, geneticists and nematologists have evaluated peanut genotypes for root-knot nematode resistance based on indices of root galling and/or egg mass production (Holbrook et al., 1983, 2000a, 2000b; Timper et al., 2000) or egg counts (Abdel-Momen et al., 1998; Choi et al., 1999). Others also have used gall counts to evaluate resistance to root-knot nematodes in plants (Harris et al., 2003). Gall number and the degree of galling may be used to reflect the ability of a plant to lessen or overcome the attack by the root-knot nematode. However, they do not indicate nematode reproduction directly, while egg mass number, egg mass index and egg number per gram root do.

In our experiments, numerous eggs were collected from the root before egg masses became readily visible to the naked eye. Use of eggs per gram root also separated the three resistance levels correctly in more combinations of inoculum level × harvest date than the use of egg masses. Therefore, we agree with Luzzi et al. (1987) that, for advanced breeding lines, the quantitative data on egg numbers will give a better indication of root-knot nematode resistance than egg mass numbers. In comparison with gall number or gall index 1 (based on gall number), gall index 2 (based on percentage of infested root) was more robust, in that it separated the genotypes into their appropriate resistance categories. Additionally, it was time-consuming and difficult to count the galls at later harvest dates, since galls are usually conjunct. Therefore, we consider gall index 2 to be a better indicator of the resistance level than gall index 1 and gall number.

Harvest date had significant effects on galling and egg production in peanut roots. Galls on roots were visible two weeks after inoculation, and gall index 2 could be successfully used to separate the different resistance levels in the selected peanut genotypes at that time. The different levels of resistance in this study were not correctly separated by eggs per gram root until eight

weeks after inoculation with 8,000 eggs/plant, although nematode eggs in peanut roots have been observed as early as 22 days after inoculation (Timper et al., 2000). This is likely due to the high variability in egg numbers at the earlier harvest dates. Based on these observations, we concluded that 8 WAI is necessary to detect differences in the ability of peanut genotypes to restrict nematode reproduction. Temperature has significant influences on penetration, development and reproduction of nematodes (Noe, 1991). Degree-days would have been more accurate than days after inoculation for determining resistance in plants, especially for early assessment dates. During these experiments, the temperature was at 20 to 35°C in the greenhouse, which is the optimum temperature for nematode infection and development. The soil temperature was recorded by a temperature recorder. The degree-days, which used 12.2°C as the threshold temperature (Trudgill and Perry, 1994), were 150 and 695 at 2 and 8 WAI, respectively (data not shown).

The size of galls as well as the number of galls is related to the number of nematodes infecting roots, although the inoculum concentration may have less effect at later evaluation stages (Abdel-Momen et al., 1998; Vovlas et al., 2005). Our results demonstrated that the later the harvest date, the lower was the inoculum level needed to separate the different levels of resistance. Based on gall index 2, the initial inoculum level could be as low as 1,000 eggs/plant to separate the three resistance levels at 8 WAI, or 8,000 to 16,000 eggs/plant could be used to separate the genotypes as early as 2 WAI. Therefore, if a rapid evaluation is required, higher inoculum levels can be used to achieve reliable results, and, if inoculum is a limiting factor, the screening period can be extended. However, to confirm the resistance by egg production level, over 8,000 eggs/plant as initial inoculum and eight weeks from inoculation to harvest are still needed.

J2, intact egg masses or egg suspensions can be used as inoculum for resistance screening tests (Hussey and Janssen, 2002). Intact egg masses are typically not used for inoculum because they are difficult to collect, quantify and disperse in the soil. Only J2 and egg suspensions were compared in our experiments, and both produced similar results. No significant differences in gall index and nematode reproduction were found at 2 and 10 WAI assessments using either type of inoculum. Compared with egg inoculum, no advantages were seen with J2 inoculum. However, three to five additional days were needed to collect the juveniles in the mist chamber.

Infection by root-knot nematodes begins with penetration of the roots by the J2 at the zone of elongation. In small pot tests, root growth is limited at later plant growth stages, which may reduce availability of suitable penetration sites. Our results showed that later inoculation (40 days after planting) resulted in fewer galls and less egg production on peanut, especially on the susceptible genotypes. This reduced the ability to separate susceptible and moderately resistant genotypes.

In summary, we showed that a gall index based on percentage of the root system with galls was a reliable indicator of the level of resistance on early harvest dates (as early as two weeks) after inoculation with 8,000 or more eggs/plant during 10 to 30 days after planting. If the nematode population is the limiting factor, as few as 1,000 eggs/plant could be used to separate the different levels of resistance on late harvest dates (8 WAI) either based on the gall index or eggs per gram root. This is important because we have identified a rapid method for assessing resistance in peanut genotypes. The selected genotypes could then be assessed for eggs per gram root at eight weeks after inoculation with 8,000 eggs/plant to verify the resistance level based on egg production.

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Table 2.1. Effect of inoculum level of *Meloidogyne arenaria* from 0 to 16,000 eggs/plant on gall index 2[a] on four peanut genotypes when tested in two greenhouse trials.

Inoculum	Peanut genotypes											
	AT201			C209-6-37			C209-6-60			NemaTAM		
0	0.03[b]	a[c]	k[d]	0.07	a	k	0.00	a	k	0.00	a	k
1,000	1.66	b	k	0.40	ab	l	0.62	b	l	0.18	ab	l
2,000	1.92	b	k	0.86	b	l	0.90	b	l	0.34	abc	m
4,000	2.79	c	k	1.35	c	l	1.41	c	l	0.63	bcd	m
6,000	3.00	c	k	1.48	c	l	1.42	c	l	0.69	cd	m
8,000	3.51	d	k	1.70	c	l	1.79	c	l	1.08	de	m
16,000	3.99	e	k	2.30	d	l	2.28	d	l	1.47	e	m

[a] Gall index 2: 0 = no galls, 1 = trace infection with a few small galls, 2 ≤ 25% roots galled, 3 = 26-50%, 4 = 51-75%, and 5 > 75% of root galled.

[b] Data presented are means of 12 replications (six replications/trial) combined across harvest dates (2, 4, 6 and 8 wk after inoculation).

[c] Numbers within columns followed by different letters (a-e) are significantly different ($P \leq 0.05$).

[d] Numbers within rows followed by different letters (k-m) are significantly different ($P \leq 0.05$).

Table 2.2. Gall index 2 and number of eggs per gram root on four genotypes of peanut using seven inoculum levels of eggs of *Meloidogyne arenaria* at four harvest dates when tested in two greenhouse trials.

Criterion	Harvest date	Genotype	Inoculum level						
			0	1,000	2,000	4,000	6,000	8,000	16,000
Gall index 2[a]	2 WAI	AT201	0[b] a[c]	0.83 a	1.17 a	1.83 a	1.92 a	2.50 a	3.08 a
		C209-6-37	0 a	0.17 b	0.50 bc	0.58 b	1.00 b	1.25 b	1.75 b
		C209-6-60	0 a	0.25 b	0.67 ab	1.00 b	0.83 bc	1.25 b	1.50 b
		NemaTAM	0 a	0 b	0 c	0.42 b	0.30 c	0.42 c	0.50 c
	4 WAI	AT201	0 a	1.64 a	1.91 a	3.00 a	3.09 a	3.82 a	3.91 a
		C209-6-37	0 a	0.25 bc	0.55 bc	1.55 b	1.45 b	1.91 b	2.45 b
		C209-6-60	0 a	0.64 b	0.91 b	1.45 b	1.64 b	2.18 b	2.64 b
		NemaTAM	0 a	0.18 c	0.17 c	0.5 c	0.42 c	1.17 c	0.83 c
	6 WAI	AT201	0 a	2.00 a	2.25 a	3.33 a	3.56 a	3.75 a	4.08 a
		C209-6-37	0 a	0.92 b	1.00 b	1.82 b	2.08 b	2.75 b	3.18 b
		C209-6-60	0 a	0.67 bc	1.42 b	1.50 b	2.25 b	2.58 b	3.58 b
		NemaTAM	0 a	0.25 c	0.83 b	0.83 c	1.08 c	1.91 c	1.92 c
	8 WAI	AT201	0 a	3.17 a	3.58 a	3.91 a	4.08 a	4.25 a	4.75 a
		C209-6-37	0 a	1.55 b	2.00 b	1.73 b	2.27 b	2.82 b	3.18 b
		C209-6-60	0 a	1.08 b	1.67 b	1.33 b	2.08 b	2.67 b	3.00 bc
		NemaTAM	0 a	0.36 c	0.67 c	1.25 b	1.25 c	1.64 c	2.42 c

Table 2.2 continued

Egg/g root	6 WAI	AT201	0	a	303	a	482	a	711	a	1,002	a	2,395	a	3,744	a
		C209-6-37	0	a	92	b	183	b	296	b	380	b	547	b	702	b
		C209-6-60	0	a	49	b	70	b	146	bc	185	bc	329	b	599	b
		NemaTAM	0	a	7	b	2	b	7	c	19	c	29	b	34	c
	8 WAI	AT201	4.9	a	3,183	a	3,778	a	6,499	a	9,847	a	9,660	a	10,402	a
		C209-6-37	1.5	a	673	b	1,118	b	1,732	b	2,521	b	3,255	b	3,723	b
		C209-6-60	0	a	947	b	1,873	ab	1,344	b	2,191	b	3,205	b	3,315	b
		NemaTAM	0	a	46	b	52	b	52	b	83	b	121	c	123	c

[a] Gall index 2: 0 = no galls, 1 = trace infection with a few small galls, 2 ≤ 25% roots galled, 3 = 26-50%, 4 = 51-75% and 5 > 75% of root galled.

[b] Data presented are means of 12 replications (six replications/trial).

[c] Numbers in columns within the same harvest date followed by different letters are significantly different ($P \leq 0.05$) based on Fisher's LSD test.

Table 2.3. Summary of the evaluation results under seven inoculum levels of *Meloidogyne arenaria* at four harvest dates by six assessment criteria when tested in two greenhouse trials.

Criterion	Harvest date	Inoculum level eggs/plant						
		0	1,000	2,000	4,000	6,000	8,000	16,000
Gall number	2 WAI	N[a]	N	N	N	N	N	N
	4 WAI	N	N	N	N	N	N	Y
	6 WAI	N	N	N	N	N	N	Y
	8 WAI	N	N	N	N	N	N	N
Gall index 1[b]	2 WAI	N	N	N	N	N	Y	Y
	4 WAI	N	N	N	Y	Y	Y	Y
	6 WAI	N	Y	N	N	Y	Y	Y
	8 WAI	N	N	N	N	Y	Y	Y
Gall index 2[c]	2 WAI	N	N	N	N	N	Y	Y
	4 WAI	N	N	N	Y	Y	Y	Y
	6 WAI	N	N	N	Y	Y	Y	Y
	8 WAI	N	Y	Y	N	Y	Y	N
Eggs/g root	6 WAI	N	N	N	N	N	N	Y
	8 WAI	N	N	N	N	N	Y	Y
Mass number	8 WAI	N	N	N	N	N	N	N
Mass index[d]	8 WAI	N	N	N	N	Y	Y	N

[a] Y: The four peanut genotypes AT201 (susceptible to *M. arenaria*), C209-6-37, C209-6-60 (moderately resistant to *M. arenaria*), and NemaTAM (highly resistant to *M. arenaria*) were separated in their appropriate resistance categories in the combination of inoculum level × harvest date; N: The four genotypes were not separated in their appropriate resistance categories in the combination of inoculum level × harvest date.

[b] Gall index 1: 0 = no galls; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; and 5 = more than 100 galls.

[c] Gall index 2: 0 = no galls, 1 = trace infection with a few small galls, 2 ≤ 25% roots galled, 3 = 26-50%, 4 = 51-75%, and 5 >75% of root galled.

[d] Mass index: 0 = no egg masses; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; and 5 = more than 100 egg masses.

Table 2.4. Gall index 2[a] and eggs per gram root of four peanut genotypes inoculated either with *Meloidogyne arenaria* eggs (E) or juveniles (J2).

Genotype	2 WAI Gall index 2		10 WAI Gall index 2		10 WAI eggs/g root	
	2,000 J2	8,000 E	2,000 J2	8,000 E	2,000 J2	8,000 E
AT201	2.92[b] a[c]	3.45 a	4.89 a	4.40 a	3,426 a	3,419 a
C209-6-37	2.40 ab	2.00 b	2.40 b	3.18 b	304 b	1,132 b
C209-6-60	2.08 b	1.64 b	2.36 b	2.60 b	338 b	1,102 b
COAN	0.09 c	0.27 c	0.67 c	0.75 c	10 c	53 c
Mean[d]	1.87	1.84	2.58	2.73	1,018	1,427
CV%	48.1	57.4	41.3	37.1	49.2	76.4

[a] Gall index 2: 0 = no galling, 1 = trace infection with a few small galls, 2 ≤ 25% roots galled, 3 = 25-50%, 4 = 51-75%, and 5 > 75% of roots galled.

[b] Data presented are means of 12 replications (six replications/trial).

[c] Values for AT201, C209-6-37, C209-6-60 and COAN in each column followed by the same letter do not differ significantly ($P > 0.05$).

[d] Means across all genotypes, no significant differences between any pair of the means wihin on harvest date and the same critierion.

Table 2.5. Effects of plant age at inoculation on reproduction (eggs/g root) of *Meloidogyne arenaria*[a] in six peanut genotypes with different levels of resistance to *M. arenaria* when tested in two greenhouse trials.

Genotype	0 DAP[b]			10 DAP			20 DAP			30 DAP			40 DAP		
D098	2,525[c]	b[d]	m	3,134	a	ml	4,002	a	kl	7,115	a	k	2,186	ab	m
AT201	7,383	a	k	3,287	a	k	4,469	a	k	5,579	a	k	3,016	a	k
C209-6-37	917	b	l	3,044	a	k	2,199	ab	kl	2,742	b	k	1,480	ab	l
C209-6-60	1,480	b	k	1,501	ab	k	1,945	ab	k	2,626	b	k	1,854	ab	k
D099	629	b	k	783	b	k	695	b	k	845	bc	k	729	b	k
COAN	202	b	k	444	b	k	539	b	k	201	c	k	177	b	k

[a] Inoculation level was 8,000 eggs/plant.

[b] Days after planting.

[c] Results of eggs/g root are the means of 12 replications (six replications/trial).

[d] Values in each column followed by the same letter (a – f) and values in each row followed by the same letter (k – m) do not differ significantly ($P > 0.05$) by Fisher's least significant difference (LSD) test.

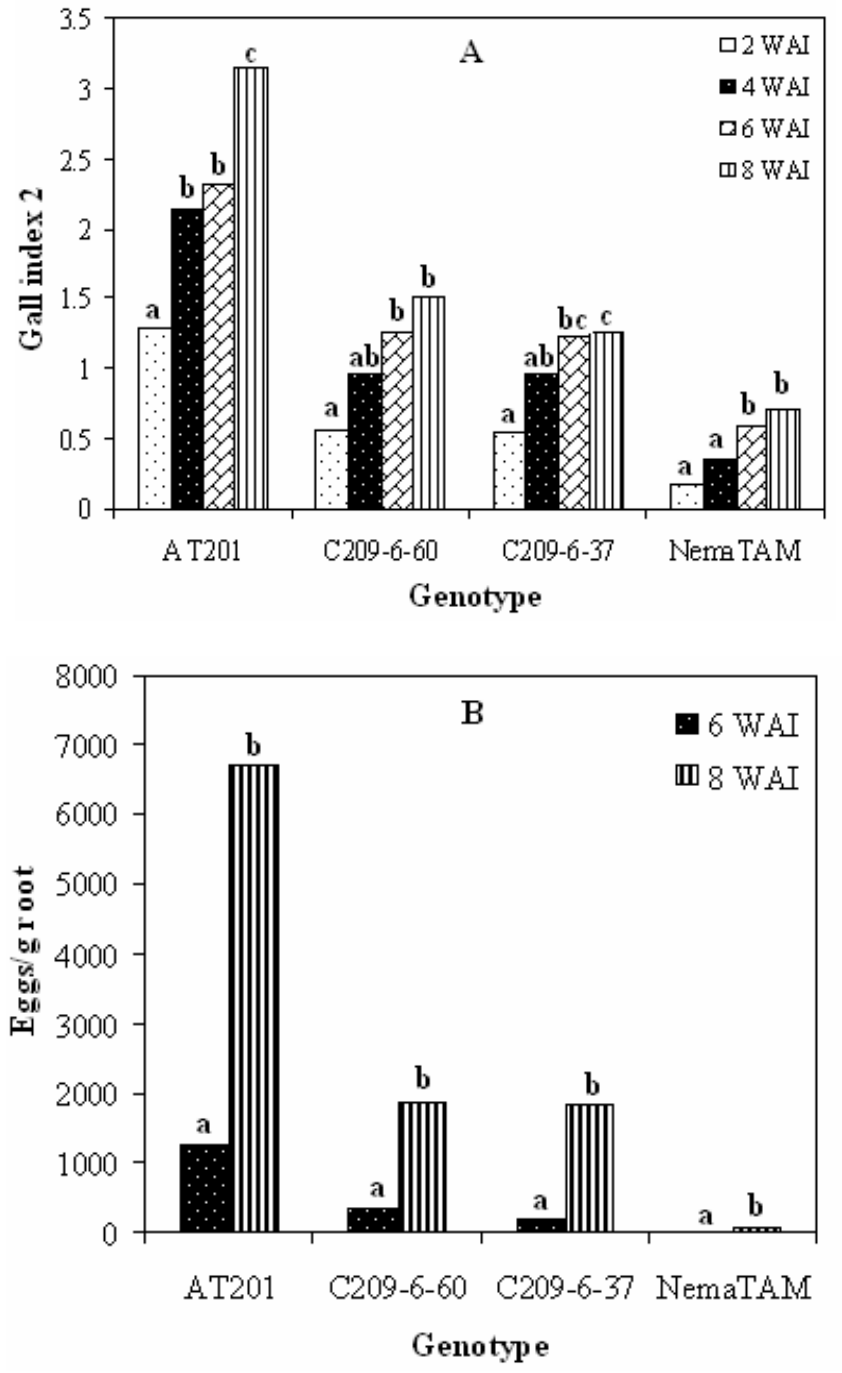

Figure 2.1. Effect of harvest dates on root galling (A) and egg production (B) in different peanut genotypes. Gall index 2: 0 = no galling, 1 = trace infection with a few small galls, 2 ≤ 25% roots galled, 3 = 25-50%, 4 = 51-75% and 5 > 75% of root galled. Bars within a genotype with the same letter are not significantly different ($P > 0.05$).

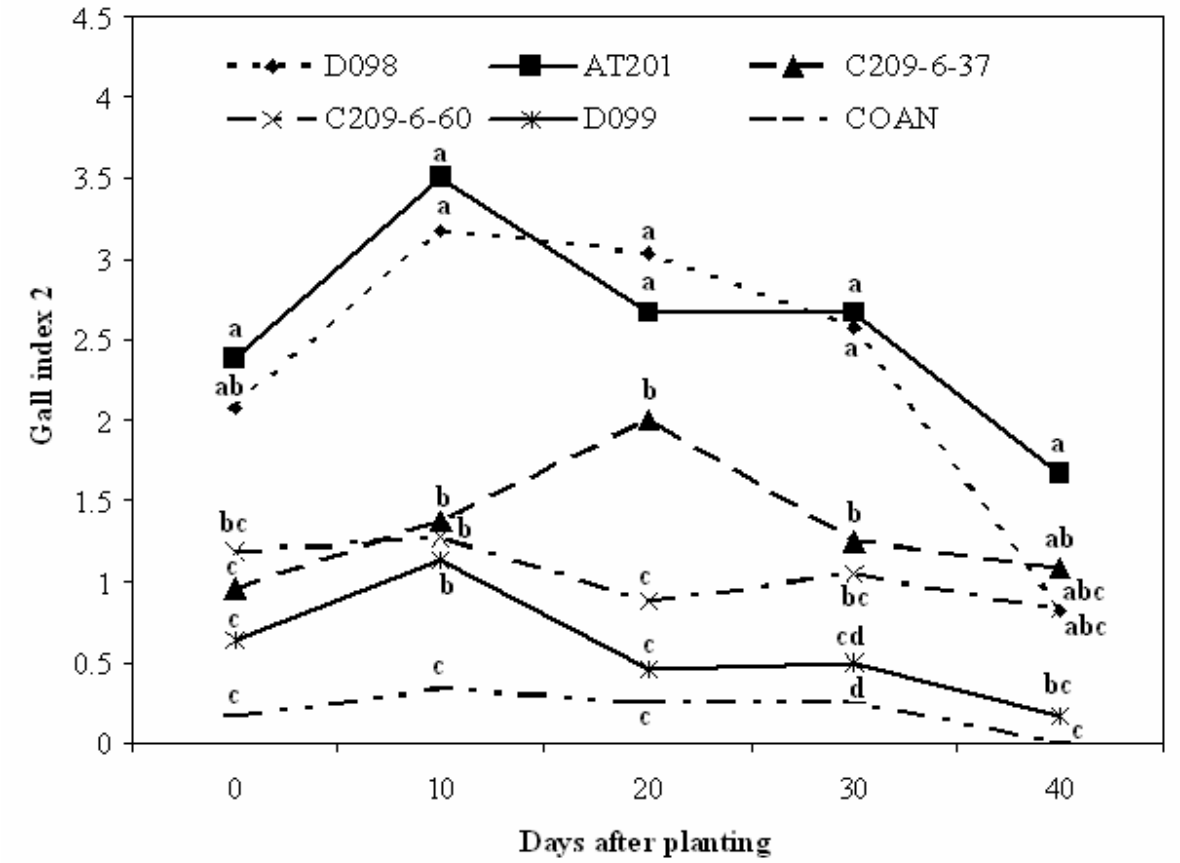

Figure 2.2. Root galling on six peanut genotypes inoculated with *Meloidogyne arenaria* infection at different days after planting (DAP). Gall index 2: 0 = no galling, 1 = trace infection with a few small galls, 2 ≤ 25% roots galled, 3 = 25-50%, 4 = 51-75% and 5 > 75% of root galled. For each genotype, gall index 2 at each date interval that differ ($P \leq 0.05$) according to Fisher's least significant difference (LSD) test are indicated by different letters around the symbols.

APPENDIX TO CHAPTER 2

Table 2A.1. Comparison of the results from the quick screening method and the traditional method

Entry	Quick method		Traditional method	
	Gall index 2	Resistance level[a]	Egg mass	Resistance level[b]
C686-1-2	3.3	S	1.3	MR
C686-1-4	3.5	S	3.3	S
C686-1-7	3.6	S	2.8	MR
C686-1-8	2.6	MR	1.3	MR
C686-4-1	3.0	S	1.3	MR
C686-4-4	1.8	MR	2.6	MR
C686-5-7	4.4	S	3.6	S
C689-19-11	2.2	MR	1.8	MR
C689-19-4	2.0	MR	1.0	MR
C689-19-6	3.2	MR	1.4	MR
C689-32-11	2.5	MR	2.0	MR
C689-32-23	1.4	MR	1.4	MR
C689-32-24	1.4	MR	0.8	HR
C689-32-5	2.5	MR	1.0	MR
C689-6-6	0.3	HR	0.5	HR
C724-19-1	3.4	S	3.8	S
C724-19-10	2.0	MR	2.0	MR
C724-19-11	2.2	MR	2.4	MR
C724-19-12	3.2	S	1.2	MR
C724-19-15	2.8	MR	1.8	MR
C724-19-18	3.2	MR	1.4	MR
C724-19-25	4.0	S	4.0	S
C724-19-5	3.3	S	3.3	S
C724-19-9	2.4	MR	2.6	MR
C724-25-12	1.8	MR	0.8	HR

Table 2A.1 continued

C724-25-15	0.5	HR	0.8	HR
C724-25-17	0.0	HR	1.0	MR
C724-25-21	1.6	MR	1.2	MR
C724-25-28	0.8	HR	1.0	MR
C724-25-30	2.5	MR	2.3	MR
C724-25-4	1.4	MR	0.5	HR
C724-25-8	1.5	MR	2.0	MR
C724-31-12	1.8	MR	2.3	MR
C724-31-20	2.3	MR	2.3	MR
C724-31-26	1.2	MR	2.6	MR
C732-1-7-3	2.0	MR	1.3	MR
C732-1-7-5	2.8	MR	0.8	HR
COAN	0.5	HR	1.5	MR
Georgia Green	4.0	S	4.0	S

[a] in Quick method: S; gall index 2 ≤ 1; MH: 1 < gall index 2 ≤ 3; HR: gall index 2 > 3.

[b] in Traditional method: S: egg mass ≤ 1; MH: 1 < egg mass ≤ 3; HR: egg mass > 3.

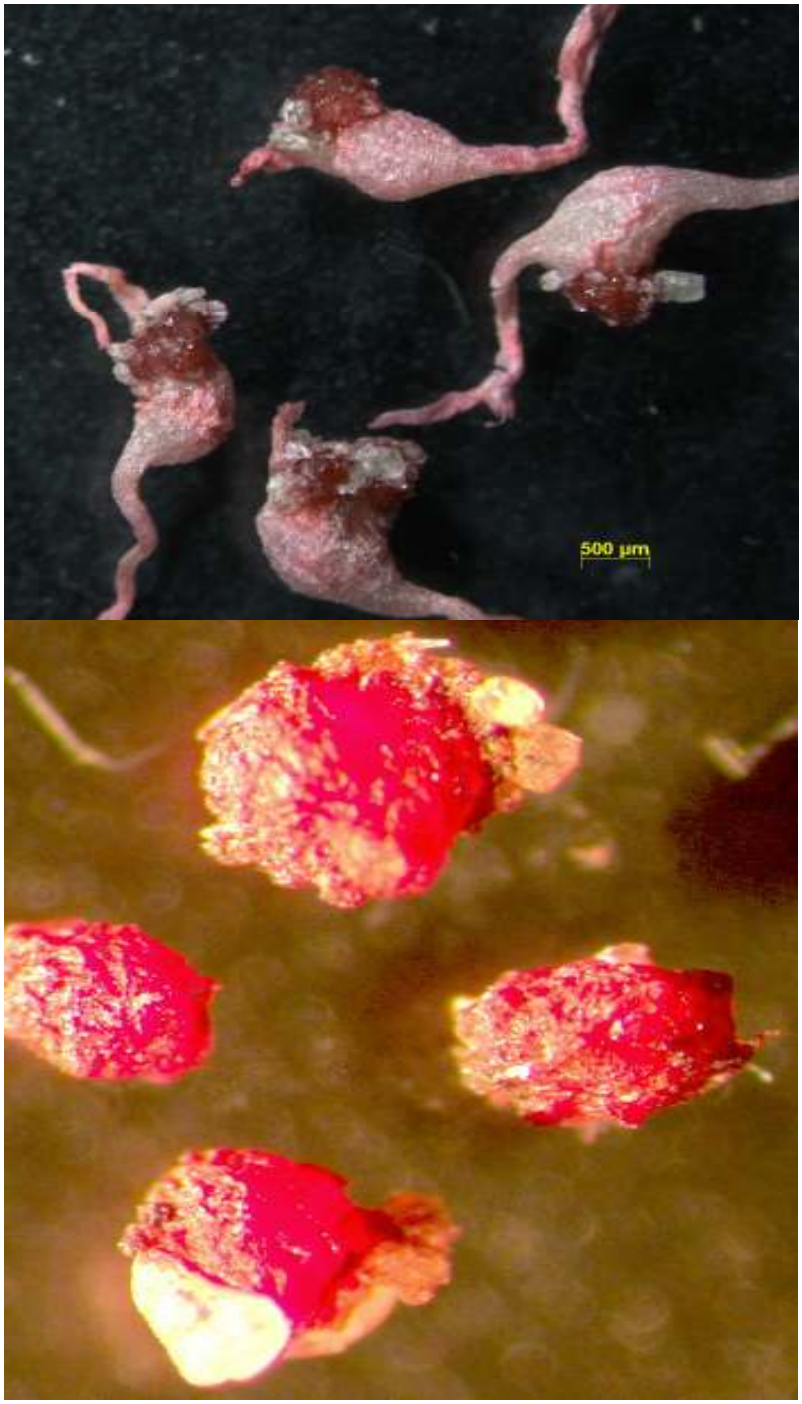

Figure 2A.1. Egg masses of *Meloidogyne arenaria* on tomato (upper) and peanut (lower) root.

CHAPTER 3

RESISTANCE IN PEANUT CULTIVARS AND BREEDING LINES TO THREE ROOT-KNOT NEMATODE SPECIES[1]

[1]W. B. Dong, C. C. Holbrook, P. Timper, T. B. Brenneman, Y. Chu, and P. Ozias-Akins. Submitted to the Plant Disease, 11/20/ 2007. In review.

ABSTRACT

Three major species of root-knot nematode infect peanut: *Meloidogyne arenaria* race 1 (Ma), *M. hapla* (Mh), and *M. javanica* race 3 (Mj). Sources of resistance to all three nematodes are needed for developing novel peanut cultivars with broad resistance to *Meloidogyne* spp. Cultivars and breeding lines of peanut were evaluated for resistance to Ma, Mh, and Mj in the greenhouse and in the laboratory. Twenty-six genotypes with some resistance to Ma, Mj, or Mh were identified from 60 accessions based on average eggs/g root and gall index relative to a susceptible control. Among these, fourteen genotypes were moderately to highly resistant to all three species, five genotypes were resistant to Ma and Mj, two genotypes were resistant to Mj and Mh, one genotype was resistant Ma alone, and four genotypes were resistant to Mh alone. Reproduction of Ma on lines NR 0817, C724-19-11, and D108 was highly variable indicating that these genotypes were likely heterogeneous for resistance. COAN, NemaTAM, C724-25-8, and the Ma-resistant plants of C724-19-11 contained the dominant SCAR marker (197/909) for nematode resistance. Results with the molecular markers indicate that the high resistance to Ma in GP-NC WS 6 may be different from the resistance in COAN, NemaTAM, and C724-25-8. Resistance to Ma was correlated with resistance to Mj in peanut, whereas resistance to Mh was not correlated with the resistance to either Ma or Mj. The resistant selections should be valuable sources for pyramiding resistance genes to develop new cultivars with broad and durable resistance to *Meloidogyne* spp.

Additional keywords: *Arachis hypogaea,* greenhouse screening, molecular marker

Root-knot nematodes (*Meloidogyne* spp.) are among the most serious plant pests in the world. Several species of root-knot nematodes are pathogenic on peanut (*Arachis hypogaea* L.)

and cause considerable yield loss annually. Of these, *M. arenaria* race 1 (Neal) Chitwood, *M. hapla* Chitwood, and *M. javanica* race 3 (Treub) Chitwood are the major pathogenic species of peanut (Abdel-Momen and Starr, 1997; Minton and Baujard, 1990). These three species are known to occur in many peanut-producing regions, including North, Central and South America, Africa, Asia, Europe, and Australia (Sasser, 1980; Song et al., 1992). *Meloidogyne arenaria* and *M. javanica* are common in warm peanut-growing regions whereas *M. hapla* occurs mainly in cool regions. In the U. S., *M. arenaria* and *M. hapla* exist throughout the peanut-producing areas. *Meloidogyne arenaria* is the predominant species parasitizing peanut in the southern regions, especially in Alabama, Florida, Georgia, Texas, and South Carolina, where up to 40% of the fields are infested and yield losses in heavily infested fields can exceed 30% (Koenning et al., 1999; Minton and Baujard, 1990; Wheeler and Starr, 1987). All three species may cause significant losses in yield and quality of peanut (Abdel-Momen and Starr, 1997). *Meloidogyne hapla* is the most prevalent species in more northerly states, including North Carolina, Virginia, and Oklahoma (Anon, 1987; Koenning and Barker, 1992). Populations of *M. javanica* parasitic on peanut are common in Egypt (Tomaszewski et al., 1994) and India (Sharma et al., 1995), but they are rare in the U. S., having been described only from a few fields in Florida, Georgia, and Texas (Lima et al, 2002; Minton and Baujard, 1990; Wheeler and Starr, 1987).

Developing cultivars with host resistance to nematodes, which can be defined as the suppression of nematode reproduction by the resistant plant relative to reproduction on a susceptible genotype of the same species (Williamson and Hussey, 1996), is a desirable approach to manage nematodes. Many sources of moderate resistance to *M. arenaria* have been identified from *A. hypogaea* in the U. S. germplasm collection (Holbrook and Noe, 1992; Holbrook et al., 2000a). High levels of resistance to *M. arenaria* exist in *Arachis* spp. (Holbrook and Noe, 1990;

Nelson et al., 1989), and resistance has been introgressed into *A. hypogaea*. Currently, there are six registered interspecific germplasm lines with resistance to *M. arenaria*: TxAG-6 and TxAG-7 (Simpson et al., 1993), GP-NC WS 5, GP-NC WS 6 (Stalker et al., 2002), and NR 0812 and NR 0817 (Anderson et al., 2006). A backcrossing program was used to introgress the root-knot nematode resistance from TxAG-7 into peanut breeding populations (Starr et al., 1995). This work resulted in the release of cultivars COAN and NemaTAM, which are highly resistant to *M. arenaria* and *M. javanica* (Simpson and Starr, 2001; Simpson et al., 2003). The resistance in the two cultivars is governed by a single dominant gene (Choi et al., 1999; Starr et al., 1990). However, neither COAN nor NemaTAM has been widely used in agricultural practice due to the low yield potentials relative to the recurrent parent Florunner in noninfested fields (Starr et al., 1999) and high susceptibility to tomato spotted wilt virus (TSWV) (Holbrook et al., 2000b). No peanut cultivars or interspecific germplasm have high levels of resistance to *M. hapla* (Timper et al., 2003), although resistance to *M. hapla* has been identified in *Arachis hypogaea* and related species (Castillo et al., 1973; Dong et al., 2001; Subramanyam et al., 1983).

Planting cultivars with currently available sources of nematode resistance may be effective in managing *M. arenaria* and *M. javanica*; but the presence of *M. hapla* throughout the peanut-growing region raises concerns about durability of resistance. Planting cultivars with resistance genes to *M. arenaria* and *M. javanica* may provide a competitive advantage to *M. hapla* and lead to a species shift. In an analogous situation, planting potato cultivars with resistance only to *Globodera rostochiensis* led to a rapid increase in *G. pallida*, a species unaffected by the resistance (Cook and Evans, 1987). Reliance on a single gene for resistance to nematodes can also lead to selection of virulent biotypes. Several populations of *M. incognita* have recently been identified as virulent on tomato with *Mi* in regions where tomato is a major crop (Eddaoudi et al., 1997; Kaloshian et al., 1996). Additional sources of resistance to root-knot

nematodes in peanut are needed to develop new cultivars with broad and durable resistance to *Meloidogyne* spp.

In order to expedite breeding for nematode resistance, several molecular markers have been developed (Burow et al., 1996; Chu et al., 2007; Church et al., 2000; Garcia et al., 1996). One RAPD Marker Z3/265 was developed from an F_2 population of GA6 (*A. hypogaea* (PI261942) x *A. cardenasii* Krapov. & W.C.Gregory) backcrossed with PI261942 (Garcia et al., 1996). A 265bp fragment derived from *A. cardenasii* was linked at 10±2.5 cM and 14±2.9 cM from the putative nematode resistance genes *Mag* and *Mae*, respectively. It was successfully converted into a SCAR (sequence characterized amplified region) marker (Garcia et al., 1996). Choi et al. (Choi et al., 1999) found that the single dominant resistance gene in COAN was linked to RFLP markers R2430E and R2545E. Burow et al. (1996) identified three RAPD markers (RKN 229, RKN 410, and RKN 440) linked to *M. arenaria* resistance in several breeding populations derived from TxAG-7 in the fifth backcross generation. Marker RKN 440 was identified in the backcross population with a 5.8 ± 2.1% recombination rate with the resistance gene, derived from either *A. cardenasii* or *A. diogoi* Hoehne. Based on the sequence of the RAPD fragment that originated from COAN, a new SCAR marker 197/909 was recently developed (Chu et al., 2007). This SCAR marker amplifies fragments from both susceptible and resistant plants, but of different molecular weights, thus avoiding false negative classifications caused by failed reactions with dominant markers.

The objectives of this study were to 1) use greenhouse screening methods and molecular markers to identify new resistance sources to *M. arenaria* (Ma), *M. javanica* (Mj), and *M. hapla* (Mh); and 2) determine the correlations between the resistances to different species of *Meloidogyne*.

MATERIALS AND METHODS

Peanut genotypes. A total of 60 peanut cultivars and breeding lines were evaluated in this study. These include nini cultivars from China, nine cultivars from the U.S., twelve and five breeding lines from China and the U.S., repectively, four released germplasm lines from the U.S., and twenty-one selections from Chinese peanut germplasm and local cultivars (Table 3.1). Of these, five accessions were reported to have moderate resistance to *M. hapla* (D009, D029, D031, D040, and D099) (Dong et al., 2001; Song et al., 1995); eight accessions [NR 0817 (Anderson et al., 2006), GP-NC WS 5, GP-NC WS 6 (Stalker et al., 2002), COAN (Simpson and Starr, 2001), NemaTAM (Simpson et al., 2003), C209-6-37 (Holbrook et al., 2003), C724-19-11, C724-25-8 (Holbrook, *unpublished*)] had high or moderate resistance to *M. arenaria*; and COAN (Simpson and Starr, 2001), and NemaTAM (Simpson et al., 2003) were also reported to be resistant to *M. javanica*.

Nematode inoculum. One isolate each of *M. arenaria, M. hapla,* and *M. javanica* was used to evaluate the peanut accessions for resistance. The isolate of *M. arenaria* originated from a peanut field in Tift County, GA. *Meloidogyne javanica* and *M. hapla* were isolated from peanut in Texas. The nematodes were cultured alternately on tomato (*Lycopersicon esculentum* cv. Rutgers) or eggplant (*Solanum melongena* cv. Blackbeauty) and peanut cv. Georgia Green. Eggs used for inoculum were extracted from roots of tomato or eggplant by use of 0.05% NaOCl in water (Hussey and Barker, 1973). Species identity of the isolates was confirmed by isozyme phenotyping and by a host differential test (Robert et al., 1996).

Greenhouse resistance screening. The peanut genotypes were evaluated for resistance to *M. arenaria*, *M. hapla*, and *M. javanica* in three separate experiments in a greenhouse with six replicates in each trial. Two seeds were planted in each 10 × 10cm square pot filled with

steam-pastuerized (steam heated at 100°C for 6 hr) sandy soil (texture: 85% sand, 11% silt, 4% clay) and thinned to one plant per pot after germination. Eight thousand nematode eggs were distributed into two holes (3-cm deep) at the base of each plant 2 week after planting and covered with soil. In the greenhouse, soil temperatures varied between 20°C and 35°C, every experiment was arranged as a randomized complete block design on a bench.

Peanut plants were uprooted and washed clean of soil 60 days after inoculation. Each plant was assessed for root galling based on the following index: 0 = no galling, 1 = trace infection with a few small galls, 2 ≤ 25% roots galled, 3 = 25-50%, 4 = 51-75, 5 ≥ 75% of root galled. Roots were then cut into ca. 5-cm pieces, weighed, and agitated in 1% NaOCl solution for 5 min. Eggs were collected and rinsed with tap water on nested 150- and 25-µm-pore sieves, and a subsample was counted under 10 x magnification with an inverted microscope. The genotypes selected as resistant to nematodes, based on either gall index or egg number per gram root and the susceptible control (Georgia Green) were re-evaluated at least one more time to confirm the resistance.

Gall index and egg number data were analyzed with the PROC GLM procedure of SAS (version 9.1; SAS Institute, Inc., Cary, NC). Means for each genotype within one nematode population were compared using Fisher's protected least significant difference test at $P \leq 0.05$. The correlation coefficients of resistance to different species of *Meloidogyne* were analyzed by the PROC CORR procedure of SAS.

SCAR marker screening. Newly expanded leaf tissue samples were collected from about 30-day old peanut seedlings in the greenhouse. Total genomic DNA was extracted using the CTAB method according to Hopkins et al. (Hopkins et al., 1999), with several modifications. The DNA was dissolved in TE buffer (10 mM Tris, 0.1mM EDTA, pH 8.0) with 1% RNase and

stored at 4°C. SCAR makers Z3/265 (Garcia et al., 1996) and 197/909 (Chu et al., 2007) were used to determine if the resistance gene(s) were linked to the Ma-resistant markers in selected genotypes. One µl of the DNA extract was used for a 25 µl volume PCR reaction. Each PCR reaction was performed with 0.5 U of Hotmaster Taq DNA polymerase (Promega) using the buffer supplied by the manufacturer containing 25 mM Mg^{2+} (final reaction concentration, 2.5 mM). Amplification conditions for both sets of primers were similar: initial denaturation at 95°C for 5 min; 40 cycles of 95°C for 30 s, annealing temperature was 60.2°C for 30 s, and 72°C for 30 s; final extension at 72°C for 7 min. All PCR amplifications were performed with the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). PCR products were separated on 2% agarose gels (Invitrogen, Carlsbad, CA) and 8% polyacrylamide (PAGE) gels.

RESULTS

Meloidogyne arenaria. Fifty-seven genotypes were included in the first greenhouse screening trial for resistance to *M. arenaria*. The second greenhouse trial evaluated 28 accessions with low gall indices (≤ 3.0) or low egg numbers (≤ 50% of the susceptible control Georgia Green), as well as two genotypes that did not germinate in trial 1, three new collections, and susceptible control Georgia Green. Of these entries, 21 accessions with low gall indices or low egg numbers were selected and advanced to the third trial with Georgia Green as the susceptible control. Genotype GP-NC WS 5 was also included in trial 3, although it had a high gall index and eggs/g root in the earlier trials. There were no significant interactions of genotype × trial for gall index and eggs/g root; thus data for the genotypes included in all three trials or in the second and the third trials were pooled across trials. The data was analysed with GLM of SAS as unbalanced data set (Table 3.2). Eggs per gram root for all the selected genotypes, except GP-NC

WS 5, were lower ($P \leq 0.05$) than the susceptible control Georgia Green. Reproduction of *M. arenaria* on genotypes NemaTAM, GP-NC WS 6, COAN, and C724-25-8 were less than 10% of Georgia Green, and they were classified as highly resistant (HR). Sixteen genotypes were classified as moderately resistant (MR) because nematode reproduction was < 50% of Georgia Green. The genotypes 950213 and GP-NC WS 5 were classified as susceptible (S). On all the HR and MR genotypes, gall indices were ≤ 3.00.

The variability of egg numbers for NR 0817, C724-19-11, and D108 was extremely high, and the frequencies of individual plants with different resistance levels for these three genotypes were different from NemaTAM (highly resistant), C209-6-37 (moderately resistant), or Georgia Green (susceptible) (Fig. 3.1). Genotypes NR 0817, C724-19-11, and D108 showed high frequencies in both HR and S categories, and low in MR, although they were ranked in the MR group. This suggests that these three genotypes were segregating. The resistant phenotype of the SCAR marker Z3/265 was detected in five (62.5%), three (42.9%), and two (33.3%) individuals of NR 0817, C724-19-11, and D108, respectively. Those plants showing the marker were scored HR in the greenhouse screening, whereas the others showed a susceptible band pattern with one exception in C724-19-11 (Fig. 3.2).

Meloidogyne javanica. Fifty-seven, 34, and 24 genotypes were included in the *M. javanica* screening trials 1, 2, and 3, respectively. Trials 2 and 3 included resistant selections from the previous trial, new collections, and the susceptible control Georgia Green. Data for those genotypes that were tested in three or the last two trials were pooled and analyzed because there were no significant interactions between genotypes and trials for gall index and eggs/g root (Table 3.3). All the selected genotypes, except GP-NC WS 5, showed lower ($P \leq 0.05$) eggs/g root and gall indices than the susceptible control Georgia Green. The eggs/g root for genotypes

C724-25-8, NemaTAM, and COAN were less than 10% of the susceptible control, and these three genotypes were classified as highly resistant. Based on both eggs/g root and gall index, 18 genotypes were classified as moderately resistant. On genotypes HTS 02-01 and GP-NC WS 5, *M. javanica* produced over 50% of the eggs produced on Georgia Green; therefore these two genotypes were classified as susceptible.

Meloidogyne hapla. Fifty-seven, 37, and 26 genotypes were included in the *M. hapla* screening trials 1, 2, and 3, respectively. Trial 2 and 3 included resistant selections from the previous trial, new collections, and a susceptible control Georgia Green. Data for those genotypes, which were included in all three or in the last two trials, were combined for analysis. Compared with *M. arenaria* and *M. javanica*, the galls caused by *M. hapla* were smaller and the symptoms on root systems were less severe. Therefore, the gall index standard which was used to identify the resistant genotypes to *M. hapla* was modified from that for *M. arenaria* and *M. javanica*. The gall indices for the genotypes that were classified as highly or moderately resistant to *M. hapla* were ≤ 2 rather than ≤ 3.0 for Ma and Mj. The genotypes GP-NC WS 5, HTS 02-01, D108, and NemaTAM supported the same amount of *M. hapla* reproduction as the susceptible control Georgia Green (Table 3.4). The genotypes D031, 970105, and 990304 were highly resistant to *M. hapla* with < 10% than eggs/g root of Georgia Green. Eighteen genotypes with ≤ 50% of the eggs/g root on Georgia Green and low gall indices (≤ 2.0) were moderately resistant.

SCAR Marker Phenotypes. A total of 27 genotypes, including 26 selected genotypes with high or moderate resistance to one or more of the three *Meloidogyne* species and the susceptible control (Georgia Green), were evaluated with the SCAR markers Z3/265 and 197/909. On 2.5% agarose gel, the marker Z3/265 produced a strong resistance band at 265 bp on the genotypes COAN, NemaTAM, C724-25-8, GP-NC WS 6, and some individuals of C724-19-11, D108, and NR 0817 (Fig. 3.3). This set of primer also produced a faint resistance band on the genotypes

970105, 991219, D040, D031, HTS 02-01, and some individuals of C724-19-11, which were moderately resistant or susceptible to *M. arenaria* in the greenhouse (Fig. 3.2A & Fig. 3.3). The susceptible control Georgia Green and the other genotypes did not show any specific bands.

On 8% polyacrylamide gel, the marker 197/909 amplified the Ma-resistant band on NemaTAM, COAN, C724-25-8, and some individuals of C724-19-11 with high resistance to *M. arenaria* and *M. javanica*, but not on GP-NC WS 6 and some individuals of NR 0817 and D108 that also showed high resistance to *M. arenaria* in the greenhouse. The PCR product from NR 0817 was approximately 260 bp, even smaller than that from Ma-susceptible genotype Georgia Green. The genotype 970105 showed two major bands, which might be an indication of heterozygosity. The other genotypes with moderate resistance to *M. arenaria*, or with moderate to high resistance to *M. javanica* and *M. hapla* showed the Ma-susceptible band pattern (Fig. 3.4).

Correlations for Resistance to *M. arenaria, M. javanica*, and *M. hapla*. Twenty-six out of 60 genotypes were identified with some resistance to *M. arenaria, M. javanica,* or *M. hapla*. For the 26 resistance selections and Georgia Green, gall indices caused by *M. arenaria* were correlated with gall indices caused by *M. javanica* ($r = 0.7498$, $P = 0.0015$). However, the resistance to *M. hapla* did not show any significant correlations to resistance to either *M. arenaria* ($r = 0.2188$, $P > 0.05$) or *M. javanica* ($r = 0.3256$, $P > 0.05$).

DISCUSSION

Some of the peanut genotypes evaluated in this study were known to be resistant to one or more *Meloidogyne* species. For the most part, our results support previous findings, such as the high resistance to *M. arenaria* and *M. javanica* in COAN and NemaTAM (Simpson and Starr, 2001; Simpson et al., 2003), high resistance to *M. arenaria* in GP-NC WS 6 (Stalker et al., 2002),

and moderate resistance in C209-6-37 (Holbrook et al., 2003). Our results also indicated that there existed moderate resistance to *M. hapla* in COAN, which is consistent with the finding of Timper et al. (Timper et al., 2003). NemaTAM was classified as susceptible to *M. hapla*, although NemaTAM was derived from the same backcross introgression pathway as COAN and the same resistance gene(s) to Ma and Mj. The resistance to Mh in COAN may have been lost in NemaTAM during the two additional backcross generations with Florunner. We also obtained some conflicting results from previous reports. The genotype GP-NC WS 5 (Stalker et al., 2002) was released as a Ma-resistant breeding line, and has been used as the resistant parent to develop additional germplasm with nematode resistance (Anderson et al., 2006). However, in our study it was extremely susceptible. Perhaps GP-NC WS 5 was not completely homogeneous when it was released, and the seeds that we used in this study were collected from susceptible plants. The genotype NR 0817 (Anderson et al., 2006) was also released as a Ma-resistant breeding line; however, in this study it was classified as moderately resistant, mainly due to a mixture of resistant and susceptible individuals. The results indicate that the genotypes NR 0817, C724-19-11, and D108 are still segregating for resistance; therefore, further selection is needed before they would be good sources of nematode resistance for peanut breeding programs.

High levels of resistance to *M. arenaria* have been identified in wild *Arachis* spp. (Nelson et al., 1989). The different mechanisms of resistance that exist in the wild *Arachis* species suggest different genes for resistance exist in different wild species (Nelson et al., 1990; Starr et al., 1990). High levels of resistance to *M. arenaria* in *A. cardenasii* were reported to be conditioned by at least two dominant genes (Garcia et al., 1996) and are expressed as a hypersensitive-like reaction with few J2 showing signs of development (Nelson et al., 1989). The germplasm TxAG-6, an interspecific *Arachis* hybrid developed from a cross made in Texas

between *Arachis batizocoi* Krapov. & W. C. Gregory x (*A. cardenasii* x *A. diogoi*), was the source of the resistance in COAN, NemaTAM, C724-25-8, and C724-19-11 (Simpson and Starr, 2001; Simpson et al., 2003; Holbrook, *unpublished*). The results of RFLP markers indicated that the resistance in COAN and NemaTAM was derived from *A. cardenasii* and segregates as a single dominant gene (Choi et al., 1999; Church et al., 2000). However, the resistance in COAN does not appear to involve a necrotic hypersensitive response, and may be due to constitutive factors in the roots (Bendezu and Starr, 2003). The gene(s) conditioning hypersensitive response in *A. cardenasii* may not have been introgressed into COAN. In addition, a recessive gene for resistance to *M. arenaria* has been identified in TxAG-6 (Church et al, 2005); however, it is not known if this recessive gene exists in COAN and NemaTAM. The resistance in GP-NC WS 6 and NR 0817 originated from a cross made in North Carolina between *A. cardenasii* and *A. hypogaea* (Anderson et al., 2006; Stalker et al., 2002). Two dominant genes conferring resistance to *M. arenaria* were identified in GP-NC WS 6: *Mae* conditions resistance to egg production and *Mag* conditions resistance to gall formation (Garcia et al., 1996; Stalker et al., 2002). There is no evidence that *Mae* is the same gene that suppresses egg production in COAN. We have shown the presence of SCAR marker Z3/265 in both COAN and GP-NC WS6 and the absence of SCAR marker 197/909 in GP-NC WS 6 suggesting that the resistant genes in the genotypes derived from the two different introgression pathways may be different. Moreover, resistance in other genotypes which were moderately resistant to *M. arenaria*, such as D054, D031, D040, and D099, etc., may also be conditioned by different genes since all these genotypes are *A. hypogaea* without any introgression from wild species.

The SCAR marker Z3/265 was developed from an F_2 population of GA6, which originated from a cross made in North Carolina. In our results, this marker exists in the

genotypes with resistance gene(s) derived from both the North Carolina cross and Texas cross. It is also present in some Ma-susceptible genotypes with or without any introgression from wild species. This confirmed a previous finding that Z3/265 can produce a false positive score (Chu et al., 2007). The SCAR marker 197/909 was developed based on the sequence of a RAPD fragment that originated from COAN (Chu et al., 2007). This marker shows a high correlation with the greenhouse phenotype data. It amplifies fragments from both susceptible and resistant samples, but of different molecular weights, avoiding false negatives. However, this marker is only present in the genotypes directly related with the resistance gene derived from the TxAG-7.

In this study we found that resistance to *M. arenaria* and to *M. javanica* was highly correlated, indicating that in many peanut genotypes the same gene(s) may confer resistance to both species, or the resistance gene(s) for each species are closely linked. This was different from the previous observations with the interspecific hybrid TxAG-7, in which the resistance to *Meloidogyne* spp. segregated independently (Abdel-Momen et al., 1998). Our study also showed that resistance to Mh was not correlated with resistance to Ma or Mj. The mechanisms of resistance to *M. hapla* may be different from that of *M. arenaria* and *M. javanica*. The difference between resistance to *M. arenaria* and resistance to *M. hapla* may not be caused by differences in their parasitism gene. Recent evidence suggests that Ma, Mj, Mh, and Mi (*M. incognita*) may have identical parasitism gene (s) (Huang et al., 2006; Huang et al., 2006). Therefore, the resistance gene(s) in peanut may be related to differential recognition by the plant of the three *Meloidogyne* species.

In summary, the data presented in this report showed that resistance to all three *Meloidogyne* spp. exist within cultivated peanut (*Arachis hypogaea*), either with or without introgressed genes from wild species. We identified several additional genotypes with moderate

resistance to *M. arenaria* and *M. javanica*, such as D009, D031, D040, D054, D099, D998, D999, 950521, 950530, 970101, and 990304. The level of resistance in these genotypes was as good as or better than the resistance in the moderately resistant genotype C209-6-37. These genotypes may have different resistance gene(s) from released Ma-resistant germplasm (Anderson et al., 2006; Simpson et al., 1993; Stalker et al., 2002) because they do not have introgressed genes from wild species. We identified peanut genotypes with high and moderate resistance to *M. hapla*. The three highly resistant genotypes and most of the 18 moderately resistant genotypes do not have introgressed genes from wild species, whereas some moderately resistant genotypes, such as C724-19-11, COAN, NR 0817, GP-NC WS 6, and C724-25-8, have introgressed genes. Although the genetics of the resistance to *M. hapla* in peanuts has not been determined, we believe the selected genotypes will be valuable for developing new peanut cultivars with broad and durable resistances to *Meloidogyne* spp.

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Table 3.1. Peanut (*Arachis hypogaea*) genotypes evaluated for resistance to three species of *Meloidogyne.*

Name/Code	Origin and type of line	Known resistance[a]	Parent species/ resistance source
9509[b]	China; breeding line	N	*A. hypogaea*
950213	China; breeding line	N	*A. hypogaea*
950404	China; breeding line	N	*A. hypogaea*
950521	China; breeding line	N	*A. hypogaea*
950530	China; breeding line	N	*A. hypogaea*
950536	China; breeding line	N	*A. hypogaea*
961308-1	China; breeding line	N	*A. hypogaea*
961308-2	China; breeding line	N	*A. hypogaea*
970101	China; breeding line	N	*A. hypogaea*
970105	China; breeding line	N	*A. hypogaea*
990304	China; breeding line	N	*A. hypogaea*
991219	China; breeding line	N	*A. hypogaea*
Baisha 1016	China; cultivar	N	*A. hypogaea*
C209-6-37	GA, USA; released germplasm line	Ma	*A. cardenasii*
C724-19-11	GA, USA; breeding line	Ma	COAN
C724-25-8	GA, USA; breeding line	Ma	COAN
C-99R	FL, USA; cultivar	N	*A. hypogaea*
COAN	TX, USA; cultivar	Ma & Mj	*A. cardenasii*
D002[c]	China; selection	N	*A. hypogaea*
D009	China; selection	Mh	*A. hypogaea*
D013	China; selection	N	*A. hypogaea*
D0206	China; selection	N	*A. hypogaea*
D029	China; selection	Mh	*A. hypogaea*
D031	China; selection	Mh	*A. hypogaea*
D040	China; selection	Mh	*A. hypogaea*
D054	China; selection	N	*A. hypogaea*
D098	China; selection	N	*A. hypogaea*
D099	China; selection	Mh	*A. hypogaea*
D108	NC, USA; breeding line	N	*A. cardenasii*
D122	China; selection	N	*A. hypogaea*
D129	China; selection	N	*A. hypogaea*
D131	China; selection	N	*A. hypogaea*
D132	China; selection	N	*A. hypogaea*

Table 3.1 continued

D133	China; selection	N	*A. hypogaea*
D134	NC, USA; breeding line	N	*A. cardenasii*
D140	China; selection	N	*A. glabrata*
D142	China; selection	N	*A. hypogaea*
D143	China; selection	N	*A. hypogaea*
D169	China; selection	N	*A. hypogaea*
D998	China; selection	N	*A. hypogaea*
D999	China; selection	N	*A. hypogaea*
Florunner	FL, USA; cultivar	N	*A. hypogaea*
Georgia Green	GA, USA; cultivar	N	*A. hypogaea*
Georgia-01R	GA, USA; cultivar	N	*A. hypogaea*
Georgia-02C	GA, USA; cultivar	N	*A. hypogaea*
GP-NC WS 5	NC, USA; released germplasm line	Ma	*A. cardenasii*
GP-NC WS 6	NC, USA; released germplasm line	Ma	*A. cardenasii*
HT 02-01	NC, USA; breeding line	N	GP-NC WS 5
Huayu 17	China; cultivar	N	*A. hypogaea*
Huayu 21	China; cultivar	N	*A. hypogaea*
Huayu 22	China; cultivar	N	*A. hypogaea*
Lianhua 2	China; cultivar	N	*A. hypogaea*
Luhua 9	China; cultivar	N	*A. hypogaea*
Luhua 11	China; cultivar	N	*A. hypogaea*
Luhua 14	China; cultivar	N	*A. hypogaea*
NemaTAM	TX, USA; cultivar	Ma & Mj	*A. cardenasii*
NR 0817	GA, USA; released germplasm line	Ma	GP-NC WS 5
Qinglan 2	China; cultivar	N	*A. hypogaea*
Southern runner	FL, USA; cultivar	N	*A. hypogaea*
Tifrunner	GA, USA; cultivar	N	*A. hypogaea*

[a] Ma, Mj, and Mh—resistant to *Meloidogyne arenaria, M. javanica, and M. hapla*, respectively; N—unknown.

[b] 95xxxx—the cross was made in 1995.

[c] the numbers started with a D were selections from Chinese germplasm and local cultivars.

Table 3.2. Root galling, reproduction of *Meloidogyne arenaria*, and resistance classification for selected peanut genotypes tested in the greenhouse.

Genotype	Number of replicates[a]	Gall index[b]	Eggs/g root[c]	Resistance[d]
NemaTAM	17	0.64 ± 0.25	586 ± 1041	HR
GP-NC WS 6	12	0.37 ± 0.31	875 ± 1249	HR
COAN	16	1.15 ± 0.27	889 ± 1074	HR
C724-25-8	18	0.78 ± 0.25	1073 ± 1011	HR
D054	16	1.92 ± 0.25	1341 ± 1074	MR
D031	16	1.42 ± 0.25	1428 ± 1074	MR
D040	16	1.47 ± 0.25	1879 ± 1074	MR
D998	16	1.50 ± 0.26	2386 ± 1074	MR
D099	16	0.95 ± 0.27	2439 ± 1074	MR
D009	16	1.79 ± 0.26	2491 ± 1073	MR
950530	12	2.23 ± 0.31	2836 ± 1248	MR
D108	18	1.00 ± 0.26	2917 ± 1041	MR
990304	15	1.69 ± 0.28	3393 ± 1110	MR
950521	11	1.16 ± 0.33	3705 ± 1304	MR
970101	10	1.97 ± 0.34	3852 ± 1367	MR
NR 0817	11	1.86 ± 0.33	4166 ± 1304	MR
C724-19-11	17	1.94 ± 0.25	4597 ± 1011	MR
C209-6-37	16	2.45 ± 0.26	4609 ± 1073	MR
D999	10	1.85 ± 0.31	4611 ± 1367	MR
HTS 02-01	10	2.34 ± 0.34	4670 ± 1369	MR
950213	10	3.89 ± 0.31	6337 ± 1367	S
GP-NC WS 5	11	3.45 ± 0.33	11046 ± 1304	S
Georgia Green	18	4.14 ± 0.25	11069 ± 1011	S

[a] Data from three trials (six replicates/trial) were combined for analysis as unbalanced data.

[b] Gall index, where 0 = no galling, 1 = trace infection with a few small galls, 2 ≤ 25% roots galled, 3 = 25-50%, 4 = 51-75%, 5 > 75% of root galled. Data were LSMEANs ± Standard errors.

[c] Data were LSMEANs ± Standard errors.

[d] HR--highly resistant, eggs/ g root ≤ 10% of Georgia Green; MR—moderately resistant, eggs/ g root ≤ 50% of Georgia Green, and gall index ≤ 3.00; S—susceptible, eggs/g root > 50% of Georgia Green and/or gall index > 3.00.

Table 3.3. Root galling, reproduction of *Meloidogyne javanica*, and resistance classification for selected peanut genotypes tested in the greenhouse.

Genotype	Number of replicates[a]	Gall index[b]	Eggs/g root[c]	Resistance[d]
C724-25-8	15	0.59 ± 0.29	519 ± 939	HR
NemaTAM	16	0.62 ± 0.28	703 ± 908	HR
COAN	17	0.60 ± 0.27	899 ± 880	HR
991219	8	0.96 ± 0.40	1560 ± 1299	MR
950530	12	1.34 ± 0.32	1757 ± 1057	MR
D009	13	1.57 ± 0.31	1782 ± 1011	MR
D031	13	1.44 ± 0.30	2204 ± 1009	MR
950521	18	1.17 ± 0.26	2497 ± 854	MR
D099	16	1.57 ± 0.28	2657 ± 908	MR
D040	16	1.77 ± 0.27	2996 ± 908	MR
D108	18	0.86 ± 0.26	3000 ± 854	MR
D998	10	2.47 ± 0.32	3013 ± 1158	MR
990304	13	1.85 ± 0.31	3050 ± 1009	MR
D054	18	1.67 ± 0.26	3283 ± 854	MR
D999	12	1.72 ± 0.32	3343 ± 1057	MR
GP-NC WS 6	10	1.29 ± 0.35	3544 ± 1161	MR
NR 0817	15	2.05 ± 0.29	3624 ± 937	MR
970101	9	1.81 ± 0.37	3841 ± 1221	MR
C724-19-11	11	1.91 ± 0.33	4070 ± 1100	MR
D002	18	1.83 ± 0.26	4237 ± 854	MR
C209-6-37	18	2.53 ± 0.26	4804 ± 854	MR
HTS 02-01	12	2.58 ± 0.32	6996 ± 1057	S
GP-NC WS 5	10	2.88 ± 0.35	8708 ± 1160	S
Georgia Green	14	4.49 ± 0.28	10318 ± 972	S

[a] Data from three trials (six replicates/trial) were combined for analysis as unbalanced data.

[b] Gall index, where 0 = no galling, 1 = trace infection with a few small galls, 2 ≤ 25% roots galled, 3 = 25-50%, 4 = 51-75%, 5 > 75% of root galled. Data were LSMEANs ± Standard errors.

[c] Data were LSMEANs ± Standard errors.

[d] HR--highly resistant, eggs/ g root ≤ 10% of Georgia Green; MR—moderately resistant, eggs/ g root ≤ 50% of Georgia Green, and gall index ≤ 3.00; S—susceptible, eggs/g root > 50% of Georgia Green and/or gall index > 3.00.

Table 3.4. Root galling, reproduction of *Meloidogyne hapla* reproduction, and resistance classification for selected peanut genotypes tested in the greenhouse.

Genotype	Number of replicates[a]	Gall index[b]	Eggs/g root[c]	Resistance[d]
970105	9	0.31 ± 0.39	550 ± 1745	HR
D031	12	0.43 ± 0.34	631 ± 1505	HR
990304	10	0.25 ± 0.37	811 ± 1653	HR
950530	11	0.68 ± 0.35	985 ± 1574	MR
D099	14	0.46 ± 0.34	1087 ± 1505	MR
D009	12	0.38 ± 0.34	1149 ± 1505	MR
COAN	9	0.87 ± 0.39	1180 ± 1745	MR
Luhua 14	12	1.88 ± 0.34	1712 ± 1505	MR
961308-1	11	0.71 ± 0.34	1769 ± 1574	MR
D999	12	0.87 ± 0.34	1790 ± 1505	MR
Huayu 21	11	0.91 ± 0.35	1879 ± 1574	MR
C724-19-11	11	1.54 ± 0.34	1904 ± 1574	MR
991219	16	0.50 ± 0.34	1988 ± 1505	MR
D054	11	0.51 ± 0.35	1997 ± 1574	MR
D040	12	0.38 ± 0.34	2084 ± 1505	MR
D998	14	0.50 ± 0.34	2456 ± 1505	MR
970101	12	0.87 ± 0.34	3350 ± 1505	MR
D002	12	0.83 ± 0.34	3376 ± 1505	MR
NR 0817	8	1.07 ± 0.41	4074 ± 1848	MR
GP-NC WS 6	12	1.50 ± 0.34	4103 ± 1505	MR
C724-25-8	13	1.25 ± 0.34	4393 ± 1505	S
D108	13	1.04 ± 0.34	7695 ± 1505	S
NemaTAM	12	2.17 ± 0.34	7706 ± 1505	S
Georgia Green	15	2.87 ± 0.34	8739 ± 1505	S
HTS 02-01	10	3.02 ± 0.37	9192 ± 1653	S
GP-NC WS 5	12	2.83 ± 0.34	11348 ± 1505	S

[a] Data from three trials (six replicates/trial) were combined for analysis as unbalanced data.

[b] Gall index, where 0 = no galling, 1 = trace infection with a few small galls, 2 ≤ 25% roots galled, 3 = 25-50%, 4 = 51-75%, 5 > 75% of root galled. Data were LSMEANs ± Standard errors.

[c] Data were LSMEANs ± Standard errors.

[d] HR--highly resistant, eggs/ g root ≤ 10% of Georgia Green; MR—moderately resistant, eggs/ g root ≤ 50% of Georgia Green, and gall index ≤ 2.00; S—susceptible, eggs/g root > 50% of Georgia Green and/or gall index > 2.00.

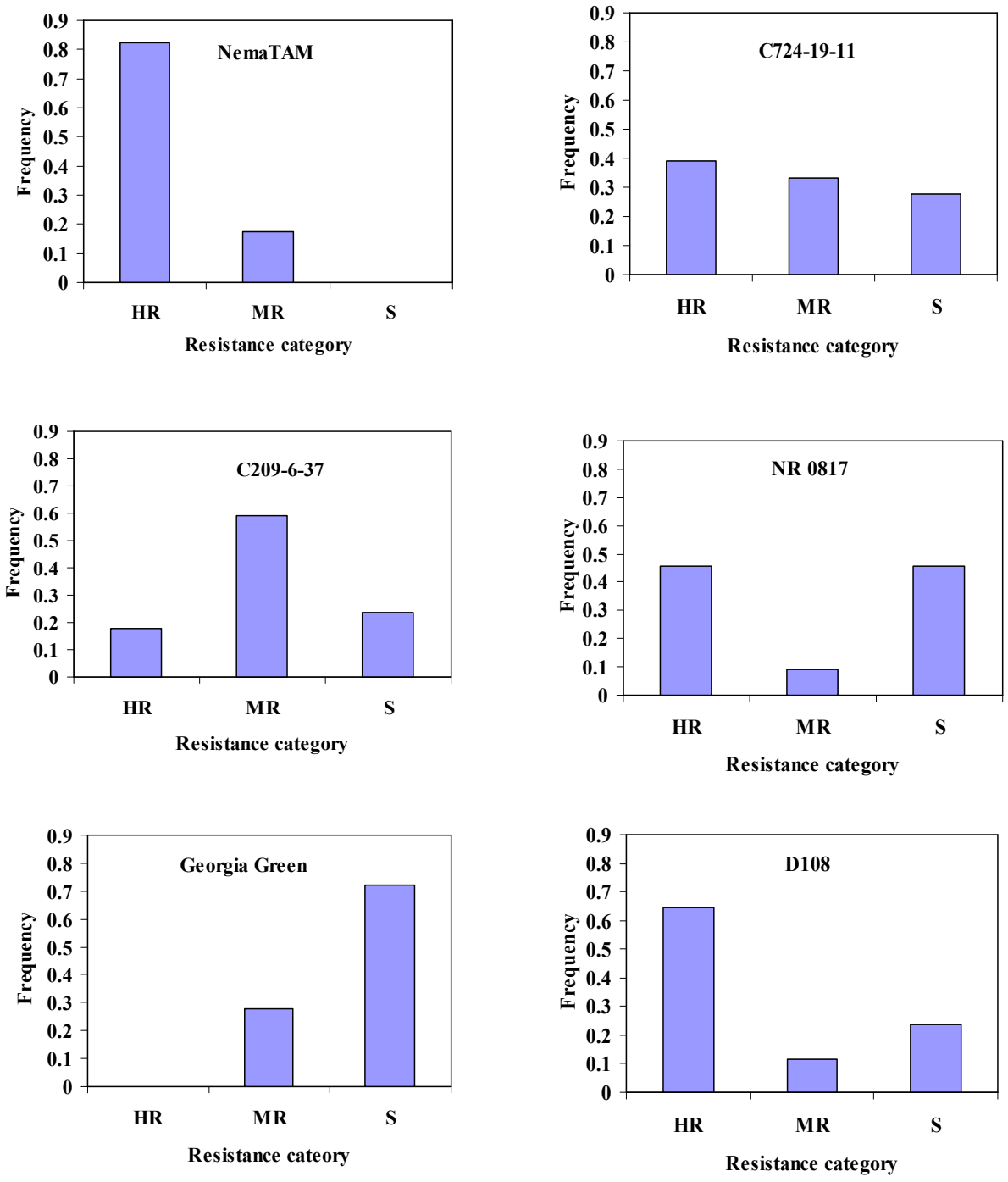

Figure 3.1. Frequency of individuals with different levels of resistance to *Meloidogyne arenaria* (Ma) in peanut genotypes. NemaTAM was classified as highly resistant; C209-6-37 was classified as moderately resistant; Georgia Green was the susceptible control.

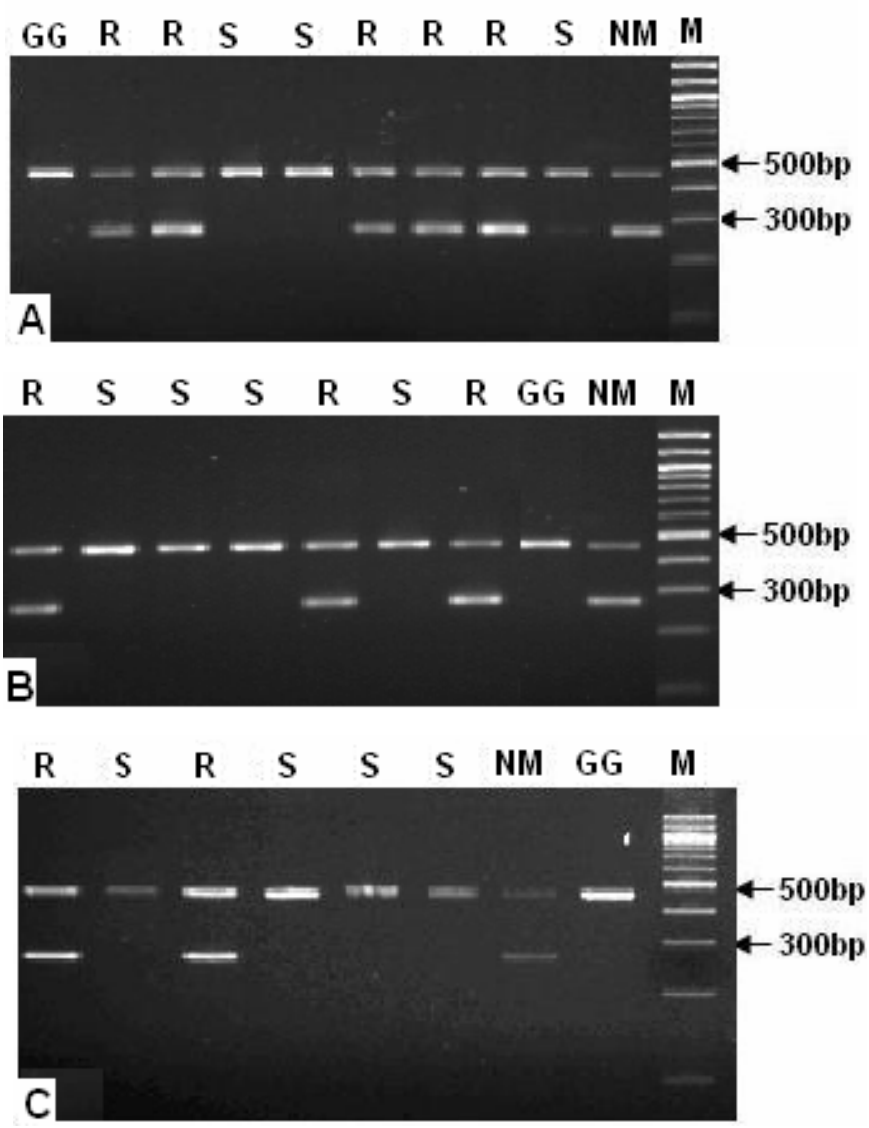

Figure 3.2. PCR amplification by primer sets 400/401 and 199/200 for individuals of C724-19-11 (A), NR 0817 (B), and D108 (C). 400/401 amplified actin deploymerizing factor an endogenous gene. It serves as a positive control for sources of PCR amplification. 199/200 was a *Meloidogyne arenaria* resistant SCAR marker (Z3/265). GG: Georgia Green (susceptible control), NM: NemaTAM (resistant control), R: highly resistant individual in greenhouse screening, S: susceptible individual in greenhouse screening.

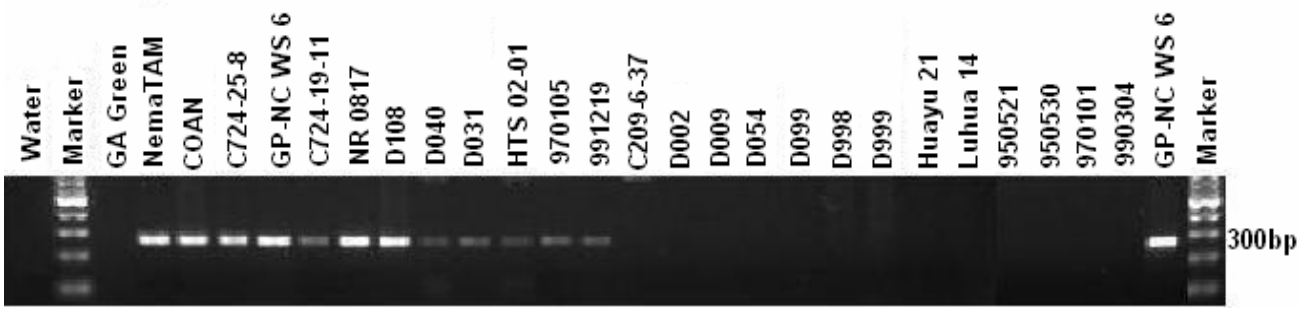

Figure 3.3. Separation of amplicons from selected peanut genotypes with primers 199/200 on 2.5% agarose gel.

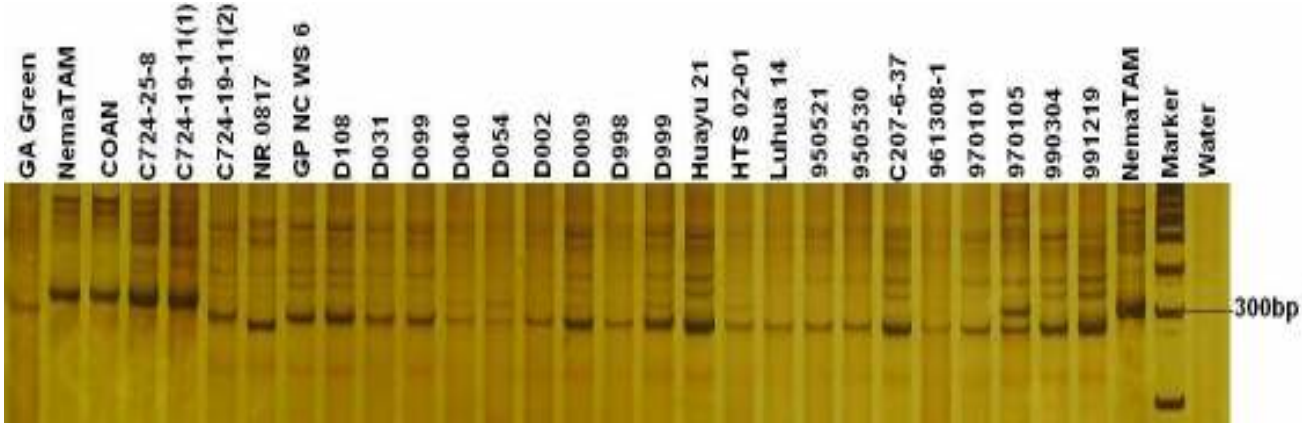

Figure 3.4. Separation of amplicons from selected peanut genotypes with primers 197/909 on 8% polyacrylamide gel. C724-19-11(1) and C724-19-11(2) were two individuals of the same breeding line.

APPENDIX TO CHAPTER 3

Figure 3A.1. Screening of resistance to *Meloidogyne* spp. in peanut in greenhouse.

Figure 3A.2. Galls on peanut pods and root systems, caused by *Meloidogyne arenaria*.

Figure 3A.3. Galls on peanut pods and root systems, caused by *Meloidogyne hapla*.

Figure 3A.4. Galls on peanut pods and root systems, caused by *Meloidogyne javanica*.

CHAPTER 4

EVALUATION OF RESISTANCE TO *CYLINDROCLADIUM PARASITICUM* IN PEANUT IN THE GREENHOUSE AND IN INOCULATED OR NATURALLY INFESTED FIELDS[1]

[1]W. B. Dong, T. B. Brenneman, C. C. Holbrook, and A. K. Culbreath. 2007. To be submitted to Peanut Science.

ABSTRACT

Screening and utilization of peanut cultivars with resistance to Cylindrocladium black rot (CBR) is a desirable approach to manage this disease. The objectives of this study were to improve greenhouse and field screening techniques for resistance to CBR, and to evaluate resistance in six runner peanut genotypes. Two peanut cultivars, Georgia-02C (CBR-resistant) and C-99R (CBR-susceptible) were used to compare the effectiveness on different inoculation methods in the greenhouse. Six runner type genotypes with varying resistance to CBR were evaluated in a naturally infested field, inoculated fields, and in greenhouse trials. Greenhouse screening experiments in soil infested with 1-5 microsclerotia (ms)/g soil could separated the CBR-resistant cultivar Georgia-02C from the susceptible C-99R correctly, based on root rot rating. The overall results indicated that different levels of resistance to CBR existed in runner type peanuts. The genotypes Georgia-02C and Georganic had low plant mortalities, whereas C-99R and DP-1 always had high mortalities in a naturally infested field in 2005 and 2006. Plant mortalities were moderate in Georgia-01R in both years, but were inconsistent in C34-24-85. Georgia-02C and Georganic also showed partial resistance to CBR in greenhouse and inoculated field experiments in both 2006 and 2007. The root rot ratings and percentage of black pods for genotypes Georgia-02C and Georganic were both lower relative to those for C-99R and DP-1. Dead and diseased plants after digging and entire plant disease severity were the better variables for evaluating CBR resistance in peanut in naturally infested fields or inoculated field, and greenhouse experiments, respectively. Plant mortality in the naturally infested field tests was significantly correlated with CBR incidence in the inoculated field tests ($P \leq 0.01$), but neither was correlated with the disease ratings for greenhouse experiments. Peanut genotypes are most reliably screened in inoculated fields or uniformly infested fields, but greenhouse evaluations may be useful to identify and characterize components of resistance.

Additional key words: Arachis hypogaea, Cylindrocladium parasiticum, groundnut, inoculation method

Cylindrocladium black rot (CBR) of peanut (*Arachis hypogaea* L.), caused by the soilborne fungus *Cylindrocladium parasiticum* Crous, M.J. Wingf. & Alfenas (Crous, 2002), was first found in 1965 in Georgia (Bell and Sober, 1966), and it threatens peanut production throughout the southeastern United States (Bell et al., 1973; Harris and Beute, 1982). The pathogen causes serious peg, pod, and root necrosis of peanut. Symptoms of the disease, which can appear in the field as early as July, include chlorosis and wilting of the main axis followed by complete wilting of the remaining foliage and death of the plant. Lateral roots are either blackened or completely severed from the taproot. Pod development is greatly reduced and existing pods may be severely rotted (Bell and Sober, 1966; Johnston and Beute, 1975). Yield loses were approximately 250 to 450 kg/ha for each 10% increase on CBR incidence (Pataky et al., 1983).

Cultural practices, fumigants, fungicides, and resistant peanut genotypes have been evaluated in the effort to develop CBR control programs. The ineffectiveness and inconsistency of cultural practices such as sanitation and rotation, and even chemical treatments (Bell at al., 1973; Black et al., 1984; Rowe et al., 1974) have stimulated breeding programs to identify and evaluate CBR-resistant peanut genotypes (Beute et al., 1976; Coffelt and Culbreath, 1982; Isleib et al., 1997, 2003; Wynne and Beute, 1983; Wynne et al., 1991).

Results from several screening tests have shown that spanish-type peanuts are the least susceptible to *C. parasiticum*, valencia-type peanuts the most susceptible, and virginia-type peanuts intermediate (Hammons et al., 1981; Harris and Beute, 1982; Phipps and Beute, 1997). However, large differences were observed in the susceptibility of cultivars within each group

(Wynne et al., 1975). NC 3033, a virginia type whose pedigree includes spanish types, was released in 1976 as the first CBR-resistant germplasm (Beute et al., 1976). Although NC 3033 has a high level of resistance to CBR, it has small seeds and is low yielding. The use of NC 3033 in breeding programs resulted in the release of partially CBR-resistant virginia-type cultivars, 'NC 12C' (Isleib et al., 1997), and 'Perry' (Isleib et al., 2003). Recently, the first runner peanut cultivar with moderate resistance to CBR was released as Georgia-02C (Branch, 2003).

A reliable and rapid technique for identifying resistance to CBR is very important for screening large numbers of lines in a breeding program. Several evaluations of CBR resistance based on disease incidence, severity, or microsclerotium production have been conducted in naturally infested fields, microplots, and greenhouses (Coffelt and Garren, 1982; Green et al., 1983a & b; Pataky et al., 1983). However, inconsistencies frequently occur in these evaluations. Field evaluations of CBR resistance are not only time consuming, but also have sometimes resulted in large error components in the analysis of variance. This is due to the nonuniform spatial pattern of microsclerotia (Culbreath et al., 1991a; Hau et al., 1982; Pataky et al., 1983), the primary inoculum of *C. parasiticum*, in field soils and is related to the inoculum density-dependent nature of CBR resistance (Black et al., 1984; Harris and Beute, 1982; Hau et al., 1982). Although inoculum density is more uniform in greenhouse screening, results do not always reflect susceptibility in the field (Pataky et al., 1983). Additionally, most of the evaluations of CBR resistance have been done with virginia, spanish, and valencia-type peanut, whereas few studies have been done with runner-type peanut (Coffelt, 1980; Coffelt and Garren, 1982; Garren and Coffelt, 1976; Pataky et al., 1983), which is the major type of peanut in Georgia.

Therefore, the objectives of this study were 1) to improve greenhouse and field screening techniques for resistance to CBR; 2) to evaluate the response of six runner peanut genotypes with varying resistance to CBR in a naturally infested field, inoculated fields, and in greenhouse trials, and 3) to compare the effectiveness of different methods for evaluating CBR resistance in runner peanuts.

MATERIALS AND METHODS

Genotypes evaluated. One CBR-resistant peanut cultivar Georgia-02C and one susceptible cultivar 'C-99R' (Gorbet and Shokes, 2002) were used to evaluate greenhouse screening techniques. Six runner-type peanut genotypes, five cultivars Georgia-02C, 'Georgia-01R' (Branch, 2002), C-99R, 'Georganic' (Holbrook and Culbreath, 2007), and 'DP-1' (Gorbet, 2003), and one breeding line C34-24-85 were evaluated in a naturally infested field, inoculated fields, and greenhouse tests. Georgia-02C and C-99R were considered as resistant and susceptible controls, respectively (Branch, 2003; Gorbet and Shokes, 2002). Georgia-01R, recently released as a commercial cultivar, has shown some resistance to CBR (Branch, 2002). DP-1 (Gorbet and Shokes, 2002) and Georganic have previously shown multiple resistances to several major diseases of peanut, such as early leaf spot, late leaf spot, and tomato spotted wilt virus (TSWV). C34-24-85 is a runner-type breeding line.

Inoculum production. The isolates CBR041, CBR0410, CBR0414, and CBR0418 of *C. parasiticum*, were obtained from infected peanut plants in south Georgia in 2004, were used to inoculated field tests and greenhouse tests. To obtain microsclerotia, the isolates were grown on potato-dextrose agar (PDA) for 6-7 wk, after which the cultures of each isolate were comminuted separately in a Waring Blendor for 2 min and passed through nested sieves. The 53-425 μm

microsclerotia were rinsed into small beakers, suspended in water, and stored at room temperature for short periods until use.

Greenhouse inoculation technique. Cultivars (Georgia-02C and C-99R) were grown in all combinations of four microsclerotia (ms) size ranges (250, 150, 75, and 53 μm) and five inoculum densities (0, 0.5, 1.0, 5.0, and 10.0 ms/g soil). The microsclerotia of each isolate were separated with four nested sieves with 250, 150, 75, and 53 μm pores. The numbers of microsclerotia collected on each sieve were determined and for each of the four size ranges, equal numbers of microsclerotia from each isolate were combined to give inoculum densities of 0.5, 1.0, 5.0, and 10.0 ms/ g soil. Appropriate amount of suspensions were added to 3000 g premixed PRO-MIX 'BX' and Robin Hood top soil (1:1) in polyethylene bags. The infested soil was thoroughly mixed by shaking the soil in bags for 2 min. For each microsclerotia size × inoculum density combination, 14 plastic cone-tainers (Stuewe & Sons, Inc., Corvallis, OR) (21 by 3.8-cm) with mesh on one end were filled with infested soil. A randomized complete block design with seven replications was used. One germinated seed was planted into each cone-tainer which was placed in a rack. The bottom one-third of the cone-tainers was submerged in water for the duration of the experiment to provide a conducive environment for the disease. Plants were harvested after growing for 8 wk in a greenhouse at ~25^{o}C. Root rot ratings were visually estimated on a 0 to 5 scale, where 0 = no symptoms; 1 = some root discoloration, primarily on secondary roots; 2 = significant root browning and some necrosis, usually on secondary and tap root, with < 25% of roots affected; 3 = moderate root rot, 25-75% of roots affected; 4 = severe root rot, > 75% of roots affected; and 5 = dead plant. Crown rot ratings were assessed on a 0 to 3 scale, where 0 = no symptoms and 3 = completely rotted. Fresh root weight, shoot weight, and main stem height were measured. The test was repeated once.

Greenhouse experiment. The six genotypes asseded in the field were also evaluated for resistance to CBR in the greenhouse. Equal numbers of microsclerotia with sizes of 150-250 μm from each of the four isolates were combined to infest soil at 1.0 ms/g soil as previously described. A randomized complete block design with six replicates was used for this experiment and each replicate contained seven plants. Ten seeds of each of the six genotypes were planted in ten cone-tainers with noninfested soil as noninoculated controls. Root rot rating was estimated as described previously at 8 weeks after inoculation. Fresh root weight, shoot weight, and main stem height were measured. Plant height reduction and whole plant weight reduction were calculated by comparing the data of each inoculated treatment and the means of the noninoculated plants of each genotype. This experiment was conducted twice.

Inoculated field experiment. Inoculated field tests were conducted at two sites in 2006 and 2007. Site 1 was at the Blackshank farm in Tifton, GA and the soil was a Tifton loamy sand (fine-loamy, siliceous, thermic plinthic kandiudults). It was previously planted to peanut and fumigated with metam sodium (Vapam) at 93.5 liters per hectare 2 weeks prior to planting. Six peanut genotypes were planted on 19 May 2006 and 16 May 2007. Subplots were two rows 0.91 m wide and 6.1 m long with 120 seeds per row. A split-plot design was used. Main plots for genotypes were random and subplots were inoculated or not inoculated with *C. parasiticum*. Entire subplots were inoculated at 50 days after planting with 480 ml of microsclerotium suspension containing 50 ms/ml with equal numbers of microsclerotia (150-250 μm) from each of the four isolates. A beaker was used to distribute the suspension of microsclerotia on the soil surface around the peanut plants, and then overhead sprinklers applied 20 mm of water to facilitate movement of the inoculum down to the peanut roots. Recommended cultural practices were followed for land preparation and fertilization and for control of weeds and insects. Leaf

spots caused by *Cercospora arachidicola* and *Cercosporidium personatum* were controlled by regular foliar application of chlornthahonil (1.2 kg a.i. /ha). Stem rot, caused by *Sclerotium rolfsii,* was controlled by flutolanil (1.57 kg a.i. /ha) at 60 days after planting. The numbers of dead and wilted plants in each subplot were counted on 5 October 2006, and 22 October 2007. The total plant numbers and the numbers of diseased plants per subplot were counted after digging, and the CBR incidence calculated by dividing the number of diseased plants per plot by total plant numbers. Ten plants were randomly sampled from each inoculated subplot. Root rot ratings were visually estimated on a 0 to 5 scale as described previously. The taproots were collected and three pieces from each were surface sterilized in 0.25% sodium hypochlorite and plated on PDA to detect *C. parasiticum*. Colonies of *C. parasiticum* were counted after 5 days incubation at 25^{o}C. Pods were weighed after drying to about 15% moisture. The percent yield reduction was calculated from the yields of the inoculated and noninoculated subplots of each genotype. The total number of pods and the number of rotted pods were counted, then the percentage of pod rot was calculated as: pod rot (%) = (number of rotted pods/total number of pods in the sample) × 100.

Site 2 was on the Tifton campus of the University of Georgia. These plots were 170 × 140 cm^2 microplots, filled with Tifton loamy sand (fine-loamy, siliceous, thermic plinthic kandiudults) to a depth of 100 cm. Each plot was fumigated with metam sodium (Vapam) at 9.35 ml/m^2 two weeks prior to planting. Peanut seeds were planted on 16 May 2006, and 23 May 2007. Plots were two rows wide (85 cm between rows) and 140-cm long with 25 seeds per row. A split-plot design was used with genotypes as main plots and inoculation status as subplots. Fifty days after planting, eight plants were selected from one row of every main plot to be inoculated with a suspension of ms at 100 ms/plant, while the other row was kept uninoculated. Two 5-cm deep

holes around each selected plant were made with a stick (approximately 0.8-cm-dia.), and 5 ml of a microsclerotia suspension (10 ms/ml) was added to each hole. A flag was used to mark the inoculated plants. Chlorothalonil (1.2 kg a.i. /ha) was applied to foliage at 2- to 3-wk intervals to control leaf spot. Plots were drench-irrigated as needed to maintain soil moisture at favorable levels. The total numbers of diseased plants in each subplot were counted after digging, and the CBR infection success rate for every subplot was then calculated by dividing the number of diseased plants by the number of inoculated plants. Root rot was rated for all inoculated plants on the 0 to 5 scale described previously. Peanut yield was obtained after drying to about 15% moisture. The percentage yield reduction was obtained by comparing the yields of the inoculated and noninoculated subplots of each genotype. Taproots of all inoculated plants in each inoculated subplot were collected and plated as described previously.

Naturally infested field assessment. One field in the Southwest Research and Education Center in Plains, GA was used for identifying resistance to CBR in 2005 and 2006. The field was a Greenville sandy clay loam soil and was planted to cotton in 2004, but previously was in peanut and had a history of CBR. Entries were planted in four or five replicate subplots, each in two-row by 4.6-m plots with 80 plants per row in May 2005 or in May 2006. Rows were 0.91 m apart and treatments were arranged in randomized complete block design. Recommended cultural practices were followed for land preparation and fertilization and for control of weeds and insects. Leaf spots caused by *Cercospora arachidicola* and *Cercosporidium personatum* were controlled by regular foliar application of recommended fungicides. Stem rot, caused by *Sclerotium rolfsii,* was controlled by flutolanil (1.57 kg a.i. /ha) at 60 days after planting.

The numbers of dead and wilted plants in each plot were counted on 14 September 2005 (115 days after planting), and 4 October 2006 (126 days after planting). Entire plots were

evaluated for incidence of CBR after inverting with a commercial digger (29 September 2005 and 7 October 2006). Ten plants were collected randomly from each plot to rate CBR root rot on a scale of 0 (no visible symptoms) to 5 (completely decayed). All peanuts were picked by combine and weighed 10 days after digging.

Data analysis. Data from the greenhouse as well as the naturally infested and inoculated field experiment were subjected to analysis of variance using PROC GLM of SAS (version 9.1; SAS Institute, Inc., Cary, NC). Means were separated using Fisher's Protected LSD test. Differences referred to in the text were significant ($P \leq 0.05$) unless otherwise stated. Pearson correlation coefficients between various assays were computed based on data from treatment means from individual experiments and pooled experiments using the PROC CORR procedure of SAS.

RESULTS

Greenhouse inoculation technique. In the greenhouse experiments, *C. parasiticum* mainly infected below-ground parts of the plants, causing root tips to slough off and taproots to be blacken necrotic. Obvious crown rot symptoms were also observed at harvest; and there were no differences in crown rot ratings between C-99R (1.32) and Georgia-02C (1.24). The root rot rating on the susceptible genotype C-99R (2.03) was significantly higher ($P \leq 0.05$) than that on the resistant genotype Georgia-02C (1.27), which validated previous work. Root rot rating was apparently a better indicator for CBR resistance than was crown rot in the greenhouse screening.

The size of microsclerotia and inoculum density had significant effects on root rot ratings on both resistant and susceptible peanut genotypes (Table 4.1). Generally, the root rot rating was increased as the inoculum density increased within the same size of ms. At the same inoculum density, the bigger the ms the higher the root rot rating. The large ms (250 μm) could cause high

root rot severity on the resistant genotype Georgia-02C at high inoculum densities (5-10 ms/g soil). Only three out of the sixteen combinations of size of ms × inoculum density separated the moderately resistant genotype Georgia-02C from the susceptible C-99R. The smaller ms (53 and 75 μm) could not separate the two genotypes correctly at any of the inoculum densities.

Greenhouse experiment. There were no significant interactions between the two trials; therefore, the data were combined for analysis. Root rot rating, CBR incidence, plant height, and plant weight were used to assess the response of the peanut genotypes to *C. parasiticum* infection. At 8 weeks after inoculation, CBR incidences for all the genotypes were over 90%, and only Georgia-02C was lower ($P < 0.05$) than the others (Table 4.2). Georgia-02C also had less root rot than all other genotypes, while DP-1 had lower ratings than the most susceptible genotypes C34-24-85 and Georgia-01R.

For all the genotypes, CBR infection reduced plant main stem height and plant fresh weight compared to noninoculated plants (Table 4.2). The greatest impact on main stem height was for Georgia-01R, which was 44.5% shorter than the average plant without CBR. The other genotypes had generally similar reductions in plant height, but Georganic was less than C34-24-85. The genotypes Georgia-01R and C34-24-85 also showed the highest percentage weight reductions from CBR at 61.0% and 53.4%, respectively. Other genotypes were less and statistically similar.

In the greenhouse, there was a positive correlation between root rot and both height ($r = 0.3399$, $P = 0.0425$) and weight ($r = 0.5838$, $P < 0.0001$). Root rot was also correlated with CBR incidence ($r = 0.4511$, $P < 0.0001$); however, incidence was not correlated with the growth parameters, plant height and weight.

Inoculated field experiments. At site 1, the entire subplots were inoculated with suspension of ms followed by watering with sprinkler for 20 min. Overall CBR incidences were

lower in 2007 than in 2006. The final incidences (CBR2, based on below ground symptoms) were 24.4% to 67.9% for the six tested genotypes in 2006, while they were 11.6% to 52.5% in 2007 (Table 4.3). In both 2006 and 2007, Georgia-02C showed the lowest CBR2, and was significantly lower than C-99R while was the highest among the six genotypes. CBR2 values of the other genotypes were intermediate and often statistically similar to both C-99R and Georgia-02C. Cultivars C-99R and DP-1 were among the genotypes with highest aboveground incidence of CBR (CBR1), and those two along with Georgia-01R had the highest root rot ratings. Georgia-02C had fewer black pods than did Georganic or C-99R, but no other differences were significant for that rating. The percentage yield reductions from CBR for all genotypes were not significantly different from each other at site 1 (Figure 4.1).

At site 2 eight plants per plot were inoculated. The inoculation success rates indicate that with susceptible genotypes one or more plants per site had symptoms, but with the more resistant genotypes there was less than one diseased plant per inoculation. The responses of the selected genotypes to CBR inoculation were similar to those observed at site 1 (Table 4.4). The peanut cultivars Georgia-02C and Georganic showed higher resistance than cultivars C-99R and DP-1 with Georgia-01R being intermediate in both 2006 and 2007. The inoculation success rates of C34-24-85 were inconsistent between years. The root rot ratings of the inoculated plants of genotypes Georgia-02C, Georganic, and C34-24-85 were relatively lower than those for C-99R and DP-1, but the differences were not always significant. As with site 1, there were no differences among pod yield reductions (Figure 4.1). In both 2006 and 2007, there were no significant differences among genotypes for *C. parasiticum* isolation rates from the taproots of inoculated plants in the two inoculated field tests (Table 4.3&4.4).

Correlation analysis demonstrated a high correlation ($P < 0.01$) between disease incidence after digging (CBR2) and root rot rating and the percentage of black pods (Table 4.5). However,

CBR2 was correlated with root isolation frequencies of *C. parasiticum* only at site 2, and there were no significant correlations with yield reduction. Root rot rating was not correlated with the percentage of black pod in site 1 (Table 4.5).

Naturally infested field. Both root rot rating and incidence of CBR was greater in 2006 compared to 2005; however, pod yields of the genotypes were not lower in 2006 than in 2005 (Table 4.6). Actually, the pod yield of Georgia-02C was dramatically higher in 2006 than in 2005 (Table 4.6). Based on the number of dead and wilted plants before harvest (CBR1), only genotype C34-24-85 showed more disease than other cultivars in 2005, whereas C-99R was higher than the others in 2006. Incidence of CBR at harvest (CBR2) was higher in 2006 than 2005, but genotypes were similarly ranked in both years, except for C34-24-85. This genotype showed the greatest number of diseased plants and the highest root rot rating among the six genotypes in 2005; however, it showed the highest resistance to CBR in 2006. In both years, the CBR2 and root rot rating of Georgia-02C were significantly lower than those of C-99R. Georgia-02C also showed higher resistance than GA-01R and DP-1 based on the root rot rating in 2006, while C-99R showed higher susceptibility than Georganic, GA-01R, and C34-24-85.

In both 2005 and 2006, CBR1, CBR2, and the root rot rating were significantly positively correlated with each other. The coefficients of CBR2 and root rot rating were 0.8025 ($P < 0.01$) and 0.7784 ($P < 0.01$) in 2005 and 2006, respectively, and were higher than those of CBR1 and root rot rating (r = 0.6842, $P < 0.01$ in 2005; r = 0.4938, $P < 0.05$ in 2006). CBR2 also had a relatively lower coefficient of variation (CV%) than CBR1 (Table 4.6), and it was easier to assess than root rot ratings.

Correlation between the parameters evaluated in field and greenhouse experiments. The means of root rot ratings for greenhouse tests, CBR2 for inoculated field tests, CBR2 and percentage of yield reduction for naturally experiments in infested fields were analyzed by

PROC CORR of SAS. It showed that CBR2 for the naturally infested field experiment was significantly correlated with that for the inoculated field experiment ($P < 0.01$), but neither was correlated with the root rot ratings for greenhouse experiments (Table 4.7). There were no significant correlations observed between root rot rating, CBR2 for inoculated and naturally infested field, and the percentage of yield reduction in naturally infested field experiments.

DISCUSSION

Resistance to CBR in peanut is density dependent (Black and Beute, 1984). High inoculum density can cause serious damage even on resistant peanut genotypes. It is critical to standardize the size and density of the inoculum to obtain reproducible results in greenhouse screening tests. Our results showed that not only inoculum density, but also the size of microsclerotia was related to root rot ratings. At the same inoculum density, root rot was more severe for large microsclerotia. This was consistent with previous reports (Black and Beute, 1984). The most effective inoculum density for detecting differences in the degree of susceptibility was 1-5 ms/ g soil (150-250 μm).

Resistance of plants to pathogens is often defined as the ability of the plant to lessen, inhibit, or overcome the attack by the pathogen (Wingard, 1953). Selection for resistance implies being able to measure one or more of these. One method is to measure the amount of pathogen present at a given moment compared with the amount present on, or in, an extremely susceptible cultivar (Parlevliet, 1989, 1992); the larger the difference in amount, the greater the level of resistance (Ribeiro do Vale, et al., 2001). Another approach is to evaluate the direct or indirect effects of the pathogen on the host even if the pathogen itself is not visible (Parlevliet, 1993). CBR is a typical soilborne disease on peanut. It is very difficult to measure the amount of pathogen, because *C. parasiticum* is present primarily as microsclertotia (ms) within discard

plants or in the soil. Number of microsclerotia per gram root can be determined by use of elutriation and semiselective medium assay procedure, and populations have been shown to increase with root rot rating (Green et al., 1983a). Nevertheless, ms/g of root is not considered as a good criterion for which to select in a breeding program because 1) it is extremely difficult to assay microsclerotia in hundreds to thousands of progenies in a breeding program; and 2) many factors beyond resistance could result in large impacts on ms/g of root. For example, highly susceptible lines may have such badly decayed roots that much of the root system remains in the soil when the plant is dug. It is also possible that the roots of highly susceptible lines are killed rapidly by CBR infection. *C. parasiticum* is a poor saprophyte and is unable to produce ms on decayed roots (Crous, 2002). Also, selection based on ms production may omit those genotypes with tolerance to CBR, since high densities of microsclerotia could be recovered from the roots if they remain healthy and produce large root systems (Green et al., 1983a). In the greenhouse experiments, some peanut plants showed obvious crown rot symptoms. Such symptoms usually occur in the field only after significant root colonization, but because this represents a potentially different component of resistance it was also evaluated. Some crowns were completely rotted, while the root systems were still relatively unaffected. Overall, crown rot ratings were not a good criterion for resistance identification, due to the lack of difference between genotypes.

For peanut resistance breeding to CBR, host response to the pathogen is more meaningful. Researchers have used percent dead and diseased plants, root rot rating (Coffelt, 1980; Green et al., 1983b; Pataky et al., 1983), and pod damage (Coffelt and Garren, 1982; Wynne et al., 1975) to screen for resistance to CBR in peanut. In inoculated field experiments, several variables for disease assessment were investigated, including CBR incidence before and after harvest, root rot rating, and pod damage. Based on CBR incidence after harvest and root rot rating, the overall

evaluation of results from the two inoculated field tests were similar. The CBR incidence and root rot ratings were lower for Georgia-02C than for the susceptible cultivar C-99R, but *C. parasiticum* was isolated from 45% of the inoculated plants of Georgia-02C, which was similar to that from C-99R (48%). This indicates that Georgia-02C may be infected as readily as C-99R by *C. parasiticum,* but apparently the disease development is slower on Georgia-02C. We evaluated the degree of yield reduction from CBR by comparing the yield of inoculated subplots with the noninoculated subplots of the same genotype. This allows us to identify those genotypes with tolerance to CBR, ie those which may produce high yield in spite of having high disease incidence and severity. In our tests, there were no observed differences in yield reduction among the cultivars, suggesting a level of tolerance in the more susceptible lines.

We assessed the incidence of diseased and dead plants both before (CBR1) and after harvest (CBR2), and rated root rot on plants selected at random in the naturally infested field tests. Tomato spotted wilted virus (TSWV) infection can cause similar symptoms as CBR on peanut roots (Culbreath et al., 1991b), and may confound root rot rating assessment. Therefore, we also evaluated incidence of TSWV based on the above ground symptoms and analyzed the correlation coefficients between TSWV incidence and CBR2 and root rot rating. There were no significant correlations (data not shown), indicating that TSWV had little effect on CBR2 and root rot rating evaluations in our field experiments. Generally, incidence ratings after digging (CBR2) are the best variable for screening a large number of peanut lines in the field for CBR resistance. If time permits, the root rot severity rating also should be assessed because more than one resistance mechanism has been shown to exist in peanut (Coffelt and Garren, 1982).

In the naturally infested field experiments, inoculated field experiments, and the greenhouse experiments Georgia-02C and Georganic were among the genotypes with the highest

resistance to CBR. C-99R and DP-1 were the most susceptible genotypes in naturally infested and inoculated field experiments, but their root rot ratings were not the highest in the greenhouse experiments. In summary, the naturally infested field and inoculated field results were consistent, but the greenhouse results were consistent only for the most resistant and the most susceptible genotypes. Peanut genotypes are reliably screened in inoculated fields or uniformly infested natural fields, while greenhouse evaluations are quick and may be useful to identify and characterize components of resistance. Unfortunately greenhouse results are not always well correlated with field results for some genotypes. Further study is still needed to improve the current greenhouse techniques for CBR resistance screening.

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Table 4.1. Root rot ratings[x] for two peanut genotypes Georgia-02C and C-99R in the greenhouse, in relation to size of microsclerotia and inoculum density of *Cylindrocladium parasiticum*.

Genotype	CK	Size of microsclerotia (µm)							
		53				75			
		Inoculum density (ms/ g soil)							
		0.5	1	5	10	0.5	1	5	10
C99R	0.36 a[y]	0.64 a	0.79 a	0.86 a	1.32 a	1.07 a	0.79 a	1.61 a	2.00 a
Georgia-02C	0.16 a	0.50 a	1.14 a	0.29 a	1.15 a	0.57 a	0.64 a	1.07 a	1.50 a

Table 4.1 continued

Genotype	CK	Size of microsclerotia (µm)							
		150				250			
		Inoculum density (ms/ g soil)							
		0.5	1	5	10	0.5	1	5	10
C99R	0.36 a[y]	1.42 a	2.28 a	2.12 a	2.34 a	2.00 a	3.07 a	3.64 a	4.50 a
Georgia-02C	0.16 a	0.59 a	0.90b	0.79b	2.20 a	1.24 a	1.50b	2.78 a	3.64 a

[x] Data are means of two trials (seven replications/trial). Root rot rating on a scale of 0 to 5, where 0 = no symptoms, 1 = some root discoloration, primarily on secondary roots, 2 = significant root browning and some necrosis, usually on secondary and tap root, with < 25% of roots affected, 3 = moderate root rot, 25-75% of roots affected, 4 = severe root rot, > 75% of roots affected, and 5 = dead plant.

[y] Means in the same column followed by the same letter are not different ($P > 0.05$) according to Fisher's least significant difference *t* test.

Table 4.2 Responses of six peanut genotypes to *Cylindrocladium parasiticum* infection in the greenhouse[v]

Genotype	Root rot rating[w]	Incidence (%)	Height reduction[y] (%)	Weight reduction[z] (%)
Georgia-02C	1.68 c[x]	90.1 b	27.1 bc	19.7 b
Georgia-01R	2.74 a	98.6 a	44.5 a	61.0 a
DP-1	2.25 b	97.4 a	26.5 bc	34.0 b
Georganic	2.62 ab	97.9 a	22.1 c	26.3 b
C34-24-85	2.88 a	97.1 a	30.9 b	53.4 a
C-99R	2.53 ab	100.0 a	24.7 bc	20.0 b

[v] Data presented in this table are means of two trials (12 replications/trial).

[w] Root rot rating on a scale of 0 to 5, where 0 = no symptoms and 5 = dead plant.

[x] Means in the same column followed by the same letter are not different ($P > 0.05$) according to Fisher's least significant difference *t* test.

[y] Height reduce degree (%) = (Average main stem height of the noninoculated plants – Main stem height of inoculated plant)/ Average main stem height of the noninoculated plants × 100.

[z] Weight reduction (%) = (Average weight of the noninoculated plants - weight of inoculated plant) /Average weight of the noninoculated plants × 100.

Table 4.3. Cylindrocladium black rot (CBR) incidence, severity, and pod damage for different peanut genotypes in inoculated field test site 1.

Year Genotype	CBR1[a]	CBR2[b]	Root rot rating[c]	% Isolation frequency[d]	% Black pods[e]
2006					
Georgia-02C	6.5	24.4	2.0	50.0	35.7
Georgia-01R	6.1	53.2	3.3	38.9	43.9
DP-1	14.9	64.7	3.5	41.7	50.2
Georganic	7.2	36.4	1.9	38.9	53.7
C34-24-85	6.5	40.3	2.1	50.0	46.7
C-99R	15.7	67.9	3.2	44.5	58.0
LSD	9.1	22.8	1.1	34.4	14.8
cv%	63.7	31.7	26.3	52.0	20.4
2007					
Georgia-02C	0.6	11.6	1.7	48.2	21.0
Georgia-01R	4.1	46.4	2.9	34.2	25.0
DP-1	5.5	29.9	3.1	41.2	26.7
Georganic	3.0	26.7	2.1	34.2	39.9
C34-24-85	1.6	28.5	2.7	45.3	26.9
C-99R	3.4	52.5	3.7	46.9	45.9
LSD	5.7	34.8	0.8	30.5	10.1
cv%	124.3	70.9	18.7	46.7	24.2

[a] CBR1: CBR incidence before harvest (64 days after inoculation); = the percentage of dead and wilted plants of inoculated treatment - the percentage of dead and wilted plants of non-inoculated treatment.

[b] CBR2: percentage of plants with symptoms after digging (85 days after inoculation for site1, and 90 days after inoculation for site 2).

[c] Root rot rating on a scale of 0 to 5, in which 0 = no visible disease symptoms, and 5 = completely decayed.

[d] Percentage of inoculated plants from whose taproot *C. parasiticum* was isolated.

[e] Percentage of black pods in the entire inoculated subplot.

Table 4.4. Cylindrocladium black rot (CBR) incidence, severity, and pod damage for different peanut genotypes in inoculated field test site 2.

Genotype	2006			2007		
	Inoculation success rate[a] (%)	Root rot rating[b]	% Isolation frequency[c]	Inoculation success rate (%)	Root rot rating	% Isolation frequency
Georgia-02C	63.3	2.1	39.5	66.7	1.8	40.1
Georgia-01R	100.0	2.0	16.7	95.8	2.3	24.9
DP-1	126.7	2.7	37.0	87.5	2.2	35.3
Georganic	73.3	1.9	16.7	75.0	1.7	24.9
C34-24-85	73.3	1.7	27.5	100.0	2.2	34.8
C-99R	143.3	2.7	51.9	108.3	3.1	43.2
LSD	40.3	1.2	33.5	32.5	0.9	42.3

[a] Inoculation success rate= the average number of diseased plants divided by the number of inoculated plants, with one plant inoculated per site.

[b] Root rot rating on a scale of 0 to 5, in which 0 = no visible disease symptoms, and 5 = completely decayed.

[c] Percentage of inoculated plants from whose taproot *C. parasiticum* was isolated.

Table 4.5. Correlation coefficients between different variables in inoculated field experiments

	Root rot rating[a]	%Black pod[b]	%Yield reduction[c]	Isolate frequency[d]
Site 1[f]				
CBR2[e]	0.6943**	0.4469*	0.3340	-0.1812
Root rot rating		0.1694	0.3114	-0.3749
%Black pod			0.2272	-0.0956
%Yield reduction				-0.1538
Site 2[g]				
CBR2	0.7785**	--	0.3230	0.5381*
Root rot rating		--	0.1525	0.6206**
%Yield reduction				0.2329

[a] Root rot rating on a scale of 0 to 5, where 0 = no visible disease symptoms and 5 = completely decayed.

[b] Percentage of black pod in the entire inoculated subplot.

[c] Percentage of pod yield loss compared to uninoculated subplot.

[d] Percentage of inoculated plants from whose taproot *C. parasiticum* was isolated successfully.

[e] CBR2: percentage of plants with symptoms after digging (85 days after inoculation for site1, and 90 days after inoculation for site 2).

[f] Data from the whole subplot inoculation tests in 2006 and 2007 were combined for analysis by Proc CORR of SAS.

[g] Data from the 8-plant inoculation tests in 2006 and 2007 were combine for analysis by Proc CORR of SAS.

* Correlations significant $P \leq 0.05$; ** Correlations significant at $P \leq 0.01$.

Table 4.6. Cylindrocladium black rot (CBR) incidence and severity for six peanut genotypes in naturally infested field in 2005 and 2006, Plains, GA

Genotype	2005				2006			
	CBR1[x]	CBR2[y]	Root rot rating[z]	Yield (kg/ha)	CBR1	CBR2	Root rot rating	Yield (kg/ha)
Georgia-02C	0.05	0.13	0.53	2969.8	0.76	2.30	1.18	4894.0
Georgia-01R	0.15	0.50	1.60	4463.5	0.60	2.38	2.23	4767.3
DP-1	0.18	0.55	1.88	3606.0	0.72	3.38	2.78	4264.4
Georganic	0.18	0.38	1.20	4214.8	0.64	1.80	1.73	4563.9
C34-24-85	1.00	1.57	3.17	3515.7	0.38	0.94	1.10	3442.6
C-99R	0.35	0.73	2.40	3219.9	2.04	5.00	3.63	3561.1
LSD(0.05)	0.35	0.48	1.22	1062.0	1.06	2.15	0.98	1354.1
CV%	73.95	47.07	38.40	18.4	91.89	61.72	30.90	23.6

[x] CBR1 = number of plants with above ground symptoms of CBR per meter row before harvest, assessed at 115 and 126 days after planting in 2005 and 2006, respectively.

[y] CBR2 = number of plants with CBR symptoms per meter row at harvest.

[z] Root rot rating on a scale of 0 to 5, where 0 = no visible disease symptoms and 5 = completely decayed, for ten plants per plot.

Table 4.7. Correlation coefficients between variables for Cylindrocladium black rot (CBR) assessment and plant growth traits in naturally infested field, inoculated field, and greenhouse tests.

	Greenhouse	Inoculated field	Naturally infested field	
	Root rot rating[a]	CBR2 (IF)[b]	CBR2 (NF)[c]	%Yield reduction[d]
Root rot rating		0.3428	0.3271	0.7284
CBR2 (IF)			0.8369*	0.3647
CBR2 (NF)				-0.1386

[a] Root rot rating on a scale of 0 to 5, where 0 = no visible disease symptoms and 5 = completely decayed.

[b] CBR2 (IF): percentage of plants with symptoms after digging, the averages of two inoculated field tests were used.

[c] CBR2 (NF) = number of plants with CBR symptoms in per meter row at harvest in naturally infested field.

[d] Percentage of pod yield loss compared to uninoculated subplot in inoculated field tests.

* Correlations significant at $P \leq 0.05$; ** Correlations significant at $P \leq 0.01$.

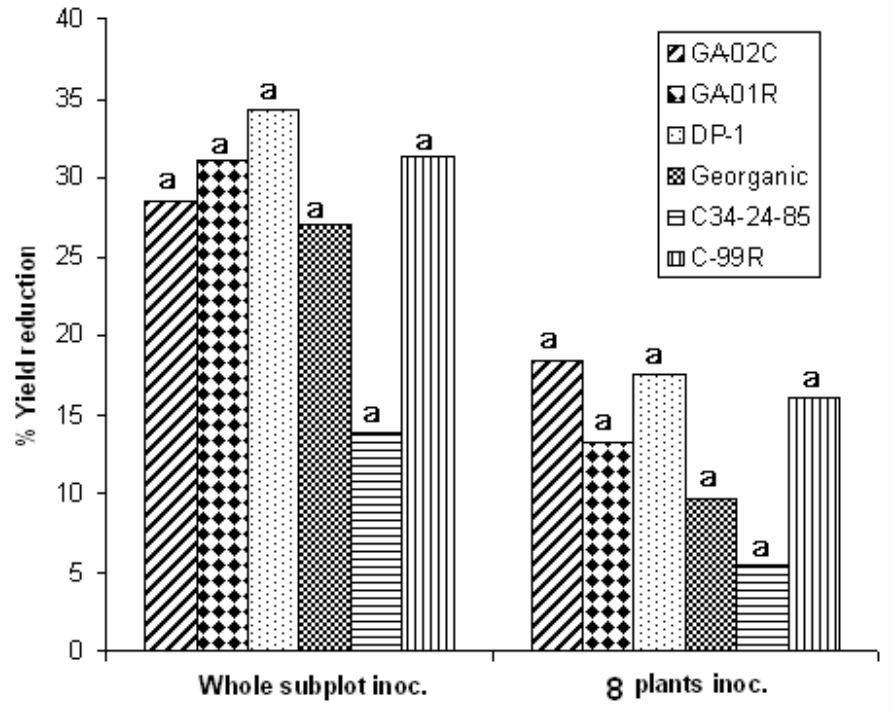

Figure 4.1. Pod yield reduction for different peanut genotypes caused by Cylidrocladium black rot (CBR) infection in two inoculated field experiments. Bars represent the averages of 2006 and 2007. Means within a test followed by the same letter are not different ($P > 0.05$) according to Fisher's least significant difference t test. %Yield reduction = 100 × (1-(yield of inoculated subplot/yield of noninoculated subplot)).

APPENDIX TO CHAPTER 4

Figure 4A.1. Identification of resistance to Cylindrocladium black rot (CBR) in the greenhouse.

Figure 4A.2. Schematic of Cylindrocladium black root rot rating system where 0=no visible disease symptoms and 5=completely decayed.

Figure 4A.3. Perithecia of *Cylindrocladium parasiticum* form on the crown of peanut plant in the greenhouse.

Figure 4A.4. Underground symptoms of Cylindrocladium black rot on peanut in the field inoculation test.

Figure 4A.5. Peanut pods rot caused by *Cylindrocladium parasiticum*.

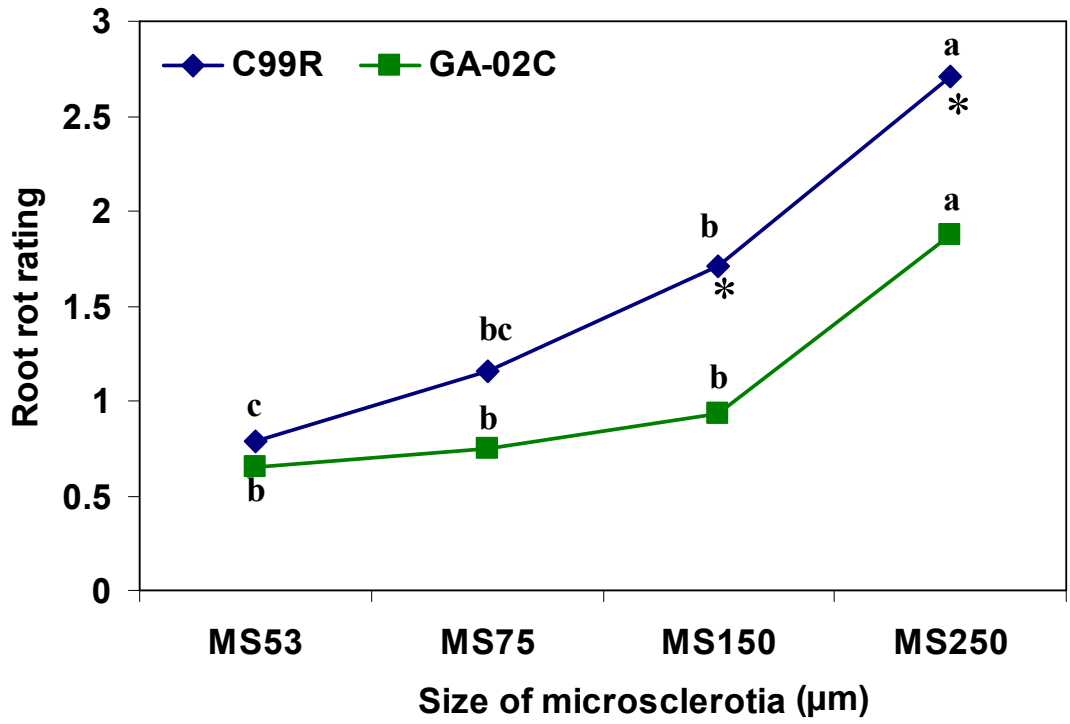

Figure 4A.6. Effects of size of microsclerotia on root rot rating in two peanut genotypes C-99R and GA-02C in the greenhouse inoculation test. Means within the same genotype followed by the same letter are not different ($P > 0.05$), according to Fisher's least significant difference t test. A star means that root rot rating in C-99R was separated from that in GA-02C ($P \leq 0.05$) when inoculated with same size of microsclerotia.

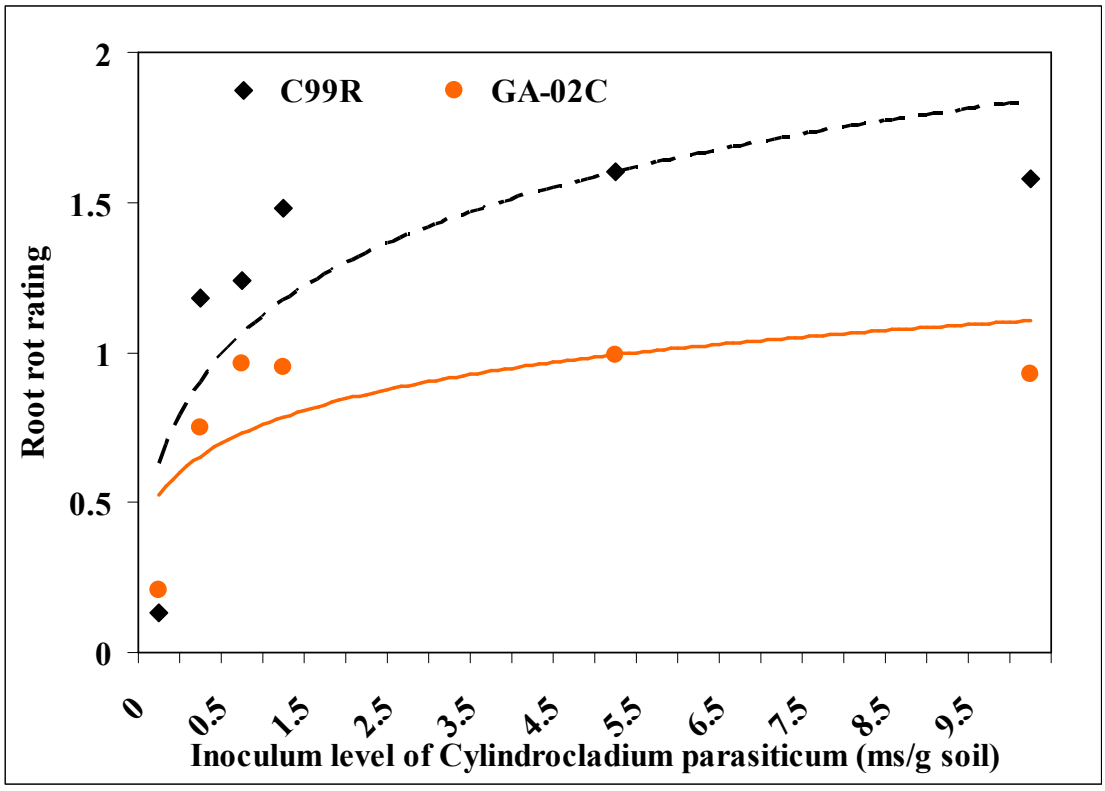

Figure 4A.7. Effects of inoculum level on root rot rating in two peanut genotypes C-99R and GA-02C in the greenhouse inoculation test, inoculated after peanut emergence.

Table 4A.1. Root rot rating and crown rot rating on two peanut genotypes with different resistance levels to Cylindrocladium black rot (CBR) in the greenhouse, inoculated with a microsclerotia suspension after peanut emergence[w].

Genotype	Root rot rating[x]	Crown rot rating[y]
C-99R	1.34 a[z]	1.07 a
GA-02C	0.88 b	0.95 a

[w] Data presented are means of two trials (seven replications/trial).

[x] Root rot rating on a scale of 0 to 5, where 0 = no symptoms, 1 = some root discoloration, primarily on secondary roots, 2 = significant root browning and some necrosis, usually on secondary and tap root, with < 25% of roots affected, 3 = moderate root rot, 25-75% of roots affected, 4 = severe root rot, > 75% of roots affected, and 5 = dead plant.

[y] Crown rot ratings were assessed on a 0 to 3 scale, where 0 = no symptoms and 3 = completely rotted.

[z] Means in the same column followed by the same letter are not different ($P > 0.05$) according to Fisher's least significant difference t test.

Table 4A.2. Pre-field inoculation test on late maturity peanut genotypes: effects of microsclerotium size on development of Cylindrocladium black rot (CBR) (Blankshank farm, Tifton, 2005)*

Ms Size (μm)	Genotype			
	C-99R		GA-02C	
	Wilted plants/plot	Efficiency (%)#	Wilted plants/plot	Efficiency (%)
40	2.5 a+	31.3 c	2.5 ab	31.3 ab
50	5.5 ab	68.8 a	2.5 ab	31.3 ab
60	5.0 ab	62.5 b	1.5 b	18.8 b
100	7.0 a	87.5 a	3.5 a	43.8 a

* Inoculated on August 19, 2005 (79 days after planting). Two 3-cm deep and 1-cm diameter holes were made around the plants. Suspension of ms with 100 ms was added to each hole. Eight plants were inoculated in each plot and marked with flags.

Efficiency (%) = wilted plants/inoculated plants × 100.

+ Data presented are means of four replications. Means in the same column followed by the same letter are not different ($P > 0.05$) according to Fisher's least significant difference t test.

Table 4A.3. Pre-field inoculation test on late maturity peanut genotypes: effects of inoculum level on development of Cylindrocladiumblack rot (CBR) (Blankshank farm, Tifton, 2005)*

Inoculum level (ms/plant)	Genotype			
	C99R		GA-02C	
	Root rot rating[@]	Incidence (%)[#]	Root rot rating	Incidence (%)
0	0.1[+]	0.2	0.6	1.9
20	1.7	11.0	1.1	6.9
100	3.2	11.4	2.4	8.7
1000	3.8	13.1	3.2	11.2

* Peanuts were inoculated on July 20, 2005 (50 days after planting) with suspension of microsclerotium. A 250 ml suspension with different level of inoculum was applied on the soil surface around plants in each row.

@ Root rot rating on a scale of 0 to 5, where 0 = no symptoms, 1 = some root discoloration, primarily on secondary roots, 2 = significant root browning and some necrosis, usually on secondary and tap root, with < 25% of roots affected, 3 = moderate root rot, 25-75% of roots affected, 4 = severe root rot, > 75% of roots affected, and 5 = dead plant.

Incidence (%) = (Number of diseased plants / Number of total plants) ×100.

+ Data presented are means of two replications.

Table 4A.4. Pre-field inoculation test: effects of microsclerotium size and inoculum level on CBR incidence in early to middle maturity peanut genotypes (Blankshank farm, Tifton, 2005)*

Inoculum level (ms/plant)	Genotype			
	Carver		Georgia Green	
	Size of ms			
	150 µm	250 µm	150 µm	250 µm
0	0.4[#]	0.8	0.4	0.8
20	9.6	7.6	11.4	6.8
100	11.2	10.8	15.9	13.4
1000	10.3	9.4	14.8	12.5

* Peanuts were inoculated on July 20, 2005 (50 days after planting) with suspension of microsclerotium. A 250 ml suspension with different levels of inoculum was applied on the soil surface around plants in each row.

[#] Data presented are means of two replications.

CHAPTER 5

THE INTERACTION BETWEEN *MELOIDOGYNE ARENARIA* AND *CYLINDROCLADIUM PARASITICUM* IN RUNNER PEANUT[1]

[1]W. Dong, T. B. Brenneman, C. C. Holbrook, P. Timper, and A. K. Culbreath. 2007. To be submitted to Plant Disease.

ABSTRACT

Cylindrocladium black rot (CBR), caused by *Cylindrocladium parasiticum*, and root-knot nematode (*Meloidogyne arenaria*) both cause significant yield loss on peanut, and both pathogens infect and cause damage to the roots of the plant. Greenhouse and microplot experiments were conducted with the runner type peanut genotypes C724-19-15 (resistant to *M. arenaria*), C724-19-25 (susceptible to *M. arenaria*), and Georgia-02C (susceptible to *M. arenaria*, partially resistant to *C. parasiticum*) to better understand the interactions between the two pathogens. In the greenhouse, root rot ratings were increased in all three peanut genotypes by addition of 500-3000 eggs/plant of *M. arenaria* with low inoculum level (1.0 microsclerotium (ms)/g soil) of *C. parasiticum*. The nematode did not affect the root rot induced by a high inoculum level (5.0 microsclerotia/g soil) of *C. parasiticum*. Infection with *M. arenaria* resulted in severe pod galling on Georgia-02C and C724-19-25, but not on C724-19-15. Gall indices were not affected by *C. parasiticum* inoculations in the greenhouse or microplots. In microplot experiments, the root rot ratings from nematode-susceptible genotypes Georgia-02C and C724-19-25 were higher in plots infested with *M. arenaria* (0.4-2.0 eggs/cm^3 soil) and *C. parasiticum* than in plots with *C. parasiticum* alone; however, *M. arenaria* did not increase the root rot ratings on the nematode-resistant genotype C724-19-15. In both 2006 and 2007, a significant interaction between *C. parasiticum* inoculum densities and nematode level was observed on plant mortality. CBR inoculum increased the mortalities on C724-19-25 and Georgia-02C, but not on C724-19-15, in the presence of *M. arenaria*. Simultaneous inoculation with *M. arenaria* decreased yield incrementally on C724-19-25 and Georgia-02C as *C. parasiticum* inoculum levels increased, but even a high level of *M. arenaria* (2.0 eggs/cm^3 soil) did not decrease yield of C724-19-15 when also inoculated with *C. parasiticum*.
Additional keywords: *Arachis hypogaea*, groundnut,

Peanut (*Arachis hypogaea* L.) is a basic source of vegetable oil and proteins world wide, and it is an important crop in the southeastern United States. The plants are unusual in that they flower above ground but the fruit (pods) develop underground. The root systems, as well as the pegs and pods, are susceptible to many soilborne pathogens. Two of the most important soilborne diseases of peanut in the southern United States are root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood race 1), and Cylindrocladium black rot (CBR), caused by *Cylindrocladium parasiticum* Crous, M. J. Wingf. & Alfenas (Martinez, 2005). *Meloidogyne arenaria* is prevalent in Alabama, Georgia, Florida, and Texas, where as many as 40% of the peanut fields are infested with this pathogen (Dickson 1998; Ingram and Rodríguez-Kábana, 1980; Koenning et al., 1999; Minton and Baujard, 1990). Yield loss in heavily infested fields can be as much as 50%. CBR was found first in 1965 in Georgia (Bell and Sobers, 1966), and it presently threatens peanut production throughout the southeastern United State (Harris and Beute, 1982). It is difficult to eradicate either of these pathogens, and the number of fields infested with CBR and/or nematodes has apparently increased in recent years (T. Brenneman, personal communication). In Georgia alone, root-knot nematode and CBR cost peanut growers $11.4 and $4.3 million, respectively, in annual losses and costs of control from 2002-2006 according to University of Georgia extension service estimates (Williams-Woodward, 2002, 2003; Pearce, 2004; Martinez, 2005, 2006); they reduced grades of peanut kernels as well.

Root-knot nematode and CBR are frequently found together in peanut fields (Culbreath etal., 1992; Diomande and Beute, 1981a), particularly, those with poor crop rotations. There are several reports documenting a disease complex between root-knot nematodes and CBR on peanut (Culbreath etal., 1992; Diomande and Beute, 1981a & b; Diomande et al., 1981). *Meloidogyne hapla* increased CBR severity on both a CBR-resistant genotype, NC 3033, and a CBR-susceptible genotype, Florigiant (Diomande and Beute, 1981a & b). Culbreath et al.

(Culbreath etal., 1992) found that severity of CBR was increased in Florigiant by either *M. hapla* or *M. arenaria* with fungal inoculum densities of 0.05 and 0.5 microsclerotia 1 gram soil. Severity of black rot was not affected by either *M. hapla* or by *M. arenaria* on genotypes moderately resistant to CBR, such as NC 10C or NC Ac 18016 in the greenhouse. However, in the microplot experiments, root rot severity was enhanced by addition of *M. arenaria* or *M. hapla* on both CBR-susceptible and resistant genotypes. Earlier work showed that race 2 of *M. arenaria*, which is not a pathogen of peanut, also promoted greater root rot from CBR (Diomande et al., 1981). The genotypes used in all of these studies were virginia type peanut. However, runner type peanuts currently account for approximately 80% of the total U.S. production (American Peanut Council, 2007), and interactions between *M. arenaria* and *C. parasiticum* on nematode-resistant or susceptible runner peanut genotypes have not been reported.

Significant progress has been made in breeding runner peanuts with resistance to *M. arenaria* and *C. parasiticum*. The first runner peanut cultivar offering partial resistance to *C. parasiticum* was released in 2002 (Branch, 2003). However, the resistance to *C. parasiticum* may be overcome when the cultivars are grown in *M. arenaria* infested fields. Cultivars with high resistance to *M. arenaria* are also available (Holbrook et al., 2008; Simpson and Starrr, 2001; Simpson et al., 2003). In order to effectively manage both the nematode and *C. parasiticum* in concomitantly infested fields, we need to better understand the interaction of *M. arenaria* and *C. parasiticum* on runner peanut with or without resistance to either pathogen.

The objective of this study was to determine the potential interactions between *M. arenaria* and *C. parasiticum* in runner peanut, particularly in CBR-resistant and Ma (*Meloidogyne arenaria*)-resistant genotypes.

MATERIALS AND METHODS

Genotypes evaluated. Three runner peanut genotypes were used in this study: Georgia-02C is moderately resistant to *C. parasiticum* and highly susceptible to *M. arenaria* (Branch, 2003), C724-19-15 and C724-19-25 are near isogenic breeding lines (Holbrook et al., 2007); C724-19-15 is highly resistant, while C724-19-25 is susceptible to *M. arenaria*.

Inocula production. Fungal inoculum for greenhouse and microplot tests was produced on potato-dextrose agar (PDA). Four isolates of *C. parasiticum* (CBR041, CBR0410, CBR0414, and CBR0418) obtained from infected peanut plants in southern Georgia in 2004, were used in all tests. To obtain microsclerotia (ms), the isolates were grown on PDA for 6-7 wk, after which the cultures were comminuted in a Waring Blendor for 2 min and passed through nested sieves with 250 µm and 150 µm openings (60 and 100 mesh, respectively). Microsclerotia in the 150-µm sieve were separated from mycelium fragments by passing a forceful stream of water through the sieve for 1 min. Microsclerotia were then rinsed into a 200-ml beaker containing a about 100 ml of water. The concentration of the microsclerotia suspension was determined and adjusted with the aid of a microscope before use. Equal numbers of microsclerotia from each isolate were used in all tests.

Meloidogyne arenaria race 1, originating from a peanut field in Tifton, GA, was cultured alternately on tomato (*Lycopersicon esculentum* cv. Rutgers) or eggplant (*Solanum melongena* cv. Blackbeauty) and peanut (cv. Georgia Green) to reduce potential contamination from *M. incognita* (a parasite of tomato and eggplant but not peanut). Eggs for inoculum for greenhouse tests were extracted from tomato or eggplant roots by agitating in 0.05% NaOCl for 2-3 min (Hussey and Barker, 1973). The eggs were then collected and rinsed with tap water on nested 150- and 25-µm-pore sieves. Inoculum of *M. arenaria* for microplot tests was prepared as

infested root tissue from cultures maintained on eggplant. After allowing 10 wk for nematode reproduction, plants were harvested and the roots washed free of soil. The infected roots were cut into segments 2 to 3 cm long and chopped in a Waring Blendor for 2 min with water. Three samples (200 ml for each one) of this suspension of infected roots were collected to estimate the number of eggs and second stage juveniles (J2). The root samples were treated with 1.0% NaOCl for 5 min. Eggs and J2 were collected in the 25- µm (500 mesh) sieve and were counted with the aid of a microscope.

Greenhouse experiment. The three genotypes (Georgia-02C, C724-19-15, and C724-19-25) were grown in all combinations of three *C. parasiticum* inoculum densities (0, 1.0, and 5.0 ms/g soil) and three nematode levels (0, 500, and 3000 eggs/pot). Appropriate amount of inoculum suspensions were added to 3000 g premixed PRO-MIX 'BX' and Robin Hood top soil (1:1) in polyethylene bags. The infested soil was thoroughly mixed by shaking the soil in bags for 2 min. A split-plot treatment design was used with genotypes as main plots. Subplots of *C. parasiticum* densities × *M. arenaria* levels were randomized within ten replicate main plots. One seed was planted in each 10 × 10-cm square pot filled with infested soil, and the pot was placed on a bench in the greenhouse. The bottom one-third of the pots were submerged in water for the duration of the experiment to provide a conducive environment for CBR. Plants were harvested after 8 wk in a greenhouse at ~25^{o}C. Root rot ratings were visually estimated for CBR symptoms based on a 0 to 5 scale, where 0 = no symptoms; 1 = some root discoloration, primarily on secondary roots; 2 = significant root browning and some necrosis, usually on secondary and tap root, with < 25% of roots affected; 3 = moderate root rot, 25-75% of roots affected; 4 = severe root rot, > 75% of roots affected; and 5 = dead plant. Gall indices were also assessed for nematode symptoms based on a 0 to 5 scale, where 0 = no galling; 1 = trace infection with a few

small galls; 2 ≤ 25% of root galled; 3 = 26-50%; 4 = 51-75%; and 5 > 75% of root galled. Whole plant fresh weight was measured, and the experiment was conducted three times with 10 replicates for each treatment.

Microplot experiment: The microplot experiment was conducted at the Tifton Campus of the University of Georgia in 2006 and 2007. These plots were 170 × 140 cm^2 concrete microplots, filled with Tifton loamy sand (fine-loamy, siliceous, thermic plinthic kandiudults) to a depth of 100 cm. Each plot was fumigated with metam sodium (Vapam) at 9.35 ml/m^2 two weeks prior to planting. Peanut was planted on 16 May 2006, and 22 May 2007. A split-plot was the experimental design, with *C. parsisticum* densities × nematode levels as main plots and genotypes as subplots over two years with eight replicates. Inoculum densities of *C. parasiticum* were 0, 0.5, and 5.0 ms/cm^3 soil (0, 3200, and 32000 ms/plant), and nematode levels were 0, 0.4, and 2.0 eggs+J2/ cm^3 soil (0, 2500, and 12700 eggs+J2/plant), calculated for the top 20 cm of soil. The microplots were hand-planted with three 5-cm-deep furrows made by hoe (46.3 cm between rows and 170 cm long) in each main plot. Twenty-five seeds were planted in each row after the appropriate inoculum densities in 1000 ml water were applied in every furrow. Seeds were covered with soil, and herbicides (Sonalan 0.80 kg a.i. /ha + Dual Magnum 1.89 kg a.i./ha) were applied within 3 days after planting for weed control. Chlorothalonil (1.2 kg a.i. /ha) was applied at 2- to 3-wk intervals to control foliar fungal diseases. Plots were drench-irrigated as needed to maintain soil moisture at conducive levels for CBR development. The total plant numbers and the numbers of dead and wilted plants per subplot were counted at harvest (28 September 2006 and 29 September 2007). Plant mortality in each subplot was then calculated by dividing the number of dead and wilted plants by total plant numbers. Ten plants were randomly collected from each subplot for root rot rating and gall index based on the scales described

previously. All peanuts were hand-picked and weighed after drying to approximately 10% moisture.

Data analysis. Data from greenhouse and microplot experiments were analyzed using Proc MIXED with ddfm = satterth option (a general Satterthwaite approximation for the denominator degrees of freedom) on the model statement (SAS v.9.1; SAS Institute, Cary, NC), unless otherwise stated. Any interaction effects that were not significant were removed and the reduced model was evaluated again. Main effects and interactions were considered significant when $P \leq 0.05$. Fisher's least significant difference (LSD) values at $\alpha = 0.05$ were computed using standard error and t values of adjusted degrees of freedom from the LSMEAN statement in Proc MIXED.

RESULTS

Greenhouse experiment. The results from three greenhouse trials were combined for analysis. Significant difference ($P = 0.0021$) was noted in root rot rating only for the main factor of *C. parasiticum* inoculum density, and all other main factors and interactions were not significant (Table 5.1). Root rot ratings generally increased in all three genotypes as the *C. parasiticum* inoculum densities increased (Table 5.2). The patterns of response to ms densities were similar at each nematode level. Root rot ratings were increased in the two nematode-susceptible genotypes by the addition of *M. arenaria* in soil with the noninoculated low inoculum level (1 ms per g soil) of *C. parasisicum*, but not in the nematode-resistant C724-19-15. At 5 ms per g soil, root rot was more severe but was not affected by nematode levels. At both inoculum levels of *C. parasiticum*, root rot ratings were generally less in Georgia-02C than in C724-19-15 and C724-19-25, however, the differences were not always significant.

Moderate root and pod galling occurred on C724-19-25 and Georgia-02C at both 500 and 3000 nematode eggs/pot levels of nematode inoculum, and gall indices increased as the inoculum level increased (Fig. 5.1A). Gall indices were significantly lower in the resistant genotype C724-19-15 than in the other two genotypes. Inoculation with 500 to 3000 eggs/pot only cause a few galls on several plants of C724-19-15. Inoculum densities of *C. parasiticum* had no significant effects on the gall index in any of the three genotypes (Fig. 5.1B).

Both CBR and root-knot nematode can damage peanut root systems and may profoundly affect the growth of the plant. In our greenhouse tests, only the main factor of *C. parasiticum* inoculum density showed an effect ($P = 0.023$) on whole plant weight (Table 5.1). The whole plant weights of C724-19-15 and C724-19-25 were decreased in plants grown in soil with 1 and 5 ms /g soil, while weights of Georgia-02C were reduced only by 5 ms/g soil (Fig. 5.2). The effects of the main factors of nematode level and genotype, and the interactions were not significant ($P > 0.05$).

Microplot experiment. In 2006, peanut genotype, *C. parasiticum* inoculum density, and nematode level, as well as genotype × nematode level interaction had significant effects on root rot ratings, whereas other interactions did not (Table 5.3). Root rot ratings on all three peanut genotypes generally increased as the *C. parasiticum* inoculum density increased (Fig. 5.3). In the absence of nematodes, there were no differences among root rot ratings on the three genotypes within the same *C. parasiticum* inoculum density. Root rot ratings on C724-19-25 and Georgia-02C plants grown in plots infested with both *C. parasiticum* and *M. arenaria* were higher than those infested with the fungus alone, especially at the higher level of *M. arenaria.* *Meloidogyne arenaria* alone did not increase root rot on C724-19-15 and C724-19-25, but the high population did on Georgia-02C.

In 2007, all the main factors and interactions showed significant effects on root rot ratings (Table 5.3). On all three peanut genotypes, root rot ratings generally increased as the *C. parasiticum* inoculum density increased from 0 to 5 ms/cm^3 soil, but often were not different ($P > 0.05$) between 0.5 and 5 ms/cm^3 soil within the same nematode level (Fig. 5.3). However, at the highest rate of nematode inoculation, root rot rating on C724-19-15 with 0.5 ms of *C. parasiticum* was less than that with 5 ms, and in the absence of the nematode, root rot rating on C724-19-25 with 5 ms was greater than that with 0.5 ms. Similarly, root rot ratings on the three genotypes at the same *C. parasiticum* inoculum density were not different in absence of nematodes. High populations of *M. arenaria* (2.0 eggs + J2/cm^3 soil) alone caused root rot on all three genotypes, and even the low nematode level (0.4 eggs + J2/cm^3 soil) significantly increased root rot ratings on C724-19-25 and Georgia-02C. Root rot ratings on plants grown in plots infested with *C. parasiticum* and *M. arenaria* were higher than those infested with the fungus alone, with the exception of nematode-resistant genotype C724-19-15. An apparently synergistic interaction between *C. parasiticum* inoculum densities and nematode levels occurred on C724-19-25, but not on C724-19-15 and Georgia-02C (Table 5.3; Fig. 5.3). On C724-19-25, root rot ratings caused by co-infestation at high levels of *M. arenaria* and *C. parasiticum* were significantly higher than the sum of those caused by the same levels of nematode and fungus alone (Fig. 5.3).

In both 2006 and 2007, the effects of genotype, nematode level, and genotype ×nematode level on gall index were significant, whereas *C. parasiticum* inoculum density and the other two-factor or three-factor interactions were not (Table 5.3). Only an occasional plant contained a low number of galls in plots without nematode inoculation, verifying that background populations of nematodes in the microplots was not an issue. In 2006, the high nematode level

caused greater ($P \leq 0.05$) root galling than the low nematode level on C724-19-25 and Georgia-02C in all but one comparison (Fig. 5.4). In 2007, even the low nematode level caused such severe galling on the nematode-susceptible genotypes that any additional effect of the high nematode level on galling was not apparent. By contrast, only a few galls were formed on the roots of C724-19-15 at the highest nematode inoculation level. Inoculum densities of *C. parasiticum* did not show significant effects on gall indices on any of the three peanut genotypes.

In both 2006 and 2007, dead and wilted plants were observed in all three peanut genotypes before harvesting, but the mortality was lower ($P \leq 0.05$) in C724-19-15 than in Georgia-02C or C724-19-25 (Table 5.4). Genotype, *C. parasiticum* inoculum density, nematode level, genotype × nematode level, genotype × *C. parasiticum* inoculum density, and nematode level × *C. parasiticum* inoculum density all affected plant mortalities in both years (Table 5.3). The three-factor interaction of genotype × nematode level × *C. parasiticum* inoculum density effect was also significant in 2007 (Table 5.3). In 2006 and 2007, *C. parasiticum* inoculum and *M. arenaria* alone or combined did not increase the mortality in the nematode-resistant genotype C724-19-15, and infestations with *C. parasiticum* inoculum alone frequently did not increase plant mortality in any of the genotypes. However, *C. parasiticum* inoculum strongly increased the mortalities in C724-19-25 and Georgia-02C in the presence of *M. arenaria*. The high level of nematode inoculum alone increased the mortality of C724-19-25 in 2006, while it increased the mortality of Georgia-02C in both 2006 and 2007. Of the main effects tested, nematode level, *C. parasiticum* inoculum density, and plant genotype explained 14.4, 8.3, and 6.4%, and 19.5, 5.1, and 14.9% of the mortality in 2006 and 2007, respectively. This indicated that the environmental conditions were more conducive for nematode, but less conducive for CBR in 2007, which in fact was observed in other trials as well (unpublished data).

In 2006 and 2007, genotype, nematode level, and *C. parasiticum* inoculum density, as well as the interaction of genotype × nematode level had significant effects on pod yield, but other interactions were not significant (Table 5.3). The three tested genotypes showed similar yields in the absence of *M. arenaria* (Fig. 5.5). The genotype C724-19-15 had much higher yield than the other two genotypes in the presence of the nematode (Figs. 5.5 & 5.6). Yield reduction of C724-19-25 was less in 2006 (11.0%) than in 2007 (49.0%) as the nematode level increased from 0 to 0.4 eggs + J2/cm^3 soil, while it was greater in 2006 (43.4%) than in 2007 (19.2%) as the nematode level increased from 0.4 to 2 eggs + J2/cm^3 soil. Similar trends were evident with Georgia-02C. Yield trends for the three genotypes in response to *C. parasiticum* inoculation were similar in 2006 and 2007: yield of C724-19-25 decreased as the *C. parasiticum* inoculum density increased in the presence of *M. arenaria* (Fig. 5.6), but there were no differences in yield for genotypes C724-19-15 and Georgia-02C. This indicates that C724-19-15 may have a degree of tolerance to CBR in addition to its nematode resistance, especially since there were no interactions with nematode injury confounding the yield data.

DISCUSSION

The main purpose of this study was to determine the interactions between *Meloidogyne arenaria* and *Cylindrocladium parasiticum* in runner peanut with different levels of nematode and CBR resistance. The three peanut genotypes were selected based on their combinations of disease resistance: Georgia-02C for moderate resistance to CBR and high susceptibility to *M. arenaria* (Branch, 2003), C724-19-15 for high nematode resistance, and C724-19-25 a near isogenic line of C724-19-15 without nematode resistance (Holbrook et al., 2007). We assumed C724-19-15 and C724-19-25 had no resistance to CBR based on their pedigree. However, we

found them have a similar level of partial resistance to CBR as found in Georgia-02C. In addition, all three genotypes have good resistance to tomato spotted wilt virus (TSWV) (Branch, 2003; Holbrook et al., 2007), which minimized the potential confounding effects of TSWV on root rot evaluation and pod yield (Culbreath et al., 1991).

Development of disease symptoms is not solely determined by the pathogen responsible, but is dependent on the complex interrelationship among host, pathogen and prevailing environmental conditions. In the case of soilborne pathogens, further opportunities exist for interactions with other microorganisms occupying the same ecological niche. The significant role of nematodes in the development of diseases caused by soilborne pathogens has been demonstrated in many crops throughout the world (Abdel-Momen and Starr, 1998; De et al., 2001; Rupe et al., 1999; Walker et al., 2000; Wheeler et al., 2000). Several fungus-nematode interaction studies have included peanut and C. *parasiticum*, and most researchers have determined the interactions between two pathogens based on symptoms (Culbreath et al., 1992; Diomande and Beute 1981a & b; Diomande et al., 1981; Starr et al., 1996; Walker et al., 2000). The final populations of pathogens have also been used to evaluate interactions (Culbreath et al., 1992; Diomande and Beute 1981b; Starr et al., 1996; Walker et al., 2000). In the current greenhouse experiment, a root rot rating, gall index, and plant weight were used to determine the individual and combined effects of *C. parasiticum* and *M. arenaria*. In the microplot experiments, we again evaluated root rot and galling as well as mortality and peanut yield to examine the potential interactions between *C. parasiticum* and *M. arenaria*.

In the current study, we did not measure the final populations of *C. parasiticum*. *C. parasiticum* is typically considered to be a monocyclic pathogen. Secondary infections originating from ascospores produced on plants can occur, but are reported to be unimportant

(Rupe et al., 1999). The initial population of *C. parasiticum* is critical for CBR development, and initial densities of *C. parasiticum* inoculum were known in our experiments. Final population of *C. parasiticum* may have added to our understanding of the epidemiology of CBR, but were not considered essential for our objectives. In addition, populations of *C. parasiticum* in roots or soil are not always highly related to disease severity (Diomande and Beute 1981b). The pathogen is a poor saprophyte and is unable to produce microsclerotia on decayed roots (Crous, 2002). If the plant is badly diseased and dies from root rot at an early growth stage, high densities of microsclerotia would probably not be recovered from the root or soil at harvest. On the other hand, high densities of microsclerotia could be recovered from the roots of plants which show relatively lower disease severity and accumulate more biomass (Green et al., 1983). Similarly, we did not use the final populations of *M. arenaria* in roots and soil to determine the effect of the nematodes. Plant growth responses have been shown to be related to initial nematode populations (Barker and Olthof, 1976; Seinhorst, 1967). It is logical to assume that initial nematode populations are most important as predisposing agents in disease complexes. In our experiments, the initial nematode populations were known, and gall index has been a reliable variable for assessing root-knot severity (Dong et al., 2007). Correlations between final populations of nematode and CBR incidence were detected by Diomande and Beute (Diomande and Beute 1981b) in peanut; however, such relationships were not always detectable (Culbreath et al., 1992).

Root rot ratings have been used as an indicator of CBR severity on peanut. In our study, root rot ratings were also significantly affected by *M. arenaria* in greenhouse and microplot experiments. Inoculating with *M. arenaria* alone also increased the root rot ratings on the nematode-susceptible genotypes, Georgia-02C and C724-19-25. This indicated that the enhanced root rot in plants inoculated with both pathogens was partly caused by the direct effect of *M.*

arenaria. We documented a synergistic interaction between *C. parasiticum* inoculum densities and nematode levels on C724-19-25 in the 2007 microplot test; however, the interaction was not significant for the greenhouse tests and the microplot test in 2006, suggesting that the root rot ratings were increased in an additive manner in these tests. Significant *C. parasiticum* inoculum density × nematode level interactions were observed on mortality in microplot tests in both years; however, no interactions were observed on yield in those tests. The high yield reductions on nematode-susceptible genotypes caused by *M. arenaria* alone may obscure the interactions of *C. parasiticum* inoculum density × nematode level. Similar inconsistent interactions have been observed in other studies (Diomande and Beute 1981b; Diomande et al., 1981). Diomande and Beute (1981b) reported significant interaction between *M. hapla* or *Mesocriconema ornata* and *C. parasiticum* in the field; however, there was no interaction between *M. ornata* and *C. parasiticum* on NC 3033 in greenhouse experiments. Diomande et al. (1981) found that root rot severity was increased in an additive manner when *M. arenaria* race 2 was combined with *C. parasiticum*.

Another factor that influenced our results was the inoculum densities used. More interactions between *M. arenaria* and *C. parasiticum* could have occurred if different nematode or fungal population densities had been tested. In the greenhouse, root rot ratings were enhanced by the addition of *M. arenaria* in soil with low *C. parasiticum* inoculum density, but not in soil with a high level of *C. parasiticum* inoculum. In similar studies evaluating interactions between *M. incognita* and *Fusarium oxysporum*, the population densities of both pathogens affect the development of the interaction; no interaction typically is observed if population densities of either pathogen are high or low (Abawi and Barker, 1984; Starr et al., 1989).

Without the nematode and *C. parasiticum* present, the yields of the three genotypes were equivalent. The nematode-resistant genotype C724-19-15 was generally less severely diseased than the two nematode-susceptible genotypes in the microplots, and therefore had higher yield in the presence of *M. arenaria* with or without *C. parasiticum*. The fact that no yield potential is lost in association with the nematode resistance makes it logical choice for infested fields with even low levels of *M. arenaria*, particularly if CBR is a potential threat as well. This breeding line has recently been released as the cultivar, 'Tifguard' (Holbrook et al., 2008).

Several mechanisms have been proposed to explain the increased susceptibility of many nematode-infected plants to certain fungal pathogens (Back et al., 2002). Wounding by the nematode (providing an entrance route for the fungus) was long considered important in increasing susceptibility to various fungi (Bergeson, 1972; Orion et al., 1999; Storey and Evans, 1987). Powell (1971), however, proposed that the increased capacity of certain *Meloidogyne spp*. to enhance Fusarium wilt on tobacco when the nematode preceded the fungus by a few weeks is an indication of more elaborate mechanisms. Most artificial wounding in these types of tests does not realistically mimic nematode injury. The feeding sites of sedentary endoparasitic nematodes (giant cells or syncytia) are zones of high metabolic activity. These nutrient-rich cells could be the substrate for fungal colonization (Abdel-Momen and Starr, 1998; McLean and Lawrence, 1993). A 3-4 week nematode preinoculation has been found to be critical in investigations of some nematode-fungus disease complexes (Golden and van Gundy, 1975; Negron and Acosta, 1989). Taylor (1990) suggested this could be linked to syncytial development which will take 3-4 weeks to reach peak activity in a susceptible host. Although the mechanisms of resistance to *M. arenaria* in C724-19-15 have not been documented, it should be similar to 'COAN' (Holbrook et al., 2007), which restricts the formation of feeding sites (Bendezu and Starr, 2003). Therefore,

the low root rot ratings in C724-19-15 cannot be completely explained by lack of wounds for *C. parasiticum* penetrating. Histological and physiological studies are needed to unveil the association between *M. arenaria* and *C. parasiticum* on peanut.

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Table 5.1. *P* values for main factors and interactions to determine effects of *Meloidogyne arenaria* (Ma) and *Cylindrocladium parasiticum* (Cp) on root rot rating, gall index, and plant weight in three greenhouse experiments[a]

Source	Root rot[b]	Gall index[c]	Plant weight
Peanut genotype	0.3305	0.0024	0.3428
Ma level	0.3705	0.0002	0.9954
Cp density	0.0021	0.1667	0.0232
Genotype × Ma level	0.5713	0.0239	0.5595
Genotype × Cp density	0.8826	0.4921	0.6698
Ma level × Cp density	0.5838	0.1561	0.1833
Genotype × Ma level × Cp density	0.4678	0.3163	0.7016

[a] Analysis based on three trials (10 replicates/trial).

[b] Root rot rating based on a scale of 0 to 5, where 0 = no symptoms; 1 = some root discoloration, primarily on secondary roots; 2 = significant root browning and some necrosis, usually on secondary and tap root, with < 25% of roots affected; 3 = moderate root rot, 25-75% of roots affected; 4 = severe root rot, > 75% of roots affected; and 5 = dead plant.

[c] Gall index based on a scale of 0 to 5, where 0 = no galling; 1 = trace infection with a few small galls; 2 ≤ 25% of root galled; 3 = 26-50%; 4 = 51-75%; and 5 > 75% of root galled.

Table 5.2. Effect of inoculum densities of *Cylindrocladium parasiticum* (Cp) and *Meloidogyne arenaria* (Ma) on root rot severity[a] in the three peanut genotypes.

Genotype	Cp inoculum density (ms/cm^3 soil)								
	0			1			5		
	Ma level (eggs/pot)								
	0	500	3000	0	500	3000	0	500	3000
C724-19-15	0.8[b]	1.2	1.1	2.1	2.5	2.8	2.9	2.5	3.2
C724-19-25	1.1	1.7	1.4	2.0	2.7	2.8	2.8	2.8	3.2
GA-02C	0.6	1.4	1.0	1.5	2.2	2.3	2.6	2.3	2.5

[a] Root rot rating was on a 0 to 5 scale, where 0 = no symptoms; 1 = some root discoloration, primarily on secondary roots; 2 = significant root browning and some necrosis, usually on secondary and tap root, with < 25% of roots affected; 3 = moderate root rot, 25-75% of roots affected; 4 = severe root rot, > 75% of roots affected; and 5 = dead plant.

[b] Data were average of three trials (10 replicates/trial); LSD = 0.5 for comparison of *C. parasiticum* and *M. arenaria* effects within and across genotypes.

Table 5.3. *P* values for main factors and interactions to determine effects of *Meloidogyne arenaria* (Ma) and *Cylindrocladium parasiticum* (Cp) on root rot rating, gall index, plant mortality, and pod yield in microplot experiment in 2006 and 2007.

Year Source	Root rot[a]	Gall index[b]	Plant mortality[c]	Pod yield
2006				
Peanut genotype	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Ma level	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cp density	< 0.0001	0.7354	< 0.0001	0.0018
Genotype × Ma level	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Genotype × Cp density	0.1188	0.5773	0.0055	0.2933
Ma level × Cp density	0.9677	0.0992	0.0004	0.0719
Genotype × Ma level × Cp density	0.4196	0.3154	0.4125	0.8211
2007				
Peanut genotype	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Ma level	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cp density	< 0.0001	0.6150	< 0.0001	0.0283
Genotype × Ma level	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Genotype × Cp density	0.0148	0.9169	< 0.0001	0.1249
Ma level × Cp density	0.0131	0.0920	0.0003	0.3789
Genotype × Ma level × Cp density	0.0433	0.0546	0.0008	0.1892

[a] Root rot rating based on a scale of 0 to 5, where 0 = no symptoms; 1 = some root discoloration, primarily on secondary roots; 2 = significant root browning and some necrosis, usually on secondary and tap root, with <25% of roots affected; 3 = moderate root rot, 25-75% of roots affected; 4 = severe root rot, >75% of roots affected; and 5 = dead plant.

[b] Gall index based on a scale of 0 to 5, where 0 = no galling; 1 = trace infection with a few small galls; 2 ≤ 25% of root galled; 3 = 26-50%; 4 = 51-75%; and 5 > 75% of root galled.

[c] plant mortality = (number of dead and wilted plants/total number of plant.) × 100.

Table 5.4. Effect of *Meloidogyne arenaria* (Ma) and *Cylindrocladium parasiticum* (Cp) on mortality[a] in three peanut genotypes in microplots in 2006 and 2007.

Year	Cp inoculum density (ms/cm^3 soil)								
	0			0.5			5		
Genotype	Ma level (eggs/cm^3 soil)								
	0	0.4	2	0	0.4	2	0	0.4	2
2006									
C724-19-15	2.7[b]	0	1.4	11.9	8.3	1.7	6.7	8.7	7.8
C724-19-25	0	1.3	19.2	6.3	19.7	33.9	8.5	34.9	54.6
GA-02C	1.3	6.2	45.7	2.9	23.0	38.1	1.8	42.6	67.0
2007									
C724-19-15	1.6	0.5	1.1	1.6	2.6	6.8	7.3	3.1	4.7
C724-19-25	0.5	4.7	8.9	0.5	54.7	80.7	1.6	38.0	59.4
GA-02C	0.5	25.5	48.4	1.1	45.8	57.3	6.8	64.1	67.2

[a] plant mortality = (number of dead and wilted plants/total number of plant.) × 100.

[b] Means of eight replicates. LSDs were 14.0 and 9.4 for comparison of *C. parasiticum* and *M. arenaria* effects within and across genotypes in 2006 and 2007, respectively.

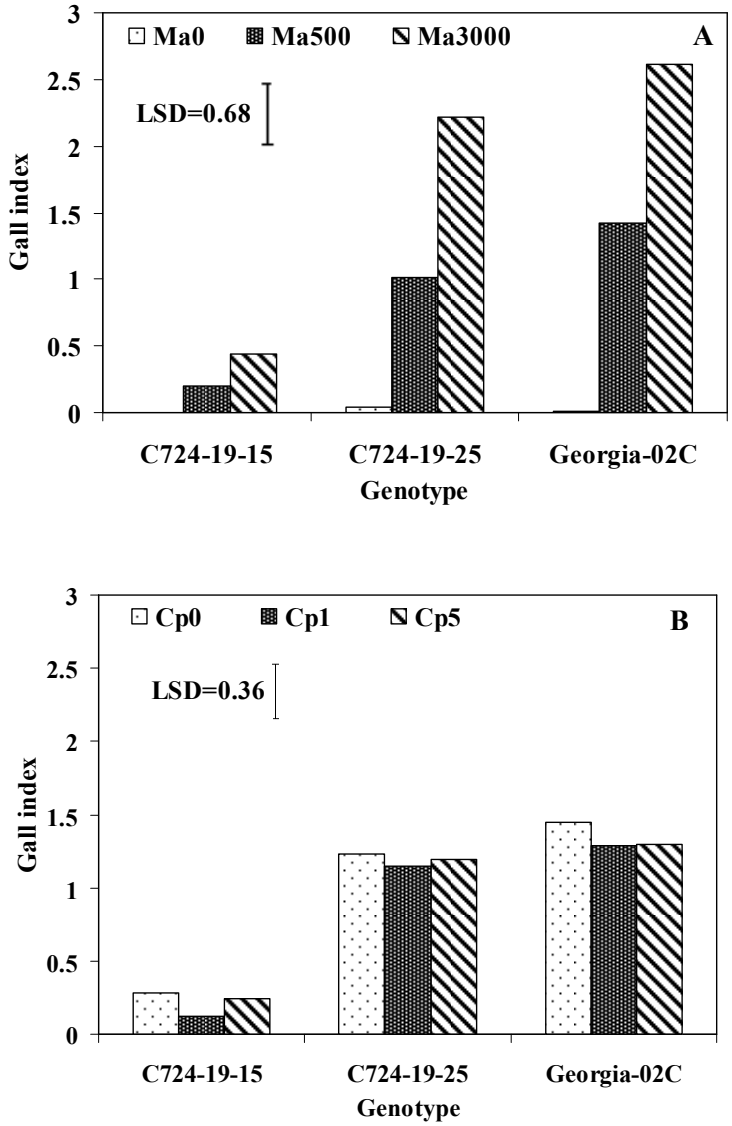

Fig. 5.1. Gall indices for three peanut genotypes in the greenhouse in relation to inoculum density of *Meloidogyne arenaria* (A) and *Cylindrocladium parasiticum* (B). Bars represent the average across trials and *C. parasiticum* densities (A) or *M. arenaria* levels (B). Ma0, Ma500, and Ma3000 mean the inoculum level of *M. arenaria* were 0, 500, and 3000 eggs/pot; Cp0, Cp1, and Cp5 mean the inoculum density of *C. parasiticum* were 0, 1, and 5 microsclerotium/cm^3 soil. Gall index based on a 0 to 5 scale, where 0 = no galling; 1 = trace infection with a few small galls; 2 ≤ 25% of root galled; 3 = 26-50%; 4 = 51-75%; and 5 > 75% of root galled.

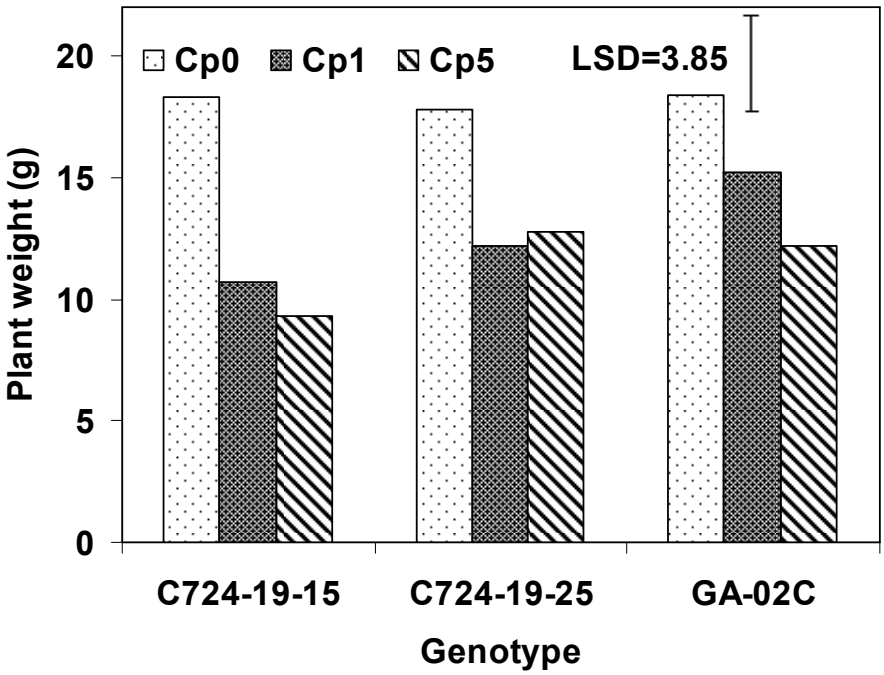

Fig. 5.2. Effect of *Cylindrocladium parasiticum* inoculum density (microsclerotia/cm^3 soil) on fresh weight of plants of three peanut genotypes in the greenhouse. Bars represent the averages across three trials (10 replicates/trial) and levels of *Meloidogyne arenaria*. Inoculum densities of *C. parasiticum* were 0 (Cp0), 1 (Cp1), and 5 (Cp5) microsclerotia /g of soil. Georgia-02C is moderately resistant to CBR and highly susceptible to *M. arenaria*, and C724-19-15 and C724-19-25 are near isogenic breeding lines (C724-19-15 is highly resistant, while C724-19-25 is susceptible to *M. arenaria*).

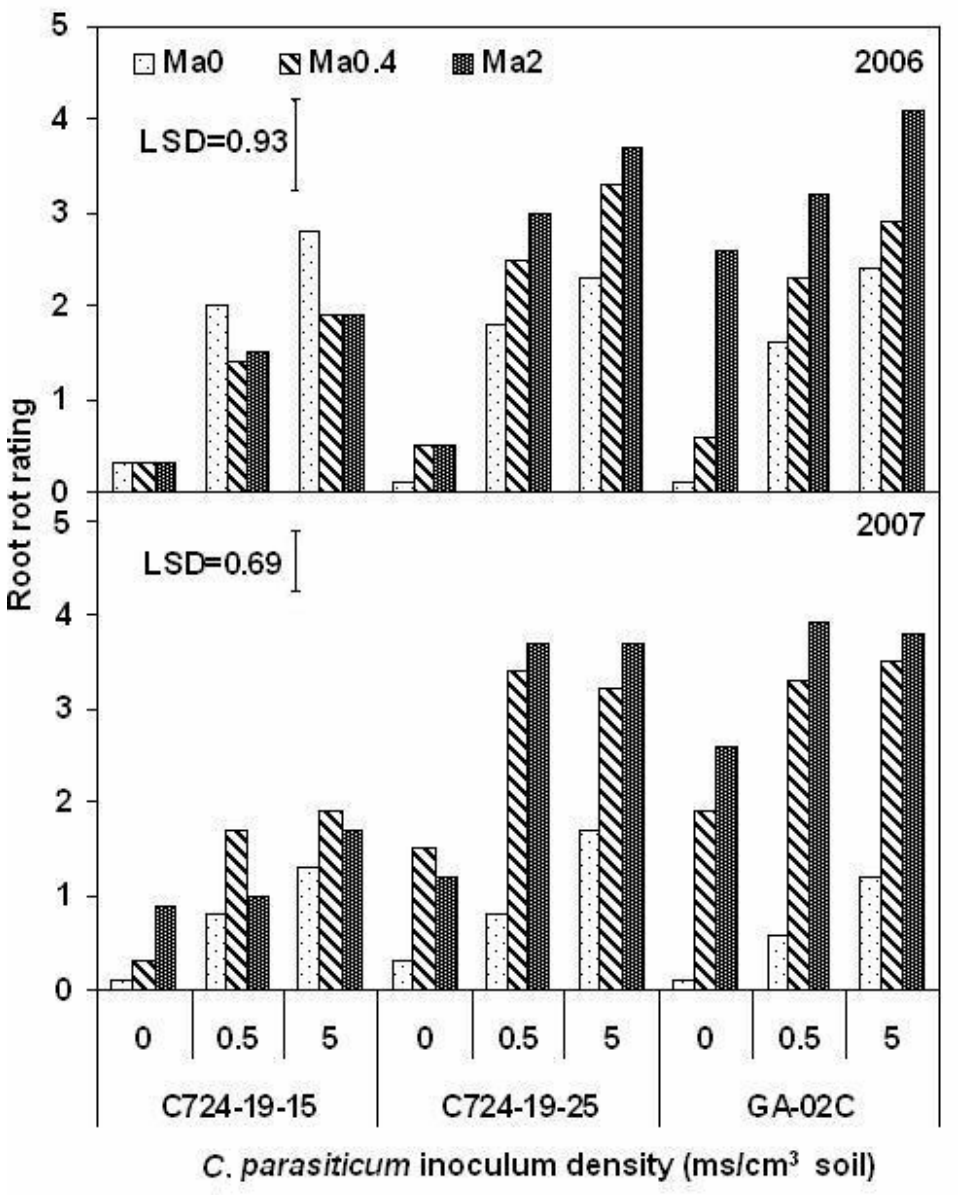

Fig. 5.3. Root rot ratings for three peanut genotypes in microplots in 2006 and 2007, in relation to inoculum density of *Meloidogyne arenaria* and *Cylindrocladium parasiticum* (0 = healthy root system, 5 = completely rotted). Bars stand for the averages of eight replicates. LSD for comparison of *C. parasiticum* and *M. arenaria* effects within and across genotypes. Ma0, Ma0.4, and Ma2 represent the inoculum levels of *M. arenaria* of 0, 0.4, and 2 eggs + J2/cm^3 soil (0, 2500, and 12700 eggs + J2/plant), respectively.

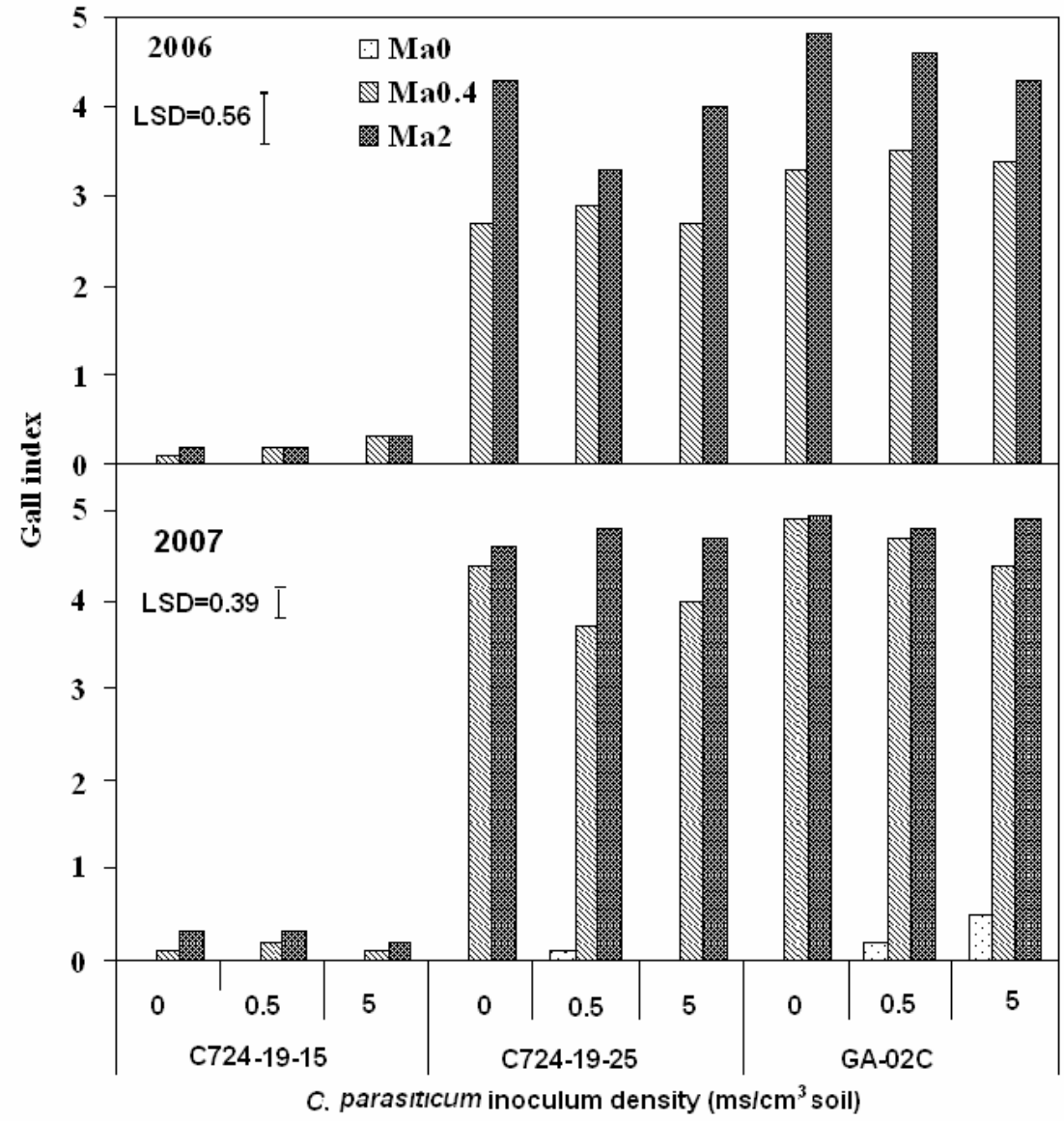

Fig. 5.4. Gall indices for three peanut genotypes in microplots in 2006 and 2007, in relation to inoculum density of *Meloidogyne arenaria* and *Cylindrocladium parasiticum*. Gall index based on a 0 to 5 scale, where 0 = no galling; 1 = trace infection with a few small galls; 2 ≤ 25% of root galled; 3 = 26-50%; 4 = 51-75%; and 5 > 75% of root galled. Bars stand for the averages of eight replicates. LSD for comparison of *C. parasiticum* and *M. arenaria* effects within and across genotypes. Ma0, Ma0.4, and Ma2 represent the inoculum levels of *M. arenaria* were 0, 0.4, and 2 eggs+J2/cm^3 soil (0, 2500, and 12700 eggs + J2/plant), respectively.

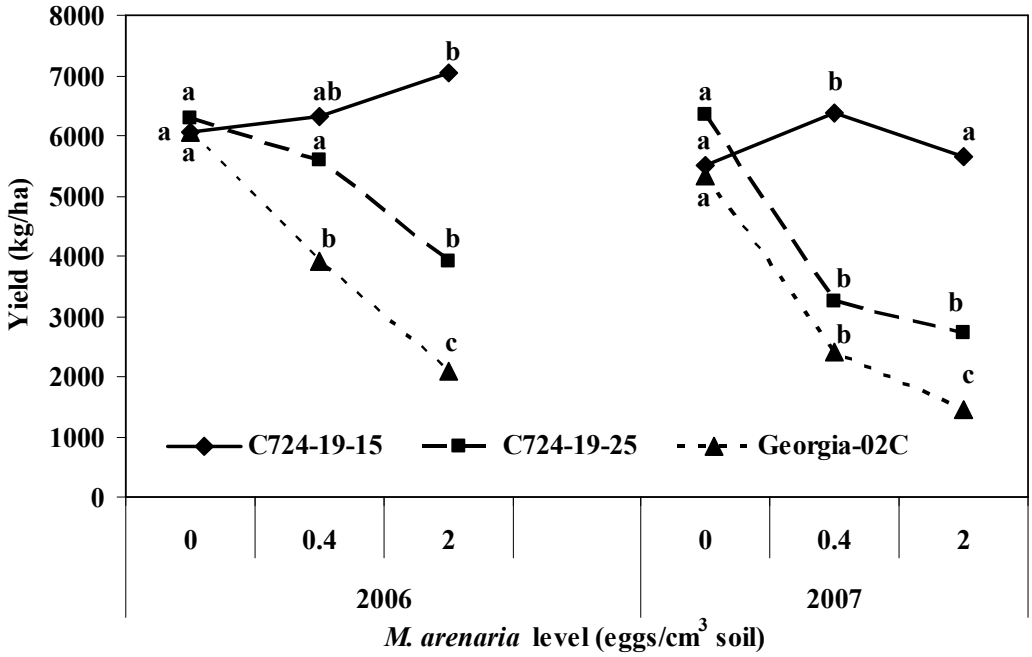

Fig. 5.5. Effect of *Meloidogyne arenaria* on pod yield of three peanut genotypes in microplots in 2006 and 2007. Data points are averaged across inoculum levels of *C. parasiticum*.

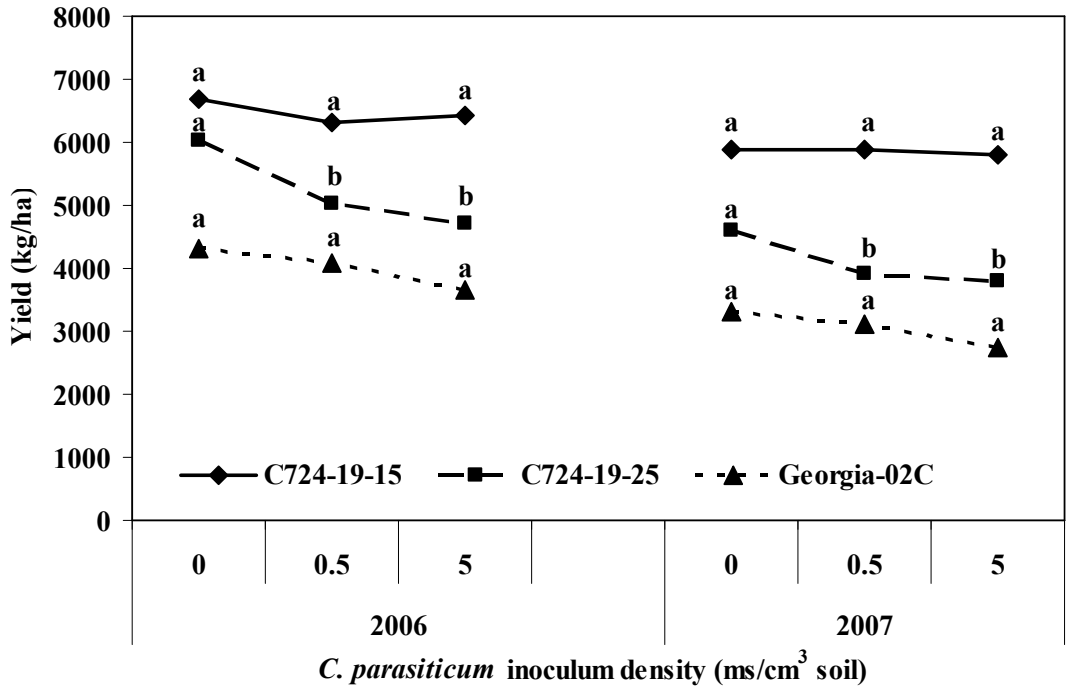

Fig. 5.6. Effect of *Cylindrocladium parasiticum* on pod yield of three peanut genotypes in microplots in 2006 and 2007. Data points are averages across inoculum levels of *M. arenaria*.

APPENDIX TO CHAPTER 5

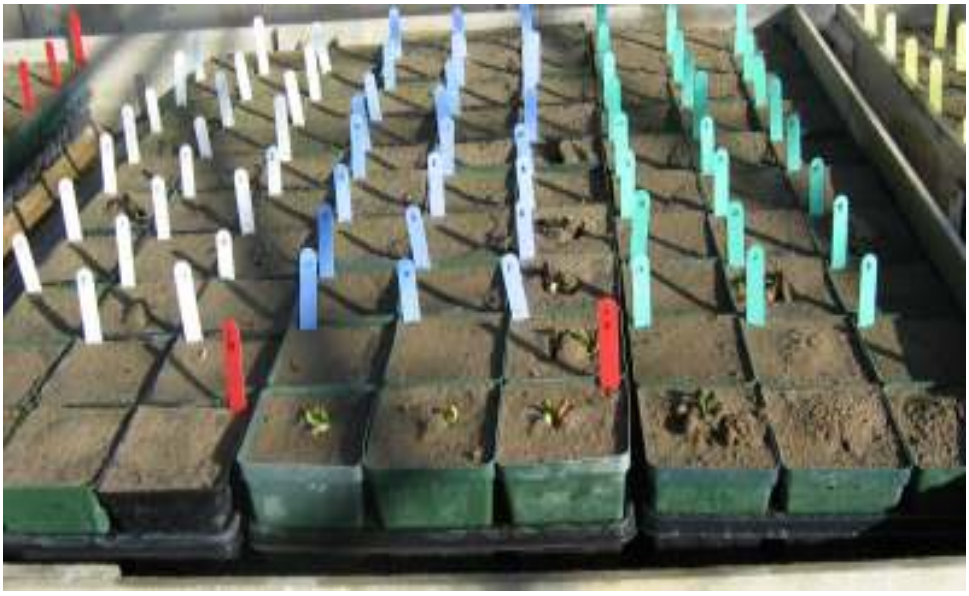

Figure 5A.1. Intreaction experiment in the greenhouse. A split-plot treatment design was used with genotypes as main plots and *C. parasiticum* densities × *M. arenaria* levels as subplots.

Figure 5A.2. Intreaction experiment in the microplots. A split-plot was the experiment degisn with *C. parasiticum* densities × *M. arenaria* levels as main plots and peanut genotypes as subplots.

Figure 5A.3. Three peanut genotypes showed similar performance in the absence of *C. parasiticum* and *M. arenaria* in the late growing season.

Figure 5A.4. In the presence of high density of *C. parasiticum* and low level of *M. arenaria,* high plant mortalities occurred on C724-19-25 and Georgia-02C, but not on the nematode-resistant genotype C724-19-15.

CHAPTER 6

CONCLUSIONS

Root-knot nematodes (RKN, *Meloidogyne* spp.) and Cylindrocladium black rot (CBR, *Cylindrocladium parasiticum*) are important soilborne diseases on peanut in many peanut producing areas of the USA. In Georgia alone, RKN and CBR cost farmers an average of $14.3 and 6.6 million in damages and control costs for each of the last 6 years. Development and utilization of peanut cultivars with resistance to both diseases is a desirable management approach in sustainable peanut producing systems. In order to enhance RKN and CBR resistance breeding in peanut, a series of greenhouse, microplot, and field studies were conducted from 2004 to 2007 to improve resistance screening techniques and identify new resistance sources. Greenhouse and microplot studies were also conducted to determine the interactions between *M. arenaria* and *C. parasiticum* in peanut.

In order to optimize the nematode resistance screening protocol, a series of greenhouse tests were conducted using seven genotypes with three levels of resistance to *M. arenaria*. The three resistance levels could be separated based on gall index as early as 2 weeks after inoculation (WAI) using 8000 eggs/plant, while 4 or more weeks were needed when 1000-6000 eggs/plant were used. High inoculum densities (over 8000 eggs/plant) were needed to separate the three resistance levels based on eggs/g root within 8 WAI. A gall index based on percentage of galled roots could separate the three resistance levels at lower inoculum levels and earlier harvest dates than other assessment methods. The use of eggs vs second-stage juveniles (J2) as inoculum provided similar results; however, it took 3-5 more days to collect J2 than to collect

eggs from roots. Plant age affected gall index and nematode reproduction on peanut, especially on the susceptible genotypes AT201 and D098. The genotypes were separated into their correct resistance classes when inoculated 10 to 30 days after planting, but were not separated correctly when inoculated on day 40. In summary, a gall index based on percentage of the root system with galls was a reliable indicator of the level of resistance on early harvest dates (as early as two weeks) after inoculation with 8,000 or more eggs/plant during 10 to 30 days after planting. If the nematode population is the limiting factor, as few as 1,000 eggs /plant could be used to separate the different levels of resistance on late harvest dates (8 WAI) either based on the gall index or eggs per gram root. This is important because we have identified a rapid method for assessing resistance in peanut genotypes. The selected genotypes could then be assessed for eggs per gram root at eight weeks after inoculation with 8,000 eggs /plant to verify the resistance level based on egg production.

Three major species of root-knot nematode infect peanut: *Meloidogyne arenaria* race 1 (Ma), *M. hapla* (Mh), and *M. javanica* race 3 (Mj). Cultivars and breeding lines of peanut were evaluated for resistance to Ma, Mh, and Mj in the greenhouse and in the laboratory. Twenty-six genotypes with some resistance to Ma, Mj, or Mh were identified from 60 collections based on average eggs/g root and gall index relative to a susceptible control. Among these, fourteen genotypes were moderately to highly resistant to all three species, five genotypes were resistant to Ma and Mj, two genotypes were resistant to Mj and Mh, one genotype was resistant Ma alone, and four genotypes were resistant to Mh alone. Reproduction of Ma on NR 0817, C724-19-11, and D108 was highly variable indicating that these genotypes were heterogeneous for resistance. COAN, NemaTAM, C724-25-8, and the Ma-resistant individuals of C724-19-11 contained the dominant SCAR marker (197/909) for nematode resistance. Results with the molecular markers

indicate that the high resistance to Ma in GP-NC WS 6 may be different from the resistance in COAN, NemaTAM, and C724-25-8. Resistance to Ma was correlated with resistance to Mj in peanut, whereas resistance to Mh was not correlated with the resistance to either Ma or Mj. Resistance to all three *Meloidogyne* spp. exists within cultivated peanut (*Arachis hypogaea* L.), either with or without introgressed genes from wild species. New genotypes with moderate resistance to *M. arenaria* and *M. javanica*, such as D009, D031, D040, D054, and D099 etc., were identified. The level of resistance in these genotypes was better than, or as good as, the resistance in the moderately resistant genotype C209-6-37. These genotypes may have different resistance gene(s) from the released Ma-resistant germplasm, because they do not have any introgressed genes from wild species. Peanut genotypes with high and moderate resistance to *M. hapla* were also identified. The three highly resistant genotypes (D031, 970105, and 990304), and most of the 18 moderately resistant genotypes do not have any introgressed genes from wild species, whereas some moderately resistant genotypes, such as C724-19-11, COAN, NR 0817, GP-NC WS 6, and C724-25-8 have introgressed genes. The resistant selections should be valuable sources for pyramiding resistance genes to develop new cultivars with broad and durable resistance to *Meloidogyne* spp.

In the greenhouse test, infesting soil with 150-250µm microsclerotia (ms) at 1-5 ms/g soil could separate the CBR-resistant genotype Georgia-02C and the susceptible one C-99R correctly, based on root rot rating. Five commercial runner peanut cultivars and one breeding line were evaluated for CBR resistance in greenhouse, inoculated field, and naturally infested field trials. The overall results indicated that differentials of resistance to CBR existed in runner type peanuts. The genotypes Georgia-02C and Georganic had low plant mortalities, whereas C-99R and DP-1 always had high mortalities in a naturally infested field in 2005 and 2006. Plant mortalities in

GA-01R were moderate in both years, but were inconsistent in C34-24-85. Georgia-02C and Georganic also showed partial resistance to CBR in greenhouse tests. In field inoculation tests, the peanut cultivars Georgia-02C and Georganic showed higher resistance than cultivars C-99R and DP-1 in both 2006 and 2007. The root rot ratings and percentage of black pods for genotypes Georgia-02C and Georganic were relatively lower than those for C-99R and DP-1. Dead and diseased plants after digging and entire plant disease severity were the better variables for evaluating CBR resistance in peanut in naturally infested fields or inoculated field tests, and greenhouse test, respectively. In conclusion, the naturally infested field and inoculated field results were consistent, but the greenhouse tests results were consistent with either the naturally infested or inoculated field tests only for the most resistant and the most susceptible genotypes. Peanut genotypes are reliably screened in inoculated fields or uniformly infested natural fields, while greenhouse evaluations are quick and may be useful to identify and characterize components of resistance. Unfortunately greenhouse results are not always well correlated with field results for some genotypes.

Greenhouse and microplot experiments were conducted with the runner peanut genotypes C724-19-15 (resistant to *M. arenaria*), Georgia-02C (partial resistance to CBR), and C724-19-25 (susceptible to *M. arenaria* and CBR) to better understand the interactions between the two pathogens. In the greenhouse, root rot ratings were increased in all three peanut genotypes by addition of 500-3000 eggs/plant of *M. arenaria* with low inoculum level (1.0 microsclerotia/g soil) of *C. parasiticum*. The nematode did not affect the root rot induced by a high inoculum level (5.0 microsclerotia/g soil) of *C. parasiticum*. Severe pod galling was present on Georgia-02C and C724-19-25, but not C724-19-15. Gall indices were not affected by *C. parasiticum* inoculations in the greenhouse or microplots. In microplot experiments, interactions

between inoculum levels of *M. arenaria* and *C. parasiticum* were observed on plant mortality in both 2006 and 2007, whereas such interactions were noted on root rot rating only in 2007, not in 2006. The root rot ratings from nematode-susceptible genotypes Georgia-02C and C724-19-25 were higher in plots infested with *M. arenaria* (0.4-2.0 eggs/cm^3 soil) and *C. parasiticum* than in plots with *C. parasiticum* alone; however, *M. arenaria* did not increase the root rot ratings on the nematode-resistant genotype C724-19-15. This was inconsistent with the greenhouse results. Simultaneous inoculation with *M. arenaria* decreased yield incrementally on C724-19-25 and Georgia-02C as *C. parasiticum* inoculum levels increased, but even a high level of *M. arenaria* (2.0 eggs/cm^3 soil) did not decrease yield of C724-19-15 when also inoculated with *C. parasiticum*.