EVALUATION OF RESISTANCE TO Meloidogyne arenaria IN THE PEANUT (Arachis hypogaea. L) cv. TIFGUARD

By

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This work is dedicated to my parents for bringing me up and educating me well.
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TABLE OF CONTENTS

ACKNOWLEDGMENTS............................................................................................................... 4

LIST OF TABLES.......................................................................................................................... 9

LIST OF FIGURES.......................................................................................................................... 11

ABSTRACT........................................................................................................................................ 13

CHAPTER

1 INTRODUCTION ......................................................................................................................... 15

   Root-knot Nematode.................................................................................................................. 15
   Economic Impact......................................................................................................................... 16
   History of the Genus.................................................................................................................... 16
   The Distribution of *Meloidogyne* Species ............................................................................ 17

   Peanut Production...................................................................................................................... 18
   Plant-parasitic Nematodes Associated with Peanut ................................................................. 20
   Species of *Meloidogyne* Parasitizing Peanut ......................................................................... 21
   Other Species of Nematodes Associated with Peanut ............................................................. 22

   Root-knot Nematode on Peanut ............................................................................................... 22
   Disease Cycle ............................................................................................................................. 23
   Parasitic Mechanism ................................................................................................................ 25
   Population Dynamics ............................................................................................................... 27
   Temperature Effects on Root-knot Nematode ......................................................................... 30
   Root-knot Nematode Management ......................................................................................... 31
   Development of Resistant Peanut Cultivars .......................................................................... 31
   Identification of Resistant Gene to Root-knot Nematode ........................................................ 33
   The Application of Host Resistance ......................................................................................... 35

2 FIELD EVALUATION OF RESISTANCE TO *Meloidogyne arenaria* IN BREEDER, FOUNDATION AND GROWER-KEPT TIFGUARD .................................... 37

   Introduction ............................................................................................................................... 37
   Materials and Methods ............................................................................................................. 38
   Field Sites and Experimental Designs ..................................................................................... 38
   Determination of Root-knot Nematode Species ..................................................................... 40
   Analysis of Resistance Gene in Infected Tifguard Plants ........................................................ 40
   Results and Discussion ........................................................................................................... 41
   464 Farm 2013-2015 ................................................................................................................. 41
   Brown Farm and AREC 2014-2015 ......................................................................................... 41
   Determination of Root-knot Nematode Species ..................................................................... 41
   Analysis of Resistance Gene in Infected Tifguard Plants ........................................................ 42
3 THE SEASONAL AND VERTICAL DISTRIBUTION OF *Meloidogyne arenaria*
FROM RESISTANT AND SUSCEPTIBLE PEANUT GROWN IN TWO SOIL TYPES .................................................................................................................. 46

Introduction .......................................................................................................................... 46
Materials and Methods ......................................................................................................... 48
Field Locations and Experimental Designs ......................................................................... 48
Seasonal and Vertical Distribution of *Meloidogyne arenaria* ........................................... 49
Statistical Analysis ................................................................................................................ 50
Determination of Effects of Soil Texture on Nematode Population ................................... 50

Results .................................................................................................................................. 51
Above and Belowground Symptoms of Root-knot Disease ............................................... 51
Seasonal Distribution of *Meloidogyne arenaria* Recovered from Georgia-06G Rhizosphere ......................................................................................................................... 51
Vertical Distribution of *Meloidogyne arenaria* Recovered from Georgia-06G Rhizosphere ......................................................................................................................... 52
Seasonal and Vertical Distribution of *Meloidogyne arenaria* Recovered from Tifguard Rhizosphere .................................................................................................................. 54
Distribution of Nematodes Associated with Soil Temperature ........................................ 54
Effects of Soil Texture on Nematode Population ............................................................... 54

Discussion ............................................................................................................................. 54
Occurrence of *Meloidogyne arenaria* in the Soil .............................................................. 54
Seasonal and Vertical Distribution of *Meloidogyne arenaria* .......................................... 56

The Influence of Soil Temperature on Nematode Population ........................................... 57
Effect of Soil Texture on Nematode Population ............................................................... 57

4 THE INFLUENCE OF TEMPERATURE ON THE SUSCEPTIBILITY OF CVs.
TIFGUARD AND GEORGIA-06G PEANUT TO *Meloidogyne arenaria* .............................. 67

Introduction .......................................................................................................................... 67
Materials and Methods ......................................................................................................... 70
Nematode Origin .................................................................................................................. 70
Penetration and Development of *Meloidogyne arenaria* ................................................ 70
Data Collection .................................................................................................................... 71
Statistical Analysis .............................................................................................................. 71
Resistance Gene Marker Analysis of Tifguard ................................................................. 71

Results .................................................................................................................................. 72
Penetration of *Meloidogyne arenaria* in the Resistant and Susceptible Peanut Cultivars ................................................................................................................................. 72
Development of *Meloidogyne arenaria* in the Resistant and Susceptible Peanut Cultivars ................................................................................................................................. 73
Reproduction of *Meloidogyne arenaria* in Different Peanut Genotypes ......................... 74

Discussion ............................................................................................................................. 74
Effect of Peanut Genotypes and Temperatures on *Meloidogyne arenaria* ...................... 74
The Function of Resistance Gene ....................................................................................... 75
Hypersensitive Reaction ...................................................................................................... 75
COMPARISON OF YIELD FROM BREEDER TIFGUARD, ISOGENIC TIFGUARD AND GEORGIA-06G TREATED AND NONTREATED WITH 1,3-DICHLOROPROPENE ................................................................. 90

Introduction ................................................................................................................................. 90
Materials and Methods .................................................................................................................. 91
  Field Design and Treatments ....................................................................................................... 91
  Peanut Harvest and Data Collection ........................................................................................... 92
  Statistical Analysis ....................................................................................................................... 92
Results ........................................................................................................................................ 92
  Peanut Growth and Root-knot Nematode Infection .................................................................... 92
  Yield Comparison ......................................................................................................................... 93
  Soil Type, Texture, Analysis ......................................................................................................... 93
Discussion .................................................................................................................................... 93

LIST OF REFERENCES .................................................................................................................. 96

BIOGRAPHICAL SKETCH ............................................................................................................ 113
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>The average percentages of three different sources of Tifguard seed infected by <em>Meloidogyne arenaria</em> at the 464 Farm, Levy Co., FL in 2013, 2014, and 2015.</td>
<td>43</td>
</tr>
<tr>
<td>3-1</td>
<td>Significance of main and interactive effects of both fixed and random variables for the vertical distribution of <em>Meloidogyne arenaria</em> extracted from 200 cm$^3$ of soil collected from plots planted Georgia-06G at the Brown Farm.</td>
<td>61</td>
</tr>
<tr>
<td>3-2</td>
<td>Significance of main and interactive effects of both fixed and random variables for the vertical distribution of <em>Meloidogyne arenaria</em> extracted from 200 cm$^3$ of soil collected from plots planted Georgia-06G at the Attapulgus.</td>
<td>61</td>
</tr>
<tr>
<td>3-3</td>
<td>Number of <em>Meloidogyne arenaria</em> second-stage juveniles extracted from 200 cm$^3$ of soil collected at four depths 0-30, 31-60, 61-90, and 91-120 cm deep from plots planted Georgia-06G at the Brown Farm (2014-2015).</td>
<td>61</td>
</tr>
<tr>
<td>3-4</td>
<td>Number of <em>Meloidogyne arenaria</em> second-stage juveniles extracted from 200 cm$^3$ of soil collected at four depths 0-15, 16-30, and 31-45 cm deep from plots planted Georgia-06G at the Attapulgus Research and Education Center.</td>
<td>62</td>
</tr>
<tr>
<td>3-5</td>
<td>Significance of main and interactive effects of both fixed and random variables for the vertical distribution of <em>Meloidogyne arenaria</em> extracted from 200 cm$^3$ of soil collected from plots planted with Tifguard at the Brown Farm.</td>
<td>64</td>
</tr>
<tr>
<td>3-6</td>
<td>Significance of main and interactive effects of both fixed and random variables for the vertical distribution of <em>Meloidogyne arenaria</em> extracted from 200 cm$^3$ of soil collected from plots planted with Tifguard at the Attapulgus.</td>
<td>64</td>
</tr>
<tr>
<td>3-7</td>
<td>Number of <em>Meloidogyne arenaria</em> second-stage juveniles extracted from 200 cm$^3$ of soil collected at four depths 0-30, 31-60, 61-90, and 91-120 cm from plots planted with Tifguard at the Brown Farm 2015.</td>
<td>64</td>
</tr>
<tr>
<td>3-8</td>
<td>Number of <em>Meloidogyne arenaria</em> second-stage juveniles extracted from 200 cm$^3$ of soil collected at four depths 0-15, 16-30, and 31-45 cm from plots planted with Tifguard at the Attapulgus Research and Education Center.</td>
<td>65</td>
</tr>
<tr>
<td>3-9</td>
<td>Sand, silt, and clay distribution at different soil depths collected from Brown Farm, Levy County, FL and University of Georgia, Attapulgus Research and Education Center, Attapulgus, GA.</td>
<td>66</td>
</tr>
<tr>
<td>4-1</td>
<td>Analysis of variance of root weight, number of <em>Meloidogyne arenaria</em> per gram root system of two peanut cultivars.</td>
<td>77</td>
</tr>
</tbody>
</table>
Effect of different treatments on plant growth, galling induced by root-knot nematodes and yield of peanut in a field trial at Plant Science Research and Education Unit, Citra, FL, Spring-Summer 2015............................... 95
LIST OF FIGURES

Figure page

2-1 The average percentages of Tifguard breeder seed infected by *Meloidogyne arenaria* was compared with Georgia-06G at two locations in 2014 and 2015... 44

2-2 The average percentages of different Tifguard seed sources that were negative for the resistance gene marker for *Meloidogyne arenaria*............................. 45

3-1 Seasonal fluctuation of *Meloidogyne arenaria* second-stage juveniles in the soil profile of Georgia-06G at four depths in a Candler sand at the Brown Farm, Levy County, FL. ........................................................................................................ 59

3-2 Seasonal fluctuation of *Meloidogyne arenaria* second-stage juveniles in the soil profile of Georgia-06G at three depths in a Norfolk loamy sand soil at University of Georgia, Attapulgus Research and Education Center, ..................... 60

3-3 Seasonal fluctuation of *Meloidogyne arenaria* second-stage juveniles in the soil profile of Tifguard at four depths in a Candler sand at the Brown Farm, Levy County, FL and at three depths in a Norfolk loamy sand soil at, ............... 63

4-1 The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 30-day period at 28 °C (Experiment 1)................................. 78

4-2 The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 30-day period at 31 °C (Experiment 1)................................. 79

4-3 The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 30-day period at 34 °C (Experiment 1)................................. 80

4-4 The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 40-day period at 28 °C (Experiment 2)................................. 81

4-5 The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 40-day period at 31 °C (Experiment 2)................................. 82

4-6 The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 40-day period at 34 °C (Experiment 2)................................. 83
4-7 The mean number of *Meloidogyne arenaria* of all development stages in roots of Tifguard and Georgia-06G recorded at 5-day intervals over a 30 day period in experiment 1. ......................................................................................................................... 84

4-8 The mean number of *Meloidogyne arenaria* of all development stages in roots of Tifguard and Georgia-06G recorded at 5-day intervals over a 40 day period in experiment 2. ......................................................................................................................... 85

4-9 The number of second-stage juveniles (J2) per gram of root system of the resistant cultivar Tifguard and susceptible cultivar Georgia-06G 5 days after inoculation in two experiments ........................................................................................................................... 86

4-10 The number of egg-laying females per gram of root system of the resistant cultivar Tifguard and susceptible cultivar Georgia-06G 30 or 40 days after inoculation in two experiments ........................................................................................................................... 87

4-11 Different developmental stages of *Meloidogyne arenaria* in peanut and tomato roots at 34 °C ........................................................................................................................................... 88

4-12 Arrows point to necrotic lesions formed around the root-knot nematode infection sites in Tifguard roots at 5 and 40 days after inoculation (DAI). ........... 89
EVALUATION OF RESISTANCE TO *Meloidogyne arenaria* IN THE PEANUT (*Arachis hypogaea* L) cv. TIFGUARD

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Chair: Donald W. Dickson
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Tifguard, which was released as a peanut cultivar resistant to root-knot nematode and tomato spotted wilt virus in 2007, was found to be heavily infected by *Meloidogyne arenaria* in several peanut production fields in Florida in 2012. The goal of this project was to determine why the cultivar that was reported to be highly resistant succumbed to root-knot nematode infection. The objectives were to evaluate the resistance of three different sources of Tifguard seeds; to determine the seasonal population changes and vertical population densities of *M. arenaria* collected from resistant and susceptible peanut rhizospheres in two different soil types; to determine the effects of high temperature on the resistance in Tifguard, and to compare the yield of Tifguard, isogenic Tifguard and Georgia-06G treated vs. nontreated with 1,3-dichloropropene. In three *M. arenaria* infested field sites, a comparison of Tifguard seed obtained from three sources showed that 2.5, 28, and 39.5% of plants that were infected by the nematode were negative for the nematode resistance gene. The seasonal distribution of second-stage juvenile (J2) of *M. arenaria* followed similar trends in two different soil types, with a peak occurring during late summer and early fall at
harvest. Number of J2 dropped following harvest and reached a density less than 10 J2/200 cm$^3$ of soil in February. Comparison of vertical population densities of J2 collected from Georgia-06G rhizosphere in two different soil types showed that greater numbers occurred in the upper 60 cm of soil during the growing season in a Candler sand, whereas in a Norfolk loamy sand greater densities were found only in the top 45 cm. The population densities of J2 collected from Georgia-06G rhizosphere at all depths were much greater in Norfolk loamy sand than that in the Candler sand. Tifguard reduced the nematode population to 1 and 28 in the Candler sand and the Norfolk loamy sand, respectively at harvest. Comparison of nematode numbers from different developmental stages at different temperatures demonstrated that the high soil temperature increased nematode infection rate and accelerated nematode development in Georgia-06G. No further development of J2 occurred in Tifguard roots at 28 or 31 °C, however at 34 °C a few J3-J4, females, egg laying females, and males of *M. arenaria* were observed. Comparisons indicated that there was no effect of 1,3-dichloropropene on root-knot nematode damage and yield of Georgia-06G, Tifguard, or isogenic Tifguard (*P* ≤ 0.05).
CHAPTER 1
INTRODUCTION

**Root-knot Nematode**

Root-knot nematode is the common name of members from the genus *Meloidogyne* (Goeldi, 1892), which is derived from a Greek word meaning of apple-shaped female. It is an economically important polyphagous group of obligate plant parasites with advanced parasitic adaptations. Root-knot nematodes have worldwide distribution and a broad host range. They are capable of infecting nearly every species of higher plants (Karssen, 2006). Typically, they parasitize internally within plant roots and feed on modified vascular plant cells, inducing small to large galls or knots on roots. This is the common underground symptom produced by the nematode (Christie, 1936). The aboveground symptoms are usually defined as a patchy distribution pattern with chlorotic and stunted plants. Both symptoms may be similar to nutrition deficiency or malfunction of root systems (Bird, 1970). These severe aboveground symptoms are explained by *Meloidogyne* infection that disrupts water and nutrient uptake and upward translocation by the root system (Bird, 1970). Hosts may be heavily infected without showing external symptoms on foliar tissue, e.g. symptomless plant leaves but galled roots, however more commonly the aboveground symptoms may be severe, especially under drought conditions. The rapid development and reproduction on good hosts result in several generations during one crop season, which leads to severe crop damage. Secondary infection by other pathogens usually causes extensive decay of nematode-infected tissues (Christie, 1950). Because of nematode infection, not only crop yield is suppressed, but also product quality is often not acceptable. Thus, this genus is of great economic and social importance.
Economic Impact

Generally, tropical regions provide more favorable environmental conditions for pathogen development, colonization, reproduction and dispersal, and as a result damage and yield losses caused by plant pathogens, including plant-parasitic nematodes are greater in tropical than in temperate regions (De Waele and Elsen, 2007). Severity of root-knot nematode damage is usually affected by specific *Meloidogyne* species. Their effects depend on susceptibility of hosts, crop history, season and soil type (Greco et al., 1992; Potter and Olthof, 1993). Similarly, economic thresholds are primarily dependent on these same factors. Based on damage thresholds established for several crops, on average, approximately 0.5 to 2 second-stage juveniles per gram of soil is enough to cause damage (Di Vito et al., 1991). There is not only a direct cost to root-knot nematode infestations, but also an indirect cost such as that cause by regulations and quarantines imposed on some species of *Meloidogyne*. For example, *M. chitwoodi*, is increasingly regulated because of its effects as a serious pathogen of potato and other economically important crops. This nematode is on the list of prohibited pathogens of many countries namely (Canada, the EU, Mexico and other countries in Latin America, and the Far East) (Hockland et al., 2006). *M. fallax* is another quarantine nematode that is being subjected to regulation. Also, the highly virulent *M. enterolobii* has been added to the EPPO alert list. Additional root-knot nematodes that might be added to the list of quarantine species include recently described *M. minor* and *M. citri*, in the USA (Viaene et al., 2007).

History of the Genus

The first root-knot disease was reported during the middle of the 19th century by a clergyman, Miles Joseph Berkeley (1855), who first attributed galls extracted from
glasshouse cucumber roots to the nematode. The nematode was first described by Cornu (1879). He based his description on nematodes found in root galls of sainfoin (Onobrychis sativus Lam.) in the Loire valley, France. In 1887, a brief description of a root-knot nematode was made by Goeldi. He illustrated a root-knot nematode extracted from coffee plants in Brazil. He named the nematode *M. exigua*. Up until 1949 all root-knot nematodes were designated as *Heterodera marioni*. Benjamin Chitwood is credited with revising the genus *Meloidogyne* (Chitwood, 1949). He placed five species and in one genus, naming them *M. arenaria, M. exigua, M. incognita, M. javanica, M. hapla* and *M. incognita* var. *acrita*. He based their identification on morphological characters, such as perineal pattern, stylet knob shape, length of stylet, and the distance of the dorsal esophageal gland orifice from the base of the stylet knob. Currently there are over 100 *Meloidogyne* spp. described (Karssen, 2002). In addition to traditional morphological identification, more modern tools are being used such as biochemical and molecular biology (Hartman and Sasser, 1985).

**The Distribution of *Meloidogyne* Species**

It is difficult to determine the origin of most *Meloidogyne* spp. Their broad host range and the worldwide movement of vegetative planting stock infected with root-knot nematodes makes it difficult to distinguish the nematode’s site of origin or whether the nematode adapted to the new site (Sasser, 1977). Some species may adapt to their new environments, whereas others may not (Sasser, 1977). *M. incognita* (Kofoid and White, 1919), the southern root-knot nematode, is one of the most ubiquitous species in the genus. It has a wider geographic range than any other species. The nematode is found from approximately 40° N latitude to 33° S (Taylor et al., 1982). The average annual temperature reported in this geographic range lies between 18 to 30 °C.
However, most populations were found where the annual temperature ranges between 24 to 30 °C. The optimum temperature for this nematode’s development is 27 °C. *M. incognita* rarely occurs in regions where the average lowest temperature is below 3 °C. The second most common species, *M. javanica* (Treud, 1885) is also distributed throughout the world. The latitude range of *M. javanica* is from approximately 33° N to 33° S (Tayler et al., 1982). It is rarely found in cold regions where monthly temperature average below 3 °C. Dryer soil conditions usually enhance survivability of *M. javanica*, and as a result it is the dominant species in regions where monthly precipitation is ≤ 5 mm for at least 3 months. Although *M. arenaria* (Neal, 1889) is not as common as the previously mentioned species, it is the most important plant-parasitic nematode worldwide on peanut. The distribution of *M. arenaria* is approximately 40° N latitude to 33° S latitude. The annual temperature range is similar to *M. incognita*. *M. hapla* (Chitwood, 1949) is less prevalent than the other three *Meloidogyne* spp. It is usually found in cool regions with latitudes between 34° N and 43° N (Taylor et al., 1982). In tropical or subtropical regions, *M. hapla* often occurs at high altitudes that are more than 1,000 m (Brown, 1955).

**Peanut Production**

The peanut, or groundnut (*Arachis hypogaea*), is an annual herbaceous plant and a member of the legume or the "bean" family (*Fabaceae*). This family is characterized by symbiosis with rhizobium which leads to production of nitrogen needed for plant growth (Nwokolo, 1996). Growth of peanut begins after the germination of a seed. The plant produces a flower above ground and ends in maturation of a fruit underground. It has at least two distinct growth habits: erect and trailing (Dickson,
1998). Appearance of flowers begins 4 to 6 weeks after planting with formation of yellow-petaled, pea-like inflorescence borne in the axillary clusters aboveground. The flowers wither responding to self-pollination and the stalks called gynophores or pegs, elongate downward into the soil. Subsequently, they turn horizontally followed by formation of a swollen ovary that develops to become a pod. The pods of most cultivated peanut contain two or three seeds and they mature at 125 to 145 days after planting (Dickson, 1998).

Peanut grows well in tropical and subtropical climates, optimally at temperatures ranging between 30 and 34 °C, although they will tolerate a range between 15 and 45 °C (Williams et al., 1978). High temperatures (above 34°C) may cause damage to peanut flowers. Plants grow best in a light, well-drained sandy loam soil with an optimal pH of 6.0-6.5 (Smith, 1950). Variable yield losses caused by drought are dependent on different factors such as duration and local environment stress (Kambiranda et al., 2011). A well distributed rainfall pattern between 50 and 60 cm over the course of the growing season is ideal for optimal peanut production (Nicholaides, 1969).

Peanut originated in South America where this species is believed to have existed for thousands of years (Hammons, 1982). Peanut played an important role in the diet of the Aztecs and other Native Indians in South America and Mexico. The Portuguese explorers may have brought peanut to Africa whose people later introduced the plant into what became the United States. On the other hand, the Spanish have been credited for bringing peanut from South America to Asia through the former colony, the Philippines (Kaprovickas, 1969).
Today, peanut is a crop of global importance to both smallholder and large commercial producers. It has been widely grown in the tropics and subtropics, where it has been classified as both a grain legume and oil crop (Hymowitz, 1990). World annual production is about 46 million tons per year. China has the largest production of peanut, a share of about 45% of the overall world production, followed by India (16%) and the United States (5%). Peanut is the 12th most valuable crop grown in the United States with a farm value of over one billion U.S. dollars. The United States produced more than 2.0 mmt from about 1,354,000 planted acres, largely from the states of Georgia, Alabama, Florida, and Texas (Anonymous, 2015). In 2014, Florida produced about 13% of the USA peanut supply, mostly in nine counties. While there are many varieties of peanut grown in the USA, Runner is by far the most commonly grown among the four market types (Tillman et al., 2015). Because of their high protein content and their chemical profile, peanut is a good food source for the human diet (Grosso et al., 1994; Nelson and Guzman, 1995). Worldwide a large percentage of peanut seed is used for the production of edible oil, whereas in the USA about 60% of the production is processed in a variety of ways and eaten as food (Allen, 1981).

**Plant-parasitic Nematodes Associated with Peanut**

Diseases pose a major threat to the production of peanut each year, and prevention of disease on peanut is a major concern. Plant-parasitic nematodes are among the most important soil borne constraints in successful peanut production. Worldwide plant-parasitic nematodes considered important on peanut include three species of *Meloidogyne*, *Pratylenchus brachyurus*, *Belonolaimus longicaudatus*, *Criconemoides ornatus*, *Aphelenchoides arachidis*, *Scutellonema cavanessi*, *Tylenchorynchus brevilineatus*, and *Ditylenchus africanus* (Dickson, 2005).
Species of *Meloidogyne* Parasitizing Peanut

Three *Meloidogyne* spp. that cause significant suppression of peanut yields and fruit quality are *M. arenaria* (peanut root-knot nematode) race 1, *M. javanica* (Javanese root-knot nematode) races 3 & 4, and *M. hapla* (northern root-knot nematode). In the United States, *M. arenaria* is the most damaging species on peanut in the more southern states, whereas *M. hapla* is the damaging in North Carolina, Virginia, and Oklahoma (Hirunsalee et al., 1995). *M. javanica* is sometimes found to be mixed with *M. arenaria* in the field (Cetintas, et al., 2003). *M. haplanaria* (Eisenback et al., 2003) was recently described as a new species parasitizing peanut in Texas, however little is known regarding its effects on peanut outside of Texas.

Cliff and Hirschmann (1985) described the morphological variations among seven populations of *M. arenaria* representing both physiological races. Race 1 infects and reproduces on groundnut, whereas race 2 does not (Taylor and Sasser, 1978). In a recent study, a race 3 of *M. arenaria* was reported. This race infects and reproduces on both resistant tobacco and pepper but not on cotton or peanut in Spain (Robertson et al., 2009). Most recently, *M. arenaria* race 3 has been found infecting peach in Florida (Sai Qiu, personal communication, 2017). The loss of peanut yield caused by the peanut root knot disease can reach up to 50% or greater in severely infested fields (Proite et al., 2008).

Peanut has been reported to be infected by *M. javanica* in Egypt (Ibrahim and El Seady, 1976), Georgia (Minton et al., 1969), and Texas (Tomaszewski et al., 1994). Other reports of *M. javanica* on peanut are from India (Patel et al., 1988; Sakhuja and Sethi, 1985), and Brazil (Lordello and Gerin, 1981). The first observation of *M. javanica* parasitizing peanut in Florida was found in a commercial production field in 2002 (Lima et
al., 2002). Later, Cetintas et al. (2003) confirmed the occurrence of *M. javanica* on peanut in Florida. Their investigation showed the ratio of *M. javanica* to *M. arenaria* race 1 in the peanut crop was approximately 29% vs. 71% respectively.

*M. hapla* has been reported damaging peanut North Carolina, Virginia, and Oklahoma (Hirunsalee et al., 1995; Machmer, 1951; Miller and Duke, 1961) and it has also been found on peanut in some southern states (Christie, 1959).

**Other Species of Nematodes Associated with Peanut**

*Pratylenchus brachyurus* is commonly found associated with peanut grown in Alabama (Steiner, 1945), Georgia (Boyle, 1950), and Florida. The extent of damage caused by this pathogen is not clear (Minton, 1984). *Belonolaimus longicaudatus* is also reported to occur on peanut depending on location (Abu-Gharbieh et al., 1969). It was reported in Florida causing extensive field damage to peanut in Levy County (Kustuwa et al., 2015). Damage on peanut by *Criconemoides ornatus* occurs especially in the southeastern United States, but only when they reach a high density (Sasser et al., 1968; Minton and Bell, 1969). The occurrences of other nematode parasites causing disease of peanut are limited in their distribution (Dickson, 2005).

**Root-knot Nematode on Peanut**

*Meloidogyne arenaria* race 1 is the dominant root-knot nematode species infecting and causing damage on peanut. The infected roots, pegs and pods have typical galls with egg masses (Sasser, 1954). Galling of the root systems inhibits plant absorbing nutrients on that leads to slow growth, stunting, and yellowish leaves. Because soil particles often adhere to egg masses, the infected roots have a crusty appearance. These diseased peanut plants are often infected by secondary soilborne pathogens such as *Sclerotium rolfsii* and *Sclerotinia minor* (Starr et al., 1996, 2002).
The nematode causes stunting of the plants and suppresses yield. Galled pods have a distorted appearance, which is not acceptable in the market. Additionally, galling severity is positively correlated to aflatoxin contamination of peanut kernels (Timper and Wilson, 2002).

**Disease Cycle**

The disease cycle of root-knot nematode in peanut starts with the penetration of roots by the second-stage juveniles (J2), which is the infective stage (Karssen, 2002). The J2 in the soil penetrates a suitable root by using its stylet to thrust repeatedly into cells at the surface. After forcing its way into the root, the juvenile moves between and through cells, eventually positioning its head adjacent to the vascular tissues. Within 2 to 3 days, the juvenile becomes sedentary, with their head embedded in the vascular cylinder to establish a feeding site. The nematode then begins to grow in diameter, loses its ability to move, and matures. On the process of establishing a feeding site, the female injects secretory proteins (from the esophageal gland) that stimulate physiological changes within the parasitized cells known as "giant-cells". When these specialized cells increase in size changes occur that allows the giant-cells to produce large amounts of proteins as food resources for the nematode (Proite et al., 2008). Because of the nematode’s esophageal gland cell secretions, the plant responds producing more growth regulators, which result in increasing cell size (hypertrophy) and number of cells within the adjacent cortex (hyperplasia) (Barker et al., 1998). Root cells around the giant-cells enlarge and divide rapidly, leading to gall formation (Hussey and Mims, 1991). The nematode goes through two additional juvenile stages interspersed by molts. The mature female is much wider but not much longer than the original second-stage juvenile. The female body becomes spherical or pear-shaped, with a
diameter of about 63.5 μm and a narrow neck embedded in the vascular tissue. The life cycle is completed when the female begins laying eggs. One single egg mass normally bears 500 to 800 eggs, but the number varies from almost none under unfavorable conditions to as many as 2,000 under highly favorable conditions (Taylor, 1978).

The development of the nematode is dependent on environmental conditions and hosts suitability (Proite et al., 2008). Eggs are deposited in a gelatinous matrix that becomes visible outside or near the galled surface of the roots. Secondary infection of peanut roots, pegs and pods occur as newly-hatched J2 move to initiate new feeding sites (Dickson and De Waele, 2005). Parthenogenesis is the main mode of reproduction of *M. arenaria*. Males are not required for reproduction and they are normally found in small numbers but may increase under adverse conditions such as food shortage, crowded population densities, and high soil temperature (Triantaphyllou, 1960). The length of the life cycle and the population densities depend upon several factors. Soil temperature, host suitability, and soil type are considered to be among the most important. At 27°C, which is usually optimum for most *Meloidogyne* spp, one generation on susceptible peanut requires approximately 25 to 35 days, whereas at 19°C at least 87 days are necessary (Tyler, 1933). Thus, three to five generations are possible in the field. The life cycle is longer on a less-suited host. Sandy, organic muck and peat soils favors population build-up more than heavier clay soils (Van, 1985).

The nematode can overwinter inside or outside of galled roots or pods in soils (Nusbaum, 1962). The second-stage juveniles (J2) are hatched from eggs when soil temperatures are around to 12°C. These J2 serve as initial inoculum for a following susceptible crop (Wallace, 1963). Soil temperature, humidity, soil type and cultivar
management are considered as main factors determining severity of root knot disease (Wallace, 1963).

The egg-mass index (EI), which was introduced in the literature by Taylor and Sasser (1978), was originally developed by members of the Southern Regional Technical Committee on Nematodes, USA. It gives an approximate measurement of root-knot nematode reproduction based on numbers of egg masses/plant instead of actual numbers of eggs and juveniles/plant. Since the number of eggs per egg mass varies, the EI is not a quantitative measurement of reproduction; still, it has been used as a basis for estimates of host efficiency. Likewise, the gall index (GI), which is based on the same scale as the EI, has been used as an indicator of plant damage even though little work has been used to correlate GI with crop yield. In the differential host test an average EI and an average GI of 2 or less are interpreted as indicating host resistance and those greater than 2 as indicating host susceptibility.

**Parasitic Mechanism**

For plant-parasitic nematodes to develop, it is necessary that they feed on certain hosts, absorbing nutrients to complete their life cycle. Two morphological characters, a stylet and an esophagus, make it possible for a nematode to be parasitic (Blaxter, 1997). Root-knot nematode is a sedentary plant-parasite with an advanced evolution of parasitism. This nematode has a typical Tylenchoida stylet and an esophagus with strong metacorpus as a pump chamber and three secretory gland cells, one dorsal (DG) and two subventral (SvG). These glands are the major source of the secretions involved in plant parasitism (Hussey, 1990). Root-knot nematodes are able to secret proteins to modify host root cells into very specialized feeding cells by the alteration of gene expression of host cells (Gheysen et al., 1997). During different
stages in the life cycle of root-knot nematodes, the morphology and function of esophageal gland cells is altered. The SvG are most functional at initial parasitism by infective J2, whereas the DG is the predominant gland in the parasitic stages (Bird, 1983). Infective J2 penetrate the host root and migrate through the cortex cells to the vascular tissue. Meanwhile, degrading enzymes for cell wall penetration are secreted from the esophageal glands through the stylet of root-knot nematodes to facilitate migration by weakening or rupturing cell walls. Giant cells induced by root-knot nematode are a result of an abnormal increase in cell number and size following repeated mitosis without wall degradation (Bird, 1996; Fenoll et al., 1997). When J2 undergo further development, the cuticle structure is altered by decreasing layers with the basement membrane left in order to absorb more nutrients from host.

The nuclear region of the gland cells is where synthesis of secretory proteins occurs. The mature enzymes are stored in Golgi-derived membrane-bounded granules that are connected with ampullae by microtubules in the gland cell (Burgess and Kelly, 1987). The number of different secretory proteins may change according to gland cell type and parasitic stages (Burgess and Kelly, 1987). After a plant-parasitic nematode penetrates the host with its stylet, enzymes are secreted from the esophageal glands and injected into plant cells through the stylet (Carneiro et al., 1996). These enzymes are able to stimulate their host to produce abnormal growth regulators that aid in the development of feeding cells thereby providing nutrients (Jones and Northcote, 1972).

Studies on the esophageal glands and stylet secretions obtained from root-knot nematodes confirmed that a group of proteins (e.g. glycosylated proteins), but not nucleic acids, were involved in the secretions (Davis et al., 2000). The composition of
secreted proteins via the nematode stylet was analyzed by designed chemical stimulation of the production of stylet secretions in a vitro system. Esophageal gland antigens have been isolated by monoclonal antibodies and used for direct analyses or screening cDNA expression libraries corresponding to secretion genes (Davis et al., 2000).

**Population Dynamics**

Plant-parasitic nematode population dynamics play an important role in plant nematology because of the relationship with the incidence of crop disease. These provide the basis for advisory programs in agriculture (Wallace, 1963). Vertical and seasonal distributions of nematodes are highly variable but they are generally related to the distribution of host roots (Wallace, 1963). In most cases, plant nematodes in soil seem to have favorable zones where they concentrate, most likely related to their survivability. It has been suggested that host distribution, root depth, height of water table, soil moisture, soil type, depth of subsoil, and temperature affect vertical distribution patterns (Ferris, 1971; Miller, 1960; 1972; O'Bannon, 1972; Potter, 1967).

There are several observations on the distribution of nematode in different soil types and different seasons (Ferris and McKenry, 1974; Norton et al., 1971). The top 45 cm of soil seems to be the optimum zone for nematodes to congregate (Barker and Nusbaum, 1971). However, different nematode species have diversity in their vertical distribution patterns.

According to Godfrey (1924), root-knot nematodes tend to concentrate at a depth where the plow can’t reach. Generally, soil samples are taken from this plow depth (15 to 20 cm deep) for determining preplant nematode population densities. Root-knot nematode populations overwintering at a deeper soil depth may be considered as a
strategy for survival. At this deeper zone they escape detection, fumigation, and are less likely to be affected by other control management tactics. This ensures their survival to serve as an initial inoculum for the next crop season (Potter, 1967). After planting a crop, nematode damage increases at a rate inconsistent with the number of nematodes found in the preplant sample.

Cobb (1914) stated that nematodes are often very numerous near the surface and gradually drop in numbers as the soil depth increases. Higher population densities are reported to be found from 20 to 50 cm deep with few being detected at 86 cm deep or greater (Godfrey, 1924). In extreme environmental conditions, nematode population densities are low or variable in the upper 15 cm of soil, with greater numbers being found at 30 to 45 cm deep. In some instances, numbers may remain relatively high down to 120 cm deep. There is much information about the influence of temperature on nematodes development; however there are no reports of nematode reactions to temperature fluctuation in the field (Ferris and McKenry, 1974).

Nematodes move in films of water that surround soil particle. Dropkin (1980) stressed that many nematode genera and species have particular soil types and climatic requirements and that certain species of nematodes prefer to live in sandy soil, whereas others prefer clay or loamy soil. Most plant-parasitic nematodes are reported to survive well in a coarse textured soil (Wallace, 1968; 1971). The relationship between nematode movement and soil texture is a function of the ratio of nematode size to pore and particle size. There are marked positive correlations between length and diameter of the nematode and optimum pore and particle sizes that allows for maximum movement (Brodie, 1975).
In the presence of host plants, root-knot nematodes can migrate towards roots of a host plant while moving up or down in the soil layers. Johnson and McKeen (1973) found that a population of *M. incognita* present in the top 15 cm can move down to a depth of 120–125 cm. According to Pinkerton and McIntyre (1987), *M. chitwoodi* J2 occurring in soil can migrate 30 cm upwards to penetrate tomato plants. Higher population numbers were reported from 20 to 50 cm deep than at shallower depths, whereas very few were detected at 86 cm deep or greater (Godfrey, 1924). Under extreme environmental conditions, the nematode population densities were low or variable in the upper 15 cm, whereas the largest numbers of nematodes were found at 30 to 45 cm deep with decreasing numbers at deeper levels and relatively high numbers down to 120 cm. However, Ferries and McKenry detected *Meloidogyne* spp. in a vineyard in California 120 cm below the soil surface and could be detected at 330 cm deep (Ferris and McKenry 1974).

Juveniles and eggs are supposed to be survival stages of nematodes in soil. Different nematode species have different survival stages that are affected by temperature. Daulton (1961) and Nusbaum (1962) stated that based on their field experiments, *Meloidogyne javanica* and *M. hapla* overwinter as eggs, which is the same survival form for *Xiphinema americanum*. According to Baunacke (1922), with enough stored food, the juveniles of sugar-beet cyst nematode can survive for months in the lab. However, their activities decreased at 15°C or lower. As the temperature increases to 25°C to 28°C, nematode activity increases so much so that the infective stage must find a suitable host or else it depletes its stored foods (Dropkin, 1963). It was reported that the higher the soil temperature, the shorter the survival period of juveniles under
fallow conditions because of increasing activity at higher temperatures (Bergeson, 1959). According to these findings, Van Gundy et al. (1976) summarized that the nematodes internal food reserves were the main factor determining the survival of many plant-parasitic nematodes. Moreover, they concluded that root-searching activity causes greater use of food reserves based on the fact that food reserves were consumed by juveniles faster in the presence of roots than in their absence.

**Temperature Effects on Root-knot Nematode**

Most *Meloidogyne* spp. are important pathogens in tropical, subtropical, and temperate regions with relatively mild winters. Thus, temperature is considered as one of the most important factors that governs the occurrence and severity of root-knot disease (Godfrey, 1926). Temperature has great bearing upon geographical distribution, population development, and survival. In southern states, long summers combined with favorable soil temperature and moisture conditions allow multiple generations of root-knot nematodes in one single growing season thereby leading to high population densities in the soil (Godfrey, 1926). In contrast, in northern areas with a shorter summer season fewer generations of nematodes and lower population densities occur. *Meloidogyne* spp. that have different climate ranges have been reported in several studies (Bird and Wallace, 1965; Olthof, 1967; Thomason and Lear, 1961).

Temperature effects on the life cycle of the root-knot nematode have been well documented. Each nematode species has its own optimum temperature range for all phases of development from the egg stage through the egg-laying stage. Susceptible plant roots may be infected when the temperature ranges from 12 °C to 40.5 °C (Tyler, 1933). In addition, it pointed out that the temperature threshold for nematode maturity and reproduction seems to be higher than that for early stages of the life cycle, but that
no eggs were found above 31.5 °C. Bergeson (1959) indicated that most eggs hatched at a temperature of 15.5 °C and above, whereas below 10 °C, survival and egg hatch decreased rapidly. Similar reports showed that temperatures below 15.6°C and above 35°C limited the reproduction of *M. incognita* (Thomason and Lear, 1961).

**Root-knot Nematode Management**

Cultural and physical practices, biological organisms, host resistance, and nematicides are traditional tactics used to manage the peanut root-knot nematode. Crop rotations are one of the most effective cultural management methods. For example, rotation of peanut with cotton, velvet bean, or bahiagrass can mitigate *M. arenaria* population densities and increase peanut yields (Dickson and Hewlett, 1989; Johnson et al., 2000; Rodríguez-Kábana et al., 1986, 1987, 1991a, 1991b). However, long periods of growing low value crops and the nematodes ability for long-term survival in soil without hosts compromise the usefulness of crop rotation. Both fumigant and nonfumigant nematicides have been proven effective for managing root-knot disease (Dickson, 1988). The fumigant nematicide 1, 3-D is highly effective, however because of measured production costs associated with its use and other environmental concerns many growers seek other options. Though biological antagonists are effective in suppressing root-knot nematode, there are limitations to their use (Bale et al., 2008). Therefore, resistant cultivars are thought to provide a more sustainable method for managing the endoparasitic nematode pathogens such as *Meloidogyne* spp.

**Development of Resistant Peanut Cultivars**

Utilization of resistant cultivars is considered an effective component in pest management (Williamson, 1999). By definition, with plant resistance the reproduction of a nematode species should be suppressed up to 90% or more (Cook and Evans, 1987).
In response to infection by a nematode, the plant resistance gene reduces the parasitic nematode’s ability to reproduce. Moreover, compared to a susceptible plant, the galls formed on roots of a resistant cultivar may be reduced or completely lacking because of the incompatible interaction between the parasites and the resistant host (Williamson, 1999).

Numerous attempts were made to find root-knot nematode resistance in cultivated peanut germplasm. Researchers were aware that a number of other Arachis spp. were highly resistant to the peanut root-knot nematode (Simpson, 1991). In 1995 a breakthrough was made when resistance genes from wild peanut were transferred into cultivated peanut. Resistant genes to root-knot nematode from A. cardenasii were successful introgressed into A. hypogaea by a backcross breeding pathway (Starr and Morgan, 2002). Garcia et al. (1996) reported that a germplasm line called TxAG-6 with resistance was generated by interspecific hybridization [A. batizocoi x (A. cardenasii x A. diogoi)]. The germplasm line TxAG-7 was obtained from the first backcross generation of TxAG-6 and resulted in release of the first root-knot nematode resistant peanut cultivar–COAN (Simpson and Starr, 2001). In 2003, NemaTAM was the second named peanut cultivar released with resistance to root-knot nematodes. Both were released by the Texas Agriculture Experiment Station (Simpson et al., 2003). NemaTAM had the same level of resistance as COAN, but greater yield potential. Both COAN and NemaTAM are derived from the same introgression but selected from a different backcross generation (Simpson, 1991). However, neither is resistant to M. hapla. Moreover, neither of these cultivars was considered for planting in the southeastern USA because of the problems with tomato spotted wilt virus (TSWV) and
other fungal diseases endemic to the area (Rich and Tillman, 2009). In 2007, a runner-type peanut cultivar (*Arachis hypogaea* L. subsp. *hypogaea* var. *hypogaea*)-Tifguard was developed that was resistant to both root-knot nematodes and TSWV. Tifguard was released by the USDA-ARS and the Georgia Agricultural Experiment Stations (Holbrook et al., 2008). The cultivar was generated by hybridizing ‘C-99R’ (Gorbet and Shokes, 2002) with ‘COAN’ (Simpson and Starr, 2001). This new peanut cultivar exhibited a high level of resistance to TSWV, *M. arenaria*, and *M. javanica* (Holbrook et al., 2008).

**Identification of Resistant Gene to Root-knot Nematode**

It has long been a question of interest on how a single gene governs resistance to a nematode by interfering with the establishment of the elaborate changes caused by the parasite in host roots (Williamson, 1994). After being attracted to and penetrating roots, root-knot nematodes migrate to the vascular tissue in a similar manner in both resistant and susceptible plants (Dropkin, 1969; Ho et al., 1992; Paulson, 1972). However, in resistant plants, the nematode is not able to establish feeding sites. Instead, a hypersensitive response (HR) formed by a localized region of necrotic cells are induced near the anterior portion of the invading J2 (Dropkin, 1969; Ho et al., 1992; Paulson, 1972). The J2, surrounded by necrotic cells, usually die and others that fail to establish feeding sites may egress from the roots. The earliest indication of HR is visible about 12 h after inoculation (Paulson and Webster, 1972). HR does not occur when J2 are migrating through the root tissue, but does so when they attempt to establish a feeding site.

The resistance from wild *Arachis* spp. was identified and reported to be controlled by two dominant genes-Mag and Mae. The former inhibits galls formation and
the latter suppresses egg production (Choi et al., 1999). The advanced germplasm line TxAG-6 mentioned above is an F1 from the complex hybrid of \( A. \ batizocoi \ x \ (A. \ cardenasii \ x \ A. \ diogoi) \), with each one having resistant genes to \( M. \ arenaria \) (Choi et al., 1999). The resistance to \( M. \ arenaria \) in \( A. \ cardenasii \) results in the inhibition of development and is considered to be a hypersensitive host reaction that occurs near the anterior end of the nematode within a few days of infection (Starr et al., 1995). The resistant mechanism to \( M. \ arenaria \) in \( A. \ batizocoi \) was considered to involve a different mechanism. Though the J2 invaded into \( A. \ batizocoi \) roots, few of them remained and developed inside the roots (Choi et al., 1999). In addition, \( A. \ batizocoi \) resistance is reported to increase the time required for \( M. \ arenaria \) to finish its life cycle. However, no hypersensitive reaction was observed in this \( Arachis \) spp. Another germplasm line TxAG-7 is derived from the first backcross generation of \( A. \ hypogaea \) ‘Florunner’ \( x \) TxAG-6 (Starr et al., 1995). The resistance to \( M. \ arenaria \) in TxAG-7 was similar to that of \( A. \ cardenasii \) in which an apparent necrotic HR occurred. But the occurrence of host-cell necrosis of a HR reaction was not observed when J2 invaded into the root system (Starr et al., 1990). The resistance in COAN generated from TxAG-7 is controlled by a single dominant gene. Most invasive J2 emigrated from roots whereas the few remaining J2 developed to reproductive females (Choi et al., 1999). Three different experiments were designed to evaluate the mechanism of resistance to \( M. \ arenaria \) in COAN (Bendezu and Starr, 2003). The rare necrosis that occurred in host tissue in roots of COAN suggested that resistance to the peanut root-knot nematode does not involve a necrotic, hypersensitive reaction. Furthermore, resistance in COAN blocks most of the J2 from penetrating roots. Only 1 of 90 was observed within the vascular
cylinder, whereas more than 70% of J2 were observed in the vascular tissue of the susceptible cultivar (Bendezu and Starr, 2003).

A gene named Rma is assumed to be a dominant root-knot nematode resistance gene introduced from other *Arachis* species. Identifying and mapping of alien DNA sequence by codominant DNA markers, most of clones are leucine-rich repeat family of plant genes. In addition, a four-genome equivalent fosmid library was developed from the resistant cultivar, which assembled into two long contigs. NT946 has about 32,923 bp whereas NT344 is about 30,462 bp. By finer mapping with marker-assisted selection, the R gene of wild peanut was found to be located on chromosome 9A and 9B. Mag and Mae were discovered to relate to Rma. The function of Mag is reported to inhibit formation of galls, whereas Mae suppresses egg production of the root-knot nematode (Nagy et al., 2010).

**The Application of Host Resistance**

Long-term use of nematode resistant cultivars can cause extensive selection pressure on the target species and affect nontarget nematodes as well (Lawrence, 1992). Problems from planting resistant cultivars successively include shifts in nematode races or species. An example of shifts in species of root-knot nematode in response to planting resistant cultivars occurred with *M. incognita* on tobacco in North Carolina. There was a species shift from *M. incognita* with an increase finding of *M. arenaria* and *M. javanica* infecting resistant cultivars of tobacco in North and South Carolina (Barker, 1989; Johnson, 1989). This was believed to have resulted from long-term use of resistant cultivars to combat *M. incognita* problems. In California there have been reports of resistant tomato cultivars being overwhelmed by resistance-breaking nematodes within field populations. Although the Mi gene in tomato cultivars has proven
to confer effective resistance against several root-knot nematode species (Williamson, 1998), numerous reports of root-knot nematode have been identified worldwide parasitizing resistant tomato plants (Tzortzakakis et al., 1996; 2005; Carvalho et al., 2015).

Severe root-knot nematode infection on the resistant peanut cultivar-Tifguard was reported in Levy County, Florida in 2012. Root-knot disease was found on Tifguard in a total of 14 sites over the summer. Tifguard had previously been reported as nearly immune to *M. arenaria* and *M. javanica*. The reason for the break down in Tifguard was unknown.
CHAPTER 2
FIELD EVALUATION OF RESISTANCE TO *Meloidogyne arenaria* IN BREEDER, FOUNDATION AND GROWER-KEPT TIFGUARD

Introduction

Tomato spotted wilt virus and root-knot nematodes cause damage and yield suppression on peanut grown in Florida (Holbrook et al., 2008). Because of nematode problem, the US Department of Agriculture (USDA) released a runner cultivar in 2007 named Tifguard. The cultivar was reported as highly resistant to root-knot nematode. It was the first peanut cultivar with a combination of resistance to TSWV and root-knot nematodes. This cultivar provided growers with another option to manage nematode and TSWV diseases in the southeastern United States. Although, Georgia-06G became the dominant cultivar planted Tifguard gained a larger proportion of the acreage where root-knot nematode was problematic (Tillman et al., 2013). With Tifguard, root-knot nematode infested fields could be put back into production without large increases in production costs.

There are five sources of Tifguard seed - breeder, Foundation, Certified, Registered, and grower-kept. Breeders first produce a relatively small quantity of seed of any new cultivar. These seeds are called Breeder seed in the seed certification system and are directly controlled by the plant breeding institution, firm or individual that is the source for production for the certified classes (USDA, 2002). Foundation seed is the progeny of breeder seed, handled so as to maintain specific genetic purity and identity, production of which must be acceptable by the Department of Agriculture and Forestry (USDA, 2009). Registered and Certified seed are the progeny of breeder or Foundation seed, handled under the same procedures as Foundation seed to maintain satisfactory genetic purity and identity. Grower-kept seed is the progeny of peanut
produced from the previous season and used as a seed source for the following crop season.

Although Tifguard was reported to be highly resistant to *Meloidogyne arenaria* and *M. javanica*, root-knot nematode damage was observed on grower-kept Tifguard peanut in summer 2012 in production fields located primarily in Levy County, FL. Typical above ground symptoms of root-knot disease were noted. The emergence of a resistance-breaking race of *M. arenaria* was considered as a possible reason. This possibility was highlighted because of the long-term monoculture of peanut in some production fields in Levy County. The practice of continuous planting of resistant cultivars leads to the increased possibility of resistance breaking by the soilborne pathogens (Frisvold, 2010). In addition to the possible development of resistance breakings strains of the nematode, higher soil temperature also was considered as a factor that could impact root-knot disease on Tifguard. This has been demonstrated with the *Mi* gene in tomato that confers resistance to several *Meloidogyne* spp. (Williamson, 1998). The objective was to evaluate Tifguard seed obtained from three sources to determine the cause for root-knot nematode infection on the resistant cultivar.

**Materials and Methods**

**Field Sites and Experimental Designs**

Resistance to *M. arenaria* in Foundation seed and grower-kept Tifguard seed was evaluated in the same field site (464 Farm) where a large number of Tifguard plants infected with root-knot nematodes were found in 2012. The field design was a randomized complete block with four replicates. In each block two rows of Foundation Tifguard and two rows of grower-kept Tifguard, were compared with two rows of Florida-07 (susceptible) (Gorbet and Tillman, 2009). The plots were 3.0 m long and rows were
spaced 0.9 m apart, with each row seeded with 16 seeds. The experiment was conducted in 2013, 2014 and 2015 in the same blocks with the following exceptions. In 2014 six rows of breeder Tifguard were compared with two rows of FloRun 107 (susceptible) (Tillman and Gorbet, 2015). In 2015 Georgia-06G (Branch, 2007b) seed replaced Florida-07. The plots were sprayed with a back-pack sprayer every 14 to 20 days with chlorothalonil. Weeds were removed by hand pulling. After 140 days, all the peanut plants were dug to remove roots, pegs and pods. The roots were examined for galling and egg masses immediately after digging. Red-food coloring solution (0.5 L/10 L H₂O) was used as an aid to visualize egg masses (Thies et al., 2002). The assessment for peanut plants was recorded as positive for infected roots and negative for roots without infection.

In 2014 and 2015, resistance to *M. arenaria* in breeder Tifguard seed was compared with Georgia-06G in two separated locations both known to be heavily infested with *M. arenaria* - the Brown Farm, Levy County, Florida and the University of Georgia, Attapulgus Research and Education Center (AREC), Attapulgus, Georgia. The field design was a randomized complete block with five replicates at the Brown Farm and six replicates at AREC. The row length was 6 m and the row spacing was 0.9 m. The production methods followed the IFAS, University of Florida and University of Georgia Peanut Production Guides (Stephens, 1994; Lee and John, 2014). At harvest, the peanut plants were examined for root-knot nematode galling and egg masses of roots, pegs and pods. Red-food coloring solution (0.5 L/10 L H₂O) was prepared to stain egg masses. Each peanut plant was recorded as positive if infected by RKN and
negative for plants without infection. Both fields were overseeded with red crimson clover (*Trifolium cv. Dixie*) during autumn and winter seasons.

**Determination of Root-knot Nematode Species**

A total of 250 (100 from Brown Farm, 100 from AREC and 50 from 464 Farm) females were extracted from infected roots and assessed by PAGE. Each female was placed in a microtube in a 5 µl of deionized water and stored at -20 °C. Identification of the root-knot nematode species infecting Tifguard was made by analyses of isozyme phenotype following their resolution via the polyacrylamide gel electrophoresis protocol (Dickson, 1971; Ebenshade and Triantaphyllou, 1985). Electrophoresis was performed with the use of a Bio-Rad mini-PROTEAN II unit (Bio-Rad, Philadelphia, PA). Single females were homogenized individually in the microtubes. Before homogenizing 5 µl of loading dye (Bio-Rad) was added. Each gel contained 15 wells, with each one loaded separately with a female extract. A single *M. javanica* female homogenate was placed into wells 1 and 15 to serve as a control. Electrophoresis was conducted in a discontinuous buffer system with 8% acrylamide running gel, pH 8.8, and 4% acrylamide stacking gel, pH 6.8. The voltage was maintained at 80 volts for the first 15 minutes and increase to 200 volts for 35 minutes. After electrophoresis, the gels were removed and placed into an enzyme reaction mixture to resolve esterase and malate dehydrogenase isozyme patterns.

**Analysis of Resistance Gene in Infected Tifguard Plants**

A young leaf was removed from Tifguard plants displaying root-knot nematode galling or egg masses. The leaf was placed in a microtube for overnight delivery to the National Environmentally Sound Production Agriculture Laboratory (NESPAL), Tifton,
GA. They were examined for the presence of the root-knot nematode resistance gene marker (Chu et al., 2011).

**Results and Discussion**

**464 Farm 2013- 2015**

In 2013, 30% of the plants grown from Tifguard Foundation seed were found infected by *M. arenaria*, whereas 40% of peanut grown from Tifguard grower-kept seeds were infected (Table 2-1). All the peanut plants of Florida-07 were heavily galled.

In the 2014 and 2015 season, no Tifguard breeder plants had visible galls or egg masses, whereas all Georgia-06G plants were heavily galled and with numerous egg masses (Table 2-1).

**Brown Farm and AREC 2014- 2015**

In 2014 and 2015, 12% and 3.9% of Tifguard breeder plants were galled at the Brown Farm field site, respectively (Figure 2-2 A), whereas at the AREC field site 7% and 1.8% Tifguard breeder plants were galled, respectively (Figure 2-2 B). In both trials all the Georgia-06G susceptible plants were heavily galled and with numerous egg masses (Figure 2-2).

**Determination of Root-knot Nematode Species**

Based on esterase (Est) and malate dehydrogenase (Mdh) phenotypes. All the nematode samples were identified as *Meloidogyne arenaria*. These phenotypes are the most commonly found in populations of *M. arenaria* around the world infecting not only peanut but also other crops (Brito et al., 2008).
Analysis of Resistance Gene in Infected Tifguard Plants

Based on the detection of the resistance gene marker by the NESPAL, 2.5%, 28%, and 39.5% of the Tifguard breeder, Tifguard Foundation and the Tifguard grower-kept seed, respectively were negative for the resistant gene marker (Figure 2-2).

The breeder seeds had the highest proportion of plants with the resistance gene. It appears that the seed purity declined after multiple increases of peanut seed production. It was surprising that the foundation seed had a relatively high percentage of plants with the resistance gene. The grower-kept seed had the highest proportion of Tifguard plants negative for the resistance gene. Under the field condition where different peanut cultivars are planted in close proximity, it is possible that outcrossing occurred within Tifguard or that volunteers of a susceptible cultivar from the previous crop remained in the field and were mixed with Tifguard pods at harvest.

There were some false positive diagnoses in the field for galling on Tifguard. It is difficult to visualize root-knot nematode galls on peanut roots, especially when there are only a few. The large number of rhizobium nodules on peanut roots interferes with the determination of small root-knot nematode galls. It is not possible to say whether some plants categorized as positive were not actually infected. It is also possible that some Tifguard plants that had the resistance gene could have been infected by a strain of the nematode capable of infecting the resistant peanut.
Table 2-1. The average percentages of three different sources of Tifguard seed infected by *Meloidogyne arenaria* at the 464 Farm, Levy Co., FL in 2013, 2014, and 2015.

<table>
<thead>
<tr>
<th>Peanut seed sources(^1)</th>
<th>Root-knot nematode infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2013</td>
</tr>
<tr>
<td>Tifguard breeder</td>
<td>N/A</td>
</tr>
<tr>
<td>Tifguard Foundation</td>
<td>30</td>
</tr>
<tr>
<td>Tifguard grower-kept</td>
<td>40</td>
</tr>
<tr>
<td>Florida 07</td>
<td>100</td>
</tr>
<tr>
<td>FloRun 107</td>
<td>N/A</td>
</tr>
<tr>
<td>Georgia-06G</td>
<td>N/A</td>
</tr>
</tbody>
</table>

\(^1\) Tifguard Foundation and grower-kept seed were compared with Florida 07 in 2013 and Tifguard breeder seed was compared with FloRun 107 in 2014 and Georgia-06G in 2015.
Figure 2-1. The average percentages of Tifguard breeder seed infected by *Meloidogyne arenaria* was compared with Georgia-06G at two locations in 2014 and 2015. A) the Brown Farm, Levy Co., FL. B) University of Georgia, Attapulgus Research and Education Center, Attapulgus, GA.
Figure 2-2. The average percentages of different Tifguard seed sources that were negative for the resistance gene marker for *Meloidogyne arenaria*. 
CHAPTER 3
THE SEASONAL AND VERTICAL DISTRIBUTION OF *Meloidogyne arenaria* FROM RESISTANT AND SUSCEPTIBLE PEANUT GROWN IN TWO SOIL TYPES

**Introduction**

Nematode population dynamics plays an important role in plant nematology because of its association with the incidence of crop diseases and its utility as the basis for advisory services (Wallace, 1963). Vertical distribution of nematodes is highly variable but is generally related to the distribution of host roots (Wallace, 1963). In most cases, plant nematodes concentrate in a favorable soil zone. It has been suggested that host distribution, root depth, height of water table, soil moisture, soil type, depth of subsoil, and temperature affect vertical distribution patterns (Ferris, 1971; Miller, 1960; 1972; O'Bannon, 1972; Potter, 1967). There are several observations on spatial distribution of plant-parasitic nematodes in different soil types, different host crop and seasons (Ferris and McKenry, 1974; Norton et al., 1971). The top 45 cm of soil seems to be the optimum zone for most nematodes to congregate (Barker and Nusbaum, 1971). However, nematode species vary in their vertical distribution patterns.

*Meloidogyne arenaria* is a highly virulent soilborne pathogen of peanut that has the ability to produce multiple generations during the relatively long peanut growing season. Therefore, the population density of this nematode often builds to high levels. The nematode remains nearly undetectable and symptoms on peanut may be difficult to observe until 75 to 80 days after planting (Rodríguez-Kábana et al., 1986). But as the growing season progresses, stunted and yellow plants are often accompanied with increasing nematode populations in infested sites.
The pre-plant threshold for this nematode was determined to be one egg or second-stage juvenile /100 cm$^3$ of soil (McSorley et al., 1992). It is difficult for farmers to detect this low preplant threshold density by conventional extraction techniques (McSorley et al., 1992; Rodríguez-Kábana et al., 1982). Population densities of this nematode can increase exponentially as the host crop develops, driven by multiple reproduction cycles by harvest time (Rodríguez-Kábana et al., 1986). At harvest, damage is most easily observed on roots, however pegs and pods may be galled. A carrying capacity of 5,000 to 6,000 J2/100 cm$^3$ of soil was reported in microplot experiments (McSorley et al., 1992), however in field soil numbers were about 10-fold lower (Rodríguez-Kábana and Ivey, 1986).

In Alabama, the highest and most variable population density of J2 was found in the top 30 to 40 cm in a vertical distribution study (Rodríguez-Kábana and Robertson, 1987). The fluctuation of the J2 densities in the top soil layers was associated with the abundance of host roots, and a peak was observed before or at harvest. Deeper in the soil, the population densities were lower but fairly constant no matter at what time of year sampling occurred. However, this vertical distribution of J2 density observed in Alabama may different in deep sandy soil, such as those that exist in Florida. In such conditions, high densities of J2 may be recovered deeper in the soil (Dickson, 1985).

The vertical distribution of *M. chitwoodi* to a depth of 70 cm was examined for 2 years under summer barley, carrot, fodder beet, bean, marigold and black salsify in two fields with sandy soils (Wesemael and Moens, 2008). The results indicated that the vertical distribution was not significantly influenced by degree of susceptibility of
different crops. More importantly, the population density was closely related to the
different growing periods of the crops.

In microplots studies, *M. incognita* moved rapidly in both directions to a soil depth
of 150 cm (Johnson and McKeen, 1973). High nematode population densities and root
gall indices on tomato roots were recorded to depths of 150 cm in soil inoculated with
4,400 *M. incognita* kg$^{-1}$ of soil. The high population density in the top 30 cm of soil
reduced tomato yields by 20% in the first crop and 70% in the following crop.

Greenhouse evaluations demonstrated that high population densities of *M. incognita*
could be recovered as deep as where the crop roots reach (Ward, 1964; Bird, 1969).

Preplant population density is a predictor for root-knot disease damage, so data
from soil samples provide guidance for nematode management tactics (Been and
Schomaker, 2006). The nematode distribution both horizontally and vertically over the
period of a crop season or year usually fluctuates based on food source and
environmental conditions (Mojtahedi et al., 1991). To develop adequate sampling
procedures, data is required regarding the vertical distribution to determine the
approximate sampling depths for estimating population densities (Verschoor et al.,
2001). The objective of this study was to quantify the vertical and seasonal distribution
of *M. arenaria* from a susceptible and resistant peanut cultivar grown in two different soil
types.

**Materials and Methods**

**Field Locations and Experimental Designs**

Two peanut fields that were previously heavily damaged by root-knot nematodes
were selected for comparison of *M. arenaria* distribution over two crop seasons, 2014
and 2015. Site one, the Brown Farm, consists of a Candler deep sand and was located
in Levy County, Florida. Site two consisted of a Norfolk loamy sand stratified over a dense clay layer and was located at the University of Georgia, Attapulgus Research and Education Center (AREC), Attapulgus, GA. Breeder seed of Tifguard and Georgia-06G peanut cultivars were planted in paired rows with a 0.9 m row spacing. Rows were 6.1 m in length and each plot was replicated five and six times at the Brown Farm and the AREC for 2 years in a randomized complete block design. The plots were carefully marked so as to repeat the planting of Tifguard and Georgia-06G in as near as possible the same spot in both crop seasons. The Brown farm was planted on 10 April, 2014 and harvested on 8 September, 2014. In 2015, the Brown farm was planted on 1 May and harvested on 28 October. The AREC was planted on 5 June, 2014 and harvested on 30 October in 2014. In 2015, the AREC was planted on 19 May and harvested on 04 October. The production methods followed the IFAS, University of Florida and University of Georgia Peanut Production Guides (Stephens, 1994; Lee and John, 2014). During the winter season, both sites were overseeded with red clover (Trifolium cv. Dixie).

**Seasonal and Vertical Distribution of Meloidogyne arenaria**

During the peanut growing season, samples were taken in the rhizosphere of Tifguard and Georgia-06G peanut cultivars beginning in spring 2014 and ending in fall 2015. After peanut harvest samples continued to be collected within the former peanut plots marked by flags. At the Brown Farm, soil samples were collected from four depths at 30 cm increments with a 10-cm-diameter bucket auger to a depth of 120 cm. At the AREC, soil was collected from three depths at 15 cm increments to a depth of 45 cm. Sampling at the AREC was restricted to the top 45 cm depth because of a dense clay layer. The soil from each depth sample was mixed thoroughly placed in a plastic bag.
and stored at 10 °C (Hooper, 1986) until processed. At each sampling period, a flag was placed to mark the spot to ensure a new site was chosen for the next sampling date.

Nematodes were extracted from 200 cm$^3$ of soil via the centrifuge-flotation technique (Jenkins, 1964). The root-knot nematode J2 and other plant-parasitic nematodes were counted under an optical microscopy.

Temperature sensors (HOBO Tidbit Temperature Data Logger, Onset Computer Corporation, MA) were installed to measure soil temperature of each depth at both sites in 2015. A hole was dug and the sensors were inserted in a horizontal position at 18, 36, 54, and 72 cm deep at the Brown Farm and at 6, 18, 30 cm deep at the AREC. The sensors were set to record soil temperatures at 5 min intervals.

**Statistical Analysis**

The effects of soil depth, host genotype, and days after planting (DAP) on the number of J2 extracted from 200 cm$^3$ of soil were subjected to analysis of variance using the following model: nematode number = constant + depth + date + (depth × date) + error (R programming, R 3.1.2 with R studio). The means were compared by Tukey’s HSD (honest significant difference) test.

**Determination of Effects of Soil Texture on Nematode Population**

Soil chemical component analyses and pH were completed by University of Florida, Institute of Food and Agricultural Sciences Analytical Service Laboratories and soil texture analyses were performed by the Bouyoucos Hydrometer Method (Day, 1965).
Results

Above and Belowground Symptoms of Root-knot Disease

In 2014, the aboveground symptom of root-knot nematode damage on Georgia 06G was more readily observed at the Brown Farm than that at the AREC. Symptoms included stunted, chlorotic plants at the Brown Farm, whereas at the AREC there was hardly any visible symptom of stunting or chlorosis. However, in contrasts to the aboveground symptoms, the belowground symptoms of galls and egg masses were more readily apparent on Georgia 06G at the AREC than that at the Brown Farm. There were no above ground symptoms on Tifguard regarding stunting or chlorosis at either site.

In the 2015 season, there was a marked decrease in the aboveground root-knot nematode symptoms at the Brown Farm, whereas at the AREC the symptoms were unchanged from that observed in 2014.

Seasonal Distribution of *Meloidogyne arenaria* Recovered from Georgia-06G Rhizosphere

Initial population densities of J2 from all replicates and depths averaged 18 and 156/200 cm\(^3\) of soil at the Brown farm and the AREC, respectively. The trend lines of the nematode population density over the sampling period are shown in Figure 3-1. At the AREC 2014 harvest, the average population of J2 had increased up to 7,000/200 cm\(^3\) of soil, whereas at the Brown Farm the number of J2 detected at harvest was only 289/200 cm\(^3\) of soil. Similarity at the second harvest 2015, the population density of *M. arenaria* occurred much greater in the Norfolk loamy sand soil (AREC) than that in the Candler sandy soil (Brown Farm). However, at both sites the numbers of J2 recovered
in the second season was much lower than that which occurred during the 2014 crop season.

At the Brown Farm 2014, population densities of *M. arenaria* at the four depths increased slowly during the first 86 days until mid-June after which there was a rapid increase in their numbers, peaking in mid-August. This was followed with a small decrease in numbers immediately before harvest. The population numbers continued to decrease reaching a low in mid-February through April 2015. There was only a small increase in numbers in late May at time the clover was turned under in preparation for planting peanut. After peanut was planted in 2015, the population increased and reached the highest number at harvest.

Similar to the Brown Farm, population densities of *M. arenaria* at the three depths increased slowly during the first 77 days until late August after which there was a rapid increase in their numbers, peaking at harvest at the end of October (Figure 3-2). This was followed with a rapid decrease in numbers immediately after harvest. The population numbers continued to decrease reaching a low in mid-February through April 2015. There was only a small increase in numbers following the planting of clover in early April. After peanuts were planted in 2015, the population increased and reached the highest number at harvest.

**Vertical Distribution of *Meloidogyne arenaria* Recovered from Georgia-06G Rhizosphere**

Multifactorial ANOVA performed on nematode data showed significant effects of DAP, depth, and interactions among these factors at both locations (*P* ≤ 0.05) (Table 3-1-2).
At the Brown Farm, the highest numbers throughout the two seasons at most sampling dates were recorded from the 31-60 cm depth, and the lowest numbers were recorded from the 91-120 cm depth \((P \leq 0.05)\) (Table 3-3). There was only one difference in the numbers recorded at the deepest depths among the 15 sampling dates and that was the highest number recorded in September 2014 compared with the lowest number recorded July 2015 \((P \leq 0.05)\) (Table 3-3). Between January and July 2015 there were no differences in numbers recorded among the four sampling depths \((P \leq 0.05)\). The highest numbers recorded were similar in the upper two depths between mid-July and early October 2014 \((P \leq 0.05)\) (Table 3-3). However, the increase in the soil population in the upper two depths during 2015 season, between mid-August and mid-October, was much less than that occurred in 2014 \((P \leq 0.05)\) (Table 3-3).

The vertical distribution of J2 in the Georgia-06G rhizosphere was quite different at the AREC from that at the Brown farm. The number of J2 fluctuated among the three soil layers over the entire sampling period (Table 3-4). However, the highest numbers of J2 were found at the depth of 0-15 cm at the first harvest, whereas greatest number of J2 were observed at the depth of 16-30 cm at the second harvest. There was no difference of J2 numbers occurring from the end of July to late August among the three sampling depths \((P \leq 0.05)\) (Table 3-4). Also from December 2014 to February 2015 and between May and September 2015 there were no differences in numbers recorded among the three sampling depths \((P \leq 0.05)\) (Table 3-4). The highest numbers recorded were similar in the upper two depths in September 2014 and 2015 \((P \leq 0.05)\) (Table 3-4).
Seasonal and Vertical Distribution of *Meloidogyne arenaria* Recovered from Tifguard Rhizosphere

Multifactor ANOVA performed on nematode data showed significant effects of DAP, depth, and interactions among these factors at both locations ($P \leq 0.05$) (Table 3-5-6).

The number of nematodes extracted from Tifguard roots at harvest was 1/200 cm$^3$ of soil at the Brown Farm and 28/200 cm$^3$ of soil at the AREC. The highest number of nematodes was found at the soil depth of 0-30 cm over the whole season at the Brown Farm and at the soil depth of 31-45 cm at the AREC except at harvest (Figure 3-7-8). The resistant peanut cultivar indeed reduced the population density in the soil.

**Distribution of Nematodes Associated with Soil Temperature**

Based on statistical analysis, soil temperature had no significant effect on nematode distribution. Most J2 were found within the soil temperature ranges of 28-30 °C at the Brown Farm and within 22-24 °C at the AREC.

**Effects of Soil Texture on Nematode Population**

The soil at the Brown farm was classified as a Candler sand and was composed of 97.5% sand, 2% silt and 0.5% clay, whereas the soil at the AREC was a Norfolk loamy sand comprised of 84% sand, 5.5% silt and 10.5% clay (Table 3-9).

**Discussion**

**Occurrence of *Meloidogyne arenaria* in the Soil**

As an obligate parasite, the population density of *M. arenaria* is strongly influenced by the availability of a suitable host (Barker, 1976). The second-stage juvenile is the infective stage that penetrates roots in the presence of a host. About 16-33 days are required for *M. arenaria* to complete one life cycle on a host, which usually
depends on host species and environmental conditions (Machmer, 1977). The population density of *M. arenaria* at both field sites was low following the winter season, preceding the spring season for planting peanut. Subsequently there was a slow but progressive increase during the first 75 to 90 days following the planting of peanut. As the crop grows nematode infection increases and appears to reach the highest population densities during the remaining 45 days until harvest (Dickson and Hewlett, 1988). Most peanut cultivars mature around 135 after planting. The increase of nematode numbers on peanut is relatively slow compared to that which occurs on tomato (see chapter 4). Root penetration on tomato usually occurs in 2 days for *M. arenaria* and galls are detectable after 10-15 days. In contrast, galls in field planting of peanut can hardly be observed on until ca. 70 days after planting.

The highest population density of *M. arenaria* in the soil was observed at harvest at the AREC, whereas the highest population of *M. arenaria* was detected 105 days after planting at the Brown Farm. At time of harvest at the Brown Farm in 2014 the plants were in a state of decline from what was believed to be caused by root knot disease, however at the AREC site the growth of peanut was much better relatively to what occurred at the Brown Farm. Vine growth of the susceptible Georgia 06G was ca. 38 to 45 cm tall at AREC, whereas at Brown Farm peanut height was less than 20 cm tall. The disparity in growth of the heavily infected peanuts may have been because of differences in soil type that resulted in much better peanut growth and development at the AREC. At the AREC site peanut roots grew down into the underpinning of clay, which provided harbor from the nematode and provided moisture and nutrients. In contrast, peanut roots at the Brown Farm were vulnerable to infection regardless of soil type.
depth in that J2 and root-knot nematode eggs may be widely distributed through the soil profile. In the 2015 season the growth of peanut at the Brown Farm was very poor, however peanut growth at the AREC was similar to that that occurred in 2014.

**Seasonal and Vertical Distribution of *Meloidogyne arenaria***

The seasonal distribution of J2 at both field sites followed similar trends, with a peak occurring during late summer and early fall around harvest. Numbers of J2 decreased following harvest and this decrease continue during the winter season. There was only a small increase following the winter cover crop of clover. This period was followed by a significant and sharp rise in J2 numbers in September and October. Although the population density of *M. arenaria* is affected by soil temperature, which impacts hatching and development, the host plant susceptibility is another factor that strongly influences the dynamics (Bélair, 1989).

At the Brown Farm, during the crop season there was the largest increase of J2 occurring at the soil depths of 0 to 30 and 31-60 cm, which suggest that the nematode follows the roots as they move down into the soil profile, perhaps seeking a more favorable temperature for development and survival. There was also an increase of J2 at all soil depths sampled beginning in mid-July and continuing up to harvest. Starr and Jeger (1985) and Sohlenius and Sandor (1987) both concluded that root abundance or root biomass largely determine the vertical distribution of *Meloidogyne* spp. It appears that as roots grow deeper in soil the J2 follow, increasing in numbers as the season progresses. The large decrease in numbers at all depths that began in January 2015 and continue until late May was not expected. However, the seeding of crimson clover in November most likely affected the number of nematodes detected in that viable J2 would penetrate the clover roots. Surprisingly, the increase on clover was only slight.
over the 5-month period of growth. Clover roots that were observed for galls in early April showed only minimum galling.

The highest population density of J2 at the AREC was obtained in the top 15 cm depth but numbers were only slightly less in the deeper depths of 16-30 and 31-45 cm deep. The numbers reached the highest numbers at harvest followed by a gradual decrease reaching a very low number at all soil depths in mid-February. Again, this drop was likely associated with the planting of clover in November 2014. There was an increase in J2 recovered from samples taking in early April and this decline continued until early September when they spiked at all depths.

**The Influence of Soil Temperature on Nematode Population**

The soil temperature is dependent on geographic locations and seasonal fluctuations. Different *Meloidogyne* spp. have different survival temperature ranges based on their adaptation to the local environment (O'Bannon and Santo, 1984). Franklin (1937) revealed that *Meloidogyne* spp. could survive 16 months out-of-doors in the absence of a host and could withstand some freezing. Kincaid (1946) found that root-knot nematodes found in the southern states of the U.S.A. failed to survive the winter in Indiana and Wisconsin. Existence of physiological races responding differently to low temperatures could be an explanation for these contradictory findings. Generally, the higher soil temperature has positive effects on nematode reproduction within the limitation of high temperature extremes. Nevertheless, in this study, temperatures at different soil depths did not appear to impact nematode numbers.

**Effect of Soil Texture on Nematode Population**

Soil texture is considered as one of the important factors for nematode survival and movement. Sandy soil is preferred for vital activities of root-knot nematode (Prot
and Van Gundy, 1981). The soil at the Brown Farm had more than 97% sand whereas at AREC sand content was only 85%. However, much higher numbers of nematodes were observed at the AREC than at the Brown farm. Although contradictory these results might be explained by the heavy soil clay layer underpinning that occurred only 45 cm deep. As has been pointed out the initial population is usually correlated with the final population and root damage (McSorley, 1998). Garcia (2012) also suggested a strong nonlinear relationship existed between Pi and Pf as well as percentage of yield loss by studying two soybean cultivars. Although root mass was not determined at either the Brown or AREC sites it was apparent that there was greater amount of roots occurring in the upper 45 cm of soil at the AREC. This would provide for a greater carrying capacity of J2 than would occur in the deeper and sandy soil at the Brown Farm. Other possible reasons for low number of nematodes extracted from Brown Farm include potential biological agents and the poor development of peanut in 2015.
Figure 3-1. Seasonal fluctuation of *Meloidogyne arenaria* second-stage juveniles in the soil profile of Georgia-06G at four depths in a Candler sand at the Brown Farm, Levy County, FL.
Figure 3-2. Seasonal fluctuation of *Meloidogyne arenaria* second-stage juveniles in the soil profile of Georgia-06G at three depths in a Norfolk loamy sand soil at University of Georgia, Attapulgus Research and Education Center, Attapulgus, GA.
Table 3-1. Significance of main and interactive effects of both fixed and random variables for the vertical distribution of *Meloidogyne arenaria* extracted from 200 cm$^3$ of soil collected from plots planted Georgia-06G at the Brown Farm (2014-2015).

<table>
<thead>
<tr>
<th>Effects</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAP</td>
<td>3.766e-15 ***</td>
</tr>
<tr>
<td>Depth</td>
<td>&lt; 2.2e-16 ***</td>
</tr>
<tr>
<td>DAP×Depth</td>
<td>9.466e-11 ***</td>
</tr>
</tbody>
</table>

Table 3-2. Significance of main and interactive effects of both fixed and random variables for the vertical distribution of *Meloidogyne arenaria* extracted from 200 cm$^3$ of soil collected from plots planted Georgia-06G at the Attapulgus Research and Education Center (2014-2015).

<table>
<thead>
<tr>
<th>Effects</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAP</td>
<td>9.893e-14 ***</td>
</tr>
<tr>
<td>Depth</td>
<td>1.174e-08 ***</td>
</tr>
<tr>
<td>DAP×Depth</td>
<td>1.471e-10 ***</td>
</tr>
</tbody>
</table>

Table 3-3. Number of *Meloidogyne arenaria* second-stage juveniles extracted from 200 cm$^3$ of soil collected at four depths 0-30, 31-60, 61-90, and 91-120 cm deep from plots planted Georgia-06G at the Brown Farm (2014-2015).

<table>
<thead>
<tr>
<th>Date</th>
<th>0-30 cm</th>
<th>31-60 cm</th>
<th>61-90 cm</th>
<th>91-120 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 Apr 2014</td>
<td>13 Aa</td>
<td>39 Aabc</td>
<td>26 Aabc</td>
<td>8 Aab</td>
</tr>
<tr>
<td>19 May 2014</td>
<td>33 ABa</td>
<td>52 Babc</td>
<td>23 ABab</td>
<td>6 Aab</td>
</tr>
<tr>
<td>16 Jun 2014</td>
<td>37 Ba</td>
<td>28 ABab</td>
<td>12 ABab</td>
<td>3 Aab</td>
</tr>
<tr>
<td>17 Jul 2014</td>
<td>191 Ccd</td>
<td>90 Bbc</td>
<td>52 ABabcd</td>
<td>17 Aab</td>
</tr>
<tr>
<td>17 Aug 2014</td>
<td>306 Cd</td>
<td>436 Cd</td>
<td>165 Bd</td>
<td>47 Aab</td>
</tr>
<tr>
<td>08 Sep 2014</td>
<td>295 Cd</td>
<td>392 Cd</td>
<td>127 Bcd</td>
<td>58 Ab</td>
</tr>
<tr>
<td>05 Dec 2014</td>
<td>214 Bcd</td>
<td>162 Bc</td>
<td>58 Abcd</td>
<td>20 Aab</td>
</tr>
<tr>
<td>09 Jan 2015</td>
<td>16 Aa</td>
<td>27 Aab</td>
<td>19 Aab</td>
<td>8 Aab</td>
</tr>
<tr>
<td>20 Feb 2015</td>
<td>3 Aa</td>
<td>5 Aa</td>
<td>5 Aab</td>
<td>2 Aab</td>
</tr>
<tr>
<td>01 April 2015</td>
<td>2 Aa</td>
<td>2 Aa</td>
<td>2 Aab</td>
<td>2 Aab</td>
</tr>
<tr>
<td>28 May 2015</td>
<td>26 Aa</td>
<td>17 Aab</td>
<td>8 Aab</td>
<td>5 Aab</td>
</tr>
<tr>
<td>14 Jul 2015</td>
<td>4 Aa</td>
<td>2 Aa</td>
<td>2 Aa</td>
<td>1 Aa</td>
</tr>
<tr>
<td>14 Aug 2015</td>
<td>53 Bab</td>
<td>72 Babc</td>
<td>37 ABabc</td>
<td>11 Aab</td>
</tr>
<tr>
<td>14 Sep 2015</td>
<td>156 Cbcd</td>
<td>80 BCbc</td>
<td>35 ABabc</td>
<td>14 Aab</td>
</tr>
<tr>
<td>10 Oct 2015</td>
<td>59 Babc</td>
<td>36 ABab</td>
<td>17 ABab</td>
<td>9 Aab</td>
</tr>
</tbody>
</table>

$^1$Data are means of five replicates. Means within a row followed by an upper case letter are not different according to F-tests ($P \leq 0.05$). Means within a column followed by a common lower case letter are not different according to F-tests ($P \leq 0.05$). Values were transformed to arcsin ($\sqrt{x}$) before analysis.
Table 3-4. Number of *Meloidogyne arenaria* second-stage juveniles extracted from 200 cm$^3$ of soil collected at four depths 0-15, 16-30, and 31-45 cm deep from plots planted Georgia-06G at the Attapulgus Research and Education Center (2014-2015).

<table>
<thead>
<tr>
<th>Date</th>
<th>Depth$^1$</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-15 cm</td>
<td>16-30 cm</td>
<td>31-45 cm</td>
<td></td>
</tr>
<tr>
<td>30 Jul 2014</td>
<td>151 Aa</td>
<td>185 Aab</td>
<td>185 Aab</td>
<td></td>
</tr>
<tr>
<td>22 Aug 2014</td>
<td>1,260 Aabc</td>
<td>1,049 Aabc</td>
<td>1,089 Aabc</td>
<td></td>
</tr>
<tr>
<td>22 Sep 2014</td>
<td>8,615 Bde</td>
<td>6,056 Bd</td>
<td>2,555 Abcd</td>
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<td>2,274 Acd</td>
<td>3,231 Acd</td>
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<td></td>
</tr>
<tr>
<td>02 Apr 2015</td>
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<td>149 Aab</td>
<td>227 Aab</td>
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<tr>
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<td>25 Aa</td>
<td>45 Aa</td>
<td></td>
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<tr>
<td>02 Aug 2015</td>
<td>29 Aa</td>
<td>67 Aab</td>
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<td>1,173 Aabc</td>
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<tr>
<td>04 Oct 2015</td>
<td>3,759 Bcd</td>
<td>6,265 Cd</td>
<td>1,753 Abcd</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Data are means of six replicates. Means within a row followed by an upper case letter are not different according to F-tests ($P \leq 0.05$). Means within a column followed by a common lower case letter are not different according to F-tests ($P \leq 0.05$). Values were transformed to arcsin ($\sqrt{x}$) before analysis.
Figure 3-3. Seasonal fluctuation of *Meloidogyne arenaria* second-stage juveniles in the soil profile of Tifguard at four depths in a Candler sand at the Brown Farm, Levy County, FL and at three depths in a Norfolk loamy sand soil at University of Georgia, Attapulgus Research and Education Center, Attapulgus, GA.
Table 3-5. Significance of main and interactive effects of both fixed and random variables for the vertical distribution of *Meloidogyne arenaria* extracted from 200 cm³ of soil collected from plots planted with Tifguard at the Brown Farm 2015.

<table>
<thead>
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</thead>
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<td>DAP</td>
<td>4.488e-05 ***</td>
</tr>
<tr>
<td>Depth</td>
<td>&lt; 2.2e-16 ***</td>
</tr>
<tr>
<td>DAP×Depth</td>
<td>0.002946 **</td>
</tr>
</tbody>
</table>

Table 3-6. Significance of main and interactive effects of both fixed and random variables for the vertical distribution of *Meloidogyne arenaria* extracted from 200 cm³ of soil collected from plots planted with Tifguard at the Attapulgus Research and Education Center 2015.

<table>
<thead>
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</thead>
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<tr>
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</tr>
<tr>
<td>Depth</td>
<td>0.671477</td>
</tr>
<tr>
<td>DAP×Depth</td>
<td>0.035162 *</td>
</tr>
</tbody>
</table>

Table 3-7. Number of *Meloidogyne arenaria* second-stage juveniles extracted from 200 cm³ of soil collected at four depths 0-30, 31-60, 61-90, and 91-120 cm from plots planted with Tifguard at the Brown Farm 2015.

<table>
<thead>
<tr>
<th>Date</th>
<th>0-30 cm</th>
<th>31-60 cm</th>
<th>61-90 cm</th>
<th>91-120 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 April 2015</td>
<td>2 Aa</td>
<td>2 Aab</td>
<td>2 Aabc</td>
<td>2 Aab</td>
</tr>
<tr>
<td>28 May 2015</td>
<td>16 Bb</td>
<td>14 Bc</td>
<td>6 Ac</td>
<td>5 Ab</td>
</tr>
<tr>
<td>14 Jul 2015</td>
<td>3 Ca</td>
<td>1 Bca</td>
<td>1 Aba</td>
<td>0 Aa</td>
</tr>
<tr>
<td>14 Aug 2015</td>
<td>5 Ca</td>
<td>2 Ba</td>
<td>1 Bab</td>
<td>0 Aa</td>
</tr>
<tr>
<td>14 Sep 2015</td>
<td>16 Db</td>
<td>10 Cbc</td>
<td>5 Bbc</td>
<td>2 Aab</td>
</tr>
<tr>
<td>10 Oct 2015</td>
<td>1 Ba</td>
<td>1 Ba</td>
<td>1 Aba</td>
<td>0 Aa</td>
</tr>
</tbody>
</table>

¹Data are means of five replicates. Means within a row followed by an upper case letter are not different according to F-tests ($P \leq 0.05$). Means within a column followed by a common lower case letter are not different according to F-tests ($P \leq 0.05$). Values were transformed to arcsin ($\sqrt{x}$) before analysis.
Table 3-8. Number of *Meloidogyne arenaria* second-stage juveniles extracted from 200 cm$^3$ of soil collected at four depths 0-15, 16-30, and 31-45 cm from plots planted with Tifguard at the Attapulgus Research and Education Center 2015.

<table>
<thead>
<tr>
<th>Date</th>
<th>0-15 cm</th>
<th>16-30 cm</th>
<th>31-45 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 May 2015</td>
<td>11 Aab</td>
<td>7 Aa</td>
<td>14 Aa</td>
</tr>
<tr>
<td>03 Jul 2015</td>
<td>21 Aab</td>
<td>30 Aa</td>
<td>60 Aa</td>
</tr>
<tr>
<td>02 Aug 2015</td>
<td>3 Aa</td>
<td>12 Aa</td>
<td>24 Aa</td>
</tr>
<tr>
<td>01 Sep 2015</td>
<td>25 Aab</td>
<td>27 Aa</td>
<td>33 Aa</td>
</tr>
<tr>
<td>04 Oct 2015</td>
<td>46 Bb</td>
<td>30 Aba</td>
<td>10 Aa</td>
</tr>
</tbody>
</table>

Data are means of six replicates. Means within a row followed by an upper case letter are not different according to F-tests ($P \leq 0.05$). Means within a column followed by a common lower case letter are not different according to F-tests ($P \leq 0.05$). Values were transformed to arcsin (√x) before analysis.
Table 3-9. Sand, silt, and clay distribution at different soil depths collected from Brown Farm, Levy County, FL and University of Georgia, Attapulgus Research and Education Center, Attapulgus, GA.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Depth (cm)</th>
<th>Sand (%)</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown farm</td>
<td>0-30</td>
<td>96</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Candler sand</td>
<td>31-60</td>
<td>96.8</td>
<td>2.5</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>61-90</td>
<td>98</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>91-120</td>
<td>99</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Attapulgus Research and</td>
<td>0-15</td>
<td>88.7</td>
<td>4.3</td>
<td>7</td>
</tr>
<tr>
<td>Education Center</td>
<td>16-30</td>
<td>84</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Norfolk loamy sand</td>
<td>31-45</td>
<td>79</td>
<td>7</td>
<td>14</td>
</tr>
</tbody>
</table>
CHAPTER 4
THE INFLUENCE OF TEMPERATURE ON THE SUSCEPTIBILITY OF CVS.
TIFGUARD AND GEORGIA-06G PEANUT TO Meloidogyne arenaria

Introduction

Meloidogyne arenaria, the peanut root-knot nematode, is considered one of the most important soilborne pathogens affecting peanut in the southern USA. This species is prevalent in Alabama, Florida, Georgia, and Texas (Dickson, 2005; Ingram and Rodriguez-Kabana, 1980; Starr and Morgan, 2002). The suppression of peanut yields caused by this nematode can reach up to 50% or greater in heavily infested fields (Proite et al., 2008).

Resistant cultivars potentially provide the most economical and effective means of managing nematode diseases on agricultural crops. Up until 2001 there were no cultivated peanut with root-knot nematode resistance, yet a number of Arachis spp. were observed to be highly resistant to the peanut root-knot nematode (Baltensperger et al., 1986; Holbrook and Noe, 1990; Nelson et al., 1989). Genetic material from a wild type peanut, A. cardenasii was introgressed into cultivated peanut resulting in the first root-knot nematode resistant cultivar-COAN (Simpson and Starr, 2001). The second peanut cultivar NemaTam resistant to M. arenaria was released in 2002. This cultivar had the same resistant level as COAN, but had greater yield potential (Simpson et al., 2003). The resistance gene in COAN was reported to have three different functions on M. arenaria (Bendezu and Starr, 2003). The first effect was to reduce the number of the second-stage juveniles (J2) of M. arenaria that penetrated roots; second, the resistance gene prevented most J2 from developing further, and third, the resistance gene delayed development of J2. No hypersensitive reaction was observed in the early stage of M. arenaria infection in COAN roots, which is considered as a common defense
mechanism produced by resistant plants to disease pathogens (Bendezu and Starr, 2003).

Neither of these two cultivars was considered for planting in Florida because of the prevalence and severity of tomato spotted wilt virus (TSWV) and fungal diseases in the southeastern peanut growing region (Holbrook et al., 2008). In 2008, a runner-type peanut cultivar (*Arachis hypogaea* L. subsp. hypogaea var. hypogaea)-Tifguard was released by the USDA-ARS and the Georgia Agricultural Experiment Station (Holbrook et al., 2008). The breeding population was generated by hybridizing 'C-99R' (Gorbet and Shokes, 2002), a peanut cultivar with good field resistance to TSWV (Wells et al., 2002) with 'COAN'. Tifguard was reported to have a high level of resistance to both TSWV and *M. arenaria* (Holbrook et al., 2008).

Temperature is an important factor influencing the expression of resistance genes in plants to parasitic nematodes. For example, it was observed that susceptible alfalfa plants attracted more juveniles of *M. hapla* than resistant plants, but the magnitude of this difference decreased when temperature increased (Griffin et al., 1971; 1977). In addition, half of the resistant alfalfa plants grown at 32 °C had galled roots, whereas no galled roots were observed at 28 °C. Similarly, the number of mature *M. incognita* and *M. hapla* in resistant and susceptible bean increased as the soil temperature increased from 25 to 30 °C (Irizarry et al., 1971). Increased temperature also affected nematode resistance in soybean and tomato cultivars (Dropkin, 1969b; Holtzmann, 1965). Greater penetration and development of *M. incognita* was observed in resistant tomato roots at 30 and 34.5 °C than at 20 and 25 °C. In other studies, on tomato, decreased hypersensitive necrotic response to nematode infection was
reported at temperatures near or above 30 °C (Paulson and Webster, 1972). The Mi-1 gene in tomato confers resistance to root-knot nematodes; however, this gene becomes nonfunctional when the temperature is at or above 28 °C (Williamson, 1998). Changes in the response of root-knot nematode resistance by high temperature has been reported under both greenhouse and field conditions (Philis and Vakis 1977; Tzortzakakis and Gowen 1996; Noling 2000). It appears that heat stress affects the functioning of resistant genes in host plants (Ammiraju et al., 2003). On the other hand, it is well known that root-knot nematodes are dependent on temperature for vital physiological activities. Temperature affects root-knot nematode migration, development, and the nematode’s ability to sense host roots (Tyler, 1933). The optimal temperature for root-knot nematode development was reported as 28 °C, above which development was reduced. No development occurred at 36.5 °C (Tyler, 1933).

The number of the second-stage juveniles (J2) of *M. arenaria* in different peanut genotypes at 2 and 7 days after inoculation (DAI) was compared by Bendezu and Starr (2003). Higher number of J2 penetrated the roots of the susceptible peanut Florunner than the resistant COAN. Since the release of the root-knot nematode resistant peanut cultivar there has been no study about how temperature affects the number and development of *M. arenaria* in peanut with the resistant gene. The objective of this study was (i) to determine the number of *M. arenaria* that penetrated roots of resistant and susceptible peanut cultivars; and (ii) to determine the effect of temperature on *M. arenaria* development in resistant and susceptible peanut cultivars.
Materials and Methods

Nematode Origin

*Meloidogyne arenaria* was isolated from infected peanut roots from an infested farm located in Levy County, FL and maintained on the susceptible tomato cultivar (*Solanum esculentum* Mill. cv. Agriset 334) in the greenhouse. Polyacrylamide gel electrophoresis was used as an aid in identification of the species based on malate dehydrogenase and esterase phenotypes (Esbenshade and Triantaphyllou, 1985). Eggs were extracted from infected tomato roots with 0.25% NaClO (Hussey and Barker, 1973) method as modified by Boneti and Ferraz (1981). A modified Baermann funnel method was used for egg hatching (Rodriguez and Pope, 1981). Second-stage juveniles (J2) collected after a period of 48 hours were used as inoculum.

Penetration and Development of *Meloidogyne arenaria*

Peanut seed of Tifguard and Georgia-06G were provided by the peanut breeder Corley Holbrook, USDA-ARS, Tifton, GA. Tomato cv. Agriset 334 was used as a susceptible plant to ensure inoculum viability. Peanut seeds were surface-treated by soaking in 0.6% NaClO for 1 min followed by rinsing in sterile distilled water (Bendezu and Starr, 2003). They were placed in a petri dish with a piece of moistened paper towel for germination at 28 °C for 5 days. Seedlings were transplanted into 250 ml plastic cups filled with autoclaved sand and grown at 28 °C for 7 days. Holes were punched into the cup bottoms to provide drainage. All seedlings were inoculated with 2,000 freshly-hatched J2 of *M. arenaria*, and then all seedlings were transferred to an environmental growth chambers at either 28, 31, or 34 °C with a 12-h photoperiod. The seedlings were hand watered daily.
Data Collection

Three plants were removed at 5-day intervals following inoculation (DAI), washed in tap water, dried with a paper towel, and weighed. Roots were fixed, stained and cleared for microscopic examination (Byrd et al., 1983). The number and images of nematodes in the roots were recorded based on their developmental stages as follows: (i) second-stage juvenile (J2); (ii) J2 with swollen body (late-J2); (iii) the third and fourth stage juvenile; (iv) female; (vi) egg-laying female; and (vii) male. Red food coloring (20%) was used to stain egg masses once they were observed on roots (Thies et al., 2002). The experiment was repeated twice. In the experiment 1, harvest ceased at 30 DAI, whereas in the experiment 2, harvest was extended to 40 DAI in order to determine if egg masses of *M. arenaria* would form on Tifguard roots. Root-knot nematode egg massed were collected (three egg masses per repetition per cultivar) from both peanut cultivars once they were observed at 40 DAI.

Statistical Analysis

The effects of temperature, host genotype, and DAI on the number of different developmental stages of *M. arenaria* per gram of root tissue were subjected to analysis of variance using R programming software (R 3.1.2 with R studio). The means were compared by Tukey’s HSD (honest significant difference) Test. The data from tomato was not included in the statistical analyses. Values were transformed to arcsin (√x) before analysis. The number of eggs per egg mass was subjected to analysis by student’s t test.

Resistance Gene Marker Analysis of Tifguard

In the first experiment, a young leaflet from any Tifguard plant with root-knot nematode infection was collected and shipped to the National Environmentally Sound
Production Agriculture Laboratory (NESPAL), Tifton, GA to confirm whether the infected plant contained the root-knot nematode resistant gene. Any plant without the resistance gene was not included to the statistical analyses. In the experiment 2, all the Tifguard seed used had a small portion of seed coat removed for testing for the resistance gene Rma by the NESPAL laboratory (Nagy et al., 2009). Only seed that were positive for the resistant gene were used in experiment 2

Results

Penetration of *Meloidogyne arenaria* in the Resistant and Susceptible Peanut Cultivars

The root systems from Georgia-06G were generally 1 to 1.5 fold greater in size than that from Tifguard (*P* ≤ 0.05) (Table 4-1). Because of this, the number of each developmental stage of *M. arenaria* per gram of roots was determined in the two peanut cultivars. In both experiments, tomato was heavily infected assuring inoculum viability (Figure 4-1-6). The development of J2 was consistently faster in tomato than that which occurred in peanut roots regardless of cultivars. Galls and egg masses were readily apparent on tomato roots at 15 DAI, whereas galls and egg masses first became apparent on Georgia-06G roots at 20 DAI (Figure 4-1-6).

Both peanut genotypes and temperature affected the number of nematodes in roots (*P* ≤ 0.05) (Table 4-1). In both experiments, relative to the number of J2 found in roots at 5 DAI, the numbers in Tifguard and Georgia-06G decreased over the 30 to 40 period at all three temperatures (Figure 4-7; 4-8). There was an increase in numbers of J2 infecting roots in both experiments in response to temperatures above 28 °C. In experiment one, at day 5 the initial penetration of *M. arenaria* in Georgia-06G was greater at 31 °C than at 28 °C or 34 °C (*P* ≤ 0.05). Similarity in experiment two, there was
an increase in the number of *M. arenaria* found in Georgia-06G roots between 28 and 31 or 34 but there was no difference in numbers between 31 and 34 °C (*P* ≤ 0.05) (Figure 4-9). The number of nematodes in Tifguard was greater at 31 °C, but no differences occurred between 31 and 34 °C in either experiment. In experiment 1, there were higher numbers of *M. arenaria* J2 in resistant Tifguard roots at 34 °C than in susceptible Georgia-06G, whereas a higher number of nematodes was observed in susceptible Georgia-06G roots at 28 °C (*P* ≤ 0.05). There was no difference in nematode number found in the susceptible and resistant peanut roots at 31°C. There was a difference of penetration between susceptible and resistant cultivars at 28 and 34 °C (*P* ≤ 0.05).

**Development of *Meloidogyne arenaria* in the Resistant and Susceptible Peanut Cultivars**

In both experiments, females and males of *M. arenaria* were observed in Tifguard roots only at 34 °C (Figure 4-11). There was no development of *M. arenaria* in Tifguard roots at 28 or 31 °C (Figure 4-1-2; 4-4-5). Although females were found in Tifguard at 34 °C, no egg-laying females were observed in experiment one over the 30-day period (Figure 4-3), whereas on average of three females with small egg masses were found in experiment two at 40 DAI (Figure 4-6; Figure 4-11).

In the susceptible peanut cultivar, egg-laying females were first observed at 25 DAI at 28 °C and 20 days after inoculation at 31 °C in both experiments (Figure 4-1-2; 4-4-5). However, there was a delay in the development of nematode at 34 °C compared with that at 31 °C in both experiments (Figure 4-2-3; 4-5-6). Lowest number of egg-laying females was found in Georgia-06G roots 30 DAI at 34 °C, whereas the highest number was found at 31 °C. The numbers were similar to that at 28 °C in the first
experiment (Figure 4-10). In experiment two, highest number of egg-laying females was observed at 31 °C at 40 DAI (P ≤ 0.05) (Figure 4-10).

Necrotic lesions were formed in Tifguard roots in response to *M. arenaria* infection at all sampling dates (Figure 4-12). Cell necrosis was found in the tissue surrounding the swollen J2, which apparently stopped further development of the nematode.

**Reproduction of Meloidogyne arenaria in Different Peanut Genotypes**

Numbers of eggs per egg mass from Tifguard roots were different from those from Georgia-06G at 40 DAI (P ≤ 0.05). The average number of eggs per egg mass isolated from Tifguard was 13, whereas from Georgia-06G it was 244.

**Discussion**

**Effect of Peanut Genotypes and Temperatures on Meloidogyne arenaria**

There was a strong effect of peanut genotype on the response to *M. arenaria* infection. Tifguard was reported to be highly resistant to *M. arenaria* infection (Holbrook et al., 2008). Although nematode development occurred readily in Georgia-06G, there was only a small rate of development at 34 °C in Tifguard. In Georgia-06 the first egg-laying females were observed at 20 or 25 DAI whereas J2 were not able to develop after penetration of Tifguard (except at 34 °C).

Temperature is considered to be an important factor on egg hatching, nematode migration, root invasion and development in host roots (Tyler, 1933). At 31 °C more J2 entered Tifguard and Georgia-06G roots compared with that at 28 and 34 °C. In addition, final numbers of *M. arenaria* infecting Georgia-06G also was influenced by higher temperature. In this study, the temperature of 31 °C was the optimal for J2 to penetrate and parasitize the susceptible peanut. This result was different from Tyler’s
report where 28 °C was reported as the optimal temperature for nematode penetration and infection. Moreover, only 6 out of 26 root systems were reported to be infected by *M. arenaria* at 33 to 35 °C (Tyler, 1933), whereas all Georgia-06G and tomato plants were galled and had egg masses at 34 °C in this study. The discrepancy may indicate that root-knot nematodes in Florida have adapted to high soil temperatures.

**The Function of Resistance Gene**

Based on the data from this studies, the number of egg-laying females and eggs per egg mass were much less in the resistant cultivar Tifguard than that in the susceptible cultivar Georgia-06G. This confirms results reported by Garcia et al., 1996 about the functions of Mae and Mag genes found in the wild type *Arachis* spp. which were resistant to *M. arenaria*. These two genes were discovered to be linked to *Rma* which is the dominant root-knot nematode resistant gene in cultivated peanut (Nagy et al., 2010). Mae was reported to suppress egg production of *M. arenaria*, whereas Mag is reported to inhibit formation of galls.

**Hypersensitive Reaction**

The presence of host necrosis near the site where J2 were observed in the early stages of the host-parasite interaction was contradictory to the observation by Choi et al. (1999), and Bendezu and Starr (2003). This hypersensitive reaction (HR) mechanism has been well documented in the case of the *Mi* resistance gene in tomato to root-knot nematode infection (Paulson and Webster, 1972; Williamson, 1999). After recognition of the nematode by the plant bearing a single resistance gene, host defenses respond and are activated, leading to necrotized cells surrounding the nematode.

A resistant plant provides the most effective and environmentally safe means to manage root-knot nematodes, thus understanding the mechanisms involved in
*Meloidogyne* virulence is critical for the sustainable management of these pathogens. In summary, this research demonstrates that soil temperature is not likely to be a factor in preventing the resistant gene in Tifguard from functioning to protect this peanut from root knot disease induced by *M. arenaria.*
Table 4-1. Analysis of variance of root weight, number of *Meloidogyne arenaria* per gram root system of two peanut cultivars.

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root weight</td>
<td></td>
</tr>
<tr>
<td>Cultivar</td>
<td>2.744e-06 ***</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.6528</td>
</tr>
<tr>
<td>DAI</td>
<td>&lt; 2.2e-16 ***</td>
</tr>
<tr>
<td>Cultivar ×Temperature</td>
<td>0.7204</td>
</tr>
<tr>
<td>Cultivar ×DAI</td>
<td>1.896e-08 ***</td>
</tr>
<tr>
<td>Temperature ×DAI</td>
<td>0.8505</td>
</tr>
<tr>
<td>Cultivar ×Temperature ×DAI</td>
<td>0.7657</td>
</tr>
</tbody>
</table>

**Experiment 1**

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td>0.0009106 ***</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.0059029 **</td>
</tr>
<tr>
<td>DAI</td>
<td>&lt; 2.2e-16 ***</td>
</tr>
<tr>
<td>Cultivar ×Temperature</td>
<td>0.0374213 *</td>
</tr>
<tr>
<td>Cultivar ×DAI</td>
<td>0.0005185 ***</td>
</tr>
<tr>
<td>Temperature ×DAI</td>
<td>4e-07 ***</td>
</tr>
<tr>
<td>Cultivar ×Temperature ×DAI</td>
<td>0.0197020 *</td>
</tr>
</tbody>
</table>

**Experiment 2**

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td>6.651e-07 ***</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.0001727 ***</td>
</tr>
<tr>
<td>DAI</td>
<td>&lt; 2.2e-16 ***</td>
</tr>
<tr>
<td>Cultivar ×Temperature</td>
<td>0.0109771 *</td>
</tr>
<tr>
<td>Cultivar ×DAI</td>
<td>3.001e-09 ***</td>
</tr>
<tr>
<td>Temperature ×DAI</td>
<td>&lt; 2.2e-16 ***</td>
</tr>
<tr>
<td>Cultivar ×Temperature ×DAI</td>
<td>6.126e-06 ***</td>
</tr>
</tbody>
</table>
Figure 4-1. The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 30-day period at 28 °C (Experiment 1).
Figure 4-2. The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 30-day period at 31 °C (Experiment 1).
Figure 4-3. The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 30-day period at 34 °C (Experiment 1).
Figure 4-4. The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 40-day period at 28 ℃ (Experiment 2).
Figure 4-5. The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 40-day period at 31 °C (Experiment 2).
Figure 4-6. The effect of temperature on the number of different developmental stages of *Meloidogyne arenaria* in roots of two peanut cultivars and tomato recorded at 5-day intervals over a 40-day period at 34 °C (Experiment 2).
Figure 4-7. The mean number of *Meloidogyne arenaria* of all development stages in roots of Tifguard and Georgia-06G recorded at 5-day intervals over a 30-day period in experiment 1.
Figure 4-8. The mean number of *Meloidogyne arenaria* of all development stages in roots of Tifguard and Georgia-06G recorded at 5-day intervals over a 40-day period in experiment 2.
Figure 4-9. The number of second-stage juveniles (J2) per gram of root system of the resistant cultivar Tifguard and susceptible cultivar Georgia-06G 5 days after inoculation in two experiments. Different letters over bars from the same experiment indicate significant differences at 0.05 level.
Figure 4-10. The number of egg-laying females per gram of root system of the resistant cultivar Tifguard and susceptible cultivar Georgia-06G 30 or 40 days after inoculation in two experiments. Different letters over bars from the same experiment indicate significant differences at the 0.05 level.
Figure 4-11. Different developmental stages of *Meloidogyne arenaria* in peanut and tomato roots at 34 °C. Photos courtesy by author.
Figure 4-12. Arrows point to necrotic lesions formed around the root-knot nematode infection sites in Tifguard roots at 5 and 40 days after inoculation (DAI). Photos courtesy by author.
CHAPTER 5
COMPARISON OF YIELD OF BREEDER TIFFGUARD, ISOGENIC TIFFGUARD AND GEORGIA-06G TREATED AND NONTREATED WITH 1,3-DICHLOROPROPENE

Introduction

Yield drag, an 'old idea' in plant breeding, is used in comparison of genetically engineered crop with nongenetically engineered crops (Oplinger, 1999). Yield drag refers to a negative effect on yield of crop plants that have a specific gene or a specific trait (Benbrook, 1999). A 'drag' on yield has been reported in breeding soybeans selected for higher protein levels for decades. Though with continued breeding efforts, this drag has been overcome to a certain extent by the development of high yielding and high protein lines, it remains a constant challenge (Hain and Lee, 2010).

Genetically modified crops are unique because of an additional gene being placed into one of the chromosome. Yield drag can occur if the transgene inserts in the coding region of a native gene and interrupts coding of gene products which might be associated with yield. On the other hand, genetically modified plants are also being manipulated to produce a new protein in large quantities. The new protein could be made at the cost of the proteins that are normally produced because the pool of protein building blocks (amino acids) is limited. This could result in a shortage of other proteins and cause yield drag (Hain and Lee, 2010).

Although not transgenic, Tifguard was generated by interspecific crossing and insertion of gene(s) coding for root knot nematode resistance. It is not known whether the gene insertion(s) cause yield drag in Tifguard. The yield produced by Tifguard was compared to other peanut cultivars planted in Florida from 2010 to 2013 (Tillman et al., 2013). On average, the yield of Tifguard was ranked at 11th among 15 peanut cultivars.
The objective of this experiment is to compare yield from breeder Tifguard, isogenic Tifguard and Georgia-06G treated and nontreated with 1,3-dichloropropene (1,3-D).

**Materials and Methods**

**Field Design and Treatments**

The trial was located at the University of Florida Plant Science Research and Education Unit, Citra, FL. An arbitrarily selected preplant soil sample from each plot was collected to determine the initial population density of nematodes. A 2.5 cm-diameter cone-shaped sampling tube was used to collect soil samples according to a zig-zag pattern in each plot (Barker and Campbell, 1981). Six soil cores from each plot were mixed thoroughly and 200 cm³ processed to extract second-stage juveniles of *M. arenaria*. 1,3-D (Dow AgroSciences Inc., Indianapolis, IN) was applied at rates of 150 L/ha 10 days preplant by a Yetter Coulter Rig. The chemical was injected 20.3 cm deep using a 63.5-cm-diameter coulter and trailing chisel shank on the tractor mounted applicator. Ten days after chemical application, Tifguard breeder, isogenic Tifguard, and Georgia-06G peanut cultivars were planted. The planting and harvesting methods including preplant fertilizer and herbicide application normally followed the IFAS Peanut Production Guide (Stephone, 1994). The field design was arranged in randomized complete block with two factors, chemical treatment and three different peanut cultivars (Tifguard breeder+1,3-D; Tifguard breeder; isogenic Tifguard+1,3-D; isogenic Tifguard; Georgia-06G+1,3-D; Georgia-06G ). The treatments were replicated four times, with each plot measuring 9.14 m long and 0.9 m row spacing.

A preplant commercial fertilizer mix including minor elements (3-9-18) was applied broadcast (560 kg/ha) and incorporated into soil before seeding. Gypsum was
applied at 2,242 kg/ha at early peg initiation. Irrigation was applied via a pivot overhead traveling system on an as needed basis. Prowl was applied preplant to suppress early weed emergence. An over-the-top application of paraquat + 2, 4-D and Select were applied to suppress broad weeds and grasses, respectively. During the growing season, fungicide was applied every 14 to 20 days.

**Peanut Harvest and Data Collection**

The peanuts were dug and inverted 130 days post harvest. Peanut growth were assessed at harvest based on a subjective rating scale from 1 to 10, where 1 refers the very poor growth and 10 refers the best growth in terms of height, width and general appearance of the plants (Turner and Backman, 1991). Six groups of peanut roots were arbitrarily selected from each treatment at harvest to determine root-knot nematode infection. The assessment index for galls and egg mass was based on the following scale: 0 = no gall or egg mass, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = ≥100 per root system (Taylor and Sasser, 1978). After field digging for 3 days the plants were combined, the pods collected in cottage bags, and placed in peanut wagons for drying to 10 % moisture. Each sample was weighted and recorded.

**Statistical Analysis**

The SAS proprietary statistical package was used to subject data to analysis of variance (ANOVA) and mean differences were separated by Duncan's multiple-range test.

**Results**

**Peanut Growth and Root-knot Nematode Infection**

Georgia-06G had lower plant growth ratings than Tifguard or isogenic Tifguard (P ≤ 0.05). There were no differences in peanut growth between Georgia-06G treated and
nontreated with 1,3-D (P ≤ 0.05), nor was there an effect of 1,3-D on the peanut growth or yield of Tifguard and isogenic Tifguard (P ≤ 0.05) (Table 5-1).

A relatively small amount of galling was observed on roots from Georgia-06G and isogenic Tifguard without 1,3-D treatment. Both of them are susceptible to Meloidogyne arenaira. There was relatively little infection observed on Georgia-06G without 1,3-D whereas more than 30 galls or egg masses were found on isogenic Tifguard without 1,3-D treatment (Table 5-1).

**Yield Comparison**

Georgia-06G had the lower yield than Tifguard or isogenic Tifguard (P ≤ 0.05). However, there was no difference of yield between Georgia-06G treated or nontreated with 1,3-D (P ≤ 0.05). The 1,3-D had no effect on yield produced by Tifguard treated and nontreated with 1,3-D (P ≤ 0.05). Similarity, there was no difference of yield between isogenic Tifguard treated and nontreated with 1,3-D (P ≤ 0.05) (Table 5-1).

**Soil Type, Texture, Analysis**

The soil was a mixture of fine sandy Arredondo and span type (sand 95%, silt 3%, clay 2%; organic matter 1.5%; pH 6.5). Moisture content of the soil at field capacity averages 13.5% to 30-cm deep and bulk density of the planting beds average 1.4 g/cm³. Drainage was excellent with very little underlying clay.

**Discussion**

Yield is a key factor for profitability expectations and results. Yield drag is often used in comparing crop with genetic engineering to crops without genetic engineering or crop with application of herbicide or without herbicide (Oplinger, 1999). Tifguard is the first peanut cultivar with resistance to two pathogens. The yield performance of Tifguard was conducted and compared with other peanut cultivar in 2010-2013. Based
on the pod yield from different peanut varieties, Tifguard is a good peanut cultivar with high yield compared with other peanut cultivars (Tillman et al., 2013).

In this study, however, Tifguard had higher pod yield compared with Georgia-06G. The isogenic Tifguard is also a peanut cultivar susceptible to *M. arenaria*. The untreated isogenic Tifguard had the highest pod yield among the six treatments albeit the highest infection was also found on it. Based on the statistical analysis, there is no significance of estimated yield among Tifguard and isogenic Tifguard with or without treatment. It seems that yield drag was not observed on Tifguard performance. Moreover, the chemical treatment was not effective management for root-knot nematode since the treated Georgia-06G and untreated Georgia-06G had the same yield performance.

This is single year trial and results from any single year may not be sufficient to determine the yield performance of peanut variety. The results from one-year trial are simply a reflection of the growing season that occurred in this year. The multi-year results are better for evaluation of the yield performance of peanut varieties.
Table 5-1. Effect of different treatments on plant growth, galling induced by root-knot nematodes and yield of peanut in a field trial at Plant Science Research and Education Unit, Citra, FL, Spring-Summer 2015.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant growth rating&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Gall index&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Georgia-06G without 1,3-D</td>
<td>6.5 a</td>
<td>0.8 a</td>
<td>3,064 a</td>
</tr>
<tr>
<td>2. Georgia-06G with 1,3-D</td>
<td>6.8 a</td>
<td>0 a</td>
<td>2,957 a</td>
</tr>
<tr>
<td>3. Tifguard without 1,3-D</td>
<td>8.5 b</td>
<td>0 a</td>
<td>4,462 b</td>
</tr>
<tr>
<td>4. Tifguard with 1,3-D</td>
<td>9.4 c</td>
<td>0 a</td>
<td>4,731 b</td>
</tr>
<tr>
<td>5. Isogenic Tifguard&lt;sup&gt;3&lt;/sup&gt; without 1,3-D</td>
<td>9.8 c</td>
<td>3 b</td>
<td>4,785 b</td>
</tr>
<tr>
<td>6. Isogenic Tifguard&lt;sup&gt;3&lt;/sup&gt; with 1,3-D</td>
<td>9.9 c</td>
<td>0 a</td>
<td>4,408 b</td>
</tr>
</tbody>
</table>

Data are means of four replications. Means within a column followed by a common letter are not different according to Duncan’s multiple-range test ($P \leq 0.05$).

<sup>1</sup> Scale 1-10, where 1 = very poor plant growth, 10 = very good plant growth

<sup>2</sup> Scale 1 – 4, where 0 = 0 galls on roots or pegs and pods, 1 = 1 – 10 galls on roots and < 10 on pegs and pods, 3 = 11 – 100 galls on roots and 10 – 50 on pegs and pods, 4 = > 100 galls on roots and > 50 on pegs and pods.

<sup>3</sup> Root-knot nematode susceptible Tifguard.
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BIOGRAPHICAL SKETCH

Weimin Yuan was born in 1985 to Mr. Youjun Yuan and Mrs. Hua Li in Jiangsu Province, P.R. China. She grew up in City of Nantong in China where she also obtained her primary and secondary education in Nantong No.1 Middle School of Jiangsu Province. In 2004, she was accepted in the B. S. plant protection program in Nanjing Forestry University. For her master degree in Nanjing Forestry University, she majored in Microbiology under the supervision of Dr. Xiaoqin Wu and studied the endophytic bacteria present in *Bursaphelenchus xylophilus*. She worked as a research assistant on project dealing with identification and function of endophytic bacteria using molecular and culture-based methods. She was awarded as an outstanding student and got scholarship three times during seven years in Nanjing Forestry University. After finishing, she moved to Shanghai and worked as a professional clerk in two private companies. Realizing the need to get more professional training and knowledge, she went to pursue a doctoral program at the University of Florida where she studied root-knot nematodes under the supervision of Dr. Donald W. Dickson. Her research project was focused on evaluation of resistance to *Meloidogyne arenaria* in the peanut cultivar Tifguard.