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Effects of Meloidogyne Incognita, Soil Physical Parameters, and Thielaviopsis Basicola on Cotton Root Architecture and Plant Growth

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of the requirements for the degree of
Doctor of Philosophy in Plant Science

By

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ABSTRACT

The root-knot nematode, *Meloidogyne incognita*, and the seedling pathogen, *Thielaviopsis basicola*, commonly co-exist in Arkansas cotton fields and may interact resulting in increased losses. The primary objective of this research was to evaluate the effects of soil physical parameters on these soilborne pathogens and cotton growth in controlled environmental, field, and microplot studies. Controlled environmental experiments used two soil bulk densities and four pathogen treatments: non-infested soil, soil infested with *M. incognita* or *T. basicola* and soil infested with both pathogens. The results indicated bulk density generally did not affect seedling growth or disease since soils had low penetration resistance under well-watered conditions. The combination of *M. incognita* with *T. basicola* reduced seedling stands and root volume more than either pathogen alone. Both *M. incognita* and *T. basicola* reduced root topological characters, but only *M. incognita* changed the root topological index. The effects of subsoiling and application of the nematicide *1,3-dichloropropene* (Telone II®) on root system development and plant growth were investigated from 2009 to 2011 in a cotton field in northeastern Arkansas. Subsoiling did not consistently affect early season growth. Nematicide treatment consistently improved seedling growth for one or more parameters in 2010 and 2011. Root galling and the population of *M. incognita* were suppressed by Telone II®. Neither subsoiling nor nematicide application affected cotton development or root topology. The effects of a soil hard pan (HP) and *M. incognita* on cotton root architecture and plant growth were evaluated in a microplot study in 2010 and 2011 at Hope, Arkansas. An artificial HP was created 20 cm below the soil surface in half of the microplots. Pathogen treatments included soil infested with *T. basicola* plus four different *M. incognita* levels (0, 4, 8, 12 eggs/cm^3 soil). Generally, soil HP improved seedling growth due to higher soil water contents above the HP layer. *M. incognita*

reduced taproot length, delayed cotton maturity and reduced seed cotton yield. Root topology provides a new approach to quantify the changes caused by soilborne pathogens and soil physical factors and will help in crop management in the future.

This dissertation is approved for recommendation
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Chapter I

Introduction and Literature Review

Cotton. Cotton is an important economic commodity that has been associated with human activity since before recorded history (Stewart, 2001). Cotton is important as a fiber crop, but cotton seed is also used for edible vegetable oil and protein in animal feed worldwide. Lint production in the U.S. accounts for nearly 25% of the world supply. According to USDA's forecast in August 2011, U.S. cotton production reached 16.55 million bales planted on about 13.7 million hectares (USDA, 2011). The production of cotton seed varies from 4.9×10^9 to 5.9×10^9kg annually. In 2011, Arkansas was the third largest upland cotton-producing state in U.S. with about 1.4 million bales produced on 2.67×10^5 hectares (USDA, 2011).

Cotton is a perennial plant grown as an annual crop, and various disease, weed, and insect pests can be economically significant in cotton production. However, eradication of the boll weevil (*Anthonomus grandis* Boheman) from most U.S. production areas and wide scale adoption of insect and herbicide-resistant cultivars has greatly lowered the need for pesticides and allowed growers to focus on a more limited number of pests (Koenning et al., 2004).

Plant parasitic nematodes on cotton. Nematodes are microscopic roundworms and have been described as being the most numerous and widely distributed group of multi-cellular organisms in the world (Bogoyavienskii et al., 1974). Plant-parasitic nematodes, cause substantial economic losses in many crops including cotton (Overstreet and McGawley, 2001). Nematodes were recognized as a serious problem on cotton as early as the 19th century, but there was little research on nematodes of cotton until the 1950s (Koenning et al., 2004). The estimated losses in yield caused by nematodes on cotton have increased from 2% in 1990 to more than 4% in 2010 (Blasingame and Patel, 2011). Collectively nematodes are responsible for more loss to the cotton industry than any other pathogen group (Blasingame and Patel, 2011).

The four most damaging nematode species on cotton in the U.S. are southern root-knot (*Meloidogyne incognita* Kofoid &White (Chitwood)), reniform (*Rotylenchulus reniformis* Linford & Oliveira), Columbia lance (*Hoplolaimus columbus* Sher), and sting (*Belonolaimus longicaudatus* Rau) (Overstreet and McGawley, 2001; Sasser, 1972). In Arkansas, the most economic important nematodes on cotton are *M. incognita* and *R. reniformis* (Overstreet and Kirkpatrick, 2011).

Meloidogyne incognita. The southern root-knot nematode has been recognized as an important pathogen on cotton since 1889 (Atkinson, 1889; Starr et al., 2005). This nematode receives its common name due to the characteristic formation of root galls in response to infection. Although there are over 30 *Meloidogyne spp.* worldwide (Poinar, 1983), only two species, *Meloidogyne incognita* and *M. acronea* Coetzee, can reproduce on cotton. *M. acronea* is important on cotton only in parts of Africa, while *M. incognita* is distributed throughout most cotton production regions worldwide (Bateman et al., 2000; Thomas and Kirkpatrick, 2001).

Meloidogyne incognita is a sedentary endoparasite that reproduces by mitotic parthenogenesis (Triantaphyllou, 1985). The life cycle of *M. incognita* is characterized by an egg and four juvenile stages. Only the second juvenile stage (J2) is infective (Thomas and Kirkpatrick, 2001). Juveniles may infect root hairs or at root tips and migrate to the zone where the phloem and xylem tissues are differentiating to establish a permanent feeding site (Huang, 1985). Salivary secretions from the esophageal glands stimulate the formation of specialized feeding cells known as giant cells (Hussey et al., 2002). Once feeding has been initiated, J2 become sedentary within the root and develop through the third and fourth stage at the feeding site. As the giant cells enlarge, the root tissue proliferates, and the feeding sites become visible as knots or galls characteristic of the *Meloidogyne* species (Tang, 1993).

Symptoms of *M. incognita* infection may include stunted or chlorotic plants that appear water-stressed even with adequate soil moisture. Nematode infection reduces both the maximum rate and cumulative amount of water flowing through the cotton plant, while decreasing leaf transpiration rates and stomatal conductance, and increasing leaf temperature (Kirkpatrick et al., 1995). Similar nematode-induced decreases in root hydraulic conductance have also been reported on tomato and bean (Meon et al., 1978; Wilcox-Lee and Loria, 1986).

Ecologically, both soil temperature and texture are important factors that determine the survival and pathogenicity of *M. incognita*. The optimal temperature for *M. incognita* ranges from 25 to 30 °C (Eisenback and Triantaphyllou, 1991; Van Gundy, 1985) and the nematode completes its life cycle in 20 days at 29 °C. Egg hatch is inhibited below 10 °C (Goodell and Ferris, 1989). *M. incognita* was most damaging in a low clay-content soil mixture (Koenning et al., 1996) and population densities of *M. incognita* were strongly associated with soil texture, with higher populations in sandy soils than where the percentage of silt and clay was higher (Jaraba-Navas et al., 2007; Monfort, 2005). Reproductive potential of *M. incognita* is greater in soil with a sand content from 72% to 91% than in soil with clay content near 30% (Koenning et al., 1996; Prot and Van Gundy, 1981). Root-knot nematodescan survive in the soil up to 10 years even in a dry environment. The gelatinous matrix of the egg mass acts as a barrier to water loss from eggs and to protect the eggs from predators (Lee, 1972). Some *Meloidogyne* species can enter a state of anhydrobiosis in dry soil as J2s for long-term survival (Van Gundy, 1985).

Current nematode management practices for *M. incognita* on cotton rely to a great extent on nematicides because highly resistant cultivars are not available commercially (Koenning et al., 2004). Widely-used nematicides for controlling *M. incognita* in cotton include aldicarb which is a nonfumigant nematicide marketed as Temik 15G®, Bayer Cropscience, (Research Triangle

Park, NC). In addition, soil fumigants including 1, 3-dichloropropene (Telone II®, Dow AgroSciences, Indianapolis, IN and metam potassium (K-Pam, AMVAC Chemical Corporation, Newport Beach, CA) are used to some degree in areas where application equipment is available. Recently, various seed treatment combinations that include a nematicidal component have been developed (Faske and Starr, 2007; Monfort et al., 2006a). Monfort et al. (2006a) found that plants were taller and root galling severity and nematode reproduction were lower after treating cotton seeds with abamectin, suggesting that this method of control may be effective in some situations. Additional products that are now commercially available as seed treatments for nematode suppression include thiodicarb (Aeris®) and a bacterial antagonist (*Bacillus firmus*) that is marketed as Votivo® by Bayer CropScience (Research Triangle Park, NC). Regardless of the method of application or the product that is used, the cost of nematicides and their potential adverse effect on the environment have prompted considerable effort by crop breeders and nematologists to develop resistant cultivars for nematode management.

Plant resistance is the most effective and environmentally friendly approach to controlling root-knot nematodes (Roberts, 1992). Unfortunately, there are no highly resistant cotton cultivars available commercially. High levels of resistance have been reported in certain "Auburn" breeding lines (Shepherd and Huck, 1989), and highly resistant cotton germplasm lines including Auburn 623 RNR (Reg. No. GP-20, PI 529546; Shepherd, 1974), Auburn 634 RNR (Reg. No. GP-166, NSL 161720; Shepherd, 1982), and related "M" series lines (Zhang et al., 2007) have been registered. Nem-X, an Acala-type upland cotton cultivar, exhibits less root galling and higher yield in the comparison with susceptible cotton cultivars in the presence of nematodes (Ogallo et al., 1997), and has been used somewhat successfully in the more arid regions of the western U.S. where Acala types are adapted (Roberts et al., 1984).

Cultural practices that may help manage nematodes in cotton include fallowing and crop rotation. Fallowing or crop rotation may, however, be difficult to justify in some situations because of high land values and the lack of economically attractive alternative crops (Thomas and Kirkpatrick, 2001). Timper et al. (2006) found that certain winter cover crops have potential for the suppression of *M. incognita*. For example, planting rye or *Meloidogyne*-resistant legumes as winter cover crops may lower the nematode population density for a subsequent cotton crop. Precision farming is also an area of interest in agriculture, and site-specific applications of nematicides may be of value in some production systems (Koenning et al., 2004; Mueller et al., 2010).

Thielaviopsis basicola. Another soilborne pathogen that is important on cotton is *Thielaviopsis basicola*, the causal agent of black root rot of seedlings. *T. basicola* can infect over 230 plant species in 49 families (Johnson, 1916; Otani, 1962). This pathogen is widespread in many major crop production areas, affecting tobacco, citrus, several ornamental and vegetable crops, many legumes, and cotton. The pathogen was first reported on cotton in Arizona in 1939 and has since been found in many cotton-growing regions including Australia, Egypt, India, Peru, Spain, the former Soviet Union and the United States (Allen, 2001). Black root rot of cotton seedlings results in the stunting of seedling growth of cotton. Symptoms include chlorosis and wilting of the plant, generally accompanied by a black discoloration of the root system. Colonization results in a loss of the cortical tissue of roots, and under extreme cases the root system collapses (Allen, 2001).

The optimal soil temperature for black root rot is between 16°C and 20°C, and the disease is generally associated with alkaline or neutral soil pHs and is most common in wet, poorly drained soils (Allen, 2001). Chlamydospores are the primary survival structure. Rothrock (1992)

found that survival of *T. basicola* was lower at warmer soil temperatures (24°C to 34°C) than at 10°C to 18°C. Consequently, one of the most important methods to control black root rot is planting the crop after the minimal soil temperatures average 18ºC (65ºF) or warmer for 3 consecutive days (Allen, 2001). Because of its wide host range, weed control is an important component in black root rot management, particularly during crop rotations. Summer flooding can reduce the survival of the pathogen and the incorporation of a legume cover crop such as hairy vetch as a green manure also has been shown to reduce the incidence of black root rot on cotton seedlings (Rothrock et al., 1995). Yield losses caused by *T. basicola* are difficult to assess on cotton because *T basicola* resistant cultivars are not available. Lint yield increases of 160 kg or greater were obtained by eliminating *T. basicola* from fields by summer flooding (Devay and Garber, 1997). Sterol-inhibiting fungicides and SAR (systemic acquired resistance) products may reduce black root rot severity (Toksoz et al., 2009).

Interaction between *Meloidogyne incognita* and *Thielaviopsis basicola.* An interaction between *M. incognita* and *T. basicola* has been demonstrated on cotton (Starret al., 2001; Walkeret al., 1998, 1999, 2000). Increased Seedling mortality, delayed plant development, and reduced plant height-to-node ratio (HNR) was shown in the presence of both pathogens. With the combination of *M. incognita* and *T. basicola*, seed cotton yield was reduced, and the length of time required for boll maturation was lengthened. In addition, the position of the first sympodial node on the main stem was higher, indicating that maturity was delayed, and fruit set was suppressed (Walker et al., 1998).

Plant growth was suppressed in the presence of both *M. incognita* and *T. basicola* under various temperature regimes (Monfort et al., 2006b; Walker et al., 2000). Under continuous 20°C, 24°C and 28°C or two cyclic linear regimes with ranges of 14°C to 32°C or 18°C to 28°C

over 24 hours, plant height-to-node ratio and total fresh weight were reduced when the soils were infested with both pathogens. Histological examination of roots infected by both pathogens showed that *M. incognita* infection allowed *T. basicola* to colonize vascular tissue which generally is not accessible to the fungus in the absence of the nematode (Walker et al., 1999). Conversely, infection by *T. basicola* reduced root galling and reproduction of *M. incognita* (Jaraba-Navas et al., 2007; Walker et al., 1998, 2000).

The cotton root. Cotton seed germinate under favorable environmental conditions such as high soil oxygen, adequate soil water and soil temperatures above 18 $^{\circ}$C (65 ºF) (Oosterhuis and Bourland, 2001). The first organ to emerge from the seed coat is the radical or primary root. During the vegetative stage, numerous lateral roots emanate from the taproot to form the entire root system. The total root length produced by a cotton plant during the growing season can be several hundred meters. Root length decreases because of the death of roots, and root activity declines during boll development as carbohydrates are directed to the fruit. The total root weight can account for approximately 20% of the total dry weight of the plant (Oosterhuis and Bourland, 2001).

Soil environmental factors strongly influence cotton root architecture including soil temperature, moisture, aeration and fertility (Li, 2003; Li et al., 2005). Cotton plants usually produce roots more than 1 meter deep (Hons and McMichael, 1986; Oosterhuis and Bourland, 2001). Cotton grows poorly in the soil with high strength (Bennie et al., 1981; Taylor et al., 1964). Soil strength is a transient localized soil property which is a combined measure of soil subunit's solid phase adhesive and cohesive status (Soil Science Society of America, 2012).The growth of cotton roots ceases when soil resistance to penetration exceeds 3 MPa (Lowry et al., 1970; Taylor and Burnett, 1963; Taylor and Gardner, 1963; Taylor et al., 1966; Taylor and

Ratliff, 1969). McKenzie and McBratney (2001) using a resin containing fluorescent dye to study the relationship between soil structure and the root morphology of cotton found that compacted soil strongly impeded the development of taproots. The roots were also severely tapered and deflected approximately 90° at the top of the compact layer. Glinski and Lipiec (1990) reported that mechanically impeded soil reduced root size, diminished elongation rates, disturbed root distribution, increased root diameters, and reduced nutrient uptake while enhanced lateral branching.

Another important environmental factor that affects root growth and development is soil temperature. Root growth tends to increase with rising temperatures (McMichael et al., 1996). Extension of cotton roots increased with increasing temperature up to approximately 36°C and lateral root development increased with rising temperature (Galligar, 1938). In addition, the rate of cotton root growth strongly depends on soil moisture and roots cease to grow when soil water content falls below 0.06 cm^3/cm^3, equivalent to -100 Joules/kg water potential (Taylor and Klepper, 1974).

Root Architecture Research Methods. The root typically lies below the surface of the soil and functions to acquire nutrient resources from the soil (principally water and ions). Roots also anchor the plant. Characteristics of roots include diameter, color, growth rate, surface texture and certain physiological attributes such as transport ability and hormone content (Fitter, 2002). Fine roots and lateral roots are considered the primary site for water and nutrient absorption because of the relatively higher hydraulic conductivity in this area (Gordon and Jackson, 2000; Li, 2003; Rieger and Litvin, 1999), Anchorage is primarily a function of the main roots. In general, thicker roots may exert greater forces on soil and have the capacity to penetrate compacted soil more easily (Goss, 1977). Fitter (1985) suggested roots with more branching

close to the main axis have the ability to exploit the soil volume surrounding the roots more fully than less branching roots or those that branch further away from the main axis. Exploratory root systems tend to possess many branches and tips away from the main axis, thus exploring greater soil volumes (Fitter, 1985).

A. Traditional approaches. Excavation and direct monitoring *in situ* are the two primary methods that have been used to study the development and architecture of root systems. Excavation of entire root systems is a useful technique for exploring the morphological characteristics, architecture, or biomass of individual plants. Soil core sampling involves taking a cylinder-shaped core sample and using this soil core to estimate the spatial distribution and the volumetric relationship of fine roots, generally with diameters<5mm. A growth core, or a mesh bag technique, consists of a cylindrical gauze bag with a specific volume of root-free substrate. A hole drilled in the bag at the appropriate point allows the roots of neighboring plants to colonize the substrate (Polomski and Kuhn, 2002).

A number of methods have been developed to directly monitor roots *in situ*. A trench wall method is particularly useful for studying coarse roots (Polomski and Kuhn, 2002). Root windows are used to monitor the morphological development of roots as well as the phenological changes and life span or mortality of individual roots (Polomski and Kuhn, 2002). Rhizotrons and minirhizotrons allow researchers to investigate root-soil relationships under specific conditions (Polomski and Kuhn, 2002). Other methods include hydroponic approaches and root tubes or root boxes (Polomski and Kuhn, 2002). Various markers are commonly used to study root development or function. Isotopes (radioisotopes or stable isotopes), plant toxicants, dyes and fluorescence dyes have been used to assess translocation paths or changes in root tissue or the growth medium (Polomski and Kuhn, 2002).

B. Root architecture and root models. Root tips and root tissues behind the root tips shape the whole root systems through the accumulated effects of growth and branching in response to plant growth stage and soil environment (Diggle, 1988). Root function reflects the trivalent branching structure which is the most fundamental feature of root systems (Fitter, 2002). Root system architecture is crucial for nutrient acquisition from the soil (Lynch, 1995). Since the environment for root system development is highly heterogeneous both in time and space, it is important for root systems to possess plasticity leading to architectural adaptation to ensure the attainment of resources under differing conditions. Thus root system architecture can be modified to improve nutrient-acquiring capacity (Sorgonà et al., 2007).

Quantitative (topological) and qualitative (geometrical) aspects of root systems are significant in root architecture (Fitter, 1987). Due to their underground habitat, their interactions with their environment and their diverse functions have been inherently difficult to study (Brugge, 1985; Robinson, 1996). Consequently simulation modeling has provided a tool to study root systems. Several models have been developed to study root architectural characteristics. Diggle (1988) proposed a three-dimensional architectural model to simulate the growth of fibrous root systems. Based on this model, the time interval used, the numbers of axes, initiation times of axes, growth rates and branching characteristics of root systems were evaluated and all branches and root tips were recorded in three-dimensional coordinates. ROOTMAP, a model which was developed from the Diggle's three-dimensional root architectural model (Dunbabin et al., 2002), also considered root growth responses to soil water and nutrient dynamics. Two other models which primarily focus on branching structure of the root system, the development model (Rose, 1983) and topological model (Fitter, 1985) have also been useful in studying root development.

Based on Rose's (1983) development classification theory root systems are classified according to branching development orders that are categorized as axes and laterals which can be primary, secondary, or tertiary. Although the development model was suitable to describe the development of many root systems, death of a root, cessation of growth of the axial meristem, or the inordinate growth of laterals could allow changes in the direction and rate of growth of roots, reflecting root system development rather than function (Fitter, 2002).

Topological models for describing root systems, on the other hand, avoid some of the problems of the developmental model (Figure 1) (Fitter, 1985, 1987; Fitter et al., 1991). In a topological model, the basic architectural unit is the link, defined as a length of root between two nodes or the junction of two root branches (Fitter, 1986). There are two types of links: interior (I) links which join two branching points, and exterior links (E) which end at the root meristem. Exterior links are defined as external-external (EE) links and external-internal (EI) links. EE links join other exterior links at their base while EI links join interior links. These models also describe the direction of classification, and the terminal branches (exterior links) are noted as order 1. Magnitude (μ) is a topological parameter that is used to classify the interior links - the number of exterior links that it serves. Consequently, links change their "order" when the root system grows, and the magnitude of the interior link increases following the generation of a new link anywhere in the subsystem. Two other parameters that are used to quantify topological models include altitude (α) and total exterior pathlength (*Pe*). Altitude is defined as the number of links in the longest path from any exterior link to the base link. *Pe* is the sum of the number of links in all paths.

Based on this topological classification system, there are two idealized topological models - herringbone and dichotomous (Fig.1.). With herringbone root systems, branching is

confined to the main axis while a dichotomous system may show branching on all links (Berntson, 1995; Fitter, 1985; Knuth, 1968; Werner and Smart, 1973).

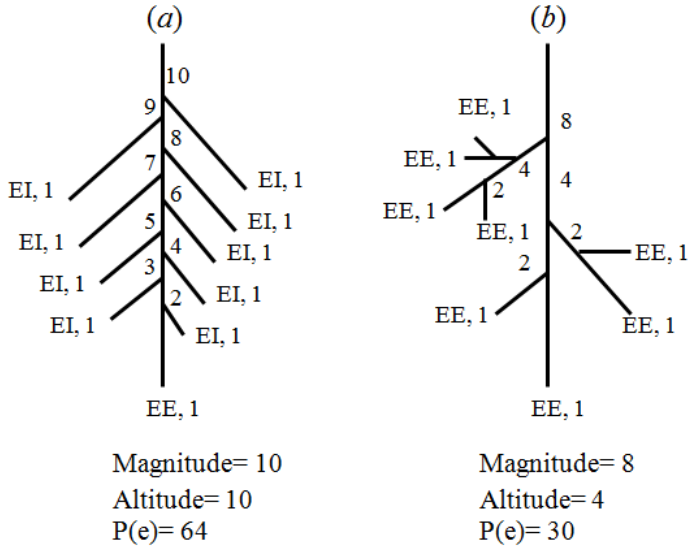

Figure 1. Diagram showing the distinction between extreme branching patterns (Fitter and Stickland, 1991; redrawn). a. Herringbone; b. Dichotomous. Magnitude (μ) is the sum numbers of exterior links. Altitude (α) means the number of links in the longest path from any exterior link to the base link; Total exterior pathlength (*Pe*) means the sum of the number of links in all paths; EI is exterior-interior and EE is exterior-exterior links; unlabelled links are interior.

Considering that the most significant functions of root systems are absorption and transportation of water and nutrients from the soil to above ground plant parts, the primary determinants of transport efficiency are the number and lengths of the interior and exterior links. Transport effectiveness and efficiency are enhanced by the first few generations of branching in the dichotomous root system. However, this may minimize *Pe* and transport distances, so in many cases, herringbone topologies are the most beneficial to the plant (Fitter, 2002). In contrast, when reviewed as the cost of root construction using carbon and water currency, dichotomous topology is cheaper to construct and maintain, and also more efficient in transporting immobile materials. In general, the "expensive" herringbone systems would facilitate the slow growth habit of perennials that absorb resources from the soil in nutrient-poor area whereas annuals and

perennials growing in a nutrient-rich environment would tend to produce nearly dichotomous systems (Fitter et al., 1988; Fitter and Stickland, 1991).

The topological index (TI) is another important feature that is considered in topological models. TI characterizes the branching structure of the whole root system and provides a way to compare and group root systems via theoretical topological extremes in branching. The TI is defined by the slope of the regression line from double-logarithmic ($\log_e$) plots of *Pe* or α against μ (Fitter, 1986, 1987; Fitteret al., 1988; Larkinet al., 1995, 1996). Values for TI usually range from 1.92 for herringbone models to 1.2 for dichotomous patterns and the TI value would be 1.52 for a root system with equal branch initiation on all root links (Fitter, 1986; Werner and Smart, 1973). Glimskär (2000) and Sorgonà et al. (2007) used a simplified formula [log(a)/log(μ)] to calculate TI and defined a herringbone structure when the values were near 1.0 and a dichotomous root structure when TI values were close to 0.5. Another index, $a/E(a)$, which describes random branching was also suggested (Fitter et al., 1991). However, the determination of this index is complicated and sometimes is not feasible if μ is larger than 500 (Werner and Smart, 1973).

The WinRHIZO image analysis system (Regent Instruments, Inc., Quebec, Canada) which was specifically designed for root measurement has been used in root morphological and architectural studies. WinRHIZO consists of a high-resolution image scanner combined with a computer program that includes image acquisition components. This system makes it possible to conduct diverse analyses of root system morphology (root length, area, volume, topology, architecture and color analyses). WinRHIZO has also been used by a few researchers to study root architecture. Tian et al. (2006) studied the genotypic differences in nitrogen acquisition ability among maize plants and reported that greater nitrogen acquisition ability depended on the

coordination of leaf and root growth. McPhee (2005) quantified the phenotypic variation in *Pisum* seedling root production and root architecture and found that one genotype (PI 261631) was superior to the others for total root length and volume. Similarly, Sorgonà et al. (2007) found that the citrus rootstock 'Cleopatra Mandarin' was less competitive than co-generic rootstocks due to nitrate acquisition based on topological index. From an experimental methodology standpoint, Crush et al. (2005) recommended using sand for evaluations of root parameters in white clover because of better root branching structure in sand.

Factors that affect root systems architecture. A. Nutrient availability. Nutrient availability can influence many features of root system morphology including root branching, root elongation, lateral root length and root area (Andren et al., 1993). Arredondo and Johnson (1999) found significant differences in root system topology among nutrient treatments, and Fitter et al. (1991) found that branching complexity of root systems (described by root topology) could be related to habitat characteristics. Some plant species may exhibit simple branching patterns (herringbone) in infertile soil, likely due to reduced competition from other plants, whereas a more complex, branching topology (dichotomous) might be exhibited in more fertile habitats (Fitter et al., 1991).

B. Soil physical conditions. Roots experience mechanical stress when elongating through the soil (Lipiec et al., 2003). In the field, soil can be compacted by heavy machinery traffic during planting, crop maintenance, and harvest (Harveson et al., 2005). Compaction, in turn, results in an increase in soil bulk density that is characterized by increased soil strength, decreased air permeability and hydraulic conductivity due to reduced number and size of macropores, and increased root penetration resistance (Allmaras et al., 1988; Coelho et al., 2000; Horn et al., 1995; Lowry et al., 1970; Russell and Goss, 1974; Whalley et al., 1995).

Increased bulk density can lead to decreased aeration and impeded root development and elongation (Greacen and Oh, 1972; Horn et al., 1995). Griffiths et al. (1991) showed a decrease in root elongation of barley (*Hordeum vulgare*) from 1.17 to 0.54 mm h^{-1} by increasing the soil bulk density from 1.0 to 1.3 $Mg \cdot m^{-3}$. Coelho et al. (2000) reported that a compaction layer with a bulk density of 1.6-1.7 $Mg \cdot m^{-3}$ reduced cotton root length, leaf area index, and evapotranspiration.

However, it is soil strength (resistance) rather than the soil bulk density that determines the critical impedance factors controlling root penetration in sandy soils. A highly significant linear correlation ($r = -0.96$) between the soil strength and the root penetration percentage has been demonstrated (Medvedev, 2009; Taylor and Gardner, 1963).Soil penetration resistance begins to inhibit root growth of most plants at about 1.5 MPa, and the roots of many plants stop growing altogether when the strength of soil reaches about 2.5 MPa (Coelho et al., 2000). Inverse linear relationships between soil strength and yield of corn, soybean, and wheat grown on coastal plain soils in the southeastern USA with compaction layers has been reported (Busscher et al., 2000).

Distinct differences in root distribution in compacted and uncompacted soils also occur (Horn et al., 1995; Pierret et al., 2007). In some regions of the southern USA, plant roots are partially or wholly confined to a shallow plowed layer above a compacted soil pan (Lowry et al., 1970). Although the total root biomass of maize (*Zea mays* L.) was similar in both compacted and uncompacted soil, the root systems in uncompacted soil had a greater proportion of deep roots (Whalley et al., 1995) whereas nearly all the roots were prevented from penetrating deeper than 10 to 15cm by a compacted layer (Taylor and Burnett, 1963). Similarly, Lowry et al. (1970) found that the soil mass was thoroughly exploited by roots growing in cylinders containing a low

density soil, but almost no roots penetrated below the high-density pans when roots were excavated at harvest time. Roots tend to have greater diameters in soil with higher mechanical impedance, than roots grown under conditions of lower mechanical impedance (Materechera et al., 1991).

C. Tillage effect on soil attributes, root development and plant growth. One of most common tillage approaches used to rectify compacted soil and improve soil physical properties is subsoiling (Busscher et al., 1986; Mullins et al., 1992; Raper et al., 2000a, b; Schwab et al., 2002; Vepraskas et al., 1995). Subsoiling may be conducted annually, although increased energy costs may discourage this tillage practice on an annual basis by some growers.

Subsoiling facilitates root distribution and penetration by disrupting compaction layers, which may in some instances help plants to overcome short-term drought conditions (Raper et al., 1998). Abu-Hamdeh (2003) suggested that subsoiling reduced soil bulk density in the top 40 cm of the soil profile. Subsoiling exhibited a significant positive effect by reducing cone penetration resistance at depths of 10-20 and 20-50cm and increased plant height and yield by improving soil physical properties. Similarly, Salih et al. (1998) demonstrated that plowing rather than disc harrowing increased cotton plant height and shoot dry biomass, and enhanced yield.

D. Biological factors. Soilborne pathogens also have the capability of suppressing or altering root growth and development. Such pathogens as the root-knot nematode elicit morphological changes in root systems due to galling that may change the growth habit or function of a root system. *Meloidogyne* species have evolved strategies to establish feeding cells in root systems. Hyperplasia and hypertrophy of surrounding cells lead to formation of root galls, the swollen portion of roots, which are the typical symptoms caused by the root-knot nematode

(Caillaud et al., 2008; Tang, 1993).The formation and development of giant cells produce to anatomical changes in roots, including disruption of the xylem, root epidermis, and cortical tissues (Shepherd and Huck, 1989). Moreover, nematode infection inhibited new root development resulting in reduced number of fine roots, degeneration of existing roots, distortion of the vascular system and disruption of the hormonal or nutritional balance (Hussey, 1985; Vighierchio, 1979). Few studies have been conducted, however, that relate root architectural changes due to pathogens to those changes due to physical factors. Root topological models have been used to study changes in alfalfa root architecture by *Pythium* spp. in the laboratory (Larkin et al., 1995) and in the field (Larkin et al., 1996). Larkin et al. (1995) used architectural analysis methods for quantitative assessment of the impact of *Pythium* spp, on root system branching structure of alfalfa (*Medicago sativa* L.). Infection reduced total root system length and total numbers of root orders. In addition, topological parameters, such as root system magnitude, altitude, and total exterior pathlength decreased simultaneously leading to a smaller root system compared to uninfected plants. In this case, infection by *Pythium* resulted in a herringbone pattern.

Other organisms also may affect root development, *Ralstonia solanacearum*, a soilborne Gram-negative plant pathogenic bacterium, disrupts petunia (*Petunia hybrida*) root architectural development by constraining the elongation of lateral roots and inducing the formation of new root lateral structures that provide new sites for extensive bacterial colonization (Zolobowska and Van Gijsegem, 2006). Arbuscular mycorrhizal fungi (AMF) can exhibit a strong impact on root morphogenesis, and may induce changes in root architecture, including increased root branching and the development of a longer proportion of smaller diameter, higher-order roots (Berta et al., 2002; Gamalero et al., 2004). Boukcim and Plassard (2003) found that mycorrhizal

fungi could decrease NO_3^- uptake rate in two contrasting *Pices abies* open-pollinated families and related this to changes in root architecture in low-growth field performance. However, Yao et al. (2009) characterized the effect of AM colonization on the root system architecture of trifoliate orange (*Ponirus trifoliate* L. Raf.) seedlings and demonstrated that AMF colonization significantly reduced the total root length, the root volume and root surface area but induced more fibrous roots and less coarse roots.

Summary There are no reports available that relate root architectural changes brought about by either *M. incognita* or *T. basicola* to cotton development and yield, and no studies have been done to quantify their impact on cotton growth and development in the presence of a compaction layer. Consequently, the objectives of this dissertation include: 1) investigating root morphological and architectural changes brought about by *M. incognita* and *T. basicola* at different soil bulk densities; 2) to determine the effects of subsoiling and application of a fumigant nematicide on root architecture and cotton growth, development and yield in the field; 3) to study the effects of a soil compaction layer, and *M. incognita*, and *T. basicola* on cotton plant growth and development in microplots.

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Chapter II

Effects of *Meloidogyne incognita* and *Thielaviopsis basicola* on Cotton Growth and Root Morphology

ABSTRACT

Effects of the root-knot nematode *Meloidogyne incognita* and fungal pathogen *Thielaviopsis basicola* on cotton seedling growth and root morphology were evaluated in controlled environmental experiments. Two soil bulk densities (BD) (1.25 and 1.50 g/cm^3) were created. Four pathogen treatments included non-infested soil, soil infested with *M. incognita* (Mi), soil infested with *T. basicola* (Tb) and soil infested with both pathogens. Plant growth and root systems were evaluated 44 days after planting. Infestation by Mi and Tb together significantly reduced seedling emergence, number of nodes, and root system volume compared to either pathogen alone. Either Mi or Tb reduced plant height, root fresh weight, top dry weight, and root topological parameters including magnitude, altitude, and exterior pathlength. A BD by Mi by Tb interaction was found for number of stem nodes. Plants grown in non-infested soils at 1.50 g/cm^3 had fewer nodes than those grown in non-infested soils at a bulk density of 1.25 g/cm^3. Both pathogens reduced the number of nodes at a bulk density of 1.25 g/cm^3. The combination of *M. incognita* and *T. basicola* reduced the number of nodes in both bulk densities compared to either pathogen alone or the non-infested control. Root colonization by *T. basicola* increased with the presence of *M. incognita*. The greater soil bulk density reduced root colonization of Tb, but increased root galling by Mi. Root topological index (TI) for all treatments was about 1.92 indicating a herringbone (less branching) architectural structure. Mi infection significantly increased TI. Studying root architecture by a topological model provides an additional approach to evaluate fungal-nematode interactions for soilborne-pathogen systems.

INTRODUCTION

The root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is an important pathogen on cotton (*Gossypium hirsutum* L.) that is distributed throughout all the cotton growing states in the U.S. and most other cotton production regions worldwide (Bateman et al., 2000; Thomas and Kirkpatrick, 2001). Feeding by the nematode stimulates the formation of specialized multinucleate giant cells which are used as feeding sites (Huang, 1985). As the giant cells enlarge, the root tissue proliferates, becoming visible as knots or galls on the roots characteristic of *Meloidogyne* species (Tang et al., 1993; Thomas and Kirkpatrick, 2001). Black root rot of cotton is caused by the fungal pathogen *Thielaviopsis basicola* (Berk. & Broome) Ferraris (syn. *Chalara elegans* Nag Raj & Kendrick).The pathogen affects the root cortical tissue primarily, causing a black discoloration of the root system (Allen, 2001). This tissue damage suppresses early-season growth of cotton seedlings, resulting in yield loss, especially where the growing season is short (Allen, 2001). *M. incognita* was detected in 30% and the *T. basicola* in 70% of cotton fields in surveys in Arkansas (Bateman and Kirkpatrick, 1997; Rothrock, 1997) and both pathogens are frequently present together in Arkansas cotton fields (Rothrock and Kirkpatrick, 1998).

An interaction between *M. incognita* and *T. basicola* on cotton has been described (Walker et al., 1998, 1999, 2000). Increased seedling mortality, reduced plant height-to-node ratio and suppressed cotton yields were reported when both organisms were present (Walker et al., 1998). Histological examination of roots colonized by both pathogens showed that in association with *M. incognita*, *T. basicola* colonized the vascular tissue, which generally does not occur in cotton in the absence of *M. incognita* (Walker et al., 1999). *T. basicola* also may

reduce root galling and reproduction of *M. incognita* (Jaraba-Navas, 2007; Walker et al., 1998, 2000).

Distribution of *M. incognita* and *T. basicola* is aggregated in cotton fields suggesting soil factors may influence pathogen survival (Monfort, 2005). Greater populations of *T. basicola* have been associated with areas with lower sand content (Monfort, 2005). *M. incognita* populations and damage in cotton fields, on the other hand, is greater in areas with greater sand content (Monfort et al., 2007). These pathogens also differ in their preferences for other soil environmental requirements. Black root rot severity increases when soils are wet and temperatures are below 24ºC (Allen, 2001; Johnson and Hartman, 1919; Walker et al., 1999), while the root-knot nematode is favored by warmer soil temperatures (25 to 30ºC) (Walker et al., 2000; Van Gundy, 1985) and soil water tensions of approximately -0.11 MPa (Towson and Apt, 1983; Van Gundy, 1985). Soil bulk density (BD), the degree of soil compaction (Soil Science Society of America, 2012), is another important soil physical parameter that could influence the distribution, population density or damage potential of these soilborne pathogens. Research in producers' fields examining areas of stunted cotton associated with damage from *M. incognita* and *T. basicola* and adjacent non-affected areas of cotton observed trend for affected areas to have greater bulk densities than corresponding non-affected areas (Jaraba-Navas, 2007). Increased soil bulk density decreases the number of large pores and porosity (Carter and Ball, 1993). Reduced soil porosity may inhibit air and water movement, modify soil thermal properties, and increase impedance for root growth, affecting crop growth and development (Carter and Ball, 1993; Taylor, 1971). *M. incognita* is affected by soil bulk density, with nematode movement decreasing as soil bulk density increases (Eo et al., 2007).

Both *M. incognita* and *T. basicola* adversely affect cotton root systems. Traditionally, the effects of *M. incognita* and *T. basicola* on cotton are evaluated by root galling severity and root discoloration, respectively. Root dry weight, root surface area and volume, have been used to estimate reductions in root development caused by these and other pathogens. However, changes induced by *M. incognita* and *T. basicola* on root system morphology and architecture are unknown.

Topological models, developed by Fitter (Fitter, 1987), provide a technique that allows the quantitative investigation of root systems. Topological models emphasize the functional significance of the root system compared to the developmental model of primary, secondary and tertiary roots (Fitter, 1985, 1987; Fitter et al., 1991). This topological technique has been used to study root architecture after inoculation with arbuscular mycorrhizal fungi (AMF) (Berta et al., 1995; Cruz et al., 2004; Orfanoudakis et al., 2010). The technique was first used to study pathogen-induced changes in root architecture by Larkin (Larkin et al., 1995, 1996), who quantified the impact of *Pythium* spp. on root system morphology of alfalfa (*Medicago sativa* L.) seedlings. These studies demonstrate the possibility of using topological models as a method to investigate soilborne pathogen-induced changes in root morphology. The objective of this study was to use morphometric and topological methods to evaluate the influence of different soil bulk density on changes in cotton seedling root architecture and plant growth in response to infection by *M. incognita*, *T. basicola*, or both. The hypotheses of this study was that the plant pathogens *M. incognita* and *T. basicola* will reduce cotton growth and alter root topology and increasing soil bulk density will increase the effects of these pathogens on cotton development.

MATERIAL AND METHODS

Pathogens. A *M. incognita* race 3 population was provided by the Arkansas Nematode Diagnostic Laboratory, University of Arkansas Southwest Research and Extension Center at Hope, AR. The nematode population originated from a commercial cotton field in Arkansas and was maintained and increased on tomato (*Lycopersicon esculentum* L. cv. Rutgers) in a greenhouse. One day prior to inoculation of plants, nematode eggs were extracted from infected tomato roots as described by Hussey and Barker (Hussey and Barker, 1973).

T. basicola isolate 3N-25B was recovered from cotton seedlings at the Delta Branch Station near Clarkedale, AR. Chlamydospore chains were obtained as described by Candole and Rothrock (1997). *T. basicola* was cultivated on 10% carrot juice agar for 6wk before cultures were flooded with sterile distilled water to dislodge endoconidia. Chlamydospores were removed from cultures with a rubber policeman and suspended in sterile distilled water. The spore suspension was passed through two monofilament nylon fabrics (Tetko, Inc., Depew, NY) with openings of 53µm and 20µm, successively. Chlamydospore chains caught on the 20µm nylon fabric were then transferred into a 50-mL sterile centrifuge tube containing 20 mL of sterile distilled water and stored in a refrigerator at 4°C. Percent germination rate was determined by placing chlamydospores on carrot juice agar for 24 hr.

Soil and experimental design. A Rilla silt-loam soil (40% sand, 56% silt, and 4% clay) from a cotton field with a history of both *M. incognita* and *T. basicola* was used in this study. Before planting, the soil was steam pasteurized for 30 min at 70°C. Soil samples were collected and oven-dried to determine soil water content.

Polyvinylchloride (PVC) pipes (15cm inside diameter and 25cm in length) were used to grow cotton. Fiberglass mesh (1.5 × 1.5mm opening) was glued to one end of the tube with silicone caulk. Prior to use, tubes were immersed for 15 min in 0.5% NaOCl solution to disinfest

them. After tubes were rinsed in tap water and dried at room temperature, four 5cm intervals were marked on the inside wall from bottom to top for each tube.

The experiments consisted of four pathogen treatments and two soil bulk densities. A randomized complete block design with a factorial arrangement of treatments and four replications per treatment was used. The four pathogen treatments included non-infested soil, soil infested with *M. incognita* (4 eggs/cm^3), soil infested with *T. basicola* (40 chlamydospore chains/cm^3) and soil infested with both pathogens (4 eggs/cm^3 + 40 chlamydospore chains/cm^3). *T. basicola* spore suspensions were diluted in 2 mL of sterile distilled water and then sprayed onto each soil portion and mixed thoroughly in a plastic bag by hand. *M. incognita* eggs were suspended in distilled sterile water, and applied into two holes (0.5cm diameter by 5 cm-deep) per pot.

Soil columns were compacted to obtain bulk densities (BD) of 1.25 or 1.50 g/cm^3. Soil for each pot was added in four portions to ensure the uniformity of soil bulk density throughout the pot. Each portion was added and packed individually until the soil reached a previously marked 5 cm-line. Twenty-four hours after pots were watered for the first time, a SC 900 Soil Compaction Meter (Spectrum Technologies, Inc., Plainfield, IL, USA) was used to measure soil strength in both bulk density treatments.

Twelve seeds of the cotton cultivar DP 555 BG/RR (Delta and Pine Land Company, Scott, MS) were planted 2.5cm deep in each pot. Pots were placed into growth chambers with nocturnal temperatures of 15ºC and a 14-hr photoperiod with day temperatures of 24ºC during the first 22 d after planting (DAP). From the 23rd day to the end of the experiment, a night temperature of 19ºC and 26ºC during day time were set using the same 14-hr photoperiod. These temperatures were used to approximate typical soil temperatures during the early cotton season

in Arkansas (Monfort et al., 2006). Soil temperature was monitored using a Model 450 WatchDog Data Logger (Spectrum Technologies, Inc., Plainfield, IL, USA). Pots were watered from the bottom when the matric potential of the soil in the pots reached -20 joules/kg by weight. Each experiment was conducted for 44d.

Seedling development. Plant emergence was assessed 12 DAP, and seedling density was thinned to 2 plants per pot. Plants were removed 44 DAP. The height from the cotyledonary node to the tip of the main stem terminal was measured, and the number of nodes was counted for each plant. The portion of the plant above the cotyledonary node was dried at 60°C and weighed. Root systems from each plant were rinsed with tap water for 20 min. Root systems were scanned to acquire root images and analyzed with the WinRHIZO Image Analysis System (Regent instruments Inc., Quebec, Canada) to assess root growth characteristics including: surface area, volume, number of links, average radius, and various topological features.

Root topology describes the non-metric aspects of branching structure that rely on a basipetal ordering system in which the exterior link has a magnitude of 1 and the magnitude of any internal link has a magnitude equal to the sum of the magnitudes of the links to which it gave rise to developmentally (Fitter et al., 1991). In this study, links were divided into interior links (the segment between two junctions) and exterior links (roots ending in a meristem). Calculated topological parameters included magnitude (μ), which is the number of exterior links; altitude (α), which indicates the number of links in the longest unique path from the base link to an exterior link; total exterior pathlength (*Pe*), which is the sum of links in all possible unique paths from the base link to all exterior links, exterior-interior links (EI), and exterior-exterior links (EE) (Fitter, 1987). Topological index (TI) characterizes the branching structure of one whole root system and allows comparisons of root systems with theoretical topological extremes in

branching (herringbone or dichotomous). The value of TI was determined by the slope of the regression line from double-logarithmic ($\log_e$) plots of *Pe* against μ (Fitter, 1987; Fitter and Setters, 1988; Larkin et al., 1995, 1996). The values for TI range from 1.92 for a herringbone model to 1.20 for a dichotomous pattern, and the TI value would be 1.52 for a root system with equal branch initiation on all root links (Werner and Smart, 1973).

Disease assessment. After scanning root systems, each plant was immersed for 2 min in 0.5% NaOCl, blotted dry in a paper towel, and weighed. Roots were rated for discoloration caused by *T. basicola* using a scale of 0 to 10, where 0 = 0%, 1 = 1 to 10%, 2 = 11 to 20%, 3 = 21 to 30%, 4 = 31 to 40%, 5 = 41 to 50%, 6 = 51 to 60%, 7 = 61 to 70%, 8 = 71 to 80%, 9 = 81 to 90 %, and 10 = 91 to 100% of the root system being discolored. Root systems were also evaluated for nematode galling on a scale of 0 to 5, where 0 = 0, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 50, and 5 = 51-100 galls/root system. Roots were placed on an amended TB-CEN medium (Specht and Griffin, 1985), which was modified by adding Penicillin G (60 mg/L), kept in the dark at room temperature (20 to 23°C), and after 12 d, the percentage of the root system with growth of *T. basicola* on the medium was rated using the same scale as for root discoloration.

Statistical analysis. The experiment was conducted twice. Mid-values for each rating scale were used for analyses. Statistical analyses were conducted using the GLM procedure with SAS 9.2 (SAS Institute Inc., Cary, NC). Treatment means were separated with Fisher's protected least significant difference (LSD) at $P \leq 0.05$. When interactions were significant ($P \leq 0.05$), appropriate interaction means were examined and LSDs were calculated.

RESULTS

Seedling growth. A number of main effects and interactions were observed for seedling growth parameters (Table 1).The only significant effect for plant stand was a *M. incognita* by *T. basicola*

interaction. Both pathogens together reduced the emergence of cotton seedlings compared to either pathogen alone (Table 2). Because of a significant run by bulk density by *M. incognita* by *T. basicola* interaction for plant height (Table 1, $P = 0.0079$), each experimental run was analyzed separately. Plant height was reduced by either *M. incognita* or *T. basicola* over both bulk densities and in both experimental runs compared to soil without the pathogen (Table 2). Plants tended to be taller in the larger bulk density soil, and differences were significant in the second experimental run. A bulk density by *M. incognita* by *T. basicola* interaction was occurred for the number of stem nodes (Table 1). Plants grown in non-infested soils at 1.50 g/cm^3 had fewer nodes than those grown in non-infested soils at a bulk density of 1.25 g/cm^3 (Table 3). Both pathogens reduced the number of nodes at a bulk density of 1.25 g/cm^3, but not at bulk density of 1.50 g/cm^3. The combination of *M. incognita* and *T. basicola* reduced the number of nodes in both bulk densities compared to either pathogen alone or the non-infested control (Table 3). *M. incognita* and *T. basicola* significantly reduced root fresh weight and top dry weight of seedlings (Table 2).

Disease. Both *M. incognita* and soil bulk density influenced the amount of root colonization by *T. basicola* (Table 4). The percentage of root area colonized by the fungus increased in the presence of the nematode and decreased for the larger bulk density (Table 5). Root discoloration was not impacted by the presence of the nematode or by soil bulk density. An experimental run by bulk density by *T. basicola* interaction was observed for *M. incognita*-induced root galling (Table 4). Galling tended to be more severe at the larger bulk density, with differences being significant in the first experimental run (Table 5). Galling was significantly suppressed in the presence of *T. basicola* in the second experimental run.

Root development and architecture. For root growth and architectural parameters, no three-way interactions of soil bulk density by *M. incognita* by *T. basicola* were observed and few two-way interactions were present (Table 6). *M. incognita* affected all root morphological parameters, except for root volume, and all the architectural parameters (Table 6). Similarly, *T. basicola* affected all morphological and architectural parameters, except average radius. Soil bulk density had a significant influence on root surface area, root volume, magnitude, and exterior pathlength (Table 6). A two-way interaction of *M. incognita* by *T. basicola* was present with seedling root volume. Root volume decreased in the presence of both pathogens compared to the reduction in root volume for either pathogen alone (Table 7).

M. incognita reduced root surface area, total root length, number of links, as well as topological parameters including magnitude, altitude, and exterior pathlength (Table 7 and 8). After infestation by *M. incognita*, root surface area and total root length were reduced to 30% and 40%, respectively, compared to soil not having *M. incognita*. The number of links for root systems in soil infested with *M. incognita* also was reduced by 36% (Table 7). The topological parameters of magnitude, altitude, and exterior pathlength decreased 41%, 27% and 45%, respectively, compared to soil without *M. incognita* (Table 8). Infestion with *M. incognita* increased the average radius of roots 34% compared with soil not infested by the nematode (Table 7).

In soil infested with *T. basicola*, the root surface area, total root length, and total number of links were 48%, 51% and 64% lower than in soil not infested with the pathogen (Table7). The magnitude and exterior pathlength of root systems in soil infested with *T. basicola* were significantly reduced by 46%, and 64%, respectively, as compared with soil not infested with the *T. basicola*. A two-way interaction of experimental run by *T. basicola* occurred for altitude ($P =$

0.0105) and average radius ($P = 0.0006$) and no consistent response was observed (Table 7 and 8). Soil with larger soil bulk density had increased root surface area and root volume (Table 7). The same trend was observed in topological parameters, where both the magnitude and exterior pathlength were greater at 1.50 g/cm^3 compared to 1.25 g/cm^3 (Table 8).

For the topological index (TI) determined by the regression slope from double-log plots of the root system topological parameters, total exterior pathlength versus magnitude for cotton seedlings was only significant for *M. incognita*. Soil infestion with *M. incognita* significantly increased TI ($P = 0.0002$) from 1.79 to 1.89, demonstrating a less branched (herringbone) root system (data not shown).

DISCUSSION

Plant growth was generally not affected by soil bulk density in this study. Previous studies have shown that cotton growth was reduced by soil bulk densities greater than 1.55 g/cm^3 or soil strength equal to or greater than 2.96 MPa (Lowry et al., 1970). However, it is soil strength (resistance) rather than the soil bulk density that produces a critical impedance factor controlling root penetration in soils (Taylor and Gardner, 1963). A highly significant linear correlation (r = -0.96) between the soil strength and the percent root penetration has been demonstrated (Taylor and Gardner, 1963). Cotton root penetration in soils is positively related to soil moisture (Taylor and Gardner, 1963), and at a given bulk density, soil resistance to root penetration increased with soil drying (Coelho et al., 2000). Taylor and Gardner (1963) reported that at a bulk density of 1.55g/cm^3 about 90% of the taproots penetrated soil at 8% water content by weight, but only 40% penetrated at 5.5% water content. Although the largest soil bulk density in this study was 1.50 g/cm^3, which is close to the 1.55 g/cm^3 that was reported to restrict root development, the mechanical impedance after watering was 2.6 MPa, considerably smaller than

2.96 MPa. Thus, under the well-watered conditions of this study, root growth and development would not likely have been impeded. *M. incognita* generally reduced plant growth in both bulk densities. *T. basicola* also decreased plant growth in bulk density treatments. These results suggest that soil bulk density may not affect damage to cotton plants caused by *M. incognita* or *T. basicola* when soil moisture is not limiting. However, the soil water potential ranges in the field and differences in soil water levels for different bulk densities may increase the differences that could be observed in field situations at these bulk densities. An increase in soil bulk density was one of the only soil parameters observed to be associated with areas of stunted cotton in producer's fields where increased damage, especially root galling, from these pathogens was observed compared to adjacent areas that were not stunted (Jaraba-Navas, 2007).

The impact of soil bulk density and *T. basicola* on nematode galling was not consistent across both experimental runs, possibly due to less severe galling on plants in the first run. Results from preliminary experiments using bulk densities of 1.25 and 1.40 g/cm^3 demonstrated that galling did not differ between these soil bulk densities (data not shown). *M. incognita* juveniles migrate in soils through pore spaces with diameters of 30 to 100 μm and >100 μm partially filled with water, and migration of *M. incognita* is negatively related to soil bulk density (Eo et al., 2007). Nematode migration in soil decreased when soil density increased from 0.60 to 0.85 g/cm^3 for a Kanto loam (humicandosol, loam) composed of 29% sand, 38% silt, and 33% clay owing to reductions in the volume of pores with diameters suitable for nematode migration in soil. Nematodes move faster in soil pores partially filled with water (approximately 40 to 60% of water holding capacity) than in saturated pores (Wallace, 1960). In this study, the air-filled porosities for both bulk densities when soils reached -20 joules/kg by weight were 0.34 $cm^3 \cdot cm^{-3}$ and 0.24 $cm^3 \cdot cm^{-3}$ for soil bulk densities of 1.25 and 1.50 g/cm^3, respectively, which are within

the ideal ranges for nematode mobility in soil. The presence of partially unsaturated pores that favor nematode migration in both bulk densities under the soil conditions in this study may explain why nematode galling was not affected by soil bulk density. The greater bulk density tended to increase root architectural parameters compared with the lower bulk density, as can be seen by larger magnitude and exterior pathlength, greater surface area and average root volume, compared with the lower soil bulk density.

The reduction of *M. incognita* galling by the presence of *T. basicola* has been documented in other studies (Walker et al., 1998, 2000). *M. incognita* has been reported to increase the root colonization by *T. basicola* (Walker et al., 1998, 2000), which is in agreement with this study. Root colonization by the fungus decreased at the larger bulk density, implying that disease development may change as soil bulk densities change. However, root discoloration was not affected by soil bulk density. These results differed from a previous study by Bhatti and Kraft (1992) who showed that black root rot in chickpea (*Cicer arietinum* L.) was more severe at 1.50 g/cm^3 than at 1.25 g/cm^3. These conflicting results may be due to differences in inoculum rate of *T. basicola*, different soil water contents, or differences in hosts used in the studies. In addition, inoculum rate in the chickpea study was 20% greater in the compacted soil than in the less compacted soil by volume since the number of propagules used was based on weight rather than on volume. Soils also were dryer in the chickpea experiment (-26 to -40 joules/kg) than in the present study (-1 to -20 joules/kg).

Pathogen-induced changes in root morphology were first reported in alfalfa by Larkin et al. (1995, 1996). In comparison with alfalfa grown in non-infested soil, total root length, total number of root segments of all morphometic orders (first, second and third-order) roots, as well as root system magnitude, altitude and exterior pathlength was reduced in soil infested with *P.*

irregulare or *P. ultimum*. Large changes in the topological index (TI), which characterizes the branching structure of the whole root system, were also observed in soil infested with *P. irregulare* (TI = 1.86) or *P. ultimum* (TI = 1.72) for alfalfa compared with the TI of healthy alfalfa roots. However, some other *Pythium* spp., such as *P. torulosum*, *P. sylvaticum,* and *P. dissotocum*, showed little ability to alter branching structure of alfalfa roots. *Pythium* spp. infect roots behind the root cap, which is similar to the preferred site of infection by *M. incognita*, and affects juvenile root tissue. The primary effect caused by *Pythium* spp. was to reduce the overall size and length of affected root systems, which may have resulted in a delay in the growth and development of the root systems or the loss of colonized root segments and branches due to necrosis and death (Larkin et al., 1995).

Feeding by the root-knot nematode results in a disruption of the vascular system that may limit nutrient and water flow in the vascular system of the plant (Koenning et al., 2004). After infection, cotton root growth was reduced and root length decreased up to 28% (Kirkpatrick et al., 1991). In addition, leaf transpiration rates and stomatal conductance decreased, while leaf temperature increased (Kirkpatrick et al., 1995). In this study, a similar reduction of root length after infestion by either *M. incognita* or *T. basicola* alone was observed. Infection by *M. incognita* significantly reduced total root length, root surface area, total number of root links as well as topological attributes, including magnitude, altitude, and exterior pathlength, compared with plant root systems in soil not having the nematode. Infection of *T. basicola* also altered root growth by reducing total root length, root surface area, total number of root links, and root magnitude, altitude and exterior pathlength. Root-knot nematodes dramatically increased the average radius of the root (up to 34%) compared with soil not infested with the nematode. Hypertrophy and hyperplasia of the root tissue induced during the formation of galls likely

increased the radius of affected root regions. Fitter et al. (1991) reported that the radius of the root could greatly influence the exploitation efficiency of the root system, but not the topology characteristics. Consequently, the increased radius due to nematode infection in our study likely reduced rather than enhanced the root exploitation ability for water and nutrients.

In contrast to *Pythium* damage in alfalfa, no TI differences were observed for *T. basicola* infestion alone. The TI for root systems for all treatments was near 1.92 indicating a low degree of branching or a herringbone structure. Infection by *M. incognita* significantly increased TI (TI = 1.89) compared with the TI of root systems grown in the absence of *M. incognita* (TI = 1.79). This lack of change in the TI for root systems affected by *M. incognita* or *T. basicola* may be the result of cotton seedlings have a strong taproot, herringbone pattern, or reflect the differences in colonization of tissue between *Pythium* spp. and the pathogens in this study. Although *M. incognita* infects the root behind the root cap, this obligate parasite takes a long period to develop a feeding site and does not likely impact the developing root system until much later in root development. The cortical colonization of *T. basicola* results in a cortical root rot and also does not affect roots near the root tip in contrast to *Pythium* spp.

The synergistic interaction between *M. incognita* and *T. basicola* reduced cotton stand, seedling growth and yield (Monfort, 2005; Monfort et al., 2006; Walker et al., 2000), results that were also observed in this study. The interaction of *M. incognita* and *T. basicola* in this experiment was not as dramatic as generally observed in the field, which may explain the lack of significant effect in some instances. In this study, the effects of the interaction between *M. incognita* and *T. basicola* on cotton was evident for stand and the number of stem nodes. *M. incognita* and *T. basicola* together significantly reduced root volume in comparison with healthy roots or roots in soil infested with either pathogen alone implying that the presence of both

pathogens caused more severe damage to root systems than either pathogen alone. However, relative to root development, each pathogen alone reduced more root parameters than the combined effects of both pathogens.

In this study, soil bulk density generally displayed no effects on both plant growth and disease severity. *M. incognita* and *T. basicola* interaction reduced height-to-node ratio and root volume more than either pathogen alone. Both *M. incognita* and *T. basicola* infection reduce root topological parameters but topological index changed only by *M. incognita*. The traditional approach to study the effect of soilborne pathogens, such as the root-knot nematode and *T. basicola*, on a host plant has been based on estimates of galling, root discoloration or biomass. However, pathogen-induced changes in root system morphology as well as root architecture have potential to provide quantitative rather than qualitative information. Root branching plays a crucial role in the exploration of the soil and water and nutrient uptake and is considered as an important adaptive strategy for survival (Fitter et al., 1991). Studying the root architecture using a topological model provides an additional approach to evaluating fungal-nematode interactions for soilborne pathogen systems.

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Table 1. *P*-values for analysis of variance of the effects of soil bulk density (BD)[v], *Meloidogyne incognita* (Mi)[w] and *Thielaviopsis basicola* (Tb)[x] on cotton seedling growth[y]

Source	Stand[z]	Height (cm)	Nodes	Root fresh weight (g)	Top dry weight (g)
Run	0.8952	0.2770	0.6875	0.0573	0.0251
BD	0.6494	0.0480	0.6875	0.0773	0.5602
Run*BD	0.9273	0.1204	0.4252	0.0573	0.9439
Mi	0.2471	<.0001	0.0002	0.0005	<.0001
Run*Mi	0.9273	0.0001	0.0592	0.2969	0.6239
BD*Mi	0.6494	0.2559	0.2382	0.6577	0.3221
Run*BD*Mi	0.9273	0.8429	0.4252	0.4021	0.9813
Tb	0.1852	<.0001	<.0001	<.0001	<.0001
Run*Tb	0.9273	0.0046	0.0592	0.1960	0.5001
BD*Tb	0.1852	0.0109	0.2382	0.6772	0.8330
Run*BD*Tb	0.9273	0.3004	1.0000	0.6011	0.1430
Mi*Tb	0.0358	0.0151	0.0054	0.2969	0.4174
Run*Mi*Tb	0.9273	0.0286	0.6875	0.4957	0.1218
BD*Mi*Tb	0.9273	0.3610	0.0024	0.9678	0.8330
Run*BD*Mi*Tb	0.9273	0.0079	0.1227	0.9249	0.6910

[v] Soil bulk densities were 1.25 and 1.50 g/cm^3.
[w] Soils were infested at planting with 4 eggs of *M. incognita* /cm^3 soil.
[x] Soils were infested at planting with 40 chlamydospore chains of *T. basicola* /cm^3 of soil.
[y] Plant variables were measured 44 days after planting. Analysis based on two runs and four replications per run. Data from Jaraba-Navas, 2011.
[z] The number of surviving seedlings 12 days after planting.

Table 2. The effects of soil bulk density (BD)[v], *Meloidogyne incognita* (Mi)[w], and *Thielaviopsis basicola* (Tb)[x] on cotton seedling growth[y]

Main effect	Stand		Height (cm) Run 1	Height (cm) Run 2	Root fresh weight (g)	Top dry weight (g)
BD						
1.25	9.3 a[z]		3.9 a	3.0 b	0.424 a	0.270 a
1.50	9.0 a		4.2 a	4.2 a	0.337 a	0.286 a
	Tb					
	0	40				
Mi						
0	9.2 ab	9.9 a	4.5 a	4.6 a	0.484 a	0.351 a
4	10.0 a	7.5 b	3.6 b	2.6 b	0.278 b	0.206 b
Tb						
0			5.6 a	4.7 a	0.534 a	0.367 a
40			2.6 b	2.9 b	0.227 b	0.190 b

[v] Soil bulk densities were 1.25 and 1.50 g/cm^3.
[w] Soils were infested at planting with 4 eggs of *M. incognita* /cm^3 soil.
[x] Soils were infested at planting with 40 chlamydospore chains of *T. basicola* /cm^3 of soil.
[y] Plant variables were measured 44 days after planting. Stand for the 12 seed planted was measured 12 days after planting. Data from Jaraba-Navas, 2011.
[z] Means in a column and main effect or interaction followed by a common letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference (LSD). Parameters means were analyzed separately if a run by main effect interaction was found.

Table 3. Three way interaction of soil bulk density (BD)[v], *Meloidogyne incognita* (Mi)[w], and *Thielaviopsis basicola* (Tb)[x] on seedling stem nodes[y]

BD	Mi	Tb	Nodes
1.25	0	0	3.7 a[z]
1.25	0	40	2.2 c
1.25	4	0	2.7 bc
1.25	4	40	1.4 d
1.50	0	0	2.7 bc
1.50	0	40	2.7 bc
1.50	4	0	3.2 ab
1.50	4	40	1.1 d

[v] Soil bulk densities were 1.25 and 1.50 g/cm^3.
[w] Soils were infested at planting with 4 eggs of *M. incognita* /cm^3 soil.
[x] Soils were infested at planting with 40 chlamydospore chains of *T. basicola* /cm^3 of soil.
[y] Main stem nodes were measured 44 days after planting. Data from Jaraba-Navas, 2011.
[z] Means in a column followed by a common letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference (LSD).

Table 4. *P*-values for analysis of variance for the effects of soil bulk density (BD)[u], *Meloidogyne incognita* (Mi)[v], and *Thielaviopsis basicola*(Tb)[w] on root discoloration and colonizatiox[y] by *Thielaviopsis basicola* or galling[y] by *Meloidogyne incognita*[z]

Source	Root discoloration (%)	Root colonization (%)	Galling
Run	0.2565	0.8833	0.0001
BD	0.3419	0.0367	<.0001
Tb			<.0001
BD*Tb			0.0031
Run*BD			<.0001
Run*Tb			0.0008
Run*BD*Tb			0.0001
Mi	0.0966	0.0023	
BD*Mi	0.5706	0.2875	
Run*BD	0.8290	0.8488	
Run*Mi	0.2424	0.9493	
Run*BD*Mi	0.7486	0.8488	

[u] Soil bulk densities were 1.25 and 1.50 g/cm^3.
[v] Soils were infested at planting with 4 eggs of *M. incognita* /cm^3 of soil.
[w] Soils were infested at planting with 40 chlamydospore chains of *T. basicola* /cm^3 of soil.
[x] Root discoloration and colonization: 0 = 0%, 1 = 1 to 10%, 2 = 11 to 20%, 3 = 21 to 30%, 4 = 31 to 40%, 5 = 41 to 50%, 6 = 51 to 60, 7 = 61 to70, 8 = 71 to 80, 9 = 81 to 90 and 10 = 91 to 100%. Analyses were conducted using mid-point values. Treatments without *T. basicola* were dropped from the analyses.
[y] Root galling: where 0 = 0, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 50, and 5 = 51 –100 galls/root. Analyses were conducted using mid-point values. Treatments without *M. incognita* were dropped from the analyses.
[z] Data from Jaraba-Navas, 2011.

Table 5. The effects of soil bulk density (BD)[t], *Meloidogyne incognita* (Mi)[u], and *Thielaviopsis basicola* (Tb)[v] on root discoloration and colonization[w] by *Thielaviopsis basicola*or galling[x] by *Meloidogyne incognita*[y]

Main effect	Root discoloration (%)	Root colonization (%)	Galling	
			Run 1	Run 2
BD				
1.25	20.8 a[z]	92.8 a	7.5 b	44.2 a
1.50	30.0 a	81.9 b	27.1 a	49.2 a
Mi				
0	33.7 a	78.7 b		
4	17.2 a	95.9 a		
Tb				
0			22.8 a	75.0 a
40			11.7 a	18.5 b

[t] Soil bulk densities were 1.25 and 1.50 g/cm^3 of soil.
[u] Soils were infested at planting with 4 eggs of *M. incognita* /cm^3 of soil.
[v] Soils were infested at planting with 40 chlamydospore chains of *T. basicola* /cm^3 of soil.
[w] Root discoloration or colonization: 0 = 0%, 1 = 1 to 10%, 2 = 11 to 20%, 3 = 21 to 30%, 4 = 31 to 40%, 5 = 41 to 50%, 6 = 51 to 60, 7 = 61 to70, 8 = 71 to 80, 9 = 81 to 90 and 10 = 91 to 100% Analyses were conducted using mid-point values. Treatments without *T. basicola* were dropped from the analyses.
[x] Root galling: where 0 = 0, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 50, and 5 = 51 –100 galls/root. Analyses were conducted using mid-point values. Treatments without *M. incognita* were dropped from the analyses
[y] Data from Jaraba-Navas, 2011.
[z] Means in a column and main effect followed by a common letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference (LSD). Parameters means were analyzed separately if a run by main effect interaction was found.

Table 6. P-values for analysis of variance of the effects of soil bulk density (BD)[w], *Meloidogyne incognita* (Mi)[x] and *Thielaviopsis basicola* (Tb)[y] for root morphological parameters[z] 44 days after planting.

	Root length (cm)	Surface area (cm^2)	Root volume (cm^3)	No. of links	Average radius (mm)	Magnitude	Altitude	Exterior pathlength
Run	0.0142	0.0321	0.3519	0.0077	0.2328	0.2713	0.0807	0.3555
BD	0.1107	0.0333	0.0104	0.1562	0.2954	0.0039	0.6477	0.0233
Run*BD	0.1388	0.2469	0.9160	0.2496	0.2949	0.6773	0.0014	0.1877
Mi	<.0001	0.0004	0.2482	0.0061	<.0001	<.0001	0.0032	0.0040
Run *Mi	0.1415	0.0755	0.0356	0.3936	0.5073	0.3999	0.4199	0.7318
BD*Mi	0.4873	0.9777	0.5458	0.6026	0.8270	0.3080	0.9987	0.2310
Run*BD*Mi	0.7819	0.4050	0.0813	0.8805	0.1071	0.5088	0.5473	0.8103
Tb	<.0001	<.0001	<.0001	<.0001	0.2356	<.0001	<.0001	<.0001
Run*Tb	0.0584	0.5445	0.2470	0.0832	0.0006	0.5623	0.0105	0.3183
BD*Tb	0.6221	0.5763	0.6122	0.1921	0.4882	0.4286	0.1393	0.1681
Run*BD*Tb	0.8352	0.8829	0.4592	0.7686	0.1973	0.6700	0.1211	0.6375
Mi*Tb	0.9427	0.1581	0.0029	0.5786	0.2555	0.9365	0.4191	0.4958
Run*Mi*Tb	0.5560	0.6990	0.7800	0.4185	0.0856	0.6850	0.8458	0.4380
BD*Mi*Tb	0.0728	0.2485	0.6583	0.5410	0.0720	0.5556	0.4855	0.4536
Run*BD*Mi*Tb	0.2747	0.5985	0.6076	0.8845	0.1864	0.7881	0.3168	0.9925

[w] Soil bulk densities were 1.25 and 1.50 g/cm^3.
[x] Soils were infested at planting with 4 eggs of *Meloidogyne incognita* /cm^3 soil.
[y] Soils were infested at planting with 40 chlamydospore chains of *Thielaviopsis basicola* /cm^3 of soil.
[z] Root morphological parameters include number of links (the length of root between two nodes or junctions of two root branches), magnitude (μ, the number of exterior links), altitude (α, the number of links in the longest path from the base link to an exterior link), and total exterior pathlength (*Pe,* the sum of links in all possible paths from the base link to all exterior links).

Table 7. The effects of soil bulk density (BD)[v], *Meloidogyne incognita* (Mi)[w] and *Thielaviopsis basicola* (Tb)[x] on cotton root growth.

Main effect	Rootlength (cm)	Rootvolume (cm^3)		Surface area (cm^2)	Averageradius (mm)		Links[y]
BD							
1.25	125.43 a	0.40 b		24.52 b	0.35 a		1313.6 a
1.50	151.69 a	0.51 a		30.26 a	0.37 a		1665.7 a
		Tb					
		0	40				
Mi							
0	172.57 a[z]	0.55 ab	0.41 b	32.01 a	0.31 b		1802.6 a
4	103.02 b	0.63 a	0.22 c	22.53 b	0.41 a		1161.0 b
Tb					Run1	Run2	
0	184.21 a			35.79 a	0.31 b	0.39 a	2166.4 a
40	91.00 b			18.63 b	0.39 a	0.35 a	785.4 b

[v] Soil bulk densities were 1.25 and 1.50 g/cm^3.
[w] Soils were infested at planting with 4 eggs of *M. incognita* /cm^3 soil
[x] Soils were infested at planting with 40 chlamydospore chains of *T. basicola* /$cm^3$of soil
[y] The length of root between two nodes or junctions of two root branches.
[z] Means in a column and main effect or interaction followed by a common letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference (LSD). Parameters means were analyzed separately if run by main effect interaction were found.

Table 8. The effect of soil bulk density (BD)[v], *Meloidogyne incognita* (Mi)[w] and *Thielaviopsis basicola* (Tb)[x] on root system topological parameters[y] of cotton seedlings

Main effect	Magnitude	Altitude		Exterior pathlength
BD		Run1	Run2	
1.25	61.3 a	89.4 a	54.2 a	2133.5 a
1.50	84.9 b	63.2 a	74.5 a	3458.1 b
Mi				
0	91.2 a[z]	80.8 a		3581.5 a
4	54.0 b	59.3 b		1963.4 b
Tb		Run1	Run2	
0	93.9 a	100.5 a	70.3 a	4073.0 a
40	51.2 b	52.0 b	57.3 a	1456.0 b

[v] Soil bulk densities were 1.25 and 1.50 g/cm^3.
[w] Soils were infested at planting with 4 eggs of *M. incognita* /cm^3 soil
[x] Soils were infested at planting with 40 chlamydospore chains of *T. basicola* /cm^{3}of soil.
[y] Root morphological parameters magnitude (μ, the number of exterior links), altitude (α, the number of links in the longest path from the base link to an exterior link), and total exterior pathlength (*Pe,* the sum of links in all possible paths from the base link to all exterior links).
[z] Means in a column and main effect followed by a common letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference (LSD). Parameters means were analyzed separately if run by main effect interaction were found.

Chapter III

Effects of Subsoiling and the Nematicide *1,3-dichloropropene* on Root Morphology and Plant Growth of Cotton

ABSTRACT

The effects of subsoiling and application of the nematicide *1,3-dichloropropene* on root system development and plant growth were investigated from 2009 to 2011 in a commercial cotton field in northeastern Arkansas. The four treatments were subsoiling with an Ecolo TIL® 2500 chisel plow, in-row application of the nematicide *1,3-dichloropropene* with a Yetter Avenger®, subsoiling plus *1,3-dichloropropene*, and a control that was neither subsoiled nor treated with the nematicide. Subsoiling did not consistently affect plant development of seedlings except in 2010 when an increase in root fresh weight and volume was observed. Nematicide application increased height-to-node ratio and plant dry weight in 2010 and 2011 and root fresh weight and taproot length was increased with nematicide treatment on seedlings in 2011. Nematicide application also increased root magnitude in 2009 and root volume in 2011 in early season samples. Subsoiling and nematicide treatments did not affect late season cotton growth or yield. Neither subsoiling nor nematicide application had much effect on root topological characters (magnitude, altitude and exterior pathlength) or root topological index using WinRHIZO® image analysis. Root galling and the population of second-stage juveniles of *M. incognita* were suppressed by Telone II®.

INTRODUCTION

The southern root-knot nematode, *Meloidogyne incognita* Kofoid & White (Chitwood), is one of the most detrimental nematode species on cotton (*Gossypium hirsutum* L.) in the U.S. (Blasingame and Patel, 2001; Overstreet and McGawley, 2001). *Meloidogyne incognita* is a sedentary endoparasite that induces gall formation around the nematodes as a response to infection (Huang, 1985). Severe galling may lead to plant stunting, and galling may limit water flux, lower transpiration, and increase stomatal resistance to opening (Kirkpatrick et al., 1995). Another soilborne pathogen that can be a significant factor on cotton seedling development is *Thielaviopsis basicola* (Berk. & Broome) Ferris (syn. *Chalara elegans* Nag Raj & Kendrick), the causal agent of black root rot of seedlings. *M. incognita* and *T. basicola* together may result in an interaction that can cause increased seedling mortality, decreased seedling growth, and lowered yields (Walker et al., 1998, 1999, 2000). The typical symptom caused by *T. basicola* infection of cotton seedlings is a cortical root rot. The colonized epidermis and cortical tissue may slough off and result in stunting of affected seedlings (Allen, 2001).

M. incognita and *T. basicola* were reported to occur together frequently in Arkansas cotton fields (Rothrock and Kirkpatrick, 1998). Both pathogens may also alter root system morphology and functionality (Kirkpatrick et al., 1995; Walker et al., 2000). Histological examination of roots indicated the infection by *M. incognita* facilitated colonization of vascular tissue by *T. basicola* that was not observed without the nematode (Walker et al., 1999). Soil texture has been shown to play a crucial role in the survival of both *M. incognita* and *T. basicola* (Monfort, 2005).

Roots are important for absorbing water and minerals from the soil environment for transport to aboveground parts, and roots serve to anchor the plant. As roots penetrate through

the soil, they encounter mechanical stress (Lipiec et al., 2003). In some cases, this stress may be related to compaction layers that form in response to planting or harvesting operations (Harveson et al., 2005). The primary features of compacted soil are increased soil strength and decreased air permeability and hydraulic conductivity (Allmaras et al., 1988; Whalley et al., 1995). Soil strength (resistance to penetration) is a key factor that impedes root penetration (Medvedev, 2009; Taylor and Gardner, 1963). A significant negative linear relationship between soil strength and the root penetration percentage has been reported (Taylor and Gardner, 1963). Soil resistance to penetration in compacted soil starts to inhibit root growth for most plants at 1.5 MPa, and roots cease to grow altogether when soil resistance is about 2.5 MPa (Coelho et al., 2000).

Soil compaction reduces crop production worldwide (Raper, 2005). Cotton grows poorly and develops slowly when grown in soil with high strength (Bennie and Burger, 1981; Taylor et al., 1964). Tillage pans, formed during tillage operations, may inhibit root proliferation (Campbell et al., 1974), although tillage may also be used to destroy tillage pans (Taylor and Burnett, 1963) through a process known as subsoiling (Raper et al., 2000a, b; Schwab et al., 2002). Subsoiling has been shown to increase hydraulic conductivity, reduce soil resistance and bulk density, increase rooting depth and enhance cotton yield (Borghei et al., 2008; Busscher et al., 1986; Mullins et al., 1992; Raper, 2005; Raper et al., 1998; Simoes et al., 2009; Vepraskas et al., 1995).

Root-knot nematode infection, black root rot, and soil compaction all may change cotton root system morphology, and all may occur in the same field. However, to date, no research has been reported on the effects of these factors collectively on cotton root morphology and plant growth. The hypothesis for this study is that a plow pan restricts cotton growth and root

development and the presence of a plow pan may increase losses from the root-knot nematode. The objective of this study was to determine the effects of subsoiling and the application of a fumigant nematicide on cotton root morphology and plant growth in a commercial cotton field.

MATERIALS AND METHODS

The study was conducted in a cotton field near the town of Leachville in Mississippi County, Arkansas in 2009, 2010 and 2011. The field was selected based on a history of both *M. incognita* and *T. basicola*, and the presence of an obvious compaction layer approximately 15 cm below the soil surface. The four treatments were subsoiling followed by Telone II® application (ST), subsoiling without Telone® II (SNT), Telone II® application without subsoiling (NST) and a control that received neither subsoiling nor Telone II® (NSNT). The subsoiling operation was conducted by the grower using his equipment. In January 2009, an Ecolo-TIL® 2500 (Case IH, CNH America LLC, Racine, WI) was used to subsoil four strips across the field. The strips were 48 rows (92-cm spacing) wide and depth of the subsoiling operation was about 39 cm. Subsoiled strips were alternated with 48 strips that were not subsoiled. Eight weeks prior to sowing, the soil fumigant, *1,3-dichloropropene,* (Telone II®, Dow AgroSciences LLC. Indianapolis, IN, USA), was applied at a rate 114 mL/m^2 to 12 of the cotton rows in the right side of each subsoiled strip and at the left side of each non-subsoiled strip using a Yetter Avenger with 25-inch coulters (Yetter Manufacturing, Inc., Colchester, IL, USA). In subsequent years the plot area was shifted 24 rows. As a result of shifts in tillage plots, the non-subsoiled plots had not been subsoiled prior to the start of the experiment in 2009. However in 2010 and 2011, the non-subsoiled plots had been subsoiled in 2009.

The commercial cotton cultivar, DPL0912 (Delta and Pine Land Company, Scott, MS), was planted in the trial all three years. The sowing dates were 13 May in 2009, 1 May in 2010

and 17 May in 2011. Weather data was obtained from the Judd Hill Plantation weather station located in Poinsett County, Arkansas, approximately 50 miles from the field. Each year, sampling locations were arbitrarily selected in each location and their latitude and longitude was marked with a Trimble® Nomad® Outdoor Rugged Handheld Computer (Trimble Navigation Limited, Sunnyvale, CA). Cotton plant samples were taken early in the season (at the seedling stage) and immediately prior to harvest. Ten, twenty and thirty seedlings for each plot and 160, 320, and 480 seedlings for the entire field with intact root systems were collected, respectively, on 11 June in 2009, 22 June in 2010, 15 June in 2011. Plant height was measured from the cotyledonary node to the tip of the main stem terminal and the numbers of nodes on the main stem were counted. Total leaf areas were determined using a LI-3100 Area Meter (LI-COR Biosciences Inc., Lincoln, NE, USA). Leaves and plant stems above the cotyledonary node were dried at 60°C in an oven for 24 hr to measure dry weights. The root system of each plant was first rinsed with tap water for 20 min, then immersed for 2 min in 0.5% NaOCl, dried in a paper towel and weighed. Each root system was rated for root discoloration using a scale of 0 to 10, where 0 = 0%, 1 = 1 to 10%, 2 = 11 to 20%, 3 = 21 to 30%, 4 = 31 to 40%, 5 = 41 to 50%, 6 = 51 to 60%, 7 = 61 to70%, 8 = 71 to 80%, 9 = 81 to 90% and 10 = 91 to 100% of the root system discolored. Root systems were evaluated for root galling caused by *M. incognita* using a scale of 0 to 5, where 0 = no galls, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = >100 galls/root system. Root systems were then scanned by a high-resolution image scanner and a WinRHIZO image analysis system (Regent instruments Inc., Quebec, Canada) was utilized to analyze root images for morphological characters (root surface areas, volume, radius, tips, links) and architectural data (altitude, magnitude and exterior pathlength). Link is defined as a length of root between two nodes or junctions of two root branches (Fitter, 1986); Magnitude (μ) is the

number of exterior links; Altitude (α) means the number of links in the longest path from any exterior link to the base link and exterior path length (*Pe*) means the sum of the number of links in all paths (Fitter, 1986).

Roots were then plated on TB-CEN medium amended with Penicillin G (60 mg/liter, ICN Biomedicals Inc., Auroro, OH) (Specht and Griffin,1985) and kept in the dark at room temperature (20 to 23°C). After 12 d, the percentage of each root system with growth of *T. basicola* in the medium was rated using the same scale as for root discoloration.

Immediately prior to harvest each year, 10, 12 and 18 cotton plants per plot were cut below the cotyledonary node and evaluated for season-long vegetative and reproductive development using COTMAP (Bourland and Watson, 1990) on 3 October in 2009; 15 September in 2010; 17 October in 2011, respectively. Root systems were carefully excavated to save as many roots as possible. Seed cotton was hand-picked and weighed. Roots from each plant were rinsed and bleached as described earlier for seedling roots, the roots were rated for galling severity, and then scanned using the WinRHIZO system.

In both early and late season, soil samples were taken at each sampling location with a 2.5 cm-d soil sampling tube. A sample consisted of a composite of 10 cores taken to a depth of about 15 cm from the root zone around the plants that had been excavated. *T. basicola* population density in the soil was determined by a pour-plate technique using amended TB-CEN medium. Twenty-seven grams of soil was added into sufficient sterile 0.15% water agar to make a suspension with a volume of 250 mL. The soil suspensions were then shaken using a wrist action shaker for 20 minutes, and a 1.0 mL aliquot from each soil suspension was pipetted into each of six petri plates (100 × 15 mm). Molten TB-CEN medium (~45°C) was poured into each plate, and the plate was swirled to distribute the medium containing the soil. Plates were

incubated at room temperature (20 to 23 °C), and the number of colonies of *T. basicola* for each plate was counted 12 d after plating. Soil populations were expressed as colony forming units (CFU) per gram of soil based on soil oven dry weight. Population density of *M. incognita* in the soil was determined by extraction with a semi-automatic elutriator (Byrd et al., 1976) and sugar floatation (Jenkins, 1964).

Soil penetration resistance was measured around cotton roots at each sampling spot with a SC 900 Soil Compaction Meter (Spectrum Technologies, Inc., Plainfield, IL, USA) to a depth of 45 cm in 2010 and 2011. The cone used had an included semi-angle of 15° and a diameter of 12.15 mm. The penetration rate was approximately 1.0 cm/s. Soil moisture content was measured by drying soil samples taken at the same time as each soil penetration resistance measurement to constant mass at 100 °C. Soil textures for different soil depths (0-15 cm, 15-30 cm and 30-45 cm) were determined in 2011as described by Arshad et al. (1996). Soil bulk densities for 0-10 cm and 10-20 cm soil depth were taken using a bulk density sampler (cylinder, 5 cm in diameter and 10 cm in length) and slide hammer (AMS, Inc, American Falls, ID) on 5 July and 24 October in 2011. Soil taken from sampler was weighed after oven drying to constant mass at 100 °C.

The experiment was analyzed as a split-plot design with cultivation (subsoiling or no subsoiling) as the main plot. Sub-plots were nematicide treatment or no nematicide treatment. Mid-point values were used for analysis of root galling by *M. incognita* (rating of 5 = 150 galls/root), root discoloration, and root colonization by *T. basicola*. A root topological index (TI) was determined by the slope of the regression line from double-logarithmic ($\log_e$) plots of exterior pathlength against magnitude (Fitter, 1986). Soil bulk density, soil penetration resistance, soil particle-size distribution were also analyzed. Statistical analyses were conducted

using GLM in SAS 9.2 (SAS Institute Inc., Cary, NC). Parameter means were separated according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$. When interactions were significant ($P \leq 0.05$), appropriate LSDs were calculated.

RESULTS

The 2009 growing season was cooler and wetter than either the 2010 or 2011 seasons (Figure 1). More rainfall occurred and mean air temperatures were lower in 2011 than 2010 except in July.

There were no subsoil by nematicide interactions in any year for seedling height to node ratio (HNR), plant dry weight, root fresh weight, or taproot length, except for taproot length in 2010 (Table 1). Subsoiling increased taproot length without Telone II® and Telone II® improved taproot length without subsoiling (Table 1, $P = 0.0306$; Table 2). Subsoiling alone did not significantly affect any of the parameters in any year except for root fresh weight in 2010 when fresh root weight was greater following subsoiling. Application of Telone II® did not affect any of the parameters in 2009, but nematicide application increased HNR and plant dry weight in 2010 and 2011, and increased root fresh weight and taproot length in 2011 (Table 2). Subsoiling and nematicide application had very little effect on root morphological or architectural characters (magnitude, altitude, exterior pathlength, root volume, total root length, or topological index) of seedlings. Telone II® resulted in a slight increase in magnitude in 2009 ($P = 0.0251$) in which magnitude were 120.6 and 83.2 for with or without Telone II® application, respectively. In 2011, root volume was 0.41 after Telone II® application in contrast to 0.34 for without Telone II® ($P = 0.0083$). Subsoiling slightly increased root volume in 2010 with the value of 1.13 comparing with 0.92 for non-subsoiling ($P = 0.0202$).

Neither subsoiling nor nematicide application affected final (at-harvest) plant height, boll production or yield consistently (Table 3). However, a subsoiling by Telone II® effect was found on the position of first sympodial node in 2010 (Table 3 and 4) in which subsoiling without Telone II® application and Telone II® application with no subsoiling lowered the position of first sympodial node. Telone II® application tended to raise the position of first sympodial node in 2011 (Table 3 and 4). Root morphological or architectural parameters did not differ late in the season (Table 5).

Effects of subsoiling and Telone II® application were independent both early in the season and at harvest, and no interactions were detected for root galling (Table 6). Telone II® suppressed galling severity ($P \leq 0.05$) of cotton seedlings in seedlings and at harvest all three years, whereas subsoiling reduced root galling in 2011 at harvest (Table 6). Subsoiling increased the population of *T. basicola* in the soil in 2010, but not in either 2009 or 2011 (Table 7). The population density in 2011 was considerably lower than either 2009 or 2010, likely due to the higher April-June temperatures in 2011 (Figure 1). Subsoiling resulted in higher root discoloration ratings in 2011 (Table 7).

Soil penetration resistance was measured in the field in the early and late season for both 2010 and 2011. In the early season, the field was irrigated with 2.9 cm of water 2 weeks prior to soil penetration measurements. In the late season, 1.5 cm and 2.7 cm of natural rainfall occurred two weeks before soil penetration measurements in 2010 and 2011, respectively. In all cases, we assumed that the field was uniform relative for irrigation or rainfall. A tillage pan was found 15 cm and 20 cm below the soil surface in the early season of 2010 and 2011, respectively, in the plots that were not subsoiled (Figure 2. A, C). In the late season, the CI tended to increase with increased soil depths (Figure 2. B, D). In the late season of 2010, subsoiling effects on soil

penetration resistance was not obvious (Figure 2. B). However, subsoiling distinctly reduced soil penetration resistance in the late season in 2011 (Figure 2 D). Soil bulk densities in 2011 indicated that subsoiling effects were not obvious and soil bulk density was significantly reduced by subsoiling at 0.1-0.2 m soil depth in the late season (Table 9). The soil particle sizes differed at different soil depths (Table 8) based on soil texture measurements from the field in 2011. Sand silt and clay were 80, 13 and 7% at 0-15 cm, 79, 13 and 9% at 15-30 cm and 71, 17 and 12% at 30-45 cm soil depths.

DISCUSSION

Subsoiling primarily reduces soil compaction and improves root development and plant growth (Borghei et al., 2008; Raper, 2005). The fumigant nematicide, Telone II®, reduces nematode populations and thus facilitates plant growth (Kinloch and Rich, 1998). In this study, few two-way interactions of subsoiling by Telone II® were present on root morphology or cotton growth. Telone II® effects were more obvious than subsoiling effects. Telone II® application reduced root galling (both early and late season), suppressed J2 population (late season) and therefore, enhanced root length (early and late season 2009), root volume (early season 2011 and late season 2010), root fresh weight (early season 2011), taproot length (early season of 2011), root dry weight (late season 2010), and plant height-to-node ratio (early seasons of 2010 and 2011). However, late season growth and yield effects were not found for Telone II® treated plots. In some cases, Telone II® application reduced soil penetrometer readings by soil.

Subsoiling tends to increase hydraulic conductivity, reduce soil resistance and bulk density, increase rooting depth and enhance cotton yield (Borghei et al., 2008; Raper et al., 1998, 2005; Simoes et al., 2009). In this field study, subsoiling was intended to reduce soil resistance and soil bulk density as was reported in other studies, but little effect was seen relative to crop

performance. Subsoiling significantly increased cotton root fresh weight in 2010 and root volume of cotton seedlings in 2011, but not in 2009, and had little impact on root architecture or crop growth or yield. However, a few differences in growth and the benefits of subsoiling were observed suggesting soil physical parameters were not the limiting factors for plant growth.

Soil penetration resistance, also referred to as cone index (CI), is strongly associated with bulk density and soil moisture content (Coelho et al., 2000; Taylor and Gardner, 1963; Vaz and Hopmans, 2001). In general, CI is positively related to soil bulk density but inversely related to soil water content (Cassel, 1983; Cruse et al., 1981). At a given bulk density, soil resistance to root penetration increased with soil drying (Coelho et al., 2000). The CI above the tillage pan (15 cm) was lower after subsoiling, which likely explained the subsoiling effects on root growth of cotton seedlings in 2010. Less obvious subsoiling effects were seen on cotton seedlings in 2011, likely due to the relatively high CI above the tillage pan. Less obvious subsoiling effects on CI were observed in the late season of 2010, which may explain why little effect of subsoiling occurred on late-season plant growth or root morphology. It is more difficult to explain the lack of subsoiling effects on plant growth and root morphology in the late season in 2011 where subsoiling distinctly reduced soil penetration resistance. However, in 2011, subsoiling effect toward soil bulk density were not obvious in the upper 0.1 m of soil, and subsoiling reductions in CI were only observed at 0.1 to 0.2 m in the late season. In the early season of 2010, nematicide application reduced soil penetrometer reading at certain soil depths (5 cm, 10 cm and 15 cm) and subsoiling by nematicide interaction on soil resistance displayed at soil depth of 30 cm (data not shown). Since nematicide was applied by a Yetter Avenger® with 25-inch coulters, the soil was likely disturbed to some degree and may have reduced soil compaction.

The relationship between soil penetration resistance, bulk density and water content is also influenced by soil texture. Bennie and Burger (1981) reported that penetration resistance increased more than 20% when clay and silt contents increased in soils at a specific bulk density and water content. Based on soil texture measurements from the field in 2011, the soil particle sizes differed at different soil depths in which sand, silt and clay were 80, 13 and 7% at 0-15 cm, 79, 13 and 9% at 15-30 cm and 71, 17 and 12% at 30-45 cm soil depths, respectively. It is possible in this site that the soil was dryer near the soil surface but wetter deeper in the soil profile. Cotton roots may have still been able to grow and maintain function even in the non-subsoiled plots.

Subsoiling effects on the root-knot nematode were not obvious based on root galling severity and only reduced galling in the late season of 2011. This may be because the establishment and development of nematode population depends on more than just soil physical environment, and soil temperature, soil water content, aeration and other factors may also involve. The population density of *T. basicola*, on the other hand, was increased by subsoiling early in the season of 2010. This may have been a result of movement and redistribution of chlamydospores by the subsoiling operation. The population of *T. basicola* was low in 2011, likely because of lower reproduction of the pathogen due to the hot weather (Rothrock, 1992).

In the controlled environmental study described in Chapter 2, root topological index (TI) indicated a herringbone branching type implying that the root system was not affected by soil physical environment. Similarly, in this field study, subsoiling failed to change the root branching pattern although a value of TI that was near 1.52 was found, indicating equal branching (Werner and Smart, 1973) in 2009 (data not shown). Herringbone patterns were shown in the early seasons of 2010 and 2011. At the late season, topological indices ranged from

1.70 to 1.83 under all the treatments which displayed a herringbone branching features in microplot study. Topological models directly relate to root function. Changes of root system morphology occur when environment changes. In the field study, more evenly distributed branching was observed in the early season of 2009 due to cool and wet weather. But in the late seasons less branching root and herringbone pattern were found because of hotter and drier environment conditions. In addition, the reason for the similar root branching in these two studies may be due to the "tap root" crop characteristic of the cotton plant.

Study of root architecture provides another method to quantify the improved root development after subsoiling or Telone II® application. Topological index (TI) enabled comparison of different root branching characteristics in response to different treatments. The smaller TI in the early season of 2009 (from 1.48 to 1.52) indicated equal branching. The topological indices either in the early seasons of 2010 and 2011 (from 1.79 to 1.85) or in the late seasons of all three years (from 1.65 to 1.71) exhibited herringbone root patterns. However, the subsoiling by Telone II® effects were not obvious on TI, which may further support the less obvious two-way interaction on root development and plant growth.

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Figure 1. Rainfall and air temperature in 2009, 2010 and 2011

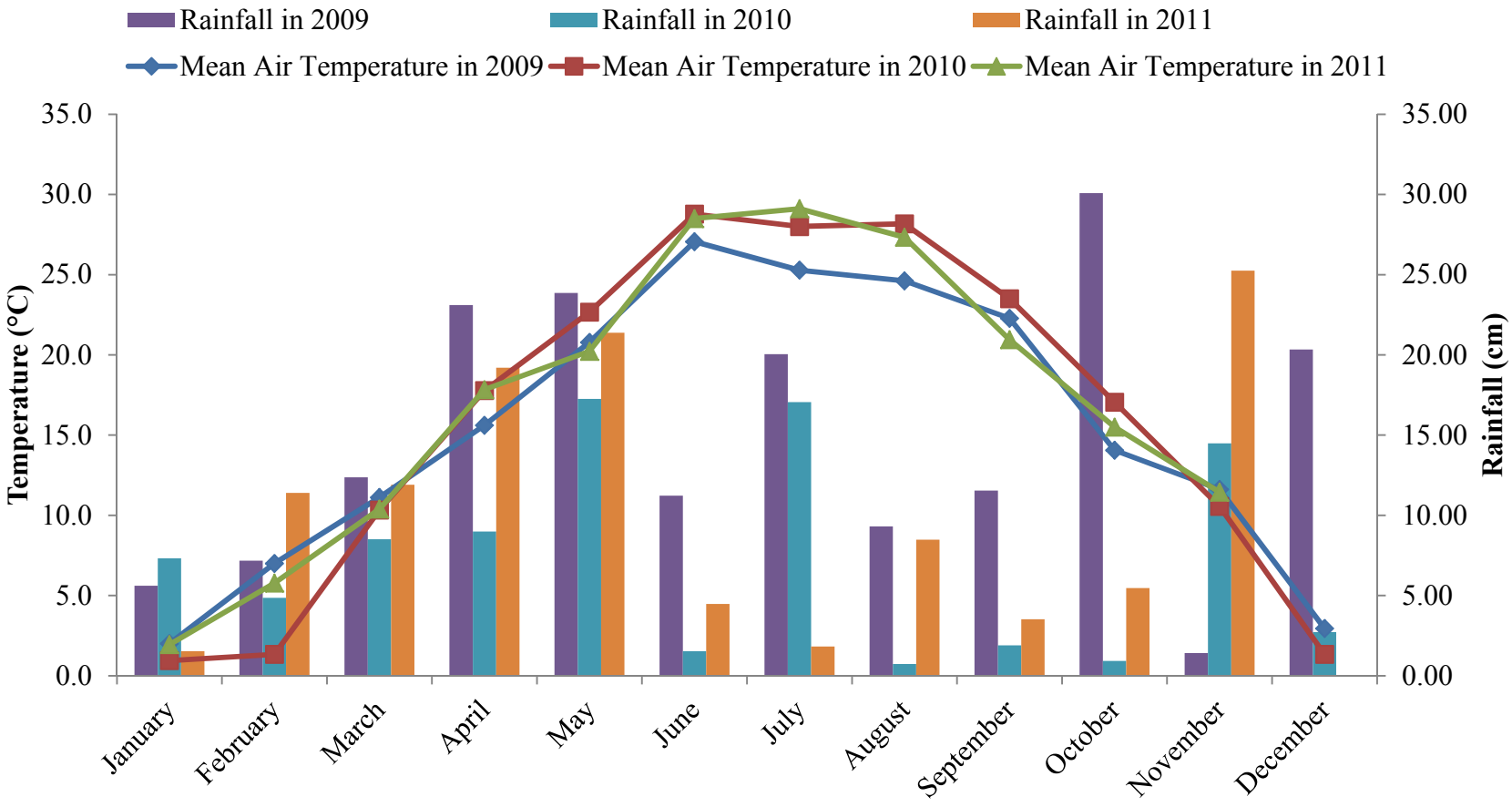
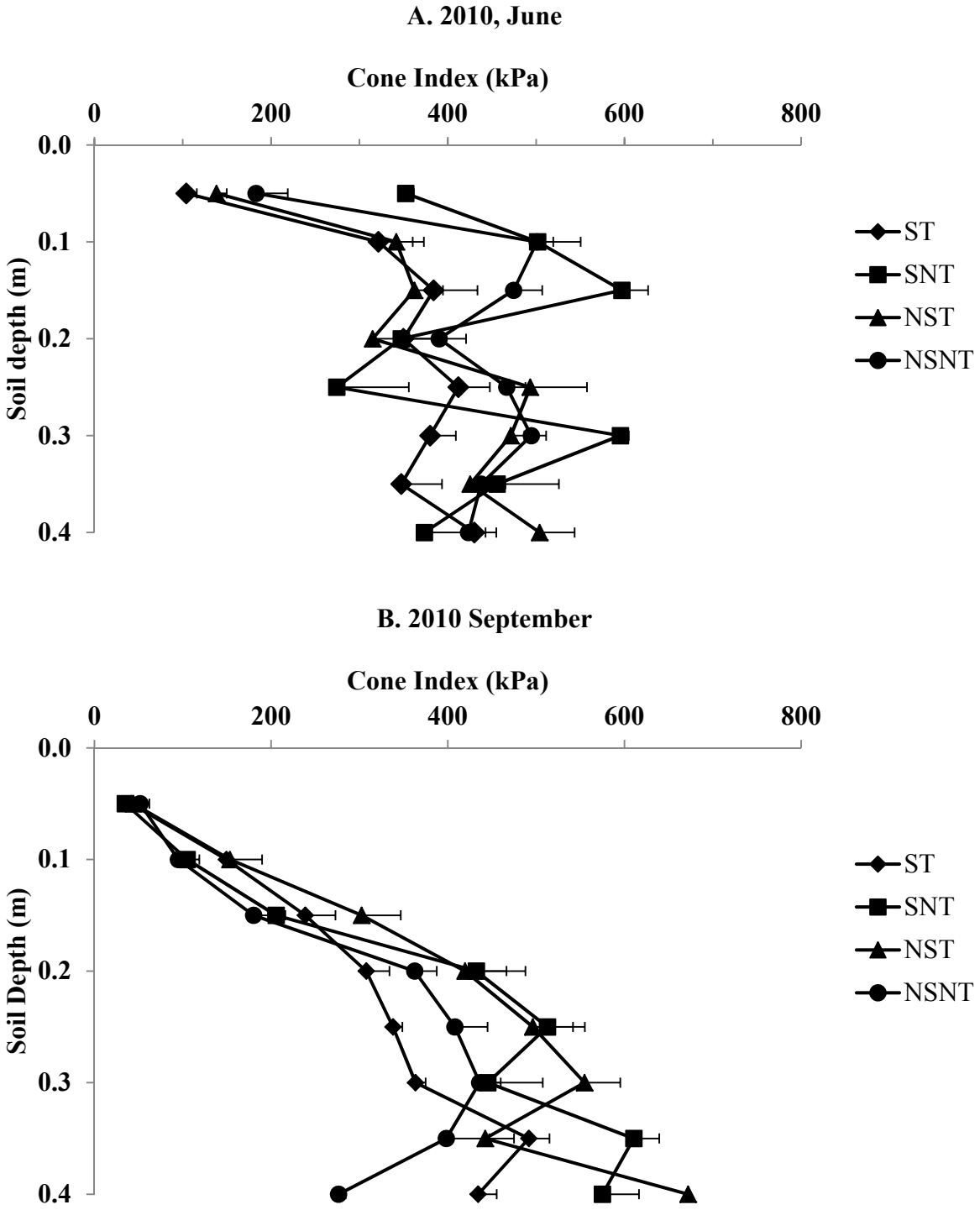
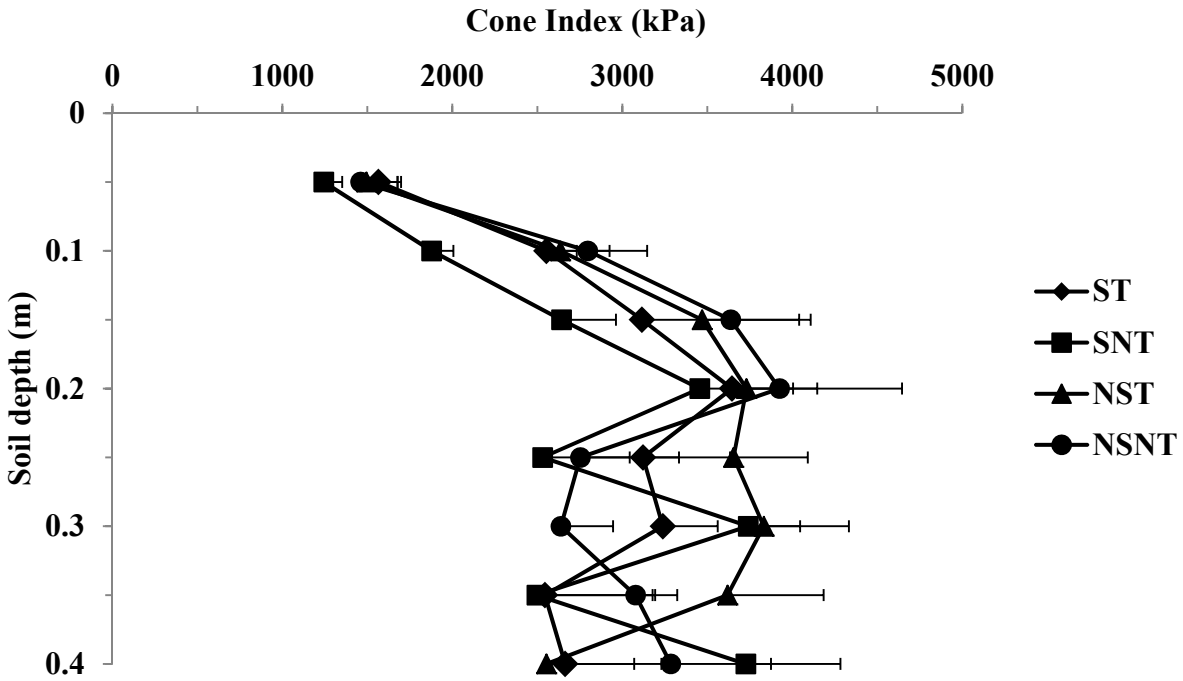


Figure 2. Cone indices in the Leachville field in the early and late season of 2010 and 2011. ST = subsoiling and Telone II® application; SNT = subsoiling, no Telone II® applied; NST = no subsoiling, Telone II® applied; NSNT = no subsoiling and no Telone II® applied (control). A. June, 2010; B. September, 2010; C. July, 2011; D. October, 2011. Mean values (n = 12) are plotted with standard error bars



C. 2011 July



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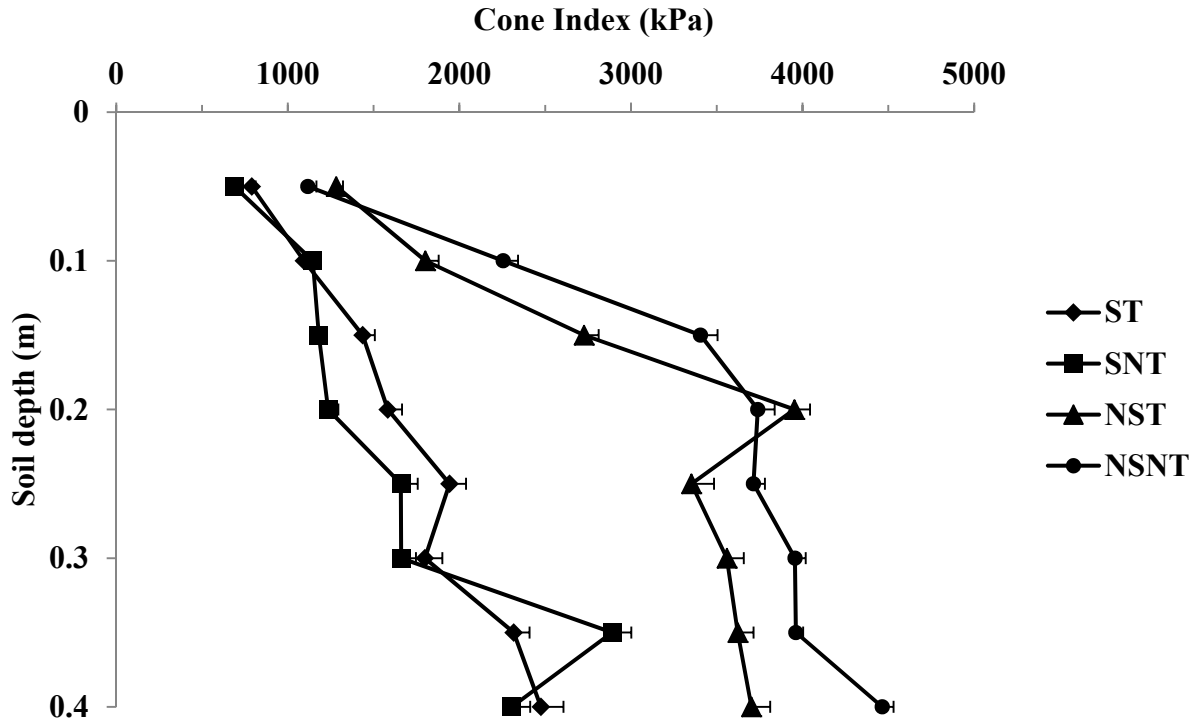

Table 1. Probability values for main and interaction effects of subsoiling and Telone II® on cotton seedling growth in cotton seedlings, 2009, 2010 and 2011

Treatment	HNR[y]			Top dry weight[y] (g)			Root fresh weight (g)			Taproot length (cm)		
	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009[z]	2010	2011
Subsoil	0.2346	0.5058	0.5077	0.5415	0.0980	0.3663	0.4733	0.0443	0.8351	-	0.0946	0.4748
Telone II®	0.1492	0.0473	0.0170	0.9470	0.0496	0.0188	0.2873	0.0659	0.0127	-	0.0306	0.0015
Subsoil* Telone II®	0.3773	0.3900	0.0935	0.1569	0.1011	0.3183	0.2395	0.0643	0.1342	-	0.0306	0.1524

[y] Height-to-node ratio (HNR) determined by plant height from the cotyledonary node/number of nodes.
[z] '-' indicates taproot length was not measured.

Table 2. Effect of subsoiling and Telone II® on cotton seedling growth in 2009, 2010 and 2011

Treatment	HNR[w]			Top dry weight (g)			Root fresh weight (g)			Taproot length (cm)		
	2009	2010	2011	2009	2010	2011	2009	2010	2011	2010		2011
Subsoil												
0	1.44 a[x]	3.08 a	2.02 a	0.79 a	6.34 a	0.63 a	0.62 a	1.24 b	0.55 a			10.29 a
1	1.49 a	3.17 a	1.94 a	0.76 a	8.16 a	0.58 a	0.59 a	1.45 a	0.53 a			11.06 a
										Subsoil		
Telone II®										0	1	
0	1.41 a	2.85 b	1.90 b	0.77 a	6.22 b	0.53 b	0.57 a	1.20 a	0.45 b	2.17	5.25	8.77 b
1	1.52 a	3.39 a	2.06 a	0.78 a	8.28 a	0.67 a	0.64 a	1.49 a	0.62 a	5.04	5.25	12.57 a
LSD A[y] =										1.77		
LSD B[z] =										2.05		

[w] Height-to-node ratio (HNR) determined by plant height from the cotyledonary node/number of nodes.
[x] Means in a column and main effect followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at P≤0.05.
[y] LSD A to compare subsoiling at the same or different nematicide treatments.
[z] LSD B to compare two nematicide treatment means for the same subsoiling.

Table 3. Probability values for main and interaction effects (*P* values) of subsoiling and Telone II® on cotton growth at harvest, 2009, 2010 and 2011

Treatment	Height[x] (cm)			First sympodial node[y]			Total boll			Yield[z] (g)		
	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
Subsoil	0.9722	0.6817	0.4136	0.4481	0.1024	0.3878	0.3917	0.4894	0.4208	0.5745	0.1579	0.4271
Telone II®	0.5297	0.7904	0.1018	0.3506	0.2214	0.0018	0.0586	0.3639	0.6470	0.0807	0.5502	0.3147
Subsoil* Telone II®	0.3627	0.5407	0.0866	0.6054	0.0114	0.9420	0.7017	0.5618	0.8371	0.2062	0.4779	0.9690

[x] Plant height measured from the cotyledonary node to the tip of the main stem terminal.
[y] Main stem node where the first sympodial branch was initiated; cotyledonary node = 0.
[z] Seed cotton, yield per plant.

Table 4. Effect of Subsoiling and Telone II®on cotton growth at harvest in 2009, 2010 and 2011

Treatment	Height[t] (cm)			First sympodial node[u]			Total boll			Yield[v] (g)		
	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
Subsoil												
0	81.99 a[x]	69.05 a	71.00 a	6.7 a		8.7 a	9.5 a	14.0 a	16.5 a	47.94 a	65.22 a	76.79 a
1	82.17 a	70.72 a	74.46 a	6.8 a		9.0 a	8.9 a	15.1 a	15.4 a	51.33 a	58.35 a	70.72 a
					Subsoil							
Telone II®					0	1						
0	80.70 a	70.27 a	71.43 a	6.9 a	6.0	5.1	8.4 b	8.3 a	13.9 a	15.7 a	44.96 a	60.14 a	71.70 a
1	83.46 a	69.51 a	74.03 a	6.6 a	5.1	5.5	9.3 a	10.1 a	15.2 a	16.2 a	54.32 a	63.42 a	75.81 a
LSD A[y] =					0.58							
LSD B[z] =					0.48							

[t] Plant height measured from the cotyledonary node to the tip of the main stem terminal.
[u] Nodes to the first sympodial branch excluding cotyledonary node.
[v] Seed cotton yields per plant were handpicked and weighed in grams.
[x] Means in a column and main effect followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at P≤0.05.
[y] LSD A to compare subsoiling at the same or different nematicide treatments.
[z] LSD B to compare two nematicide treatment means for the same subsoiling.

Table 5. Probability values for main and interaction effects of subsoiling and Telone II® on root architectural characters in cotton at harvest, 2009, 2010 and 2011

Treatment	Magnitude[x]			Altitude[y]			Exterior pathlength[z]		
	2009	2010	2011	2009	2010	2011	2009	2010	2011
Subsoil	0.9354	0.9594	0.6807	0.5364	0.7560	0.9472	0.8097	0.9334	0.7094
Telone II®	0.4705	0.9766	0.9691	0.8371	0.2447	0.9730	0.7506	0.9527	0.8431
Subsoil*TeloneII®	0.3482	0.6928	0.4409	0.8979	0.0624	0.7933	0.3501	0.4351	0.5339

[x] Magnitude = the number of exterior links.
[y] Altitude = the number of links in the longest path from any exterior link to the base link.
[z] Exterior path length = the sum of the number of links in all paths.

Table 6. Effect of subsoiling and Telone II® on root galling in the early and late season, 2009, 2010 and 2011

Treatment	Galling[y]					
	Early season			Late season		
	2009	2010	2011	2009	2010	2011
Subsoil						
0	10.2 a[z]	5.3 a	8.5 a	40.0 a	28.7 a	36.2 a
1	15.9 a	27.6 a	4.0 a	22.7 a	35.4 a	18.1 b
Telone II®						
0	24.9 a	32.8 a	11.2 a	56.4 a	54.7 a	47.6 a
1	1.1 b	0.1 b	1.2 b	6.3 b	9.5 b	6.7 b

[y] Root galling based on a 0-5 scale: 0=no galls, 1=1-2, 2=3-10, 3=11-30, 4=31-100, 5=>100 galls/root. Analyses were conducted using mid-point values.
[z] Means in a column and main effect followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.

Table 7. Effect of subsoiling and Telone II® on *T. basicola* soil population density and root discoloration severity in cotton seedlings, 2009, 2010 and 2011

Treatment	CFU/g soil[x]			Root discoloration[y] %		
	2009	2010	2011	2009	2010	2011
Subsoil						
0	25.35 a[z]	32.45 b	2.04 a	2.3 a	12.2 a	7.7 b
1	66.04 a	69.56 a	1.59 a	3.1 a	14.1 a	17.3 a
Telone II®						
0	32.53 a	54.79 a	3.00 a	2.2 a	14.1 a	16.6 a
1	58.86 a	47.23 a	0.64 a	3.3 a	12.2 a	8.4 a

[x] Colony forming units (CFU)/g of soil was determined by the pour-plate technique on amended TB-CEN medium.
[y] Root discoloration based on a scale of 0 to 10, where 0= none, 1=1-10%, 2=11-20%, 3=21-30%, 4=31-40, 5=41-50, 6=51-60%, 7=61-70%, 8=71-80%, 9=81-90% and 10=91-100%. Analyses were conducted using mid-point values.
[z] Means in a column and followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.

Table 8. Particle-size distribution at different depths for Leachville field, northeast Arkansas

Soil particle	Depth, m		
	0-0.15	0.15-0.30	0.30-0.45
Sand	0.80	0.79	0.71
Silt	0.13	0.13	0.17
Clay	0.07	0.09	0.12
Soil textures	Loamy sand	Loamy sand	Sandy loam

Table 9. Effects of subsoil and Telone II® on soil bulk densities in 2011

	Soil bulk density, g/cm^3			
Treatment	0-0.10 m		0.10-0.20 m	
	July	October	July	October
Subsoil				
0	1.30 a	1.46 a	1.27 a	1.47 a
1	1.34 a	1.51 a	1.31 a	1.41 b
Telone II®				
0	1.32 a	1.48 a	1.30 a	1.45 a
1	1.32 a	1.48 a	1.27 a	1.44 a

Chapter IV

Effects of *Meloidogyne incognita, Thielaviopsis basicola,* and a Soil Hard Pan on Cotton Root Architecture and Plant Growth in Microplots

ABSTRACT

The effects of *Meloidogyne incognita, Thielaviopsis basicola,* and a soil hard pan (HP) on cotton root architecture and plant growth were evaluated in a microplot study in 2010 and 2011 at the Southwest Research and Extension Center, Hope, Arkansas. Ninety-six microplots were used. An artificial HP was created 20 cm below the soil surface in half of the microplots. The pathogen treatments for hard pan and non-hard pan plots included soil infested with *T. basicola* (40 chlamydospore chains/ cm^3 soil) at four different *M. incognita* levels (0, 4, 8, 12 eggs/ cm^3 soil). Two additional pathogen treatments were non-infested soil and soil infested with *M. incognita* only (4 eggs/ cm^3 soil). A steam-pasteurized, fine loamy sand (87.1% sand, 6.8% silt and 6.1% clay) was filled into the top 20 cm of plots with a HP and the entire plots without HP (NHP) in both years. Soil was disinfested by drenching with Vapam® HL in 2011 before planting. Greater stand in 2010 and a greater height-to-node ratio (HNR) in HP plots in 2011 and root fresh weight in both years were found for seedlings. Nematode infestation tended to increase total root length, root magnitude, altitude and exterior pathlength at the seedling stage. *M. incognita* infestation decreased HNR in 2010. In the late growth season of 2011, both *M. incognita* infection and HP reduced taproot length and root dry weight below the HP. Root magnitude, altitude, and exterior pathlength were larger in the HP plot in 2010 but HP reduced root altitude in 2011. A HP increased the number of cracked bolls (114 days after planting (DAP)) and lowered the position of the first sympodial branch on the main stem. *M. incognita* infestation delayed crop development, decreasing number of cracked bolls and increasing first sympodial branch, and reduced plant height, and seed cotton yield. Topological indices under all the treatment ranged from 1.70 to 1.83 indicating a herringbone root branching both years. HP reduced J2 population in 2010 but increased galling in the late season of 2011. Generally, HP

improved seedling growth. *M. incognita* infection delayed cotton maturity and reduced seed cotton yield but other than decreasing taproot length did not decrease other root parameters.

INTRODUCTION

The southern root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is an important pathogen on cotton (*Gossypium hirsutum* L.) that is distributed throughout U.S. Cotton Belt (Koenning et al., 2004). The root-knot nematode causes the greatest crop loss in cotton in sandy soils (Monfort et al., 2007) and is favored by temperatures above 25 $^{\circ}$C (Thomas and Kirkpatrick, 2001). *M. incognita* may co-exist in Arkansas cotton fields with *Thielaviopsis basicola* (Berk. & Broome) Ferraris (syn. *Chalara elegans* Nag Raj & Kendrick), the cause of black root rot on cotton seedlings (Allen, 2001; Rothrock and Kirkpatrick, 1998). Where both pathogens occur together, plant growth, development and yield can be severely affected (Walker et al, 1998, 1999) and temperature may be less restrictive for disease losses (Monfort et al., 2006; Walker et al., 2000).

A functional root system is vital for anchorage and nutrient uptake from the soil environment (Lynch, 1995). Impaired root growth and development due to pathogens such as *M. incognita* or *T. basicola*, or to physical edaphic factors may limit crop growth and development. A common physical factor that may impact root growth in agricultural fields is compaction (Harveson et al., 2005). Compacted soil has a higher bulk density and soil strength (resistance) (Whalley et al., 1995). Root penetration is inhibited by high soil resistance (Medvedev, 2009; Taylor and Gardner, 1963). Compaction restricts root growth of most plants when the soil resistance reaches about 1.5 MPa; at a resistance near 2.5 MPa, most roots cease penetrating vertically (Coelho et al., 2000). Lowry et al. (1970) reported the distribution of plant roots were partially or fully aggregated to a shallow plowed layer above a compacted soil pan. An inverse linear relationship between soil strength and yield of corn, soybean and wheat grown in soils with a hard pan has been reported (Busscher et al., 2000). Both *M. incognita* and *T. basicola*

distort cotton roots by either inducing gall formation or by colonizing the cortical tissue. Reduced root growth or function is a common symptom of both pathogens, but no research has been done to quantify the nature of this reduction. Changes in root system architecture due to these pathogens have been documented in controlled environmental studies (Chapter II). The changes in root architecture, particularly in combination with changes that may occur due to increased bulk density as a result of a hard pan could be very important in limiting in crop productivity. The hypotheses for this study was that *M. incognita* and *T. basicola* will reduce plant development and alter root growth and a soil hard pan will impede root development further restricting growth of cotton.

The objective of this study was to determine the effect of a hard pan and the plant pathogens *M. incognita* and *T. basicola* on cotton root architecture and plant growth in microplots.

MATERIALS AND METHODS

Ninety-six concrete microplots (76 cm in diameter, buried 80 cm deep) located at the Southwest Research and Extension Center (SWREC), Hope, Arkansas, were used for this study in 2010 and 2011. Prior to planting in 2010, the microplots were used for a soil texture study (Jaraba-Navas, 2011). Soils in microplots were compacted due to natural forces. The soil bulk densities at 0-0.10 m soil depth ranged from 1.80 to 2.07 g/cm^3 based on soil measurements before the study was initiated. Twenty centimeter of soil was removed and the remaining compacted soil was left in 48 microplots to form the artificial soil hard pans (HP). A steam-pasteurized (30 minutes at 70°C), fine loamy sand (87.1% sand, 6.8% silt and 6.1% clay) was added to fill the microplots above the compacted zone. Another 48 microplots were dug to a depth of 80 cm and filled with the pasteurized loamy sand to form non-hard pan plots (NHP). In

2011 approximately 45 days prior to planting, soils were disinfested in all the microplots by drenching with Vapam® HL (sodium methyldithiocarbamate, Amvac Chemical Corporation, Los Angeles, CA) at 35 mL/plot in 3,785 mL water, poured uniformly on the soil surface. Immediately after application, each plot received an additional 8 liters of water to help disperse the fumigant into the soil and to provide a water seal at the surface. One week prior to planting, all plots were tested for nematodes and fungal propagules to ensure that none had survived from the previous season.

The experimental design of this study was a completed randomized design with eight replications. Treatments included non-hard pan and hard pan plots with the six pathogen treatments; a non-infested control, *M. incognita* alone (4 eggs/cm^3 soil), *T. basicola* alone (40 chlamydospore chains/cm^3) and *T. basicola* (40 chlamydospore chains/cm^3) in combination with three different densities of *M. incognita* (4, 8, and 12 eggs/cm^3 soil).

T. basicola chlamydospore chains were harvested from 6-week-old cultures grown on 10% carrot juice agar as described by Candole and Rothrock (1997). Cultures were rinsed with sterile distilled water to remove most endoconidia. Cultures were flooded with sterile distilled water and a rubber scraper used to dislodge chlamydospores. The resulting spore suspension was filtrated through two monofilament nylon fabrics (Tetko, Inc., Depew, NY) with openings of 53 µm and 20 µm, successively. Chlamydospore chains retained on the 20 µm mesh were transferred into a 500 mL sterile flask containing about 400 mL of sterile distilled water. The spore suspension was stored in a refrigerator at 4°C before infestation. The germination rate of chlamydospores was determined on carrot juice agar after 24 hours prior to use in the microplots. Soil was infested with 40 chlamydospore chains/cm^3 soil in the top 15 cm of soil both years.

Each year, the chlamydospores were applied to each plot in 200 cm^3 sterile distilled water with a sprinkle can immediately before planting and incorporated by mixing into the top 15 cm of soil.

Inoculum of *M. incognita* host race 3 was obtained from stock cultures maintained in a greenhouse on tomato (*Lycopersicon esculentum* Mill. cv. 'Rutgers'). In 2010, inoculum was prepared by cutting tomato root systems (60 days old) into segments 1-2 cm in length and mixing the root segments thoroughly with the soil in which the plants were grown. All root segments and soil were composited, and subsamples were assayed to quantify the number of nematodes that were present. Nematode egg numbers were determined by collecting all infected root segments from a standard volume of soil and extracting the eggs in 0.05% NaOCl (Hussey and Barker, 1973) for 4 min. Vermiform second-stage juveniles in the soil were assayed using a semi-automatic elutriator (Byrd et al., 1976) and centrifugal flotation (Jenkins, 1964). In 2010, the soil and *M. incognita*-infested tomato roots were added to specific microplots for the first inoculation in a sufficient volume to achieve a density of 4 eggs/cm^3 soil (in the upper 15 cm of the microplot). Control plots received root fragments and soil from healthy tomato plants. The soil-root mixture was incorporated thoroughly into each microplot with a shovel and a garden rake just prior to planting. Microplots receiving the second and third inoculation of 4 eggs/cm^3/inoculation were inoculated at 12 days-intervals to obtain a density of 8 or 12 eggs/cm^3 soil, respectively. Inoculum was applied by making two holes (0.5 cm in diameter and 5 cm in length) and adding the nematode suspension. In 2011, the same nematode population was used, but inoculum consisted exclusively of eggs that were extracted from infected tomato plants by extraction for 4 minutes in 0.05% NaOCl as described above. Nematode eggs were applied in three different events 12 days apart to achieve final densities of 4, 8 and 12 eggs/cm^3 soil. Non-infested control plots received sterile water only.

Irrometer Tensiometers (Spectrum Technologies, Inc., Plainfield, IL, USA) were placed at 10 cm and 20 cm below the soil surface to monitor the soil matric potential of HP plots and NHP plots in both years. In the spring and early summer, 6 mm of water were added to each plot when the matric potential at 10 cm reached -30 kPa. 12 mm and 24 mm of water were added to each plot at mid-summer and early fall, respectively, when matric potential reached -50 kPa. Watering was stopped on 8 September in 2010 and 10 September in 2011. Soil temperature and soil water matric potential (watermark sensor) also were recorded from arbitrary selected plots (#65(HP), #81(HP), #79(NHP) in 2010; #69(HP), #85(HP), #45(NHP) in 2011) with Model 450 WatchDog Data Loggers (Spectrum Technologies, Inc., Plainfield, IL, USA) 10 cm and 20 cm below the soil surface. Polyethylene rain shields were installed over the microplots in 2010 in an attempt to keep natural rainfall out of the plots. The covers were not used in 2011.Weather data were obtained from a weather station located at the SWREC for both years. In the early season and late season of both years, soil penetration resistance for each plot was measured with a SC 900 Soil Compaction Meter (Spectrum Technologies, Inc., Plainfield, IL, USA) to a depth of 45 cm. The soil moisture content for different soil depths (0-15 cm, 15-30 cm and 30-45 cm) for each soil penetration resistance measurement were determined in 2011.

Twenty cotton seeds that were not treated with fungicide of the root-knot susceptible cultivar DP 0935 B2RF (Delta and Pine Land Company, Scott, MS) were planted in each plot immediately after infestation. *T. basicola* infestation, first *M. incognita* inoculation and planting occurred on 29 April 2010 and 5 May 2011, when the average soil temperature at 15 cm was above 16 °C for three consecutive days. The second and third inoculum applications of *M. incognita* occurred on 11 May and 23 May in 2010 and 18 May and 30 May in 2011. Microplot soil fertility was maintained by applying Jack's Fertilizer, 20-20-20 (J. R. Peters Laboratory™)

(2.1% of nitrate nitrogen, 17.9% of urea nitrogen, 20% of P_2O_5 and 20% of K_2O) to each plot periodically throughout the growing season to maintain plant growth. Insect control was accomplished with esfenvalerate (Asana) and acephate (Orthene) based on scouting according to Arkansas Extension Service recommendations for cotton (Studebaker, 2010). Seedling stand was determined 20 DAP, and the plant population was thinned to eight plants per plot. Four seedlings with intact root systems from each plot were excavated, 31 and 34 DAP in 2010 and 2011, respectively. Plant height from the cotyledonary node to the tip of the main terminal and the number of main stem nodes were determined for each plant. Plant height to node ratio (HNR) was calculated. Leaf areas for all plant leaves were measured with a LI-3100 Area Meter (LI-COR, INC, LinColn, Nebraska, USA). Leaf and stem tissue above the cotyledonary node were dried at 60 °C in an oven for 24 hours and weighed for top dry weight.

Excavated root systems were rinsed in running tap water for 20 minutes, surface-disinfested with 0.5% NaOCl by immersion for 1.5 minutes, blotted dry and weighed. Each root system was rated for root discoloration based on a scale from 0 to 10, where 0 = 0%, 1 = 1 to 10%, 2 = 11 to 20%, 3 = 21 to 30%, 4 = 31 to 40%, 5 = 41 to 50%, 6 = 51 to 60%, 7 = 61 to 70%, 8 = 71 to 80%, 9 = 81 to 90% and 10 = 91 to 100% of root system discolored. Nematode galling was evaluated on a scale of 0 to 5, where 0 = no galls, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = >100 galls/root system.

Each seedling root was scanned by a high-resolution image scanner (Epson® Expression® 10000 XL, Epson America, Inc). The WinRHIZO image analysis system (Regent instruments Inc., Quebec, Canada) was used to analyze each root image to obtain the root morphological characters (root surface area, volume, radius, links, tips) and architectural data (altitude, magnitude and exterior pathlength) (Fitter, 1986). Roots were then plated on the selective

medium TB-CEN (Specht and Griffin, 1985) amended with Penicillin G, 60 mg/liter (ICN Biomedicals Inc., Auroro, OH) and stored in the dark at room temperature (20 to 23°C). The percentage of root system with growth of *T. basicola* in the medium was evaluated based on the scale used for root discoloration 12 days after plating.

In 2010, two leaf disks (1cm diameter; 0.785 cm^2 each) of the uppermost expanded leaf from an arbitrarily selected single plant in each plot were collected 124 DAP and placed in 5 ml of 100% EtOH in an amber vial. Total leaf chlorophyll (Knudson et al., 1977) was measured using a UV-1700, UV-VISIBLE SPECTROMETER (Pharma Spec. SHIMADZU). The youngest fully expended leaf for one plant from each plot was randomly selected to measure leaf temperature, stomatal resistance and transpiration by Steady State Porometer LI-1600 (LI-COR Biosciences, Lincoln, NE).

At maturity, cotton was harvested by hand 173 DAP in 2010 and 180 DAP in 2011. In 2010, plant height from the cotyledonary node to the tip of the main terminal for each plant was determined. Plant growth and development was recorded using COTMAP (Bourland and Watson, 1990) to describe for the position of the first sympodial node above the cotyledon, total number of sympodial branches, number of sympodial braches with two bolls and total number of bolls per plant. Days to first bloom and number of cracked bolls 114 DAP for each plot was recorded in 2010. At harvest in both 2010 and 2011, four mature plants with root systems were excavated carefully from each plot. Excavated roots were washed, surface-disinfested, and nematode galling was evaluated using the same scale as for early season samples. A Canon EOS Rebel T2i, (Canon, USA, Inc., Lake Success, New York) was used to take digital images for each root system and images were analyzed using the WinRHIZO software. Taproot length for each root was measured. Each root was cut at 20 cm below the soil line to evaluate root biomass

distribution and the two portions of roots were dried separately in an oven at 60 °C for 5 days then weighed.

Soil population densities of nematodes and *T. basicola* were evaluated at harvest both years in all plots. A composite soil sample consisting of 6 individual soil cores from each plot was removed with a soil sampling tube (2.5 cm diameter and 15cm length) and 100 cm^3 of soil was processed by sieving and centrifugal flotation (Ayoub, 1980) to extract J2 nematodes. The *T. basicola* population from each soil sample was evaluated by the pour-plate technique with the amended TB-CEN medium by adding 27 grams of soil into sufficient sterile 0.15% water agar for a final volume of 250 ml. The soil suspensions were then shaken with a wrist-action shaker for 20 minutes and1.0 ml was removed with a pipette and distributed into each of the six petri plates (100 × 15 mm) prior to pouring in the molten medium. Plates were kept in the dark at room temperature (20 to 23 °C) and the numbers of colonies of *T. basicola* for each plate was counted 12 days after plating. Numbers of colony forming units were calculated per gram of soil based on soil oven dry weight.

Statistical analyses were conducted used the GLM procedure with SAS 9.2 (SAS Institute Inc., Cary, NC) to evaluate treatment effects on root architecture and plant growth. As a result of treatments not being a complete factorial, orthogonal contrasts were used to compare *M. incognita* density levels and hard pan effects in the presence of *T. basicola* or hard pan, *M. incognita* and *T. basicola* effects at a *M. incognita* rate of 4 eggs/cm^3 of soil. Mid-values for each rating scale were used for analyses (galling > 100 = 150 galls/root). The root topological index (TI) was determined by the slope of the regression line from double-logarithmic (log_e) plots of the exterior pathlength (*Pe*) against the magnitude (μ) (Fitter, 1986).Treatment means were separated according to Fisher's protected LSD at $P \leq 0.05$.

RESULTS

In 2010 and 2011, the average monthly air temperatures were similar to previous years (Figure 1). Average weekly soil temperatures for the first six weeks (emergence to second true leaf stage) in 2011 were consistently higher than 2010 (Figure 2). The average soil temperature during the first six weeks after planting was 27.2°C in 2011 which was 4.6°C higher than the average soil temperature of 2010 (Figure 2). Soil water matric potential 10 cm below soil surface (above the hard pan) in HP plots tended to be greater than in the plots without a hard pan both years early in the season (Figure 3). The warm average soil temperature was associated with less rainfall during the study in 2011 indicating complex effects which resulted in increased plant growth and nematode activity but decreased black root rot. Due to environmental differences between 2010 and 2011, data were analyzed by individual years. The orthogonal contrast including the *T. basicola* comparison suggested few effects and root colonization of *T. basicola* was not observed in the seedling stage in both years. Thus results will only be presented for the orthogonal comparison for *M. incognita* rate and hard pan effects.

There was no soil hard pan (HP) by *M. incognita* rate interaction on cotton seedling growth in either 2010 or 2011. Seedling stand (% of surviving seedlings) was higher in the presence of a hard pan in 2010, and seedling height-to-note ratios (HNR) were significantly higher in plots with a hard pan in 2011(Table 1). Root fresh weight was higher in HP plots in both 2010 and 2011. *M. incognita* rate effects followed a linear trend on seedling HNR in 2010 ($P = 0.0369$) indicating that the ratio was reduced by *M. incognita*, and the inoculum rate of the nematode was important. *M. incognita* did not affect seedling stand or root fresh weights in either year. A soil hard pan by *M. incognita* rate interaction was found for various root morphological parameters and topological attributes such as total root length, magnitude, altitude

and exterior pathlength in 2010 (Table 2). Soil hard pan by *M. incognita* rate relationships were cubic for root magnitude ($P = 0.0022$), altitude ($P = 0.0105$), exterior pathlength ($P = 0.0013$) and total root length ($P = 0.0254$). In 2010, these root topological and morphological parameters tended to be suppressed by a soil hard pan in the absence of the nematode, and *M. incognita* tended to increase magnitude, altitude, and exterior pathlength. The interaction was a result of the nematode having a greater effect at the middle infestation rate for no hard pan and low rate for a hard pan than the other rates used. The hard pan reduced total root length in 2011 but increased root system volume in both years (Table 3). Magnitude, altitude and exterior pathlength were not affected by a hard pan in 2011 and only magnitude was increased by soil infested with the nematode.

Late in the season, both plant height and the taproot length were reduced by the presence of the nematode and the hard pan, and the presence of the nematode appeared to impact these parameters more in the absence of a hard pan (Table 4). There was no hard pan by *M. incognita* rate interactions for root parameters in the late season of 2011 but root dry weight below hard pan were significantly reduced by soil hard pan (Table 5). The hard pan significantly increased root volume by 43% and a quadratic trend of *M. incognita* rate effect also indicating increased root volume by *M. incognita* infestion ($P = 0.0176$) (Appendix, Table 2). These responses were not found in 2010

No soil hard pan by *M. incognita* rate interaction was seen for late season cotton plant development in 2010 (Table 6). The number of cracked bolls, a measure of earliness, at 114 days after planting, was greater in plots with a hard pan. However, the total number of bolls numbers at harvest (173 DAP), the number of fruiting (sympodial) branches and average seed cotton yield were similar in plots with or without a hard pan. The position of the first sympodial branch on

the main stem was higher in plots without a soil hard pan. The effect of increasing rates of *M. incognita* was linear for cracked bolls ($P<.0001$) and first fruiting node position ($P<.0001$) indicating that increased *M. incognita* delayed development of the crop and this delay increased with increasing nematode numbers. The *M. incognita* rate effect followed a quadratic trend for total boll numbers ($P = 0.0030$), number of sympodial branches ($P = 0.0239$) and average seed cotton yield ($P = 0.0473$) indicating the presence of the nematode decreased these parameters, but infestation rate did not differ.

There was no soil hard pan by *M. incognita* rate interaction with late season root morphological characters in 2010 and 2011 (Table 7). In 2010, root system magnitude and exterior pathlength were increased, but altitude was reduced by a soil hard pan. The effect of increasing *M. incognita* rate was not consistent for root morphological parameters, but magnitude and exterior pathlength generally increased for the presence of the nematode.

Root system topological indices under all the treatments ranged from 1.70 to 1.83 indicating that the root system exhibited a herringbone root branching pattern (Werner and Smart, 1973) both years. A hard pan by *M. incognita* rate effect was seen in the late season of 2010 and the early season of 2011 (Table 8). In the early season of 2010, the *M. incognita* reduced root TI, and in the early season of 2011, root system TI was also reduced by *M. incognita* with no soil hard pan. A two way interaction of soil hard pan by *M. incognita* rate was seen on root TI in the late season of 2010 in which both soil hard pan reduced TI and *M. incognita* infestion reduced TI in the absence of a hard pan ($P = 0.0231$).

There was no soil hard pan by *M. incognita* rate effect on galling or pathogen population in 2010 and 2011. Galling was numerically higher with a hard pan in both the early and late season both years although a significant difference was only found in the late season of

2011(Table 9). Nematode populations at harvest were not consistently affected by the presence of a hard pan. In the late season of 2011, the SPR at 20 cm soil depth in NHP plot was 1158.0 kPa while the SPR at HP layer was 2075.6 kPa (Figure 4),

DISCUSSION

In the early season, the soil water matric potential 10 cm below soil surface (above the hard pan) in HP plots tended to be greater than in the plots without a hard pan both years, indicating that the compaction layer trapped and held water in the upper soil profile to a greater degree than where gravitational water could move vertically to a greater extent in the absence of a hard pan. Difference in soil water availability could explain the increased height-to-node ratio early in the season of both 2010 and 2011 where a hard pan existed. The average taproot length for the non-infested plots was considerably less than 20 cm in both 2010 and 2011, indicating that the roots had not reached the hard pan which was 20 cm below the soil surface at the time the early-season data were recorded. Although seedling growth was greater in 2011 than 2010, a significant HP effect was still found and the greater seedling growth was likely due to warmer soil temperature in 2011. However, in the early season of 2011, soil penetration resistance (SPR) was 650 kPa at 10cm below soil surface, a level that is near the 720 kPa, that has been reported as the soil resistance level that is sufficient to decrease cotton root growth by 50% (Dexter, 1987). This likely occurred because of the extremely dry weather during the month of June in 2011, and may explain the lower total root length and the numerically smaller root topological parameters for the hard pan treatment early in the season compared to no hard pan for 2011.

Compacted soil tends to increase soil strength, and decrease air permeability and hydraulic conductivity (Allmaras et al., 1988; Whalley et al., 1995). The success of a cotton root in penetrating a compacted soil layer depends on its maximum axial root growth pressure

(ranging from 0.6 to 1.6 MPa) (Taylor and Raliff, 1969). Compacted soil strongly impedes the development of taproots (McKenzie and McBratney, 2001). In their study, roots that encountered a compaction layer were severely tapered and deflected approximately 90° at the top of the compact layer. These "J" shaped roots were also observed in this study. In the late season of 2011, the SPR at 20 cm soil depth in NHP plot was 1158.0 kPa while the SPR at HP layer was 2075.6 kPa, considerably greater than the 2000 kPa which has been reported to completely inhibit taproot growth (Taylor et al., 1966). The increased soil penetration resistance by soil HP in the late season, likely impeded plant taproot penetration. A highly significant linear correlation ($r = -0.96$) between the soil strength and the root penetration has been demonstrated (Medvedev, 2009; Taylor and Gardner, 1963). Increased soil resistance due to soil compaction reduces both the percentage of roots penetrating the soil and the rate of root growth through the soil. Distinct differences in root distribution in heavily compacted vs an uncompacted layer has been shown (Horn et al., 1995; Lowry et al., 1970; Pierret et al., 2007; Taylor and Burnett, 1963). Similar results were found in this study. In the late season of 2011, although the root biomasses above the soil HP layer was similar to that in the NHP plots, the soil HP significantly reduced the root portion (4%) that penetrated below the compacted layer, in contrast to 14.2% of the whole root biomass that was found at the same depth in NHP plots. Given the SPR that was measured, it is likely that the only reason root penetration was not be completely inhibited by the soil HP was because a few lateral roots penetrated the soil along the sides of the microplots at the interface between the soil and the concrete wall. Mechanical impedance due to soil compaction, while slowing the rate of root extension, may increase root diameter immediately behind the root tip (Atwell, 1988; Materechera et al., 1991). In this situation, the diameter of individual cortex cells rather than the cell number increases resulting in increased cell volume in impeded roots

(Materechera et al., 1991). Soil compaction induced the radial thickening of *Lupinus angustifolius* by 15% (Atwell, 1988). In the late seasons of 2011, root radius in our study was greater by 20.5% in plots with a hard pan (Appendix, Table 2). The impedance caused by soil compaction may also alter the pattern of lateral root initiation and sometimes induces formation of lateral roots (Crosset et al., 1975; Goss and Russell, 1980; Russell, 1977). In our study, proliferation of lateral roots occurred primarily above the soil hard pan in the late season of both years. Due to this increased lateral root formation, the topological parameters including root magnitude and exterior pathlength as well as root dry weight above soil HP layer, total root biomass and root volume were increased, particularly in 2011. Root system altitudes were lower in soil HP plots both years likely because of inhibited individual root penetration due to the hard pan,

Off-target drift of *2,4-dichlorophenoxyacetic acid* or a similar herbicide from a neighboring farm in the late season of 2011 precluded cotton development data from being collected. However, based on 2010 data, soil HP appeared to speed cotton development, as indicated by the higher number of total cracked bolls at 114DAP, and a lower fruit sympodial branch. Davidson (1969) suggests that small root systems may still support optimal plant growth when the water and nutrients resources are sufficient. Similarly, Rosolem et al. (1998) found an increased shoot to root dry weight ratio coupled with increased soil bulk density from 1.13 to 1.82 g/cm^3 indicating that a relatively small root system was able to support the same plant canopy in compacted soils. Iijima et al. (1991) also reported that shoot growth was promoted in "strong soils". In our study, root distribution during the late season of 2010 was mainly above the hard pan, where soil water and nutrients may have been near optimal as a result of water management practices in this study.

M. incognita infects the root behind the root cap which causes suppressed root growth. Feeding by the nematode can suppress cotton root growth and shortened root length (Kirkpatrick et al., 1991). Root-knot infection results in a disruption of the vascular system that may limit nutrient and water flow (Koenning et al., 2004), resulting in increased resistance to stomatal opening and suppression of leaf transpiration and photosynthesis rate (Evans et al., 1975; Kirkpatrick et al., 1995). In addition, leaf temperature after nematode infection is increased (Kirkpatrick et al., 1995). In our study, leaf temperature differences were not observed among different rates of nematode infestation either with or without a soil hard pan. However, during the growing season, reduced transpiration occurred in July in 2010 implying a reduced photosynthetic rate. These effects of the nematode on cotton growth in 2010 were similar to other reports (Kirkpatrick et al., 1995; Walker et al., 1998) with the nematode delaying harvest, decreasing cracked bolls and increasing first sympodial branch, and decreasing total bolls, sympodial branches and yield.

Results from controlled environmental studies indicated that *M. incognita* infection significantly reduced total root length, magnitude, altitude and exterior pathlength by 40%, 41%, 27% and 45%, respectively (Chapter II). However, the significant cubic trends between soil compaction and *M. incognita* rates on root magnitude, altitude and exterior pathlength in the early season of 2010 indicated that nematode infection tended to increase these parameters. Compensatory root growth in response to nematode invasion was has been documented using minirhizotron root video observation (Smit and Vamerali (1998) with the potato cyst nematode (*Globodera pallida*). In their study, compensatory root growth caused by the nematode was restricted to the top 30 cm and nematodes reduced rooting depth. De Ruijter and Haverkort (1999), on the other hand, found that nematodes prolonged root formation, and that nematode-

infected crops possessed more roots in the top 30 cm than uninfected crops. Haase et al., (2007) reported lateral roots of plants infested by a low level of *M. incognita* were elongated, a possible response to wounding and stress by the host plant after nematode invasion. The physiological reaction associated with nematode attack may involve increased production of phytohormones and ethylene in infected root tissue (Barker 1999; Bird and Koltai, 2000; Glazer et al., 1983). Soil compaction did not affect increase root galling caused by *M. incognita* consistently. Increased soil bulk density may negatively affect the migration of *M. incognita* J2 (Eo et al., 2007). However, Jaraba-Navas (2011) found no effect of increased soil bulk density on root galling when the soil water was maintained at optimal levels.

Late in the season *M. incognita* effects in root growth could be seen as reduced taproot length, but not root dry weight. The hard pan apparently impacted root growth, and the taproot length in NHP plot without the nematode was 46.6 cm, more than twice as long as in non-infested HP plots (19.7cm). Other root indices, magnitude, altitude, exterior pathlength, and total root length tended to be greater with *M. incognita* treatments, with magnitude being significantly greater in 2010 in the presence of the nematode. The mechanism caused this branching increment is still unknown. However, as a taproot crop, cotton plant depends on a primary or 'tap' root to emanate branch or secondary, tertiary roots (McMichael, 1986) to maintain a healthy function. The shape of the root system, the volume of soil explored by the roots and overall root density is dependent on the development of lateral roots which extend outward from the taproot (McMichael, 1986). The depth of root penetration depends on the taproot as well. This is similar to controlled environmental studies (Chapter II), when the taproots of cotton seedlings was shorten after the infestion of *M. incognita*. Although, in this study, the increased magnitude, altitude, exterior pathlength which resulted into increased total root length after *M.*

incognita treatments were observed. However seed cotton yield were lower after *M. incognita* infestion at all levels. The function of root system was the crucial factor to ensure the sufficient water and nutrient for supporting aboveground growth. The infection of *Meloidogyne* spp. induced anatomical changes resulting into the disruption of the xylem, root epidermis and cortical tissues in response to giant-cell development and gall formation (Bird, 1974; Meon et al., 1978; Shepherd and Huck, 1989). Wilcox-Lee and Loria (1987) reported that root damage and dysfunction due to alterations in root anatomy may affect host-plant water relations and suppress plant growth and development. Reduced leaf stomatal resistance and transpiration (Kirkpatrick et al., 1995) and water deficit stress symptoms after root-knot nematode infestion were also documented (O'Bannon and Reynold, 1965). Thus it is possible that the taproot length is an important root parameter to assure sufficient healthy lateral root branching and facilitate the entire root system to absorb enough water and nutrients.

Topological index (TI) for this microplot study ranged from 1.70 to 1.83, indicating a herringbone pattern in which branching is primarily on the main root axis (Fitter, 1986; Werner and Smart, 1973). A herringbone root branching pattern was also found in previous soil bulk density experimentsin a controlled environment (Chapter II). In this experiment, soil infested with *M. incognita* increased TI from 1.79 to 1.89. Changes in the TI in response to other root pathogens have also been reported (Larkin et al., 1995, 1996). Soil infested with *P. irregulare* (TI = 1.86) or *P. ultimum* (TI = 1.72) resulted in altered root system architecture in alfalfa compared with the TI of alfalfa roots in uninfested soil (Larkin et al., 1995).

Soil physical environment influenced seedling growth and root development was found both in controlled environmental study (Chapter II) and in this microplot study. The higher soil bulk density at 1.50 g/cm^3 in controlled environmental study tended to improve seedling growth

and root branching. Generally, the presence of soil hard pan enhanced seedling performance. The beneficial increment to seedling caused by higher soil bulk density and soil hard pan resulted from the sufficient soil water and low physical impedance since there is a complicated interaction among soil bulk density, water availability and physical resistance (Coelho et al., 2000; Taylor and Gardner, 1963).

Nematode effects toward plant growth were dissimilar. Nematode infection consistently indicated detrimental effects on plant growth and root development in controlled environmental studies (Chapter II) which agreed with previous report (Kirkpatrick et al., 1991). In this microplot study, however, based on orthogonal contrasts for nematode rate effects, nematode elucidated less or no effect on seedling growth in both years. The reasons that caused the different nematode effects between these two studies were not clear. But the different soil texture and growth environment may contribute to these different observations since host-nematode interaction involves a series of physiological reaction (Bird and Koltai, 2000; Glazer et al., 1983).

Nematode infection tended to increase root topological index (TI) were found in both studies. TI was increased from 1.79 to 1.89 after nematode infection which indicating a herringbone (less branching) architectural structure in controlled environmental study and only nematode infection significantly increased TI. Similarly, TI for early season in microplot study ranged from 1.71-1.79 exhibiting a herringbone architectural structure as well, and nematode infection tended to increase TI no matter the presence the soil HP or not. In contrast to TI for controlled environmental study, the smaller values of topological indices after nematode infection in microplot elicited the relatively abundant branching thus further confirm the compensatory root branching.

Compensatory root growth due to a soil hard pan and a low level of *M. incognita* were found in this study early in the season. However, it is root physiological function rather than root morphological features that determine plant growth. Late in the season taproot length was reduced in the presence of the nematode but not a variety of other root architectural parameters. This resulted in reduced plant height late in the season and reduced yield. The season-long effect of soil hard pan resulted in the root system being primarily above the hard pan. However, the presence of a hard pan did not affect seed cotton yields in this study, which was likely the result water and nutrient availability being near optimum.

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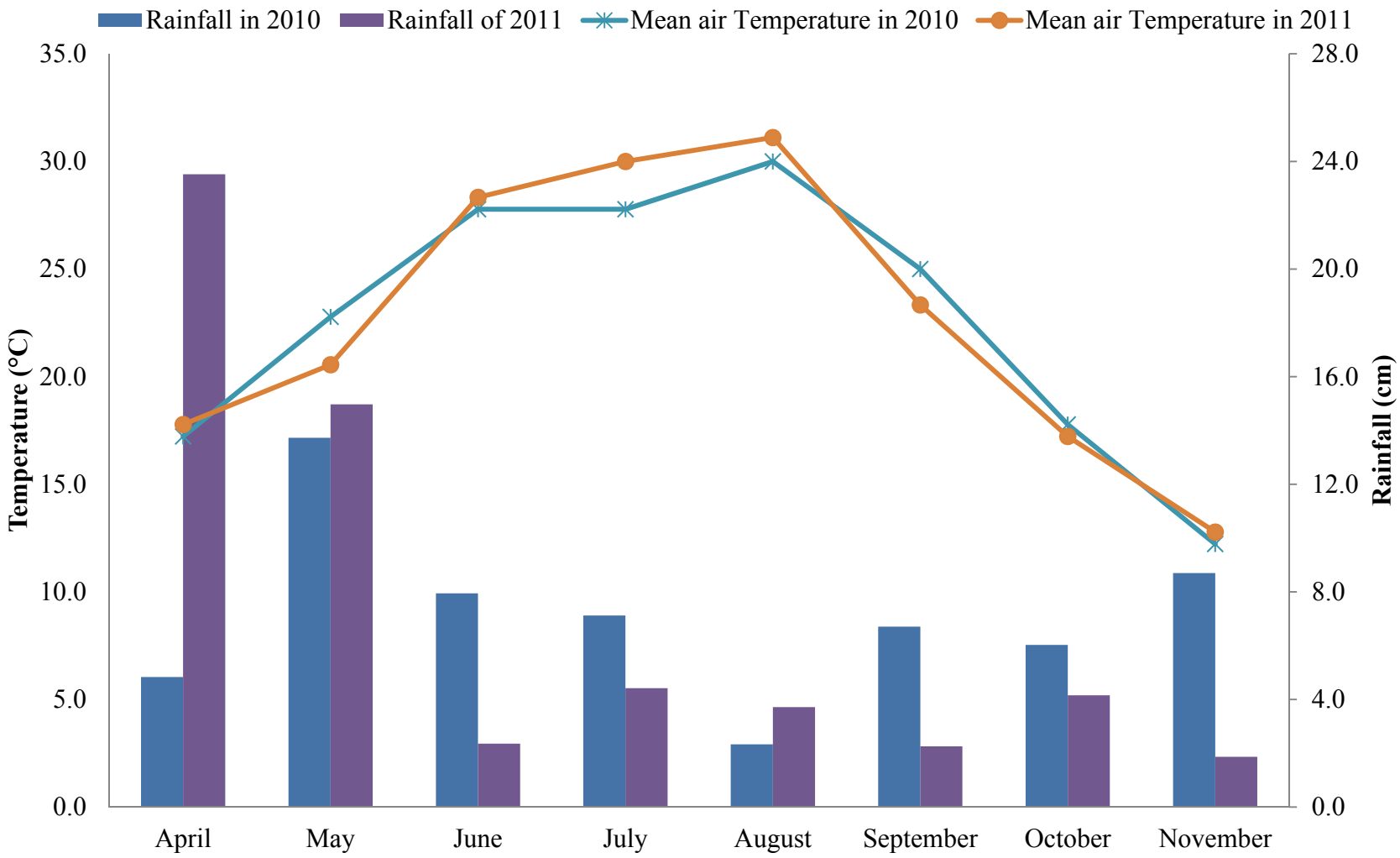
Figure 1. Rainfall and mean air temperature[x] for Hope in 2010 and 2011.



[x] Mean air temperatures were the mean values of the sums of daily maximum and minimum air temperatures.

Table 1. Effects of soil hard pan (HP)[u] and *Meloidogyne incognita* (Mi) rate[v] on cotton seedling characteristics and root fresh weight in 2010 and 2011

Treatment	Stand (%)[w]		HNR[x]		Root fresh weight (g)	
	2010	2011	2010	2011	2010	2011
HP						
0	78.2 b[y]	91.6 a	1.3 a	1.8 b	0.3 b	0.6 b
1	89.7 a	91.7 a	1.4 a	2.0 a	0.4 a	0.9 a
M. incognita rate						
0	85.9	90.3	1.5	2.0	0.5	0.7
4	87.5	93.1	1.4	1.8	0.3	0.7
8	81.6	92.5	1.2	1.8	0.3	0.7
12	83.1	93.8	1.2	1.9	0.3	0.9
Contrast[z]	----------		*P*		----------	
Mi rate Linear	—	0.3761	0.0369	—	—	—
Mi rate Quadratic	—	0.7510	0.7122	—	—	—
Mi rate Cubic	—	0.6201	0.3061	—	—	—

[u]An artificial soil hard pan (HP) was 20cm below the soil surface. 0 = no soil hard pan, 1= soil hard pan.
[v] *Meloidogyne incognita* = 0, 4, 8, 12 eggs/cm^3 soil; all treatments included *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
[w] Stand counts were recorded 20 days after planting.
[x] Height-to-node ratio (HNR) = plant height from the cotyledonary node to terminal (cm)/total number of nodes per plant.
[y] Means in a column followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.
[z] Orthogonal contrasts for Mi rate on root morphological parameters were significant if $P < 0.05$.

Table 2. Two way interaction of soil hard pan (HP)[u] by *Meloidogyne incognita* (Mi) rate[v] on cotton seedling root morphological parameters in 2010.

HP	*M. incognita* rate	Magnitude[w]	Altitude[x]	Exterior pathlength[y]	Total root length (cm)
0	0	22.6	15.0	246.6	50.08
0	4	31.7	18.7	411.6	57.12
0	8	41.4	24.0	662.6	69.91
0	12	28.2	20.5	399.3	46.67
1	0	18.8	13.3	183.1	43.67
1	4	48.7	24.5	767.5	87.61
1	8	35.8	22.2	528.4	71.99
1	12	27.1	19.0	365.1	55.97
Contrast[z]			*P*		
HP*Mi rate Linear		0.5668	0.5112	0.4355	0.7139
HP*Mi rate Quadratic		0.1347	0.0933	0.1419	0.1678
HP*Mi rate Cubic		0.0022	0.0105	0.0013	0.0254

[u] An artificial soil hard pan (HP) was 20cm below the soil surface. 0 = no soil hard pan, 1= soil hard pan.
[v] *Meloidogyne incognita* = 0, 4, 8, 12 eggs/cm^3 soil; all treatments included *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
[w] Magnitude (μ) is the number of exterior links per root system.
[x] Altitude (α) is the number of links in the longest path from any exterior root link to the base link.
[y] Exterior pathlength (*Pe*) = the sum of links in all paths.
[z] Orthogonal contrasts for HP by *Meloidogyne incognita* rate on root morphological parameters were significant if $P<0.05$.

Table 3. Effects of soil hard pan (HP)[t] and *Meloidogyne incognita* (Mi)[u] rate on cotton seedling root volume in 2010 and root morphological parameters in 2011

Treatment	Magnitude[v]	Altitude[w]	Exterior pathlength[x]	Total root length (cm)	Root volume (cm^3)	
	2011	2011	2011	2011	2010	2011
HP						
0	33.2 a[y]	19.0 a	449.9 a	73.19 a	0.16 b	0.34 b
1	29.7 a	18.6 a	380.4 a	58.50 b	0.22 a	0.61 a
M. incognita rate						
0	24.7	17.6	309.0	53.67	0.21	0.49
4	34.8	19.2	453.9	74.95	0.20	0.48
8	31.7	18.5	404.4	65.52	0.21	0.47
12	29.0	17.1	364.4	60.28	0.17	0.48
Contrast[z]	----------	----------	*P*	----------	----------	----------
Mi rate Linear	0.3864	—	0.6036	0.6593	—	—
Mi rate Quadratic	0.0121	—	0.0542	0.0055	—	—
Mi rate Cubic	0.2312	—	0.3335	0.0936	—	—

[t] An artificial soil hard pan (HP) was 20cm below the soil surface. 0= no soil hard pan, 1= soil hard pan.
[u] *Meloidogyne incognita* = 0, 4, 8, 12 eggs/cm^3 soil; all treatments included *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
v Magnitude (μ) is the number of exterior links.
[w] Altitude (α) means the number of links in the longest path from any exterior link to the base link.
[x] Exterior pathlength (*Pe*) = the sum of the number of links in all paths.
[y] Means in a column followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.
[z] Orthogonal contrasts for HP by Mi rate on root morphological parameters were significant if $P < 0.05$.

Table 4. Two way interaction of soil hard pan (HP)[w] by *Meloidogyne incognita* (Mi) rates[x] on plant growth in the late-season of 2010

HP	*M. incognita* rate	Height[y] (cm)	Taproot length (cm)
0	0	86.95	25.73
0	4	67.15	15.25
0	8	63.72	15.34
0	12	72.77	19.12
1	0	82.39	11.51
1	4	74.68	10.28
1	8	76.26	9.54
1	12	75.50	11.39
Contrast[z]		*P*	
HP*Mi rate Linear		0.0124	0.1081
HP*Mi rate Quadratic		0.0012	0.0288
HP*Mi rate Cubic		0.4836	0.3702

[w] An artificial soil hard pan (HP) was 20cm below soil surface. 0= no soil hard pan, 1= soil hard pan.
[x] *Meloidogyne incognita* = 0, 4, 8, 12 eggs/cm^3 soil; all treatments also included *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
[y] Plant height measured from the cotyledonary node to the tip of the main stem terminal.
[z] Orthogonal contrasts for HP by Mi rate on root morphological parameters were significant if $P<0.05$.

Table 5. Effects of soil hard pan (HP)[w] and *Meloidogyne incognita* (Mi) rate[x] on plant growth in late-season of 2011

Treatment	Root dry weight below HP (g)	Root dry weight above HP (g)	Root dry weight (g)
HP			
0	4.47 a	26.36 a	30.73 a
1	1.47 b	33.48 a	34.89 a
M. incognita rate			
0	3.47	25.66	29.13
4	2.05	22.99	25.04
8	2.49	24.87	27.20
12	2.28	48.88	51.01
Contrast[z]	---------- *P* ----------		
Mi rate Linear	0.1094	—	—
Mi rate Quadratic	0.1083	—	—
Mi rate Cubic	0.2503	—	—

[w] An artificial soil hard pan (HP) was 20cm below soil surface. 0= no soil hard pan, 1= soil hard pan.
[x] *Meloidogyne incognita* = 0, 4, 8, 12 eggs/cm^3 soil; all treatments also included *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
[y] Means in a column followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.
[z] Orthogonal contrasts for HP by Mi rate on root morphological parameters were significant if $P < 0.05$; '—' indicated *M. incognita* rate effects were not significant.

Table 6. Effect of soil hard pan (HP)[t] and *Meloidogyne incognita* (Mi) rate[u] on cotton growth characters for late-season of 2010

Treatment	Cracked boll[v]	First sympodial Node[w]	Total bolls	No. of Sympodial branchs	Yield[x] (g)
HP					
0	3.0 b[y]	9.6 a	22.3 a	13.3 a	101.96 a
1	8.6 a	7.7 b	23.9 a	14.6 a	112.13 a
M. incognita rate					
0	10.3	6.8	26.3	15.0	129.10
4	5.0	8.5	18.4	13.3	82.83
8	4.9	9.4	20.2	12.9	101.98
12	3.4	9.5	25.4	13.5	107.07
Contrast[z]	----------	----------	*P*	----------	----------
Mi rate Linear	<.0001	<.0001	0.7906	0.0384	0.3625
Mi rate Quadratic	0.0753	0.0103	0.0030	0.0239	0.0473
Mi rate Cubic	0.0995	0.9358	0.4630	0.8282	0.1482

[t] An artificial soil hard pan (HP) was 20cm below the soil surface. 0= no soil hard pan, 1= soil hard pan.
[u] *Meloidogyne incognita* = 0, 4, 8, 12 eggs/cm^3 soil; all treatments included *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
[v] The total numbers of cracked bolls at 114 days after planting.
[w] Nodes to the first sympodial branch excluding cotyledonary node.
[x] Seed cotton yields per plant were handpicked and weighed in grams.
[y] Means in a column followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.
[z] Orthogonal contrasts for HP by Mi rate on root morphological parameters were significant if $P < 0.05$.

Table 7. Effect of soil hard pan (HP)[t] and *Meloidogyne incognita* (Mi) rate[u] on root morphological characters in the late-season of 2010 and 2011

Treatment	Magnitude[v]		Altitude[w]		Exterior pathlength[x]		Total root length (cm)	
	2010	2011	2010	2011	2010	2011	2010	2011
HP								
0	154.5 b[y]	194.6 a	76.3 a	90.2 a	7760.1 b	10085.3 a	192.5 a	364.3 a
1	177.8 a	234.1 a	75.9 b	80.6 a	8979.4 a	11788.5 a	180.7 a	342.8 a
M. incognita rate								
0	125.8	183.9	69.3	77.2	5614.1	8289.7	170.5	341.5
4	167.0	261.9	78.4	94.4	8415.5	13709.3	180.6	383.5
8	180.3	228.2	75.5	87.6	9399.4	12702.6	187.6	355.9
12	203.2	217.0	84.0	86.1	10666.1	12712.9	202.3	346.9
Contrast[z]	----------	----------	----------	----------	*P*	----------	----------	----------
Mi rate Linear	0.0007	0.4504	0.3160	0.5082	0.0052	0.1195	0.1191	0.9047
Mi rate Quadratic	0.5839	0.0238	0.6006	0.1696	0.7353	0.1246	0.4402	0.2369
Mi rate Cubic	0.5522	0.1240	0.3885	0.3341	0.7638	0.3425	0.9892	0.3588

[t] An artificial soil hard pan (HP) was 20cm below the soil surface. 0= no soil hard pan, 1= soil hard pan.
[u] *Meloidogyne incognita* = 0, 4, 8, 12 eggs/cm^3 soil; all treatments included *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
v Magnitude (μ) is the number of exterior links.
[w] Altitude (α) means the number of links in the longest path from any exterior link to the base link.
[x] Exterior pathlength (*Pe*) = the sum of the number of links in all paths.
[y] Means in a column followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.
[z] Orthogonal contrasts for HP by Mi rate on root morphological parameters were significant if $P < 0.05$.

Table 8. Effects of soil hard pan (HP)[v] and *Meloidogyne incognita* (Mi) rate[w] on topological index (TI)[x] of 2010 and 2011

Treatment	TI				
	2010-6	2010-10		2011-6	2011-11
HP					
0	1.75 a[y]				1.74 a
1	1.77 a				1.70 b
		HP		HP	
M. incognita rate		0	1	0	1
0	1.77	1.83	1.74	1.79	1.78	1.72
4	1.72	1.77	1.77	1.71	1.74	1.71
8	1.75	1.83	1.72	1.72	1.76	1.72
12	1.80	1.75	1.73	1.79	1.73	1.74
Contrast[z]			*P*			
HP*Mi rate linear	–	0.5415		0.2464	–
HP*Mi rate Quadratic	–	0.9777		0.0069	–
HP*Mi rate Cubic	–	0.0054		0.4766	–
Mi rate linear	0.1698	–		–	–
Mi rate Quadratic	0.0044	–		–	–
Mi rate Cubic	0.5660	–		–	–

[v] An artificial soil hard pan (HP) was 20cm below the soil surface. 0 = no soil hard pan, 1= soil hard pan.
[w] *Meloidogyne incognita* = 0, 4, 8, 12 eggs/cm^3 soil; all treatments included *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
[x] Topological index (TI) is the regression slope from double-log plots of the root system topological parameters exterior patholength versus magnitude for cotton seedlings.
[y] Means in a column followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.
[z] Orthogonal contrasts for HP by Mi rate on root morphological parameters were significant if $P < 0.05$.

Table 9. Effect of soil hard pan (HP)[v] and *Meloidogyne incognita* (Mi) rate[w] on disease severity and pathogen populations in 2010 and 2011

Treatment	Galling[x]				J2[y]	
	Early-season		Late-season		Late-season	
	2010	2011	2010	2011	2010	2011
HP						
0	48.0 a[z]	69.9 a	147.4 a	103.8 b	3.343 a	2.329 a
1	58.9. a	86.0 a	150.0 a	125.4 a	2.723 b	2.459 a
M. incognita rate						
4	50.2	66.7	150.0	102.7	3.281	2.415
8	62.0	91.9	144.7	124.8	3.031	2.384
12	53.0	78.2	150.0	124.4	2.890	2.284
Contrast[z]	----------	----------	*P*	----------	----------	----------
Mi rate linear	0.8158	0.3377	1.0000	0.0533	0.0520	0.7167
Mi rate Quadratic	0.3232	0.0952	0.1081	0.1219	0.7249	0.9119

[v] An artificial soil hard pan (HP) was 20cm below the soil surface. 0= no soil hard pan, 1= soil hard pan.
[w] *Meloidogyne incognita* = 0, 4, 8, 12 eggs/cm^3 soil; all treatments included *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
[x] Root galling based on a 0-5 scale: 0=no galls, 1=1-2, 2=3-10, 3=11-30, 4=31-100, 5=>100 galls/root. Analyses were conducted using mid-point values. Treatments without *M. incognita* were dropped from the analyses.
[y] Second stage juveniles (J2) per 100 cm^3 soil were expressed as $\log_{10} + 1$.
[z] Means in a column followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.

Figure 2. Mean soil temperature for the six weeks after planting in 2010 and 2011

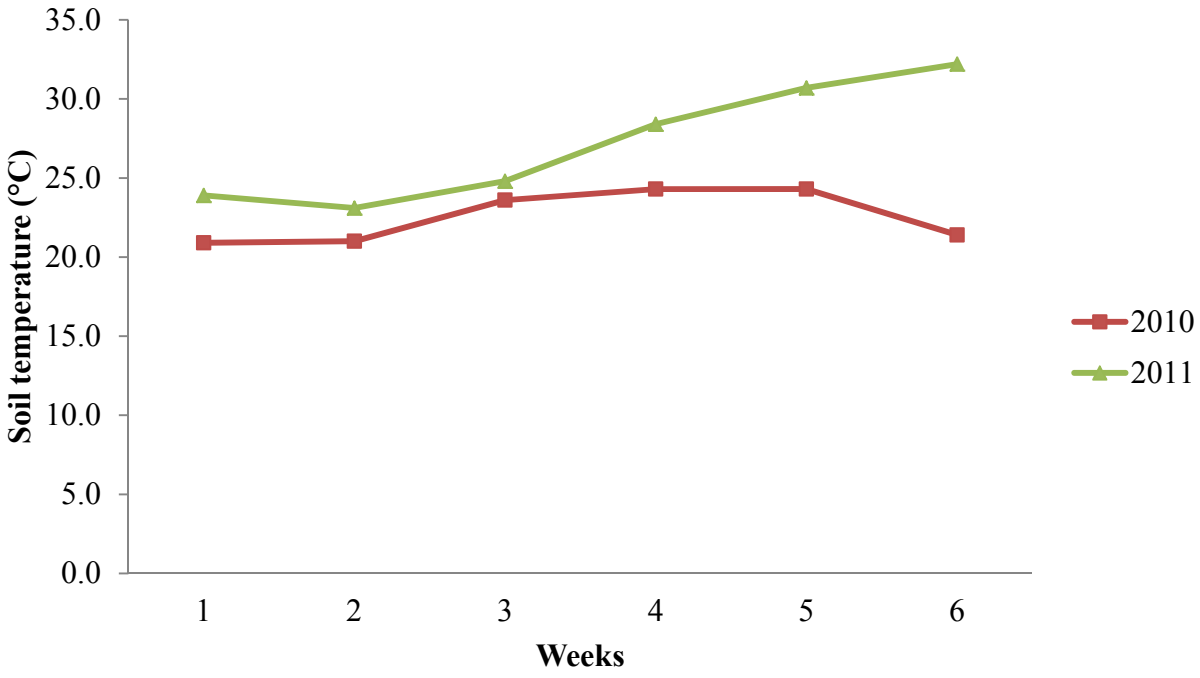
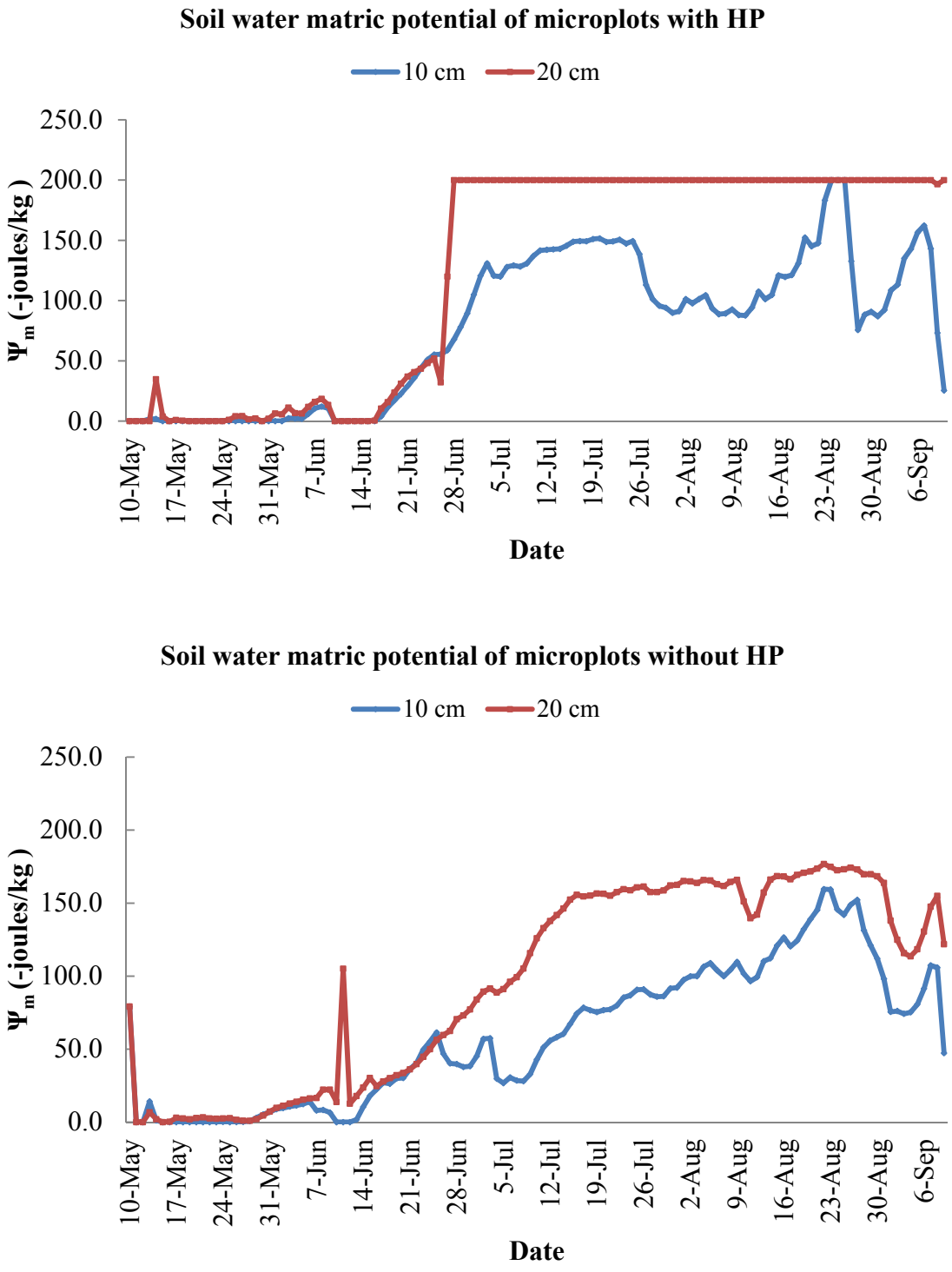


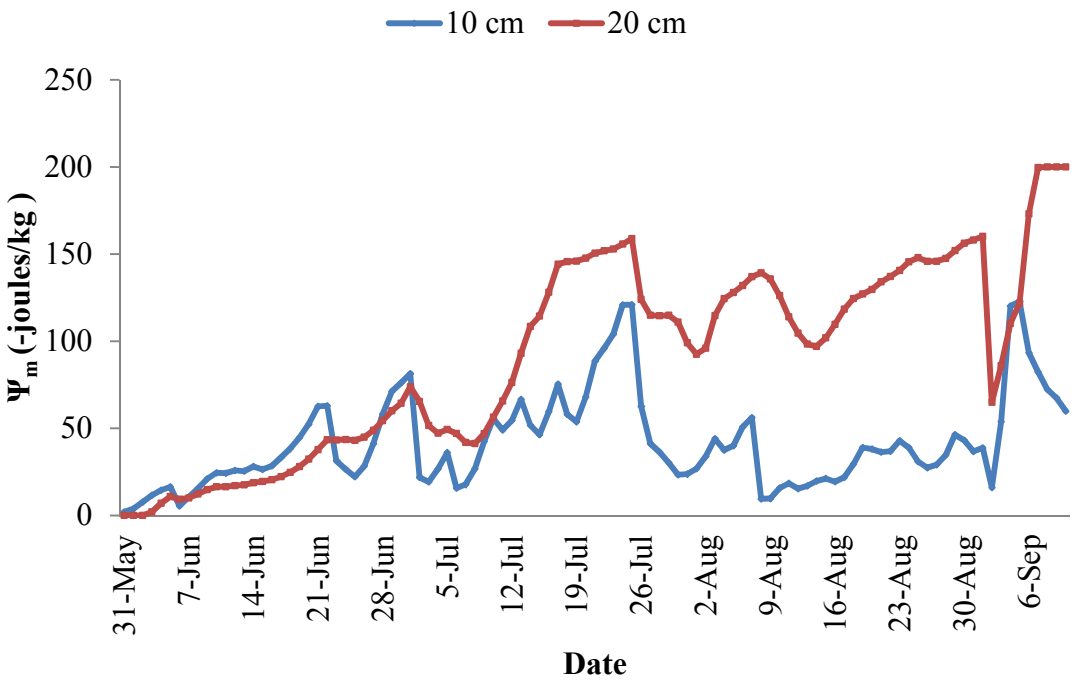
Figure 3. Hope soil water matric potential for HP and NHP plot in 2010 (A) and 2011 (B)

A.



B.

Soil water matric potential of microplots with HP



Soil water matric potential of microplots without HP

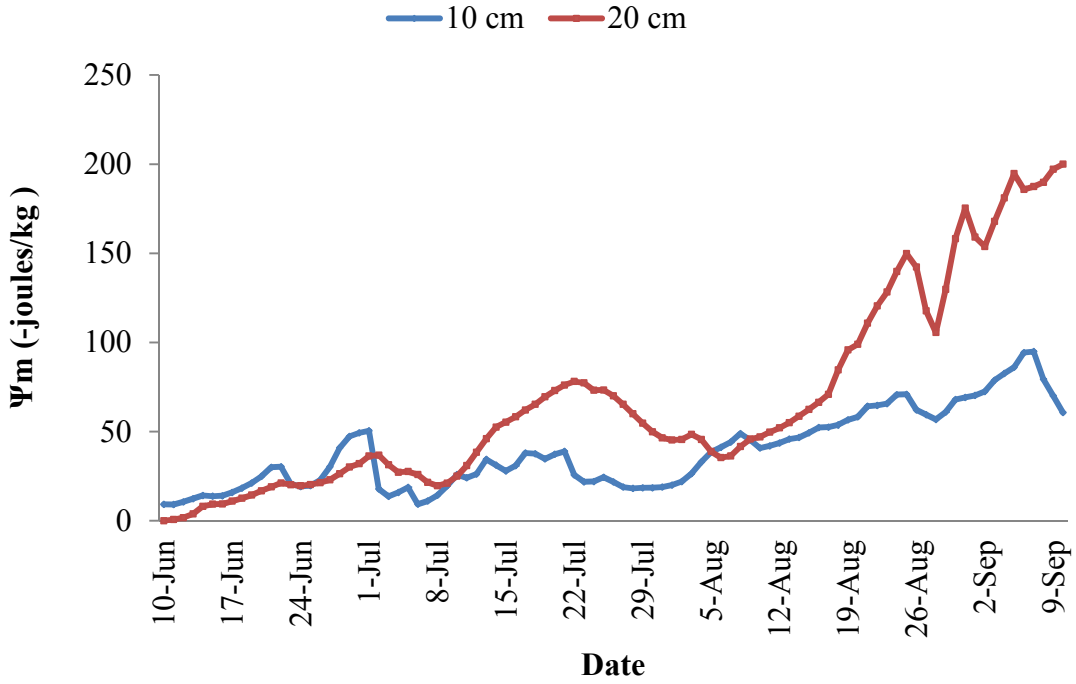
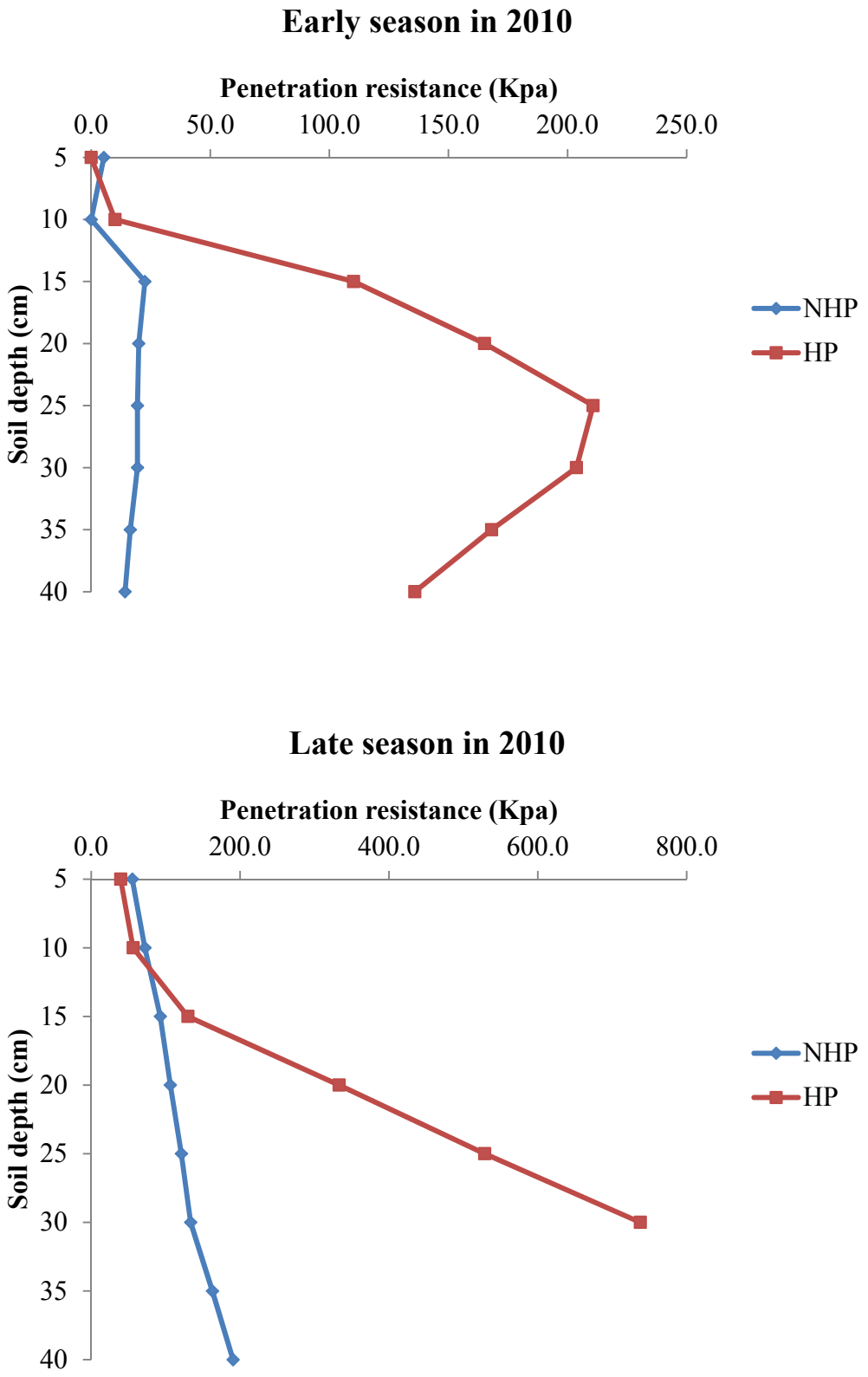
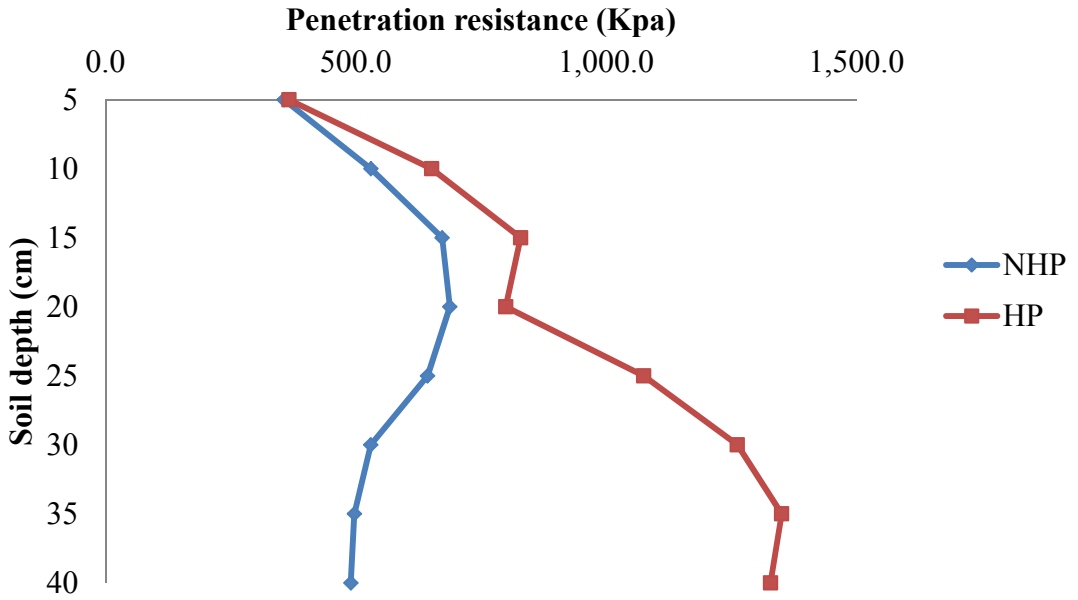


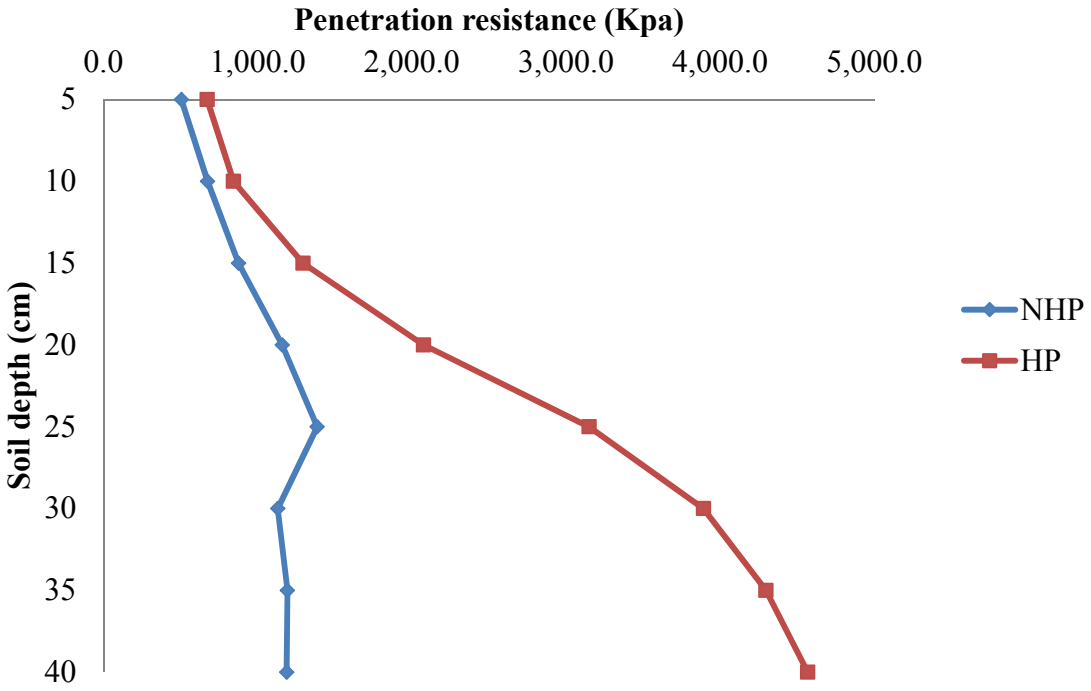
Figure 4. Soil penetration resistance for microplot in early and late season of 2010 and 2011



Early season in 2011



Late season in 2011



Appendix Table 1. Effect of soil hard pan (HP)[x] and *Meloidogyne incognita* (Mi) rate[y] on root growth in early season of 2011

Treatment	Average radius (mm)	Root surface area (cm^2)
HP		
0	0.40 a	17.20 a
1	0.60 b	20.47 b
M. incognita rate		
0	0.57	17.18
4	0.47	20.59
8	0.50	18.78
12	0.53	18.07
Contrast[z]		
Mi rate Linear	—	—
Mi rate Quadratic	—	—
Mi rate Cubic	—	—

[x] An artificial soil hard pan (HP) was 20cm below the soil surface. 0= no soil hard pan, 1= soil hard pan.
[y] Pathogen treatments included *Meloidogyne incognita* (0, 4, 8, 12 eggs/cm^3 soil) + *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
[z] Orthogonal contrasts for HP by Mi rate on root morphological parameters were significant if $P<0.05$; '—' indicated *M. incognita* rate effects were not significant.

Appendix Table 2. Effect of soil hard pan (HP)[u] and *Meloidogyne incognita* (Mi) rate[v] on root growth and plant development in late season of 2010 and 2011

Treatment	Average radius (mm)		Root surface area (cm^2)		Monopodial node	Monopodial bolls	Sympodial boll[w] (%)	Sympodial branch with two bolls	Bloom date[x]
	2010	2011	2010	2011	2010	2010	2010	2010	2010
HP									
0	0.66 a[y]	0.83 b	83.16 a	184.76 b	2.4 a	7.1 a	72 a	2.1 b	75.7 a
1	0.65 a	1.00 a	73.10 b	213.08 a	2.8 a	8.8 a	64 b	3.0 a	67.5 b
M. incognita rate									
0	0.66	0.81	69.78	171.16	3.1	9.2	63	3.1	66.8
4	0.61	0.96	71.06	221.52	2.2	4.9	75	2.4	71.8
8	0.66	0.94	77.44	211.07	2.5	7.2	66	2.3	73.1
12	0.70	0.94	88.53	204.97	2.6	9.6	67	2.1	75.2
Contrast[z]					*P*				
Mi rate Linear	0.0825	0.0492	—	0.0920	—	0.5552	—	0.0307	0.0002
Mi rate Quadratic	0.1382	0.0764	—	0.0203	—	0.0196	—	0.5371	0.3128
Mi rate Cubic	0.2945	0.3601	—	0.2258	—	0.2515	—	0.5498	0.4736

[u] An artificial soil hard pan (HP) was 20cm below soil surface. 0= no soil hard pan, 1= soil hard pan.
[v] Pathogen treatments included *Meloidogyne incognita* (0, 4, 8, 12 eggs/cm^3 soil) + *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
[w] Percentage of number of bolls from sympodial branches.
[x] First blooming day for each plot.
[y] Means in a column followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.
[z] Orthogonal contrasts for HP by Mi rate on root morphological parameters were significant if $P < 0.05$.

Appendix Table 3. Main and interaction effects (*P* values) of soil hard pan (HP)[x] and pathogen treatments (Trt)[z] on leaf temperature, stomatal resistance and transpiration of cotton grown in microplot in 2010

Treatment	Leaf temperature (°C)			Stomatal resistance (cm/s)			Transpiration (µg/cm^2/s)		
	June	July	August	June	July	August	June	July	August
HP	0.5675	0.6490	0.3559	0.0370	0.2958	0.8024	0.1454	0.0208	0.9916
Trt	0.9391	0.9209	0.2087	0.5180	0.2346	0.1971	0.3198	0.0372	0.4301
HP*Trt	0.7095	0.7049	0.7549	0.8682	0.9006	0.6166	0.9989	0.1991	0.9500

[y] An artificial soil hard pan (HP) was 20cm below the soil surface. 0= no soil hard pan, 1= soil hard pan.
[z] Pathogen treatments included *Meloidogyne incognita* (0, 4, 8, 12 eggs/cm^3 soil) + *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).

Appendix Table 4. Effect of soil hard pan (HP)[w] and *M. incognita* (Mi) rate[x] on leaf temperature, stomatal resistance and transpiration of cotton grown in microplot in 2010

Treatment	Leaf temperature (°C)			Stomatal resistance (cm/s)			Transpiration (µg/cm^2/s)		
	June	July	August	June	July	August	June	July	August
HP									
0	36.4 a[y]	33.7 a	33.8 a	1.06 b	41.08 a	3.66 a	39.02 a	36.20 b	9.29 a
1	36.6 a	33.6 a	34.3 a	1.75 a	42.24 a	3.59 a	32.25 a	45.79 a	9.28 a
M. incognita rate									
0	36.4	33.3	33.0	1.78	43.02	3.65	25.84	48.13	8.95
4	36.5	33.5	33.3	1.93	43.03	4.31	30.46	38.43	8.02
8	36.3	33.9	33.9	1.26	40.43	3.29	34.31	40.25	9.79
12	36.5	33.2	34.5	1.42	41.17	3.45	37.16	37.26	9.35
					P				
Contrast[z]									
Mi rate linear	—	—	—	—	—	—	—	0.0633	—
Mi rate Quadratic	—	—	—	—	—	—	—	0.3698	—
Mi rate Cubic	—	—	—	—	—	—	—	0.2291	—

[w] An artificial soil hard pan (HP) was 20cm below the soil surface. 0= no soil hard pan, 1= soil hard pan.
[x] Pathogen treatments included *Meloidogyne incognita* (0, 4, 8, 12 eggs/cm^3 soil) + *Thielaviopsis basicola* (40 chlamydospore chains /cm^3 soil).
[y] Means in a column followed by an identical letter are not significantly different according to Fisher's protected least significant difference (LSD) at $P \leq 0.05$.
[z] Orthogonal contrasts for HP by Mi rate on root morphological parameters were significant if $P < 0.05$.

Appendix Table 5. Effects of soil hard pan (HP)[x] on soil water contents ($cm^3 \cdot cm^{-3}$) of the microplot at the time of June 2011 and November 2011 PR measurements[y]

Treatment	Soil depth, cm					
	0-15		15-30		30-45	
	2011-6	2011-11	2011-6	2011-11	2011-6	2011-11
HP						
0	0.0876	0.0881	0.1025	0.0929	0.1027	0.0827
1	0.0914	0.1267	0.1046	0.0963	0.0954	0.0714
P value	0.3475	<.0001	0.6485	0.3798	0.2102	0.0436

[x] An artificial soil hard pan (HP) was 20cm below the soil surface. 0= no soil hard pan, 1= soil hard pan.
[y] *P* values ≤ 0.05 are significant.

Chapter V

Conclusion

The root-knot nematode, *Meloidogyne incognita*, is a detrimental soilborne pathogen on cotton (*Gossypium hirsutum*). *M. incognita* causes root galls and dysfunction of root system. Another soilborne pathogen which causes black root rot of cotton seedling is the fungal pathogen, *Thielaviopsis basicola. M. incognita* exists 30% and *Thielaviopsis basicola* found 70% of cotton field in Arkansas, and these two pathogens commonly co-exist together and interact on cotton resulting in delayed cotton development and reduced yield. In the field, the stunted areas indicating a higher soil bulk density incline to have higher *M. incognita* population. *M. incognita* prefers coarse-textured soil type with high soil temperature while *T. basicola* is favor by soil with low sand content with cool and wet soil. Based on field investigation, soil physical environmental conditions affect both these two soilborne pathogen and cotton growth. The primary objective for this research was to evaluate the effects of soil physical parameters, including soil bulk density and soil hard pans, on these two soilborne pathogens and cotton growth. Experiments have been conducted in controlled environmental, field, and microplots.

The effects of *Meloidogyne incognita* and *Thielaviopsis basicola* on cotton growth and root morphology were estimated in control environmental studies. Two soil bulk densities, 1.25 and 1.50 g/cm^3, were created and the four pathogen treatments included non-infested soil, soil infested with *M. incognita* (4 eggs/cm^3), soil infested with *T. basicola* (40 chlamydospore chains/cm^3 soil) and soil infested with both pathogens. The results elucidated the bulk density generally did not affect seedling (44 days old) growth and root galling. Low soil penetration resistance (2.6MPa) under well-watered conditions and unsaturated soil porosities which were favorable for *M. incognita* were occurred in this study. The infection caused by both *M. incognita* and *T. basicola* reduced seedling stands and root volume more than either pathogen alone. Both *M. incognita* and *T. basicola reduced* root growth and topological characters, but

only *M. incognita* changed the root topological index (TI) resulting into less branched herringbone roots.

The effects of subsoiling and application of the nematicide *1,3-dichloropropene* (Telone II®) on root system development and plant growth were investigated consecutively for three years in a commercial cotton field in northeastern Arkansas from 2009 to 2011. Treatments included subsoiling, in-row application of Telone II®, subsoiling plus Telone II®, and a control that was neither subsoiled nor treated with the nematicide. Subsoiling did not consistently affect early season growth. However, nematicide effects were more obvious in contrast to subsoiling implement. Nematicide application increased height-to-node ratio and plant dry weight in 2010 and 2011. Root fresh weight and taproot length was increased after nematicide treatment on seedlings in 2011. Nematicide application also increased root magnitude in 2009 and root volume on seedlings in 2011. Root galling and the population of second-stage juveniles of *M. incognita* were reduced by Telone II®. No subsoiling or nematicide treatment effect occurred on cotton development, seed cotton yield and root topological characters at harvest in three years.

The effects of *Meloidogyne incognita, Thielaviopsis basicola,* and a soil hard pan on cotton root architecture and plant growth were evaluated in a microplot study in 2010 and 2011 at the Southwest Research and Extension Center, Hope, Arkansas. Ninety-six microplots were used. An artificial HP was created 20 cm below the soil surface for half of the plots. The soils from another half plots were dug out to form plots without hard pan (NHP). A steam-pasteurized, fine loamy sand (87.1% sand, 6.8% silt and 6.1% clay) was filled into the top 20 cm of plots with a HP and the entire plots without HP in both years. Soil was disinfested by drenching with Vapam® HL in 2011 before planting. Pathogen treatments included soil infested with *T. basicola* (40 chlamydospore chains/cm^3 soil) plus four different *M. incognita* levels (0, 4, 8, 12 eggs/cm^3

soil). Two additional pathogen treatments were non-infested soil and soil infested with *M. incognita* only (4 eggs/cm^3 soil). Seedling stands were higher in HP plots in 2010 and greater height-to-node ratio (HNR) of seedling was observed in 2011. Root fresh weight of seedling was higher in both years in HP plot. Improved seedling growth occurred was because the higher soil water contents above the HP layer in comparison with the same soil depth in NHP plots. The soil HP enhanced cotton development in the season of 2010 expressed by the increased number of cracked bolls (114 days after planting (DAP) and lowered position of first sympodial branch. HP reduced J2 population in 2010 but increased galling in the late season of 2011. *M. incognita* infection reduced taproot length, delayed cotton maturity and reduced seed cotton yield. However, the presence of the nematode tended to increase root topological parameters including altitude, magnitude and exterior pathlength in microplot studies. Topological indices under all the treatment ranged from 1.70 to 1.83 elucidating herringbone root branching both years.

Topological models provide a new approach to quantify the changes caused by soilborne pathogens and soil physical conditions. Results provide useful crop management information for growers and also will be beneficial for studies on soilborne pathogens-host interactions.