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Screening Of New Commercial And Experimental Gossypium Hirsutum Cultivars For Tolerance To The Reniform Nematode (Rotylenchulus Reniformis)

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SCREENING OF NEW COMMERCIAL AND EXPERIMENTAL GOSSYPIUM HIRSUTUM CULTIVARS FOR TOLERANCE TO THE RENIFORM NEMATODE (*ROTYLENCHULUS RENIFORMIS*)

By

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The reniform nematode is a major pest affecting common upland cotton in the United States. Management of this pest in cotton fields only gives partial control and is sometimes neither economical nor profitable. Past research has shown no resistance to the reniform nematode in currently available commercial cotton cultivars. Screenings of several currently available cotton cultivars for tolerance to the reniform nematode were conducted in the growing seasons of 2006 and 2007 at the Delta Branch Experiment Station in Stoneville MS. Several cultivars were identified as tolerant and productive including 'Croplan Genetics 3520 B2RF,' 'DynaGrow 2520 B2RF,' and 'Stoneville 5242 BR.' Other cultivars were tolerant but less productive, including 'Deltapine 488 BG/RR,' 'Fibermax 960 B2R,' and 'Stoneville 5599 BR.' 'Deltapine 455 BG/RR,' 'Phytogen 370,' and 'Phytogen 485' were shown to be productive, but not tolerant to the reniform nematode.

DEDICATION

I would like to dedicate this research to my family, who supported me endlessly and allowed me to pursue my education.

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CHAPTER I

INTRODUCTION

Cotton cultivation in North America may be traced back 7,000 years, and evidence suggests plantings in Florida and Virginia between 1556 and 1607 [National Cotton Council (NCC), 2010c]. Figures from the National Agricultural Statistics Service (NASS) indicate that the U.S. cotton industry contributed to 27,000 businesses, furnished more than 234,000 jobs, and added more than $27 billion in revenues to the U.S. economy in 2007 (USDA-NASS, 2009).

Zhang et al. (2008) identified four agriculturally cultivated *Gossypium* species, including the tetraploid species *G. hirsutum* (Upland cotton) and *G. barbadense* (Pima, Egyptian, and Sea Island cotton), and diploid species *G. herbaceum* and *G. arboreum*. Upland cotton accounts for over 90% of the world's cotton, *G. barbadense* 8%, while *G. herbaceum* and *G. arboreum* combined comprise only 2% of world cotton (Zhang et al., 2008). The Economic Research Service (ERS) of the U.S. Department of Agriculture (USDA) estimates 2010-2011 global cotton consumption at 120.9 million bales (USDA-ERS, 2010a). Although this consumption is below the 123 million bales consumed 3 years ago, growth is expected for the second consecutive season as the global economic recovery continues (USDA-ERS, 2010a).

According to NASS, U.S. hectarage planted in upland cotton began to drop following the 2006 season (USDA-NASS, 2010b). Nationwide, this trend continued through the 2007, 2008, and 2009 growing seasons. Despite a downward trend in production the 2009 upland cotton crop was still valued at $3.5 billion (USDA-NASS, 2010a). In 2010, hectarage planted in upland cotton increased slightly, suggesting the downward trend in cotton hectares has halted. The recent downward trend in upland cotton hectarage can likely be attributed to a market favoring a switch to soybean and corn production on land historically planted in cotton. In 2010, 4.1 million hectares were planted to upland cotton in the U.S (USDA-NASS, 2010a). Texas, Georgia, Arkansas, North

Carolina, and Mississippi ranked among the highest cotton producing states in 2010 (USDA-ERS, 2010a).

In 2009, cotton ranked eighth in value among all Mississippi agricultural commodities providing 3.1% of total farm receipts and contributed 3.9% towards the total value of U.S. upland cotton (USDA-ERS, 2010c). Following national trends, Mississippi upland cotton hectarage began to decline after the 2006 season, but showed signs of leveling off in 2010 (USDA-ERS, 2010a). Mississippi hectarage devoted to cotton in 2009 was the lowest value recorded by NASS since 1953. However, production in 2009 was still quite significant for the state with an estimated value of $126 million (USDA-NASS, 2010c).

Numerous agronomic parameters such as soil fertility, weed control, disease, insects, nematodes and the environment can limit upland cotton production. Some of these limiting factors have been managed quite successfully. The introduction and wholesale adoption of genetically modified (GM) cotton cultivars have greatly reduced losses attributed to worm and weed pests. In 2010, herbicide tolerant, insect resistant, and stacked (herbicide tolerant + insect resistant) GM cultivars were planted on 93% of all U.S. cotton hectarage (USDA-ERS, 2010b). In 2010, cotton producers in Mississippi planted 9% of the hectarage to GM herbicide tolerant cultivars, 12% to insect resistant GM cultivars, and 68% to stacked GM cultivars such that GM cotton cultivars comprised 89% of the cotton hectarage (USDA-ERS, 2010b). Diseases of cotton are controlled primarily through the use of pesticides, resistant cultivars, adequate soil fertility, and proper farming methods (Dean, 2000). Other pests are often controlled through available pesticides. Environmental factors may be difficult or impossible to control, but selection of adapted cultivars, such as heat tolerant cultivars (Hall, 1992) or drought tolerant cultivars (Rosenow et al., 1983) can minimize the impact of unfavorable environments. Nematodes, however, have proven to be especially difficult to diagnose and treat. In addition, nematodes may form symbiotic relationships with other pathogens. Nematodes often live in symbiosis with fungal pathogens enhancing the effects on the host crop (Starr et al., 2001).

Advances in technology such as genetic engineering and improved management of production limiting factors have resulted in a shift in focus to controlling additional cotton pests

such as nematodes (Starr et al., 2007). Due to a lack of unique and easily recognizable above ground symptoms, identification of nematode damage in crops can be quite challenging (Koenning et al., 2004). Above ground symptoms resulting from nematode damage usually include suppressed plant growth, nutrient deficiencies, temporary wilting, fruit abortion, abnormal maturation, and ultimately, lower yields (Koenning et al., 2004). Nematode soil testing and examination of roots are often necessary to accurately diagnose a nematode problem. Living below the soil surface, nematodes will often attach to a root system and at some point during maturation begin feeding, causing cellular damage and disrupting plant growth, while the host exhibits nonspecific symptoms (Endo, 1975; Koenning et al., 2004; Robinson, 2007; Williamson and Hussey, 1996). Root galls, nematode attachment sites, and feeding sites are often visible on host crop roots (Barker et al., 1994; Bridge, 1988; Endo, 1975; Koenning et al., 2004; Robinson, 2007; Williamson and Hussey, 1996). Root systems in infected plants often suffer from a disruption of water and nutrient flow, stunting, forking of secondary roots, and shallow systems, with damage from the nematodes allowing for secondary infection entrance sites, and infection from viral vectors (Barker et al., 1994; Bridge, 1988; Endo, 1975; Koenning et al., 2004; Robinson, 2007; Williamson and Hussey, 1996). Due to such nondescript above ground symptoms, attributing symptoms to nematodes as the possible underlying cause have often been overlooked, misdiagnosed, or misidentified as other pathogens.

The primary plant-parasitic nematodes injuring cotton in the U.S. are: southern root-knot nematode(*Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949); reniform nematode(*Rotylenchulus reniformis* (Linford and Oliveira)); Columbia lance nematode(*Hoplolaimus columbus*) Sher; and the sting nematode (*Belonolaimus longicaudatus*) Rau (Koenning et al., 1999; Koenning et al., 2004; Robinson, 2007; Starr et al., 2007). Losses attributed to nematodes in U.S. cotton production peaked at 5.32% in 2006 (NCC, 2010a). Based on NASS data, this reduction in yield translated into a $5.5 million loss in value of cotton production in 2000. Such yield losses can be attributed in large part to the lack of resistant cultivars, limited crop rotation, loss of effective fumigant nematicides, recent increases in hectarage in the southeastern U.S., and increased awareness and recognition of nematode

damage (Starr et al., 2007). The root-knot nematode, historically, has been classified as the most damaging plant parasitic nematode in the cotton production region of the United States (Koenning et al., 2004); however, the reniform nematode is now considered possibly the most serious nematode threat to the upland cotton producing areas (Blasingame et al., 2002). Furthermore, it appears that the reniform nematode population will overtake other parasitic nematodes. Robinson (2007) suggested that the reniform nematode has already displaced the root-knot nematode in many cotton production areas. An increase in distribution of the reniform nematode has been well documented (Heald and Robinson, 1990; Kirkpatrick and Lorenz, 1997; Overstreet and McGawley, 2000). Displacement of root-knot nematodes may be the result of an antagonistic effect of reniform nematodes on root-knot nematode populations through competition for feeding sites (Diez et al., 2003).

According to the National Cotton Council, 36% of all nematode related cotton yield losses in the U.S. can be attributed the reniform nematode (NCC, 2010a). Based on NCC data, the percentage of nematode related yield losses attributed specifically to reniform are much more common in the southern states of Louisiana (57%), Mississippi (78%), and Alabama (94%) (NCC, 2010b). In 2007, yield losses attributed to reniform nematodes in the southern U.S. were significant. Mississippi, Alabama, and Louisiana, reported losses of 9% (142,000 bales), 8.5% (41,463 bales), and 4% (32,558 bales), respectively, in total cotton production (Blasingame et al., 2008). Surveys have indicated that the percentage of reniform infested fields and the percentage of fields above treatment threshold are growing alarmingly fast in Mississippi (Robinson, 2007). In a state where cotton is among the more valuable commodities, this pest infestation is cause for serious concern.

The first description of the reniform nematode in the U.S. was in 1940 (Linford and Oliveira, 1940). The pest was not formally identified in Mississippi until 1968 by G. W. Lawrence (Heald and Robinson, 1990). Since that time, all cotton producing counties in Mississippi have reported infestations of *R. reniformis* (Blasingame and Patel, 1987; Sciumbato et al., 2004; Blessitt and Sciumbato, 2005; Dismukes et al., 2006). The reniform nematode is an obligate parasite with a highly specialized life history (Mai et al., 1996). The infective stage and feeding

are restricted to the female. Female juveniles embed partially in the root and begin feeding. Not only does feeding destroy root tissue, but feeding also allows for secondary infection by other pathogens. As described by Linford and Oliveira (1940), “this species is highly specialized for a sedentary mode of life, that is, with a superimposed series of molts without growth intervals, degeneration of males, and transformation of adult females to a reniform shape.” Common symptoms of reniform nematode infestation include stunting, foliage discoloration, and reduced fruiting, all of which may resemble nutritional deficiencies and damage caused by other nematodes (Robinson, 1999; Schmitt and Sipes, 2000). In reniform nematode infested fields lacking irrigation, yield can be severely reduced during drought years, and any decisions regarding the use of a nematicide must be thoroughly analyzed to determine potential economic returns (Koenning et al., 2007). Studies have shown upland cotton yields increase when effective control of nematode populations occur (Lawrence et al., 1990; Rich and Kinloch, 2000). Furthermore, reniform nematode damage causes negative effects on cotton lint yield and fiber value (Cook et al., 1997; Cook and Namken, 1992).

Compounding the reniform nematode problem in cotton is the fact that many weed species, such as Florida beggerweed (*Desmodium tortuosum*), purple nutsedge (*Cyperus rotundus* L.), sicklepod (*Senna obitifolia*), and smallflower morningglory [*Jacquemontia tamnifolia* (L.) Griseb.] serve as moderate to good hosts (Davis and Webster, 2005). However, a study has shown that certain common southeastern weeds, such as ivyleaf morningglory [*Ipomoea hederaceae* (L.) Jacq.], pigweed (*Amaranthus* spp.), prickly sida (*Sida spinosa* L.), Florida pusley (*Richardia scabra* L.), cutleaf eveningprimrose (*Oenothera laciniata* Hill.), yellow nutsedge (*Cyperus esculentus* L.), and common cocklebur (*Xanthium strumarium* L.), serve as poor hosts and do not support high reniform nematode populations in the absence of a host crop (Davis and Webster, 2005). Pontif and McGawley (2007) also showed that morningglory (*Ipomoea lacunose*), hemp sesbania (*Sesbania exaltata*), and johnsongrass (*Sorghum halepense*) reduced reniform nematode reproduction compared to cotton when grown under greenhouse conditions.

Soil properties with potential to influence reniform nematode numbers have also been considered. Heald and Heilman (1970) observed no differences in symptoms when damage

caused by soil salinity was compared to damage from reniform nematodes, and found no association between the soil salinity and reniform nematode damage. The influence of soil texture on reniform nematode numbers has been attributed to a complex relationship between biotic and abiotic factors (Starr et al., 1993). A positive relationship between number of reniform nematodes and fine textured soils has been documented. Caswell et al. (1991) reported that reniform nematodes were much less common in soils with >40% sand compared to more finely textured soils. Potassium fertilization may also be positively correlated with reniform nematode populations (Pettigrew et al., 2005). However, Heald and Robinson (1990) observed no consistent relationships between reniform nematode population and soil texture, soil pH, rainfall, or irrigation regime. Furthermore, they found that reniform nematode distribution closely followed the pattern of cotton production in the southeast U.S. and major land resource regions.

Management of the reniform nematode in cotton has shown limited economic return for the grower. In 2007, approximately 18% of the U.S. cotton hectarage was treated with the nematicide aldicarb (USDA-NASS, 2008). Management of these pests has varied considerably in success. In general, nematode management has relied on treatment with in-furrow or seed-treatment nematicides, rotation with non-host crops or resistant cultivars, crop destruction, and incorporation of plant materials (Barker and Koenning, 1998; Davis et al., 2000, 2003; Koenning et al., 2003a, 2003b; Nichols, 2007; Starr et al., 2007). Research studies designed to determine the effectiveness of nematicides in controlling nematode populations have produced mixed results (Baird et al., 2000; Lawrence et al., 1990; Lawrence and McLean, 2000; Rahi and Rich, 2003). Studies found limited benefits in the use of 1,3-dichloropropene + aldicarb (Lawrence et al., 1990), oxamyl (Lawrence and McLean, 2000) and propylene oxide (Rahi and Rich, 2003) as nematcides. In a comparison of aldicarb, fenamiphos, 1,3-dichloropropene, and oxamyl at different rates and combinations for nematode control, results failed to show an advantage of any one single treatment when measured in terms of plant stand, height, and nematode populations, and only improved yield when compared to untreated control plots (Baird et al., 2000). The application of chemical or biological sources of CO_2 for altering movement and attracting reniform nematodes to a nematicide has been proposed, but has not been employed (Robinson, 1995).

Inorganic nitrogen has also been examined for potential nematicidal properties, but the rates of nitrogen required for use as a nematicide far exceed what is recommended for fertilization and could be phytotoxic (Rodriguez-Kabana, 1986).

Rates recommended for the widely used nematicides aldicarb and 1,3-dichloropropen may often be higher than what is required to obtain maximum economic returns (Zimet et al., 2002). Variable rate nematicide application has the potential to cut nematicide costs while improving yield, however, any savings may be negated by costs associated with increased sampling (Wrather et al., 2002). Furthermore, temperature and other environmental variables can influence the rate of chemical and microbial degradation of the most common nematicide aldicarb, resulting in loss of efficacy and control of reniform nematodes (McLean and Lawrence, 2003; Jones and Norris, 1998).

Alternative management options such as rotation with non-host crops and use of cover crops have also been investigated. Westphal and Scott (2005) reported that rotation to a root-knot nematode resistant soybean cultivar proved to be an economically viable alternative for controlling nematodes in cotton. Davis et al. (2003) suggested any benefit in lint yield from rotating to a non-host crop in cotton production is limited to a single year and any increase in economic return may be minimal compared to continuous cotton production with nematicides. Potential alternative fiber crops, sunn hemp (*Crotalaria juncea* L.) and kenaf (*Hibiscus cannabinus)* were shown to be nematode resistant and resulted in a substantial reduction in reniform nematode reproduction when compared to cotton (Robinson and Cook, 2001). In a study of five non-host plants, Caswell et al. (1991), found that French marigold (*Tagetes patula)* decreased the reniform nematode population below that of a “fallow-over-winter” treatment and suggested allelopathy as a possible cause. Caswell et al. (1991) also suggested that poor hosts may be as effective as non-hosts and fallow soil for reducing reniform nematode numbers. Jones et al. (2006) classified a number of different crops for potential use as a cover crop in reniform nematode infested soils. Clover (*Trifolium incarnatum, T. subterrraneum*) and vetch (*Vicia villosa*) were classified as good cover crops; rape (*Brassica rapus, B. napus* spp. *biennis*) and canola (*Brassica compestris*) were classified as bad cover crops; and ryegrass (*Lolium multiflorum*), rye

(*Secale cereale*), oat (*Avena strigosa*), lupin (*Lupinus albus*), and wheat (*Triticum aestivum*) where classified as preferred cover crops. Jones et al. (2006) did assert that choice of a cover crop must also consider potential agronomic benefits.

Use of crop rotation may be more successful than nematicides for controlling root-knot nematodes compared to reniform nematodes due to the inability of root-knot nematodes to survive more than a year without a host (Van Gundy, 1985; Koenning et al., 2004). Reniform nematodes, however, can survive rotation regimes via anhydrobiosis (Tsai and Apt, 1979), resulting in effective control for only a single year following rotation (Davis et al., 2003; Koenning et al., 2004). Although various tillage programs have been examined for use as potential control measures for nematodes, tillage in general has not been shown to significantly influence reniform populations (Robinson, 2007; Stetina et al., 2008).

The reliance on chemicals in efforts to manage nematode pests can be expensive, time consuming, and pose serious health risks. The use of host plant resistance (HPR) or cultivar tolerance would offer a low risk, economical alternative to complete reliance on chemical control and perhaps holds the greatest potential for nematode control in the future (Starr et al., 2007).

When attacked by pests such as nematodes, plants may respond by exhibiting immunity, resistance, or susceptibility. Plant immunity is simply insusceptibility or a lack of susceptibility and may include hyper-sensitive response, as in the case of *G. longicalyx* infestation with reniform nematodes (Agudelo et al., 2005). In nematology, HPR refers to plant species and cultivars which inhibit reproduction of nematodes (Roberts, 2002). Plant breeding can be used to move genes conferring HPR from cultivar to cultivar or from a wild related non-host species to a cultivar in a process referred to as trait introgression (Dighe, 2007; Koenning et al., 2004; Roberts, 1992; Robinson, 2007; Robinson et al., 2007; Sharma and Ortiz, 2002). Cook and Evans (1987) describe host susceptibility as varying levels of tolerance and intolerance, where host tolerance provides limited suppression of plant growth while being parasitized, and host intolerance allows significant suppression of plant growth during parasitism. Agrios (1997) defined tolerance as the “ability of a plant to sustain the effects of a disease without suffering serious injury or crop loss”. Plant tolerance to nematodes can be described as the capacity to grow and

yield in unfavorable conditions by supporting reproduction of nematodes independently of resistance or susceptibility (Roberts, 1992). Finally, susceptible plants are at risk of being infected and subject to damage due to feeding and reproduction of the nematode.

Numerous descriptions of cotton genotypes resistant to the root-knot nematode have been reported (Colyer et al., 2000; Cook et al., 1997; Koenning et al., 2001; Ogallo et al., 1997; Robinson and Bridges, 1998; Robinson et al., 1999; Robinson and Percival, 1997; Shepherd, 1974b; Shepherd, 1982; Shepherd, 1983; Shepherd and Huck, 1989; Shepherd et al., 1996; Starr and Smith, 1999). Root-knot nematode resistant germplasm has been publicly available over the last 30 years (Shepherd 1974a, 1974b), but has not been utilized to any extent in developing new resistant cultivars (Starr et al., 2007). Therefore, few root-knot nematode resistant cultivars have been commercially available. However, it has been shown that the commercial cultivar Acala NemX strongly suppresses root-knot nematode populations (Ogallo et al., 1997). Stoneville 5599BR, also reported to be a root-knot nematode-resistant cultivar, has been widely planted in the southeast (Starr et al., 2007). Host plant resistance to root-knot nematodes (RKN resistance) in many upland cotton cultivars can most likely be traced back to "Auburn 623 RNR", a line developed by Shepherd (1974b) which was derived from a wild accession of upland cotton known as "Mexico Wild". However, RKN resistance in many cultivars does not equal that of the original 'Auburn 623 RNR' resistant germplasm (McPherson et al., 2004; Robinson and Percival, 1997). Continued development and release of advanced germplasm lines with high levels resistance to RKN (Creech et al., 2007; Nichols, 2007) will provide new parental material for efforts directed at breeding for RKN resistance.

Molecular markers, specific sequences of DNA closely linked to a trait, allow for marker assisted selection (MAS), i.e. selection for a marker instead of a plant phenotype. The influence of environment on phenotypic expression is removed in MAS, and selection can be practiced at any stage of plant growth (Mohan et al., 1997). Several molecular markers linked to RKN resistance have been identified in upland cotton (Wang et al., 2006a, 2006b; Wang and Roberts, 2006; Shen et al., 2006; Ynturi et al., 2006; Nichols, 2007). In 2006, application of MAS for RKN resistance was made possible with the identification of a marker (CIR316) closely linked to *rkn1,*

a major RKN resistance gene in upland cotton (Wang and Roberts, 2006). In addition, two amplified fragment length polymorphism (AFLP) markers, two randomly amplified polymorphic DNA (RAPD) markers, and three resistance gene analog (RGA) markers have been identified (Niu et al., 2007). Other root-knot resistance linked markers have been discovered including BNL1231, BNL1066, and CIR003 (Wang et al, 2006b).

A source of resistance to the reniform nematode has yet to be identified in upland cotton (Cook et al., 2003; Robinson, 1999; Robinson, 2007; Robinson et al., 1999; Weaver et al., 2007). In search of sources of resistance to the reniform nematode, more than 1900 upland cotton and 900 pima cotton accessions were evaluated by comparing nematode reproduction in susceptible and moderately susceptible checks with reproduction in accessions (Robinson and Percival, 1997, Robinson et al., 2004). Although a small number of upland cotton accessions were classified as moderately resistant, only Pima accessions were classified as resistant to reniform nematodes.

Although all currently available commercial Upland cotton cultivars are considered to be susceptible to the reniform nematode, development of reniform resistant germplasm utilizing new sources of resistance has been underway for several years (Bell et al., 2009; Dighe et al., 2009; Jones et al., 1988; Robinson, 2007; Robinson et al., 2004; Romano et al., 2009; Sacks and Robinson, 2009; Starr et al., 2007). Accessions of the African species *Gossypium longicalyx* (J.B. Hutch. & B.J.S. Lee) have been described as non-hosts, immune, and highly resistant to reniform nematodes (Yik and Birchfield, 1984; Stewart and Robbins, 1996). Robinson et al. (2007) reported success in the introgression of resistance to reniform nematodes from *G. longicalyx* into *G. hirsutum* via a complex bridge cross of two trispecies hybrids. In 2007, upland germplasm lines Lonren-1 and Lonren-2 resistant to reniform nematodes were made available to breeders following a joint release by USDA, Cotton Incorporated, and Texas A&M University (non-published USDA-ARS Release Notice). Molecular marker BNL 3279 was found closely linked to the reniform nematode resistance derived from *G. longicalyx*, and the gene conferring resistance (designated as Ren^{lon}) inherited as a single dominant gene (Robinson et al., 2007, Dighe et al., 2009). While cottonseed linters are normally white in upland cotton, reniform

nematode resistance in the Lonren lines is closely linked to the fuzzy green seed trait, a trait that can be utilized as a phenotypic marker for reniform nematode resistance (Dighe, 2007). Bell et al. (2009) recently reported success in combining reniform resistance derived from the Lonren lines with root-knot resistance from Acala NemX into single genotypes. However, observation of stunted plants in agronomic evaluations of Lonren derived genotypes suggested that resistance may be associated with susceptibility to certain pre-emergence herbicides (Bell et al., 2009). Efforts are currently underway to utilize *G. aridum* as an alternate source of reniform resistance (Sacks and Robinson, 2009; Romano et al., 2009). Romano et al. (2009) reported that BNL 3279, the marker associated with reniform resistance, is also closely linked to resistance derived from *G. aridum*. It is clear that additional progress in breeding for reniform resistance is required and that commercially available reniform nematode resistant genotypes are at best, several years away.

The fact that over the last 50 years all major U.S. Upland and Pima cotton cultivars appear to be highly suitable hosts for the reniform nematode indicates the sizeable challenge faced in developing reniform resistant cultivars (Robinson et al., 1999). An absence of resistance has shifted the focus from seeking immunity or resistance to seeking tolerance to the reniform nematode in upland cotton. Tolerant plants sustain lower yield losses when compared to susceptible cultivars having equal reproduction levels and infection (Koenning et al., 2000). Koenning et al., (2004) found that tolerance may be relative to the maturity of a cotton cultivar, with tolerance favoring later maturing cultivars. There also appears to be a positive correlation between resistance and tolerance, with a wide range of variation in tolerance among moderately resistant genotypes, at least in the case of root-knot tolerance (Davis and May, 2003). Work has been underway over the last couple of years to identify reniform tolerance in upland cotton by USDA-ARS and Mississippi State University researchers at Stoneville, MS. Stetina et al, (2006, 2009) identified three cultivars with varying levels of tolerance to the reniform nematode in trials conducted over a three year period. Evaluation of breeding lines for tolerance to the reniform nematode would be beneficial to many cotton producing states, where past efforts failed to identify any appreciable tolerance to the reniform in commercial cultivars (Cook et al., 1997;

Koenning et al., 2000; Usery et al., 2005). A number of public breeding line releases have been described as “reniform tolerant” in addition to a few commercial experimental lines (Cook et al., 1997; Cook and Robinson, 2005; Stetina et al., 2006, 2009). However, these lines have not significantly changed the outlook for deploying widespread use of tolerant cultivars to help control reniform nematodes.

Readily available tolerant cultivars could provide relief for a growing economic problem at a time when resistance or immunity may be years away. Until new reniform nematode resistant germplasm becomes available, identification of the most tolerant and susceptible commercial cultivars could benefit cotton producers. Screening for reniform nematode tolerance and identifying the most tolerant commercial cultivars would aide producers in selecting cultivars best suited for reniform nematode infested fields and provide an additional management option to limit loss in production attributed to this important pest. The objectives of this research included the following: 1) identify productive cotton cultivars that exhibit a level of tolerance to the reniform nematode in west central Mississippi, 2) identify any non-productive cotton cultivars with tolerance to the reniform nematode that may be used in future breeding work, and 3) identify cotton cultivars that are very susceptible to the reniform nematode so that planting of these cultivars in reniform nematode infested soils can be avoided.

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CHAPTER II

PRODUCTIVE AND NON-PRODUCTIVE COTTON (*GOSSYPIUM HIRSUTUM*) CULTIVARS EXHIBITING TOLERANCE TO THE RENIFORM NEMATODE (*ROTYLENCHULUS RENIFORMIS*)

ABSTRACT

Field studies were conducted during the growing seasons of 2006 and 2007 at the Delta Research and Extension Center, Stoneville, MS to screen entries in the 2006 Mississippi Cotton Variety Trials for productivity and for tolerance to the reniform nematode (*Rotylenchulus reniformis)*. Trials were carried out in non-irrigated fields with primarily Bosket very fine sandy loam and Dundee very fine sandy loam soils. The 2006 season was characterized by extreme drought. Results varied between locations and years due to environmental conditions. The only cotton (*Gossypium hirsutum)* cultivars tested and considered commercially productive and tolerant to the reniform nematode were 'Cropland Genetics 3520 B2RF,' 'DynaGrow 2520 B2RF,' and 'Stoneville 5242 BR.' 'ST 5599 BR' showed tolerance to the reniform nematode but the yield was variable between environments. Additional cultivars identified as tolerant to the reniform nematode were 'Deltapine 488 BG/RR' and 'Fibermax 960 B2R,' though these were not as productive as the productive check cultivar 'Deltapine 445 BG/RR.' These cultivars can help reduce the economic losses to reniform nematode in the Mississippi Delta region.

INTRODUCTION

When planting any crop, it is important to consider potential losses due to susceptibility to pests. Upland cotton (*Gossypium hirsutum)* is an important crop in Mississippi. Based on the 2007 Census of Agriculture (USDA-NASS, 2009), cotton farms account for 2.3% of all farms and 5.7% of hectarage in the state. In 2008, cotton losses in Mississippi to nematodes were at 10%

(81,250 bales), the majority due to the reniform nematode (*Rotylenchulus reniformis:* 8%, 65,000 bales) (Blasingame et al., 2009). This estimate ranks reniform nematode as the most damaging nematode threat to cotton in the state.

Weather can affect both reniform nematodes and cotton growth and development. Primarily, temperature and drought are the major factors influencing cotton yield, along with high pest infestation in conjunction with the timing of the stress (Oosterhuis, 1999). Early planted cotton doesn't develop deep branched root systems due to cool soil temperature (McMicheal et al., 1996). High soil water may also have this effect. Soil temperature also affects cotton growth and development through impacts on yield during the boll development (August) increasingly with drought stress (Oosterhuis, 1999). The ideal temperature for cotton growth ranges from 20-30 C (Reddy et al., 1999). The weak root systems from early planting stop growing after flowering and boll set (Oosterhuis, 1999). These compromised roots cannot sufficiently take up water, which is the cooling system for the plant. In hot drought situations, stomates close, cooling stops, and plant temperature rises above optimal temperature, where plant growth actually decreases. Carbohydrate production becomes insufficient, and a myriad of yield affecting reactions occur (Oosterhuis, 1999). Furthermore, infectivity by reniform nematode is reduced when soil water content is below 7.2% or above 13% (Rebois, 1973a). Soil temperature also affected development and rate of infectivity of the reniform nematode (Rebois, 1973b). Rebois (1973a) showed decreased development/infectivity by reniform nematode at extremes of 15 C and 36 C on soybean. In the study, at 29.5 C, development/infectivity was maximum, dropping off when temperatures were lower or higher. Differences in this study may be linked also to depressed root growth. Weather affects nematicide efficacy (Kirkpatrick, 1999). Herbert et al. (1987) saw possible reduced aldicarb activity due to abnormally dry weather conditions, speculating that low soil moisture reduced the movement of aldicarb to the root zone, reducing plant uptake and systemic activity. Noling (1997) asserts that non-fumigant nematicides rely on water to carry them into the soil and disperse them into the rooting zone to be effective. Microbial degradation of

aldicarb may also be an issue in fields with a history of aldicarb usage (Lawrence et al., 2005a; McLean and Lawrence, 2003).

With nematode infestation, management through chemical treatment of infested soils remains a common control practice. According to the 2007 Census of Agriculture (USDA-NASS, 2009) a total of 394 farms used chemical nematode control nationwide, covering approximately 75,476 hectares of land. Much cost is involved with this management practice. Nematicides are dangerous to handle, can be fatal, can possibly move into ground water, and are toxic to wildlife (Chitwood, 2003). Rotation with non-host crops and cover cropping has also been used for reniform nematode population management; however, these schemes may not be profitable for the grower. Rotation to susceptible crops may be detrimental and nematode numbers in fallow fields may be lower than in fields where cover crops are used (Kirkpatrick and Rothrock, 2001; Robinson, 2007; Starr et al., 2007). If highly susceptible cultivars could be avoided in nematode-infested areas and replaced with tolerant or resistant cultivars, some of the expense and risks associated with nematicides could be avoided.

Over the last 50 years all major U.S. Upland and Pima (*Gossypium barbadense*) cultivars of cotton have appeared to be highly suitable hosts for the reniform nematode (Robinson et al., 1999). This illustrates the challenge of managing this nematode. The absence of resistance has shifted some focus to finding tolerance to the reniform nematode in *G. hirsutum*. Tolerance can be defined as the “ability of a plant to sustain the effects of a disease without dying or suffering serious injury or crop loss” (Agrios, 1997). Tolerant plants support reproduction by nematodes while sustaining lower yield losses than other cultivars having comparable reproduction and infection (Koenning et al., 2000). Koenning et al. (2004) reported that tolerance may be relative to maturity of the cotton cultivar, favoring later maturing cultivars as more tolerant. Because of the need to carry the crop to yield, tolerance must be screened in the field (Bowman and Schmitt, 1994; Cook et al., 1997; Davis and May, 2003; Hill et al., 1994; Koenning, 2002a; Koenning et al., 2000; Schmitt and Imbriani, 1987; Stetina et al., 2009; Usery et al., 2005). Stetina et al. (2009) identified three upland cotton cultivars with levels of tolerance to the reniform nematode in trials

conducted from 2003 through 2005. Germplasm lines also have been released claiming reniform tolerance (Cook et al., 1997; Cook and Robinson, 2005; Jones et al.,1988).

To identify tolerance, data on nematode reproduction and crop yield are needed. A reproductive index (RI) (Jones et al., 1967) can be calculated to establish the level of reproduction of nematodes on a cultivar and comparing cultivars with high RI's yield between infested soil and uninfested soil. A RI is equal to the final population of nematodes per a given time frame divided by the initial population, so RI values greater than 1.0 indicate that the nematode population is increasing on that cultivar.

When applied to upland cotton and the reniform nematode, cultivars are considered susceptible if the RI $\geq$1.0 and yield is negatively affected due to the larger nematode population (Usery et al., 2005). Most if not all, current cultivars of upland cotton fall into a susceptible category when this definition is followed. Intolerance, or sensitivity, to the reniform nematode of cotton cultivars affects yields negatively. For the current research, the definition of tolerance was relaxed, and included any cultivar with a TI equal to or higher than the tolerant standard, 'DES 119', described by Blasingame and Sciumbato (1991), and RI values >1.0, indicative of reniform nematode reproduction.

Several studies used only one RI over the course of the growing season (Cook et al., 1997; Davis and May, 2003) to identify host tolerance to nematodes. However, some researchers began to use two RI over the course of the growing season to further break down the reproduction (Koenning et al., 2000; Koenning and Bowman, 2005; Usery et al., 2005; Stetina et al., 2009). When populations are measured three times over a growing season, late season RI is calculated by dividing the final population by the midseason population, and early season RI is calculated by dividing the midseason population by the initial population (Seinhorst, 1967). This breakdown allowed for inference about nematode control success, host status of the cultivar tested, and relationship between population dynamics at midseason as related to tolerance (Koenning et al., 2000; Koenning and Bowman, 2005; Usery et al., 2005; Stetina et al., 2009).

Reproductive indices and yields can be compared between nematicide treated and non-treated plots to help identify high yielding tolerant cultivars (Seinhorst, 1967; Jones et al., 1967) if

nematicide treatment successfully controls nematode reproduction. Tolerance under these circumstances can be identified if a cultivar with an RI greater than 1.0 yields the same between nematicide-treated and nontreated subplots when successful control of reniform nematodes has been achieved in the nematicide-treated subplot. To compare relative levels of tolerance, a tolerance index (TI) can be calculated for each cultivar (Koenning and Bowman, 2005). The TI is calculated as TI= (yield in nontreated plot/yield in nematicide-treated plot)*100, so larger values identify the most tolerant cultivars. Using TI and RI values as well as their relationships with other, tolerance and possible resistance can be identified.

Productivity of a cultivar is the comparison of lint yield (outputs) to all costs required to get to yield (inputs). When inputs across cultivars are held constant, productivity can be inferred directly from yield, so productive cultivars are those that yield comparative to commercial standards or higher. During the years of this study, average cotton yield ranged from 940 kg/ha in 2006 to 1095.5 kg/ha in 2007, according to the National Agriculture Statistics Service Quickstats (http://quickstats.nass.usda.gov/results/2CC45D82-64FA-3C07-87E5-DD943AB52421). To be considered productive, a cultivar must yield within the range acceptable for that crop. Cook and Sundquist (1991) loosely defined productivity as yield increases achieved without comparable increases in input.

This study was undertaken to identify productive cotton cultivars that show tolerance to the reniform nematode in the Mississippi Delta. Readily available, tolerant cultivars could provide relief for a growing economic problem at a time when true resistance in commercially available lines seems in the distant future.

MATERIALS AND METHODS

A field experiment was conducted during the 2006 and 2007 growing seasons to evaluate the tolerance of selected cultivars from the 2006 Mississippi Cotton Variety Trial to the reniform nematode. Fields naturally infested with reniform nematodes near or above the threshold levels of 16.2/cm^3 soil at harvest the previous year (Overstreet, 2001; Koenning, 2002b; Sciumbato et al., 2004) were selected in this study. The experiment was conducted at two sites at the Delta Research and Extension Center (DREC), Stoneville, MS in 2006 (Field 4, Barn field) and two different sites (Field 1, Field 12) in 2007. All fields were non-irrigated. Soil type and classification data were taken from the United States Department of Agriculture Natural Resources Conservation Service (http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx), 2009. Field 4 contained both Bosket very fine sandy loam (Alfisols Udalfs Hapludalfs Mollic; fine loamy, mixed, active, thermic) and Commerce series soils (Inceptisols Aquepts Endoaquepts Fluvaquentic; fine silty, mixed, superactive, nonacid, thermic). Field 1 contained primarily Bosket very fine sandy loam. Field 12 was made up of Beulah very fine sandy loam (Inceptisols Udepts Dystrudepts Typic; coarse loamy mixed, active, thermic), Bosket very fine sandy loam, and Commerce series soils. The Barn field contained both Bosket series and Commerce series soils.

Experiments were arranged in two-factor factorial in a split-plot design. The main plot consisted of 13 cotton cultivars: 'Croplan Genetics 3520 B2RF,' 'Deltapine 20B,' 'Deltapine 445 BG/RR,' 'Deltapine 449 BG/RR,' 'Deltapine 455 BG/RR,' 'Deltapine 488 BG/RR,' 'DES 119', 'DynaGrow 2520 B2RF,' 'Fibermax 960 B2R,' 'Phytogen 370,' 'Phytogen 485,' 'Stoneville 5242 BG/RR,' and 'Stoneville 5599 BR'. DES119, a conventional cultivar developed at DREC, is considered tolerant to reniform nematodes (Blasingame and Sciumbato, 1991). Cultivar Deltapine 20B was considered the susceptible check based on previous research of Stetina et al. (2009). The commercial cultivar Deltapine 445 BG/RR was included as the productive check cultivar based on its performance in the 2005 Mississippi Cotton Variety Trials (Nichols et al., 2006). Other cultivars were chosen at random to represent the seed companies that supplied seed for the Mississippi Cotton Variety Trials. The subplot consisted of nontreated plots and aldicarb-treated plots receiving 0.84 kg a.i./ha in furrow at planting followed by 1.17 kg a.i./ha side dressed

at pinhead square. The application of aldicarb at planting under ideal conditions is sufficient to keep reniform nematode levels manageable for approximately six weeks (Blasingame et al., 2002). The subplot treatment allowed for determination of reproduction values and provided a basis for comparison of yields in aldicarb-treated versus nontreated plots within cultivars for interpolation of tolerance.

Main plots were replicated five times per environment in a randomized complete block arrangement. Main plots consisted of eight 12.2-m rows spaced one meter apart with 13 seed planted per row meter using a 4-row cone plot-type Almaco planter (Almaco, Nevada, IA). Subplots were four rows. Recommended Mississippi extension weed control guidelines were followed for preplant herbicides. None were used thereafter due to the inclusion of conventional cultivars, and weed control was achieved through cultivation when necessary. Insecticides, defoliants, and growth regulators were applied following standard production guidelines for Mississippi. Dates of importance pertaining to planting, harvest, and sampling are shown in Table 2.1.

Monthly average maximum and minimum air temperatures, soil temperature, precipitation, and pan evaporation were taken from the Delta Agricultural Weather Center at Mississippi State University (http://www.deltaweather.msstate.edu/historic_ag_weather_data/historic_ag_weather_data.htm). Thirty year averages were obtained from the weather data summary for 1964-1993 (Boykin et al., 1995).

Ten soil cores (2.5 cm in diameter and 25.4 cm deep) were collected randomly from the center two rows of each subplot at planting, midseason (8 weeks after planting in 2006 and 12 weeks after planting in 2007), and harvest. Samples were stored at the optimal storage temperature, 13°C, until extraction and counting (Barker et al., 1969), with an understanding of some population loss in storage (Lawrence et al., 2005b). Reniform nematodes were extracted from 200 cm^3 of soil by Seinhorst elutriation (Seinhorst, 1964), followed by sucrose centrifugation using a 454 g/liter sugar solution (Jenkins, 1964). Reniform nematodes per liter of soil were

calculated after counting the nematodes in one sixth of a partitioned petri dish with a stereomicroscope. Reproductive indices (RI) were calculated as:

RI=Pf/Pi, where

Pf=final population and

Pi=initial population

over a given time period. Therefore, when splitting the growing season to analyze population changes early and late in the season, late season RI is calculated using Pf/Pm for final population/midseason population, where Pm (midseason population) is substituted for Pi. Early season RI is calculated using Pm/Pi for midseason population/initial population (Seinhorst, 1967; Jones et al., 1967).

Cotton plots were harvested with a Case 2022 spindle-type picker (Case International Harvester, Racine, WI) customized for harvesting test plots. The center two rows of each 4-row plot were harvested and weighed.

Tolerance of cotton cultivars to the reniform nematode also was measured using the TI and compared to the relationship of yield to reniform nematode RI between treated and nontreated plots. A TI for each cultivar was calculated as:

Ti= (yield nontreated plot/yield nematicide treated plot)*100

(Koenning et al., 2000).

Because the 2006 trial sites were unavailable for testing in 2007, data were analyzed by environment (location-year combination). Data were evaluated for environmental differences and interactions between environment, cultivar, and nematicide. Environmental effects were tested and data were combined across environments only when no statistically significant interactions involving environments were detected. Statistical analysis was done using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC) and using the macro PDMIX800 (Saxton, 1998) to convert mean separation output to letter groupings. Means separation using differences of least squares means at $P \leq 0.05$ or $P \leq 0.10$ level of significance was accomplished with PDMIX800. Environment was considered a random-effect parameter while testing all possible interactions of fixed effects. Assuming environment was random allowed inferences over a range

of experimental conditions (Carmer et al., 1989). Replications (nested within environment) and associated interactions were considered random effects. Tolerance indices were analyzed with fixed effects of cultivar.

RESULTS

Weather

The 2006 and 2007 growing seasons at the Delta Research and Extension Center were very different from each other in terms of weather. Figure 2.1 shows maximum air temperature, minimum air temperature, and soil temperature for 2006, 2007, and 30-year averages (Boykin et al., 1995) at this research station. Figure 2.2 shows the difference between precipitation and pan evaporation for 2006, 2007, and 30-year averages (Boykin et al., 1995).

Maximum air temperature (Figure 2.1) stayed above the 30-year average for the entire growing season of 2006. In 2007, monthly maximum air temperature averages were higher than both the 30-year average and the 2006 monthly averages until June. In July the maximum air temperature dropped below that of 2006 and the 30-year average. However, in August the maximum air temperature exceeded that of 2006 and the 30-year average. September and October 2007 were similar to 2006 maximum air temperature but still higher than the 30-year average.

Minimum air temperatures (Figure 2.1) for both 2006 and 2007 were similar to the 30-year average through July. After July, both 2006 and 2007 minimum air temperatures were higher than the 30 year average for the rest of the growing season.

Average soil temperature (Figure 2.1) in 2006 stayed several degrees higher than 30-year average throughout the growing season. The 2007 soil temperature was higher than the 30-year average through June. A decrease in soil temperature then depressed the average below the 30-year average for July. The soil temperature average for August 2007 was higher than the 30-year average, and then dropped below the 30-year average again in September and October.

Both the 2006 and 2007 growing seasons started with far less precipitation than pan evaporation as compared to the 30-year average, although all three were below zero (Figure 2.2). The 2006 growing season showed less precipitation than evaporation until September of that year, which was the only month that showed a positive precipitation-pan evaporation value. July and October of 2007 were the only months of that growing season to show positive values. The

30 year average never crossed over to positive values, although much less extreme in either direction as compared to 2006 and 2007.

Treatment effects

The significance levels for main effects and their interactions are given in Table 2.2 for early and late season RI, seed cotton yield, and TI. Environmental differences were detected for all parameters evaluated at $P \leq 0.05$. Differences among cultivars with respect to early season RI and seed cotton yield were significant at $P \leq 0.05$, and differences in TI were significant at P 0.10. Significant effects of nematicide application on early season RI ($P \leq 0.10$) and seed cotton yield ($P \leq 0.05$) were noted. Environment x cultivar and environment x nematicide interactions were significant at P 0.05 only with respect to seed cotton yield. Late season RI was not significantly affected by either cultivar or nematicide.

Environmental differences

Early season RI in Environment 1 was significantly lower than in Environments 2 and 4 (Table 2.3). Early season RI in Environment 3 was significantly lower than Environment 2, but did not differ from the other environments.

The same trend was not evident with respect to late season RI. In this case, Environment 1 had significantly higher late season RI than Environments 3 and 4, but was in the same class as Environment 2 (Table 2.3). Late season RI for Environment 4 was significantly lower than both Environments 1 and 2, but did not differ from Environment 3.

Seed cotton yield for Environment 4 was significantly higher than all other environments (Table 2.3). Environment 3 ranked second and was significantly higher than both Environments 1 and 2.

Tolerance indices were highest in Environment 1 and lowest in Environment 3 (Table 2.3). Environments 2 and 4 were intermediate with respect to tolerance index.

Cultivar response

The early season RI were highest on productive standard 'Deltapine 445 BG/RR,' 'Deltapine 449 BG/RR,' and 'Stoneville 5242 BR' (Table 2.4). Only 'Deltapine 449 BG/RR' supported a significantly higher early season RI than the remaining cultivars. 'Phytogen 370' had the lowest early season RI, but it differed significantly from only two cultivars, 'Deltapine 449 BG/RR' and 'Stoneville 5242 BR.'

'Croplan Genetics 3520 B2RF,' the commercial productive standard 'Deltapine 445 BG/RR,' 'Phytogen 370,' Phytogen 485,' and 'Stoneville 5242 BR,' were the highest yielding cultivars (Table 2.4). The lowest yielding cultivar was 'DES 119', an older cultivar, which was significantly lower in yield than all other cultivars except 'Fibermax 960 B2R.' The commercial productive standard yielded higher than the other Deltapine varieties except 'Deltapine 449 BG/RR;' yielded higher than 'Stoneville 5599 BR,' and was equivalent 'DynaGrow 2520 B2RF.'

Tolerance indices shown in Table 2.4 show 'Deltapine 20B,' the susceptible control, in the same tolerance class as 'DES 119' and 'Dynagrow 2520 B2RF.' It alone, is significantly different than all other cultivar entries. Other cultivars are all statistically the same.

Table 2.5 shows seed cotton yield differences between nematicide-treated and untreated subplots for each cultivar averaged across environments. Cultivars showing no statistical differences in yield between nematicide treatments include 'Croplan Genetics 3520 B2RF,' 'Deltapine 20B,' 'Deltapine 488 BG/RR,' DES 119,' 'DynaGrow 2520 B2RF,' 'Fibermax 960 B2RF,' 'Stoneville 5242 BR,' and 'Stoneville 5599 BR.'

Because a significant interaction between environment and cultivar was detected, seed cotton yields were broken down further by environment (Table 2.6). The lowest yielding cultivars in Environment 1 were susceptible cultivar 'Deltapine 20B,' tolerant cultivar 'DES119,' 'Fibermax 960 B2R,' and 'Stoneville 5599 BR.' The highest yielding cultivars for Environment 1 were 'Croplan Genetics 3520 B2RF,' the commercial productive cultivar 'Deltapine 445 BG/RR,' 'Deltapine 449 BG/RR,' 'Deltapine 488 BG/RR,' 'DynaGrow 2520 B2RF,' 'Phytogen 485,' 'Phytogen 370,' and 'Stoneville 5242 BR.' The range of yields for this environment varying from 2363.4 kg/ha to 1337.7 kg/ha.

Environment 2 showed no significant difference between cultivars for yield. The range of yields at this environment was 2194.5 kg/ha to 1615.2 kg/ha.

The lowest yielding cultivars in Environment 3 were 'Deltapine 488 BG/RR,' 'DES 119,' and 'Fibermax 960 B2R.' The highest yielding cultivars in this environment were 'Croplan Genetics 3520 B2RF,' 'Deltapine 445 BGRR' the commercial productive standard, 'Deltapine 455 BG/RR,' 'DynaGrow 2520 B2RF,' 'Phytogen 485,' 'Phytogen 370,' 'Stoneville 5242 BR,' and 'Stoneville 5599 BR.' The yield range for this environment was 3225.2 kg/ha to 2154.6 kg/ha.

Environment 4 again showed 'DES 119' as the lowest yielding cultivar. 'DES 119' was significantly lower than all other cultivars tested in that environment. The highest yielding cultivars were 'Croplan Genetics 3520 B2RF,' 'Phytogen 370,' 'Phytogen 485,' and 'Stoneville 5242 BR.' The range of yields for environment 4 was 3955.7 kg/ha to 2608.7 kg/ha.

'Croplan Genetics 3520 B2RF,' 'Phytogen 370,' 'Phytogen 485,' and 'Stoneville 5242 BR' yielded in the same or higher seed cotton yield significance class as the productive standard in three environments. 'DES 119' produced less seed cotton than the productive standard in all three environments where significant differences among the cultivars were noted. 'Deltapine 488 BG/RR' yielded less seed cotton than the productive standard in two environments. 'Deltapine 20B,' 'DynaGrow 2520 B2RF,' 'Fibermax 960 B2R,' and 'Stoneville 5599 BR,' all yielded significantly lower than the productive standard in one of the four environments.

Nematicide effects

When averaged across all environments and cultivars, the early season RI for reniform nematode was higher in plots that had not been treated with nematicides (Table 2.7). Seed cotton yield was higher in plots treated with nematicide (Table 2.7). Together, these effects indicate that conditions conductive to evaluating tolerance have been created. However, because a significant nematicide x environment effect was detected, the effect of nematicide on seed cotton yield in each environment was examined independently (Table 2.8). Of note, yields in treated and non-treated plots in environment 1 were not significantly different. The other three environments all indicated significantly higher seed cotton yield in treated plots than in nontreated plots.

DISCUSSION

Cultivars identified as both tolerant and productive in this study were 'Croplan Genetics 3520 B2RF,' 'DynaGrow 2520 B2RF,' and 'Stoneville 5242 BR.' For these cultivars, the TI was equal to or above the tolerant standard 'DES 119,' yield was not positively affected by addition of nematicide to subplots, yield was not significantly different compared to the commercial productive standard 'Deltapine 445 BG/RR' in most environments, and reniform nematode early season RI values were 1.0 or greater. The cultivar 'Stoneville 5599 BR' was similar to these in its response to reniform nematode, but yielded similar to the productive check in only half of the environments tested. Additional evaluation is needed to determine whether 'Stoneville 5599 BR' is truly a tolerant, productive cultivar. Cotton growers in the Mississippi Delta who have fields infested with reniform nematode can benefit immediately from using these tolerant, productive cultivars.

Additional cultivars identified as tolerant to reniform nematode were 'Deltapine 488 BG/RR' and 'Fibermax 960 B2R.' However, seed cotton yields for these cultivars were lower than that of the productive check. In situations where pressure from reniform nematode is the most significant stressor limiting yield, growers may benefit from their use. The use of a tolerant cultivar in combination with other management techniques such as nematicide application (Starr et al., 2007) or crop rotation (Robinson, 2007; Stetina et al., 2007) could improve the utility of these tolerant but nonproductive lines in minimizing cotton losses to reniform nematode in this region.

The cultivars 'Deltapine 455 BG/RR,' 'Phytogen 370,' and 'Phytogen 485' were demonstrated to be productive, but did not exhibit tolerance to reniform nematode. These cultivars could be good choices for fields with little or no reniform nematode pressure.

When compared to past studies of tolerance, these results are not surprising. Identifying tolerance to the reniform nematode in breeding lines, current cultivars, and even older lines has proven difficult. Koenning et al. (2000) tested lines and found that there was no appreciable tolerance and indicated more tolerance was needed. Usery et al. (2005) saw some possible tolerance in two cultivars, Stoneville 4793 and Suregrow 521R, but their results were inconsistent and they concluded that true tolerance was not present in those cultivars tested. Cook et al.

(1997) identified possible tolerance in only a few breeding lines tested. Stetina et al. (2009) identified three possible tolerant cultivars ('Suregrow 215 BR', 'Paymaster 1218 BR', and 'Deltapine 449 BR') out of the 39 lines tested, but overall indicated tolerant lines were not widely available.

Environmental conditions including drought and late season rains affected tolerance and productivity assessments. Environments 1 and 2, which were plagued with drought during the early growing season, were different in some parameters measured compared to Environments 3 and 4. The primary difference separating these environments was precipitation, with Environments 1 and 2 being drier from May through August. Available soil moisture likely contributed to the differences in cultivar seed cotton yield and reniform nematode RI during these growing seasons. Hutton (1978) showed reniform nematode populations fluctuated with number of days of precipitation and total rainfall in heavy clay soils and in well drained clay loams there was a negative correlation between reniform nematode populations and total rainfall and number of days of precipitation. Studies have shown drought negatively affects stem height, stem dry weight, leaf area, leaf dry weight, node number, and shoot to root ratios at 49 and 59 days after planting; while positively affecting taproot length, secondary root length, and secondary root dry weight (Pace et al., 1999). The lack of irrigation in these fields during the drought periods had the potential to profoundly affect cotton yield and may have masked reniform nematode damage. Daniel et al. (1999) showed drought can result in yield decreases via smaller size and growth of sympodial leaves. Furthermore, Oosterhuis (1999) identified temperature and drought to be among the main deterrents to high yields. Secondarily, the later planting date in 2006 may have negatively affected yield (Poter et al., 1996). Poter et al. (1996), tested six cultivars over five different planting dates ranging from mid-April to mid-June, and concluded later planting dates increased plant height, fiber strength, and fiber elongation; but decreased lint percentage and micronaire. A drought experienced during one of the three years of their study also limited yield (Poter et al., 1996).

The reniform nematode has been shown to go into anhydrobiosis under drought conditions (Wang, 2001). During this period, the free-living soil nematodes exhibit a tightly coiled

body form. However, reniform nematodes in this anhydrobiotic state were not identified in the samples collected from our trials.

The effectiveness of the aldicarb treatment on reniform nematode suppression in plots allowed an inference of tolerance from both the direct analysis of seed cotton yield between treated and nontreated yields of cultivars, reniform nematode RI, and the TI. However, aldicarb is also used for control of thrips, aphids, fleahoppers, leafminers, mites, overwintering boll weevil, plant bugs, and whiteflies (Slosser, 1993). Reddy et al. (1997b) showed aldicarb has a direct regulatory effect on plant growth when pests are absent. The addition of aldicarb also changes root distribution, with deeper root systems under ideal water and nutrient environment (Reddy et al., 1997a). These additional aldicarb properties may influence seed cotton yield in this trial to favor aldicarb treated plots. However, differences in early season RI between treated and untreated plots indicate success in suppressing reniform nematode reproduction with the addition of aldicarb.

Until cultivars with resistance to reniform nematode are commercially available, tolerant cultivars could serve to reduce economic losses associated with cotton production in reniform nematode infested fields. As such, there is a need to continue evaluation programs to identify current cultivars with tolerance to reniform nematode.

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TABLES

Table 2.1 Planting, nematicide application , harvest, and nematode sampling dates for field trials evaluating cotton cultivars for tolerance to reniform nematode conducted in four environments at the Mississippi State University Delta Research and Extension Center in Stoneville, MS.

Activity	Environment 1 (Field 4, 2006)	Environment 2 (Barn, 2006)	Environment 3 (Field 1, 2007)	Environment 4 (Field 12, 2007)
Planting, initial nematicide application, initial reniform nematode sampling	22 May 2006	24 May 2006	7 May 2007	7 May 2007
Second nematicide application	10 Jul 2006	10 Jul 2006	18 Jun 2007	18 Jun 2007
Midseason reniform nematode sampling	15 Jul 2006	15 Jul 2006	2 Aug 2007	2 Aug 2007
Harvest	6 Sep 2006	27 Sep 2006	12 Nov 2007	12 Nov 2007
Final reniform nematode sampling	18 Oct 2006	18 Oct 2006	12 Nov 2007	12 Nov 2007

Table 2.2 Significance levels for main effects and their interactions for early and late season reniform nematode reproductive indices (RI), seed cotton yield, and tolerance index (TI) in a field trial at Stoneville MS.

Main effect[a]	Early season RI [b]	Late season RI [c]	Seed cotton yield[d]	TI[e]
Environment (E)	0.0038	0.0092	<0.0001	0.0005
Cultivar (C)	0.0357	0.3410	<0.0001	0.0821
E x C	0.1824	0.3114	0.0014	0.1874
Nematicide (N)	0.0594	0.4338	<0.0001	-
E x N	0.3922	0.6995	<0.0001	-
C x N	0.5128	0.7363	0.3237	-
E x C x N	0.1937	0.2647	0.4574	-

[a]Fixed effects are the main effects of cultivar (C) and nematicide (N) and all interactions of those main effects. The random variables are environment (E), E x C, E x N, and E x C x N.
[b]Early season RI = Pm/Pi = (midseason reniform nematode count)/(planting reniform nematode count)
[c]Late season RI = Pf/Pm = (harvest reniform nematode count)/(planting reniform nematode count)
[d]Seed cotton yield taken from the middle two rows of subplots.
[e]TI = [(seed cotton yield in nontreated plots)/(seed cotton yield in nematicide-treated plots)]*100

Table 2.3 Reproductive indices (RI), seed cotton yield, and tolerance index (TI) for the four environments included in the study at the Delta Research and Extension Center in Stoneville, MS; data averaged across cultivars and nematicide treatments.

Environment[a]	Early season RI [b]		Late season RI [c]		Seed cotton yield (kg/ha) [d]		TI[e]	
1	1.51	C[f]	2.48	A	1936.2	C	109.5	A
2	2.74	A	2.00	AB	1964.1	C	97.8	B
3	1.77	BC	1.37	BC	2851.8	B	84.9	C
4	2.40	AB	0.95	C	3457.7	A	95.6	B

[a]Environment 1 was a 2006 trial in Field 4, environment 2 was a 2006 trial in the Barn field, environment 3 was a 2007 trial in Field 1, and environment 4 was a 2007 trial in Field 12 all at the Delta Research and Extension Center in Stoneville, MS.
[b]Early season RI = Pm/Pi = (midseason reniform nematode count)/(planting reniform nematode count)
[c]Late season RI= Pf/Pm = (harvest reniform nematode count)/(planting reniform nematode count)
[d]Seed cotton yield taken from the middle two rows of subplots.
[e]TI = Tolerance Index = (seed cotton yield in nontreated plots)/(seed cotton yield in nematicide-treated plots)*100
[f]Means followed by the same letter in the same column are not significantly different at the 0.05 level based on differences of least squares means.

Table 2.4 Early season reniform nematode reproductive index (RI), seed cotton yield, and tolerance index (TI) for cultivars; data averaged across environments and nematicide treatments.

Cultivar	Early season RI [a]		Seed cotton yield (kg/ha) [b]		TI [c]	
Croplan Genetics 3520 B2RF	1.69	BC[d]	2772.0	AB[d]	92.5	B[e]
Deltapine 20 [f]	1.82	BC	2356.6	DE	111.9	A
Deltapine 445 BG/RR[g]	2.38	ABC	2718.4	AB	90.0	B
Deltapine 449 BG/RR	3.27	A	2654.1	BC	91.8	B
Deltapine 455 BG/RR	2.34	BC	2465.8	CD	95.1	B
Deltapine 488 BG/RR	1.91	BC	2356.7	DE	92.1	B
DES 119[h]	1.92	BC	2059.7	F	100.4	AB
DynaGrow 2520 B2RF	2.20	BC	2651.3	BC	100.7	AB
Fibermax 960 B2R	1.95	BC	2231.2	EF	99.3	B
Phytogen 370	1.56	C	2852.9	AB	93.6	B
Phytogen 485	2.14	BC	2798.2	AB	95.7	B
Stoneville 5242 BR	2.51	AB	2876.2	A	99.4	B
Stoneville 5599 BR	1.63	BC	2388.9	DE	97.8	B

Table 2.4 continued

[a] Early season RI = Pm/Pi = (midseason reniform nematode count)/(planting reniform nematode count)
[b]Seed cotton yield taken from the middle two rows of subplots.
[c]TI = (seed cotton yield in nontreated plots)/(seed cotton yield in nematicide-treated plots)*100.
[d]Means followed by the same letter in the same column are not significant at the 0.05 level based on differences of least squares means.
[e]Means followed by the same letter in the same column are not significantly different at the 0.10 level based on differences of least squares means.
[f]Cultivar considered susceptible to the reniform nematode (Stetina et al., 2009).
[g]Cultivar considered to be commercially productive (Nichols et al., 2006).
[h]Cultivar reported to have some level of tolerance to the reniform nematode (Blasingame and Sciumbato, 1991).

Table 2.5 Seed cotton yield in plots with or without aldicarb treatment at Stoneville, MS; data are averaged across four environments

Cultivar	Seed cotton yield (kg/ha)			
	No aldicarb		Aldicarb application[a]	
Croplan Genetics 3520 B2RF	2647.2	A[b]	2896.9	A
Deltapine 20B[c]	2339.1	A	2374.1	A
Deltapine 445 BG/RR[d]	2564.0	B	2872.8	A
Deltapine 449 BG/RR	2493.6	B	2814.5	A
Deltapine 455 BG/RR	2370.4	B	2561.1	A
Deltapine 488 BG/RR	2226.9	A	2486.5	A
DES 119[e]	2045.1	A	2074.3	A
DynaGrow 2520 B2RF	2623.2	A	2679.4	A
Fibermax 960 B2R	2164.3	A	2298.2	A
Phytogen 370	2723.1	B	2982.7	A
Phytogen 485	2697.1	B	2899.4	A
Stoneville 5242 BR	2840.3	A	2912.1	A
Stoneville 5599 BR	2329.4	A	2448.4	A

[a]Aldicarb applied in furrow at planting at 0.84 kg a.i./ha. Aldicarb applied at 1.17 kg a.i./ha side dress at pinhead squared knifed in with custom applicator.
[b]Means followed by the same letter in the same row are not significantly different at the 0.05 level based on ANOVA.
[c]Cultivar considered susceptible to the reniform nematode (Stetina et al., 2009).
[d]Cultivar considered to be commercially productive (Nichols et al., 2006).
[e]Cultivar reported to have some level of tolerance to the reniform nematode (Blasingame and Sciumbato, 1991).

Table 2.6 Seed cotton yield for cultivars in four environments at Stoneville, MS; data are averaged across nematicide treatments.

Cultivar	Seed cotton yield (kg/ha)[a]						
	Environment 1[b]		Environment 2[b]	Environment 3[b]		Environment 4[b]	
Croplan Genetics 3520 B2RF	2242.6	AB[f]	1971.1	3052.3	AB	3822.1	ABC
Deltapine 20B[d]	1602.6	CD	1863.4	2803.8	B	3156.6	FG
Deltapine 445 BG/RR[e]	2015.3	ABC	2111.1	3225.2	A	3521.9	C-F
Deltapine 449 BG/RR	2140.9	AB	2149.7	2833.5	B	3492.1	C-F
Deltapine 455 BG/RR	1943.7	BC	1615.2	2960.7	AB	3343.5	EFG
Deltapine 488 BG/RR	2103.9	AB	1942.9	2298.8	C	3081.1	G
DES 119[f]	1686.0	CD	1789.6	2154.6	C	2608.7	H
DynaGrow 2520 B2RF	1960.7	ABC	2097.1	3006.7	AB	3540.9	B-E
Fibermax 960 B2R	1438.9	D	1787.5	2391.7	C	3306.7	EFG
Phytogen 370	2187.0	AB	2184.4	3084.4	AB	3955.7	A
Phytogen 485	2147.8	AB	2020.2	3107.4	AB	3917.8	AB
Stoneville 5242 BR	2363.4	A	2194.5	3170.5	A	3776.3	A-D
Stoneville 5599 BR	1337.7	D	1806.6	2983.9	AB	3427.2	D-G

[a] Seed cotton yield taken from the middle two rows of subplots.
[b]Environment 1 was a 2006 trial in Field 4, Environment 2 was a 2006 trial in the Barn field, Environment 3 was a 2007 trial in Field 1, and Environment 4 was a 2007 trial in Field 12 all at the Delta Research and Extension Center in Stoneville, MS.
[c]Means followed by the same letter in the same column are not significantly different at the 0.05 level based on differences of least squares means.
[d] Cultivar considered susceptible to the reniform nematode (Stetina et al., 2009).
[e]Cultivar considered to be commercially productive (Nichols et al., 2006).
[f]Cultivar reported to have some level of tolerance to the reniform nematode (Blasingame and Sciumbato, 1991).

Table 2.7 Early season reproductive index and seed cotton yield in a field at Stoneville, MS; data averaged across environments and cultivars.

Nematicide	Early season RI [a]		Seed cotton yield (kg/ha) [b]	
No aldicarb	2.28[c]	A	2466.4	B[d]
Aldicarb applied[e]	1.93	B	2638.5	A

[a]RI early season = Pm/Pi = (midseason reniform nematode count)/(planting reniform nematode count))
[b]Seed cotton yield taken from the middle two rows of subplots.
[c]Means followed by the same letter in the same column are not significantly different at the 0.10 level based on ANOVA.
[d]Means followed by the same letter in the same column are not significantly different at the 0.05 level based on ANOVA.
[e]Aldicarb applied in furrow at planting (0.84 kg a.i./ha)+aldicarb side dress at pinhead square knifed in with custom applicator (1.17 kg a.i./ha).

Table 2.8 Effects of nematicide on seed cotton yield in four environments in a field trial at Stoneville, MS; data are averaged across cultivars.

Nematicide	Seed cotton yield (kg/ha)[a]							
	Environment 1[b]		Environment 2[b]		Environment 3[b]		Environment 4[b]	
No aldicarb	1980.9		1897.7	B[c]	2617.8	B	3369.4	B
Aldicarb applied[d]	1891.4		2030.6	A	3085.8	A	3546.1	A

[a]Seed cotton yield taken from the middle two rows of subplots
[b] Environment 1 was a 2006 trial in Field 4, environment 2 was a 2006 trial in the Barn field, environment 3 was a 2007 trial in Field 1, and environment 4 was a 2007 trial in Field 12 all at the Delta Research and Extension Center in Stoneville, MS.
[c]Means followed by the same letter in the same column are not significantly different at the 0.05 level based on ANOVA.
[d]Aldicarb applied in furrow at planting (0.84 kg a.i./ha)+aldicarb side dress at pinhead square knifed in with custom applicator (1.17 kg a.i./ha).

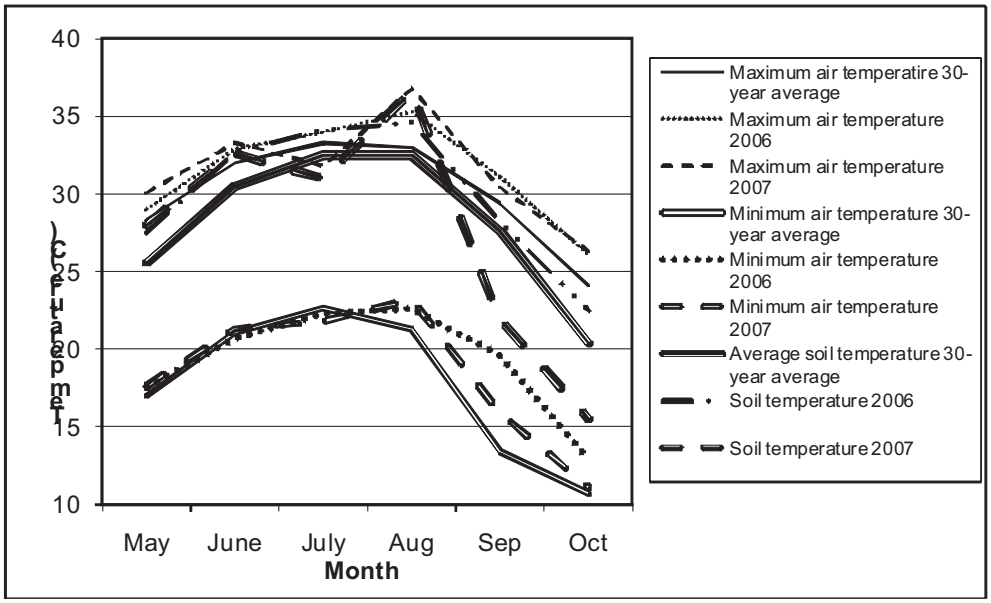

Figure 2.1. Mean soil (5 cm depth) and air temperatures for the 2006 and 2007 growing seasons[a] compared to the 30-year averages (Boykin et al., 1995) at Stoneville, MS.

[a] Monthly averages obtained from Delta Agricultural Weather Center, Delta Research and Extension Center, Mississippi State University.

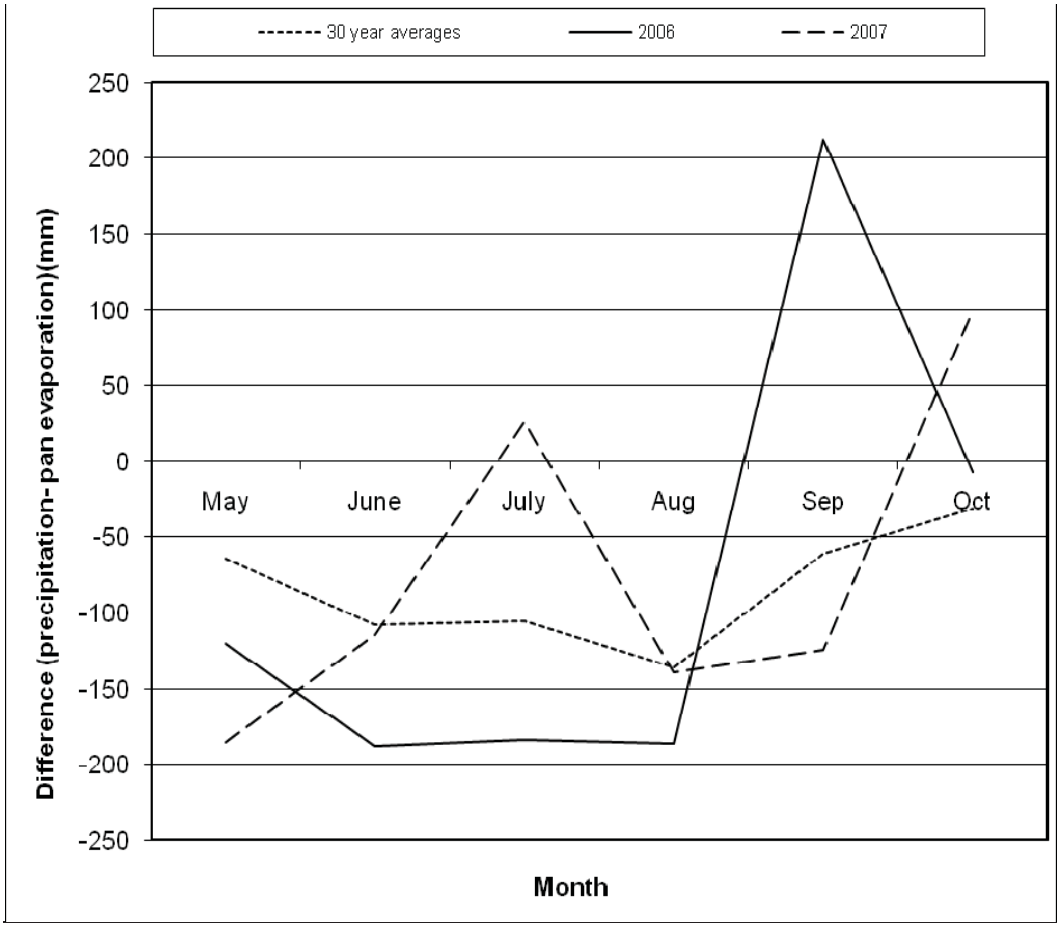

Figure 2.2. Difference between precipitation (mm) and pan evaporation (mm) for the 2006 and 2007 growing seasons[a] compared to the 30-year average (Boykin et al., 1995) for Stoneville, MS.

[a] Monthly averages obtained from Delta Agricultural Weather Center, Delta Research and Extension Center, Mississippi State University.