1320 Development of Reniform Nematode Resistance in Upland Cotton

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The purpose of this review is to assess development of resistance to the reniform nematode (*Rotylenchulus reniformis*) in upland cotton (*Gossypium hirsutum*). Cotton cultivars with reniform nematode resistance are needed. The development of resistant cultivars appears possible but presents a significant research challenge, primarily for two reasons. First, the best sources of resistance occur within diploid species that are genetically incompatible with cotton. Second, without molecular markers, reniform nematode resistance can be detected only by lengthy nematode reproduction assays. Thus, it is absolutely essential to discover a molecular marker for each resistance gene to provide seed companies with a tool for monitoring inheritance of resistance as they proceed through the cultivar development process. Simultaneous introgression of resistance from sources within several species is the wisest approach, because incomplete expression or incorporation of closely linked deleterious genes is possible in all cases, and in each case an investment of many years is required before success can be gauged by field testing of elite resistant breeding lines at multiple sites. Currently, researchers at more than a dozen USDA laboratories and state

supported universities in the United States have projects targeted at the introgression of reniform nematode resistance into agronomic upland cotton, from primitive tetraploid accessions of *Gossypium hirsutum* and *G. barbadense*, and diploid *G. arboreum*, and *G. longicalyx*. This task will take many years to complete. However, significant progress has been made toward developing cultivars carrying resistance from each source, and the first resistant cultivars could appear within three years, with committed follow through by commercial planting seed companies.

Keywords: cotton, *Gossypium hirsutum*, introgression, molecular marker, reniform nematode, resistance, *Rotylenchulus reniformis*

The reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira) (Gaur and Perry, 1991; Lawrence and McLean, 2001; Starr et al., 2002) is an increasing problem in cotton (*Gossypium hirsutum* L.) production in the eastern half of the United States Cotton Belt. It is estimated to incur annual losses of approximately $130M, with major impact in the states of Mississippi, Louisiana, and Alabama (Blasingame, 2006; Koenning et al., 2004; Robinson, 2007). Because there are no cultivars with high levels of resistance to this nematode (Robinson et al., 1999), management options are limited primarily to nematicides (Kinloch and Rich, 2001; Lawrence et al., 1990; Overstreet and Erwin, 2003; Zimet et al., 1999) and crop rotation (Davis et al., 2003; Gazaway et al., 1998, 2000), which often return only a fraction of the profits lost to reniform nematode damage. Most rotational crops are less profitable than cotton and provide cotton yield boosts only during the first year that cotton is grown following the rotation (Davis et al., 2003). Another, partial solution has been to identify tolerant genotypes that suffer less damage than do typical cultivars. A drawback of tolerance is that tolerant genotypes typically support high levels of nematode reproduction, and thus cannot be used to reduce the nematode population density in the soil. A second drawback is that tolerance appears to be highly environment-dependent, making the development of widely adapted tolerant cultivars unlikely (Koenning et al., 2000). More than a dozen breeding lines and cultivars exhibiting some degree of reniform nematode tolerance have been identified (Cook et al., 1997a, 1997b, 2003; Cook and Robinson, 2005; Jones et al., 1988; Koenning et al., 2000; Sciumbato et al., 2005; Stetina et al., 2006; Usery et al., 2005). Because discovery of tolerance provides an immediate solution to the problem, the search for tolerance remains an important research priority. Recent studies have identified several breeding lines and cultivars with potential for use in the important Mississippi Delta production region (Sciumbato et al., 2005; Stetina et al., 2006).

Reniform nematode reproduction has been evaluated on more than 3,000 genotypes of plants in the genus *Gossypium* in order to discover sources of resistance for developing highly resistant cultivars (Beasley and Jones, 1985; Carter, 1981; Muhammad and Jones, 1990; Robinson, 2002; Robinson et al., 1999, 2001, 2004, 2006; Robinson and Percival, 1997; Stewart and Robbins, 1995, 1996; Weaver et al., 2007; Yik and Birchfield, 1984). Only weak to moderate resistance has been reported in *G. hirsutum*, but high to very high levels of resistance have been found in several *Gossypium* species, including *G. anomalum* Wawr. & Peyr., *G. arboreum* L., *G. barbadense* L., *G. herbaceum* L., *G. longicalyx* Hutch. & Lee, *G. raimondii* Ulbr., *G. somalense* (Gurke) Hutch., *G. stocksii* Mast. In Hook., and *G. thurberi* Tod. (Yik and Birchfield, 1984; Robinson et al., 2004; Stewart and Robbins, 1995).

The most information regarding resistance to the reniform nematode is available for *G. hirsutum*, *G. barbadense*, *G. arboreum*, and *G. longicalyx*. Most accessions of *G. barbadense*, a species which freely hybridizes with *G. hirsutum*, are susceptible to the nematode and resistant *G. barbadense* accessions usually suppress nematode populations by only 70-90% (Robinson et al., 2004). In contrast, many accessions of *G. arboreum*, from

which genes are introgressed via bridging species, are highly resistant to the reniform nematode (Stewart and Robbins, 1995), and the most resistant of *G. arboreum* accessions suppress nematode reproduction 95% or more compared to susceptible *G. hirsutum*. As the extreme case, *G. longicalyx*, from which genes can be transferred only with great difficulty, is virtually immune. This apparent inverse relationship between compatibility and resistance within *Gossypium* greatly confounds strategies and funding for developing resistant cultivars.

Discussions among public and private cotton breeders in the United States (personal communication, R. L. Nichols, Cotton Incorporated) revealed that once genetic introgression of nematode resistance into *G. hirsutum* is achieved and breeding lines carrying nematode resistance are thereby made available, it will be essential to also provide the cotton seed industry with molecular, or readily observable phenotypic, markers for nematode resistance. Without markers for resistance, seed companies would not undertake breeding programs aimed at incorporating nematode resistance, because of the resources it would require of their breeders to conduct nematode reproduction assays. As a result, marker discovery becomes an essential component of resistance introgression research by public researchers.

A related factor is that a single gene, or a cluster of genes that is inherited simply and thus that can be monitored with one or a small number of molecular markers, is generally more desirable from the standpoint of costs of cultivar development than are multigenic sources of resistance. Because genetic recombination rates generally decrease with increasing genetic distance, introgression of resistance from some alien sources of resistance could involve small or large haplotypes inherited as a single alien segment, that would be trackable within progeny by a single marker. If resistance is due to an R-gene that is part of an R-gene cluster, then the patterns of resistance to certain other pathogens and pests might be inadvertently affected. In cotton, genes for resistance to Fusarium wilt, Verticillium wilt, bacterial blight, and the cotton root-knot nematode [*Meloidogyne incognita* (Kofoid & White) Chitwood race 3] would be of particular concern. Also, if the reniform nematode R-gene is embedded in a relatively large haplotype that encompasses genes that deleteriously affect other traits of economic importance, introgression of the R-gene might deleteriously affect overall performance.

Resistance from *G. longicalyx* and *G. arboreum* appears to be inherited as a single gene (Avila et al., 2006; Robinson et al., 2007). In contrast, inheritance of resistance from the *G. barbadense* accession, TX 110 (PI 163608), appears to be polygenic (J. L. Starr, personal communication). Thus, marker-assisted manipulation of TX 110-derived resistance might require the discovery and simultaneous monitoring of multiple markers during cultivar development. With increasing numbers of loci, the likelihood increases of encountering complications from R-gene clustering and/or haplotype based negative traits. Multigenic resistance also means that only a small number of progeny in a segregating population are likely to retain the full level seen in parents. However, multigenic resistance to pathogens in general, is more durable than monogenic resistance, because it is more difficult for pathogen populations to overcome resistance based on multiple genes.

There are several nematode-crop systems where nematode-resistant cultivars have been deployed. Nematodes in general do not overcome resistance as fast as fungi and bacteria, probably because parasitic nematodes reproduce much more slowly than fungi and bacteria, but eventual development of resistance-breaking nematode populations is common, especially among sexually reproducing species, such as the reniform nematode (Niblack et al., 2002; Plantard and Porte, 2004; Cook, 2004; Bakker et al., 1993; Roberts, 1995; Williamson, 1999; Castagnone-Sereno, 2002; Roberts, 2002; Robbins et al., 2001). An

alternative approach to multigenic resistance within a single cultivar, is use of multiple cultivars with different R-genes, that can be utilized in rotation. This approach has been employed in soybean and potato (Bakker et al., 1993; Niblack et al., 2002).

In sum, all evidence available indicates that the best approach is to work toward the development of reniform nematode resistance in Upland cotton simultaneously through introgression of resistance from multiple known sources. Failure to develop multiple sources of resistance could lead to a situation wherein the only source available carries unacceptable vulnerabilities or is of short duration due to development of resistance-breaking nematode populations.

ONGOING PROJECTS IN THE UNITED STATES

G. hirsutum. About 20 *G. hirsutum* accessions with weak to moderate levels of resistance to the reniform nematode have been reported. Resistance within *G. hirsutum* appears highly sensitive to environment and nematode population. In the following three cases, weak to moderate levels of resistance that were reproducible in one environment were not observed when tested in a different environment or against a different population of nematodes: 1) The accessions TX 20 (PI 163608), TX 69 (PI 153964), TX 709 (PI 265146), TX 834 (PI 529833), TX 874 (PI 529852), TX 893 (PI 529860), and TX 903 (PI 529864) that were scored by Yik and Birchfield (1984) in replicated experiments as moderately resistant were later scored by Robinson et al. (1997) as susceptible, because in the latter study they supported 17- to 64-fold increases in nematode populations in pots within a 7-week period. 2) Of 6 primitive *G. hirsutum* accessions that were scored by Robinson et al. (2004) as moderately resistant, only TX 1828 (PI 530459) and TX 1586 (PI 530217) were classified by Weaver et al. (2007) as resistant, or possibly resistant. 3) Of seven accessions [TX 245 (PI 165358), TX 378 (PI 165321), TX 500 (PI 209305,), TX 1419 (PI 530110), TX 1472 (PI 530151), TX 1565 (PI 530196), and TX 1765 (PI 530396)] observed by Weaver et al. (2007) to consistently support lower nematode populations than the control, all but one (TX-500) had been tested previously (Robinson et al., 2004), and only TX 1565 had been scored as possibly resistant. Its nematode population value in the 2004 study was 21% of the susceptible control, contrasted with 39 to 133% for the other five accessions.

In some cases, moderately to highly resistant primitive accessions of *G. hirsutum* from the USDA Cotton Collection have been found to have flower and leaf traits like *G. barbadense* [for example, TX 110, TX 500 (reclassified by A. E. Percival as GB 1032), TX 502 (PI 153878), TX-1347 (PI 530075, reassigned by A. E. Percival as GB 1042), TX-1348 (PI 530076, with origin near that of TX 1347), TX-2468 (PI 607773), and TX-2469 (PI 607774)] and to have been collected in areas (Guatemala: TX 110 and TX 502; Veracruz State of Mexico: TX 1347 and TX 1348; Brazil: TX 2467 and TX 2469) where introgression between *G. hirsutum* and *G. barbadense* occurs naturally. Thus, the question remains as to whether some of the resistant accessions that have been identified in the TX collection are *G. hirsutum* or *G. barbadense*. Comparison of reniform nematode reproduction on 850 accessions of *G. barbadense* and 1,419 of *G. hirsutum* (Robinson et al., 2004) clearly showed that although there is great variation and a wide range in the ability of accessions in both species to support reniform nematode reproduction, susceptible *G. barbadense* accessions on average support less reproduction than do typical *G. hirsutum* accessions, and resistance is more common in *G. barbadense*. In *G. barbadense*, the incidence of accessions detected with significantly less than 1/3 the reniform nematode reproduction measured on susceptible cv. Deltapine 16 was 2.1%, compared with 0.4% for *G. hirsutum* (Robinson et al., 2004).

At least four projects are actively pursuing development of reniform nematode resistant breeding lines from primitive accessions that either are *G. hirsutum*, or are maintained in the USDA Cotton Collection as *G. hirsutum*.

S. Stetina, L. D. Young, and W. R. Meredith, Jr. (USDA at Stoneville, MS) , and J. T. Johnson (currently Bayer CropScience) are working with TX 19 (PI 549146), TX 1347 and TX 1348. Crosses of three adapted *G. hirsutum* lines, DES 119B, DES 119H and 55-3, with nine primitive day-neutral *G. hirsutum* lines from the TX 19, TX 1347 and TX 1348 series are being evaluated. Sampling from the field is done by digging up five randomly selected plants in the progeny rows and counting the number of mature females on the tap and secondary roots. Data are expressed as nematodes/gram of root. The sampling and counting procedures are destructive. Only those progeny rows with low counts on at least four of the five root systems examined are selected for advancement. In 2003, 889 $F_{2:3}$ progeny rows and 150 parent rows were screened in the field. Of these, 117 progenies and 53 parental lines were selected and advanced for field evaluation in 2004 (Young et al., 2004).

Subsequent field and greenhouse evaluations at Stoneville in 2005 and 2006 resulted in just 24 of the original progenies and 14 of the original parental lines remaining for evaluation in 2007. Two additional sets of crosses were made to characterize the moderate level of resistance in these lines. Crosses among selected day-neutral TX 19, TX 1347 and TX 1348 lines (diallel crossing scheme) will determine if genes from all three lines are unique. Crosses between these lines and three adapted *G. hirsutum* lines (MD 9, Tamcot 98-99 Ne, and FiberMax 966: North Carolina design II) will allow estimates of heritability and gene action. In anticipation of the development of molecular markers for reniform nematode resistance by other researchers, DNA is being collected from all parents and progeny for future evaluation.

D. B. Weaver, K. S. Lawrence and L. Mangineni at Auburn University are exploiting two of seven resistant accessions that they identified in an exhaustive, replicated evaluation of 1,973 *G. hirsutum* accessions in the USDA Cotton Collection. They are concentrating on TX 245 (PI 165358) and TX 1419, which stood out early in their screenings and thus these accessions were among the first available for breeding efforts. These accessions have been crossed with several adapted cultivars, such as Fibermax 966, Paymaster 1218, Delta Pearl, and Suregrow 747. At present they have $F_{2:3}$ lines from these crosses and expect to begin evaluation of these materials soon. They also have made backcrosses to both parents, but have concentrated on backcrossing to the adapted parent. They have BC_1F_1 seed of these same populations, and will be growing these populations in the field the summer of 2007 to create BC_1F_2 populations for evaluations. They hope to shed light on heritability of the trait, and at the same time develop germplasm with reniform resistance that has better adaptability than the donor parents. A second focus of their work is to better characterize the resistance at the molecular level using gene-expression profiling. By exposing susceptible and resistant genotypes to nematode infestation, and extracting proteins from infected plants, they hope to identify proteins that are being differentially expressed in the resistant and susceptible plants.

In a collaborative project by USDA scientists at College Station, TX and Mississippi State, a half-diallel cross was made among the six most resistant primitive *G. hirsutum* accessions [TX 25 (PI 154035), TX 748 (PI 190112), TX 1586, TX 1828, TX 1860 (PI 530459) and TX 2469] identified by Robinson et al. (2004). No F_1 plants were scored as resistant but 24 F_2 plants had only 7.5 to 20% of the nematode population measured on susceptible controls. These F_2 plants were backcrossed onto root-knot nematode-resistant M-315 RNR (Shepherd

et al., 1996), LA887 or NemX, and BC_1F_2 seed have been collected for nematode resistance testing to try to recover the resistance level observed in the F_2 plants from the original cross.

At the USDA laboratory at Mississippi State, J. N. Jenkins, O. A. Gutierrez, and J. C. McCarty have begun the process of generating recombinant inbred lines (RILs) from F_2 populations from two crosses, each of which involves a root-knot nematode-resistant and a reniform nematode-resistant genotype, in order to provide tools useful to distinguish between the genes involved in root-knot and reniform nematode resistance. The root-knot nematode species to which resistance has been developed in cotton is *Meloidogyne incognita* (Koifoid & White) Chitwood race 3). Both crosses utilize M-315 RNR as the root-knot nematode-resistant genotype and either a day-neutral converted (DN) selection developed from TX 2468 (PI 607774) or the primitive *G. barbadense* accession GB 713 (PI 608139) as the reniform nematode resistance source. Plans are to evaluate RILs for both root-knot and reniform nematode resistance as well as for linked markers. This laboratory has been highly successful with this approach in the identification of markers for root-knot nematode resistance in cotton on chromosomes 11 and 14 (Ynturi et al., 2006).

In a complementary project, J. C. McCarty crossed DN TX-2468 with Deltapine 61 and evaluated day-neutral F_2 plants for reniform nematode resistance in pots. Several promising plants were selected and carried to the F_4 generation, where additional selections were made based on phenotype. Ten or 20 F_5 plants from each selection were evaluated for nematode resistance during 2006 and 2007 and on average supported only 18% to 22% of the level of nematode reproduction measured for susceptible controls. Additional selections were made at the F_5 and further crosses are planned during the 2007 growing season to obtain a better measure of plant-to-plant variability in resistance.

G. barbadense. J. L. Starr and C. W. Smith at Texas A&M University are working with progeny from a cross between root-knot nematode-resistant *G. hirsutum* M-315 RNR and reniform nematode-resistant *G. barbadense* TX 110 (PI 163608), originally reported as moderately resistant by Yik and Birchfield (1984). Numerous F_1 plants tested separately against either the reniform or the root-knot nematode consistently showed high resistance to the nematode used as inoculum, indicating that all F_1 plants were resistant to both nematodes. Based on failure of an F_2 population to fit either a one- or a two-gene model, resistance was assumed to be a polygenetic trait. Using a pedigree breeding system, lines were advanced to the F_5 generation following selection for day-neutrality in the F_2 generation, and for reniform nematode resistance in the F_3 and F_5 generations. Unfortunately, most F_5 generation lines had very low fertility due apparently to nonviable pollen. Eleven F_6 generation lines with apparently normal levels of fertility were selected and screened for reniform and root-knot nematode resistance. Three lines with resistance to both nematodes were then advanced to the F_7 generation. In a single test for seed cotton yield, in replicated single-row plots, all of the nematode-resistant selections had yields that were slightly better than M-315 RNR but not equal to those of three high yielding cultivars.

In another collaborative project between USDA scientists at College Station and Mississippi State, efforts are being made to backcross resistance from *G. barbadense* GB 713 (PI 608139) into several root-knot nematode-resistant breeding lines, including Auburn A623, M-315 RNR, Paymaster La887, and Acala NemX, as well as the obsolete but once extensively planted cultivar Deltapine 16. GB-713 was by far the most resistant *G. barbadense* accession identified in the extensive evaluation of *G. barbadense* in the USDA Cotton Collection conducted by Robinson et al. (2004) and it has been used as a resistant

experimental control in more than 30 replicated growth chamber experiments at College Station where it consistently suppresses reniform nematode reproduction, usually by 90 to 98% compared to susceptible Deltapine 16.

As a first step in this study, a reciprocal cross was made at College Station between GB 713 and Acala NemX, followed by generation of F_2 seed and seed of the BC_1 with each parent. Reniform nematode resistance was then measured in the same experiment for 30 individuals of the GB 713 parent, 30 of the Acala NemX parent, 60 F_1, 300 F_2, and 150 BC_1 with each parent. DNA was extracted from selected individual F_2 plants that showed either high or low levels of reniform reproduction. These DNAs were then screened with more than 700 SSR markers using a bulked segregant analysis methodology. Preliminary results indicated three SSR markers for resistance. Nematode reproduction on F_1 plants was uniform and intermediate between that on the two parents. Generation means analysis of nematode reproduction data from parents, F_1s, F_2s, and backcrosses onto both parents, indicated genetic control by a single partially dominant gene with additive effects. Thus inheritance indicated the trait was amenable to backcrossing into a root-knot nematode-resistant recurrent parent, by selecting for reniform but not root-knot nematode resistant backcrossed progeny at each generation. In practice, however, the level of reniform nematode resistance in the segregating progeny at each backcross has been low relative to environmental variation, throwing doubt on this direct approach. It may be necessary to self the plants after each backcross and select for reniform nematode resistance in BC_nF_2 progeny, where highly resistant homozygous plants are expected. So far, fourth and fifth backcross generation plants with nematode reproduction levels 3 to 19% of that on the susceptible control have been produced by making selections only from among F_1's at each backcross. However, the mean level of nematode reproduction on putatively heterozygous resistant plants is so high that it is impossible to conclude a 1:1 segregation pattern. F_2 progeny from the most advanced plants have not yet been tested against either nematode.

G. arboreum. In a project under the direction of E. Sacks with at the USDA at Stoneville, MS, introgression of resistance to the reniform nematode from *G. arboreum* into Upland cotton was initiated by crossing accession A2 190 (Burma C19) (Stewart and Robbins, 1995) with a $2[(AD_1)D_4]$ hexaploid bridging line named G 371. A single hybrid plant was obtained. The A2 190 × G 371 hybrid was subsequently crossed with Deltapine 16 and MD51ne to develop pseudo-backcross populations for nematode screening. In three resistance screens conducted in growth chambers using 500 ml pots, 174 backcross plants were evaluated along with 33 pots of susceptible control plants. The number of motile vermiform nematodes recovered per gram of soil confirmed that resistance in A2 190 was strong and similar to that of GB 713 (8% and 6% of the cultivar controls, respectively). Nematode reproduction in the backcross population, expressed as a percentage of the upland cultivar controls, had a bimodal distribution, suggesting the action of a dominant gene. The peak of the resistant class of the backcross population (heterozygous for resistance) was at about 15% of the cultivar controls. In addition to the A2 190 source of resistance, the hexaploid bridging line was subsequently used to obtain fertile hybrids with the resistant accessions A2 100 and A2 113. The new hybrids are currently being crossed with MD51ne to develop pseudo-backcross populations for nematode screening.

At the University of Arkansas, C. A. Avila, J. McD. Stewart, and R. T. Robbins have projects underway exploring at least four different aspects of reniform resistance introgression from *G. arboreum*. Initial inheritance studies evaluated plants in F_1 and F_2 generations from a cross between a highly resistant and a susceptible accession of *G. arboreum* to estimate the number of genes involved. The data indicated that a single gene confers reniform nematode resistance, because the F_2 generation segregated in a ratio of 3 resistant individuals for

each susceptible one ($\chi^2 < 0.12$). The assumption of a single gene controlling resistance would suggest that the gene effect is additive. This is supported by the observation that the mean of the F_1 generation was not significantly different ($P < 0.5535$) from the average of the two parental means.

Transfer of resistance into Upland cotton was accomplished by crossing resistant *G. arboreum* (A genome) with a D-genome species to produce an interspecific hybrid that upon doubling the chromosome number gave a synthetic allotetraploid (Avila et al., 2006). The resulting plant was backcrossed with upland cotton ($2AD_1$) and evaluated for resistance. Screenings of the BC_2F_1 and the BC_2F_2 for the ability of the nematodes to reproduce on the plants showed plants having resistance with population mean reproduction ratios intermediate between the resistant A-genome donor and the $2AD_1$ recurrent parent.

An AFLP marker linked to resistance was identified (Avila et al., 2005) and tested in the BC_2F_1 generation. This resulted in a good correlation of marker presence and resistance. However, in the BC_2F_2 generation the marker was not highly correlated with the resistance gene. New markers linked to resistance have been found, and their usefulness for marker-assisted selection is currently being evaluated.

Host gene response to reniform nematode infection in *G. arboreum* was evaluated through cDNA-AFLP analysis 16 days after inoculation using a resistant and susceptible accession. After sequencing of the differentially expressed transcripts, the sequences were used to search (BLAST) GenBank databases for homologous sequences. Transcripts for which homologous sequences were identified, were grouped according to their putative biological function. Transcripts of genes associated with cellular transport, cell cycle and DNA were up-regulated more in the susceptible than in the resistant accession. These investigators hypothesize that those processes are related to plant syncytium formation at the nematode feeding site. Some of the transcripts may be potential targets for iRNA silencing as a mechanism for transgenic resistance. Cellular processes that may be involved in natural resistance mechanisms, such as cellular rescue, defense, and transcription had the greater number of up-regulated transcripts in the resistant accession.

G. longicalyx. Virtual immunity to the reniform nematode in *G. longicalyx* (Yik and Birchfield, 1984) has been confirmed in various laboratories. In a project conducted by USDA and Texas A&M University scientists at College Station, TX, two tri-species hybrids of *G. hirsutum, G. longicalyx*, and either *G. armourianum* or *G. herbaceum* (Bell and Robinson, 2004; also see Brown and Menzel, 1950, and Konan et al., 2007) were utilized as bridges to introgress resistance to the nematode from *G. longicalyx* into *G. hirsutum*. Introgression was accomplished by recurrent backcrosses to agronomic *G. hirsutum* with cytogenetic analysis of early backcross generations to assess progress toward the euploid state ($2n = 52$), selection for nematode resistance at each generation, and examination of self progeny at the first, third, sixth, and seventh backcross to identify and eliminate lineages with undesired recessive traits (Robinson et al., 2007). Altogether, 689 BC_1 progeny were generated from the two male-sterile hybrids. Introgression was pursued from 28 resistant BC_1 plants, each of which was backcrossed four to seven times to *G. hirsutum* to derive agronomically suitable types. More than 2,500 backcross and selfed progeny were evaluated for nematode resistance. The resistance trait segregated (resistant:susceptible) 1:1 in backcross progeny and 3:1 in self progeny from putatively heterozygous resistant plants. There was no obvious diminution of the resistance across backcross generations. Advanced backcross plants were indistinguishable from agronomic cotton under greenhouse conditions. Comparisons of 240 homozygous resistant BC_6S_2 plants with heterozygous, susceptible, and recurrent parent plants in field plantings in 2006 showed normal lint quality

and quantity. Two BC_7 lines, LONREN-1 and -2, were released by USDA in April of 2007 and additional lines are being developed and evaluated through cooperative research agreements between USDA and Bayer CropScience, or Delta and Pine Land Company, or Mississippi State University.

Other research in this project has focused on localization and mapping of the responsible gene(s), and identification of markers sufficiently close for marker-assisted selection (Dighe et al., 2007). Marker discovery initially emphasized representation of all A-subgenome linkage groups, a wide separation of loci, and more than 1,000 phenotyped plants spanning 7 backcross and 3 self generations. Three panels were used for marker screening and evaluation, i.e., polymorphism, trait-association and linkage-detection. SSRs identified as polymorphic were screened against the trait-association panel of DNA samples from 12 highly resistant and 12 highly susceptible plants. The results associated the resistance with BNL1066 and linkage group (LG) A03 (chromosome-11), which led to testing of 14 additional markers from public maps of A03 and its homeologue, D02. Association analysis and linkage estimation was extended to 88 classified selfed progeny (BC_1-BC_6) and 984 classified backcross hybrids (BC_2-BC_8) for BNL3279_114, BNL1066_156, BNL836_215, and green fuzz locus (Fz^{lon}) respectively. The results indicated that BNL3279_114, BNL1066_156, BNL836_215 are mapped on one side within 1.4, 2.0, 4.4 cM, respectively, while, Fz^{lon} is on the opposite side of the resistance locus with a linkage estimate of 4.5 cM. Release of the germplasm and marker information should facilitate incorporation of the trait into new cotton cultivars.

In a new collaborative project, A. A. Bell with USDA at College Station has made crosses between breeding lines carrying resistance from *G. longicalyx* and six elite Mississippi State University breeding lines, including PST006, PST246, Miscot 8824, Miscot 0141-15ne, Miscot 0110-1ne, and Miscot 0023-11ne. Miscot 8824 is considered root-knot nematode resistant. P. Thaxton and T. Wallace at Mississippi State University will plant F_1 seed from these crosses in the greenhouse in 2007 to generate F_2 seed, and in 2008 six F_2 populations will be grown in 6-8 progeny rows at Stoneville, MS. B. Scheffler with USDA at Stoneville will utilize molecular markers to select resistant progeny. Plants also will be selected for the nectariless trait. Homozygous positive lines will be tested as F_2:F_3 progeny rows at Stoneville, MS in 2009 to confirm purity. Non-segregating progeny rows uniform in appearance will be harvested in bulk (F_2:F_4) to comprise a new reniform-resistant breeding line available for further testing. Heterozygous plants will be individually selfed in 2008, and plants within progeny rows will again be screened with codominant markers to determine homogeneity in 2009.

MECHANISMS, DURABILITY, AND GENETIC ENGINEERING

P. Agudelo at Clemson University has conducted several projects examining variability among reniform nematode populations, and the morphological aspects of the histopathological response characterizing resistance to the nematode in cotton (Agudelo et al., 2005a, 2005b, 2005c). Agudelo summarizes current and future research directions on the reniform nematode as: 1) characterization of virulence groups in *R. reniformis*; 2) histological characterization of resistance to *R. reniformis* on cotton genotypes, and characterization of post-penetration biochemical responses; and 3) assessment of host-induced selection on geographic isolates of the nematode.

R. Kantety and colleagues at the Center for Molecular Biology at Alabama A&M University are studying the morphometric as well as molecular variation of reniform nematode collections from several regions in Alabama. Their results indicate considerable variation

within and among reniform nematode populations collected from more than 10 locations in Alabama and one location in Mississippi. Their studies indicate that two major variants of the 18S ribosomal RNA gene sequences exist within individual nematodes while several nucleotide polymorphisms exist between multiple copies of each of the variants. They are actively developing RN-specific PCR primers for exclusive amplification of RN from soil DNA extractions. These RN-specific primers will be used in developing a quantitative real-time PCR (qRT-PCR) assay for direct detection and quantification of RN directly from soil samples. Future studies will also focus on developing alternative methods to sequencing for the identification of variation within and among reniform nematode collection sites.

The Alabama A&M University group is also undertaking an effort to generate a significant amount of genomic sequence of the reniform nematode through various approaches. The annotated reniform nematode genome sequence and comparative genome analyses will be made available to the public.

A third focus at Alabama A&M University concerns functional genomic analysis of reniform nematode infection in cotton. They are studying the molecular interactions through the analysis of gene expression as well as proteomics. The main objective is to study the species level differences as well as specific resistance introgressed from *Gossypium longicalyx*. They are developing several cDNA and serial analysis of gene expression (SAGE) libraries from reniform nematode-treated and control plants belonging to *G. hirsutum*, *G. barbadense*, *G. arboreum* and *G. longicalyx*. Upcoming studies will focus on the gene/protein expression during various stages of reniform nematode infection of resistant (R) as well as susceptible (S) genotypes of cotton. In addition, functional genomic analyses of specific infection sites will be conducted.

M. Wubben, with the USDA at Mississippi State, has recently achieved reproduction by root-knot and by reniform nematodes in vitro on hairy root cultures provided by B. A. Triplett, USDA-ARS, New Orleans, as a first and essential step toward characterization of functional genomics of successful and unsuccessful feeding site establishment, respectively, in susceptible and resistant plants. Retention of root-knot nematode resistance by M-315 RNR and of reniform nematode resistance by GB 713 in hairy root culture has been confirmed and similar experiments are underway or planned for the reniform nematode utilizing breeding lines carrying resistance from *G. longicalyx*. These achievements poise Wubben for creation of cDNA libraries and subsequent investigations leading to identification of parasitism- and/or reproduction-related genes that could be targeted for RNA-interference.

DISCLAIMER

REFERENCES

Agudelo, P., R. T. Robbins, K. S. Kim, and J. McD. Stewart. 2005a. Histological changes in *Gossypium hirsutum* associated with reduced reproduction of *Rotylenchulus reniformis*. *J. Nematol.* 37:185-189.

Agudelo, P, R. T. Robbins, J. McD. Stewart, A. A. Bell, and A. F. Robinson. 2005b. Histological observations of *Rotylenchulus reniformis* on *Gossypium longicalyx* and interspecific cotton hybrids. J. Nematol. 37:444-447.

Agudelo, P., R. T. Robbins, J. McD. Stewart, and A. L. Szalanski. 2005c. Intraspecific variability of *Rotylenchulus reniformis* from cotton-growing regions in the United States. *J. Nematol.* 37:105-114.

Avila, C. A., J. McD. Stewart, and R. T. Robbins. 2005. Development of a molecular marker linked to reniform nematode resistance in cotton. *In:* D. M. Oosterhuis (ed.). Summaries of Arkansas Cotton Research 2004. Ark. Agri. Exp. Sta., Research Series 533.

1Avila, C. A., J. McD. Stewart, and R. T. Robbins. 2006. Introgression of reniform nematode resistance from *G. arboreum* to upland cotton: 2005 advances. Pp. 122-127. *In:* D. M. Oosterhuis (ed.) Summaries of Arkansas Cotton research 2005. Ark. Agri. Expt. Sta. Research Series 543.

Bakker, J, R. T. Folkertsma, J. N. A. M. Rouppe van der Voort, J. M. de Boer, and F. J. Gommers. 1993. Changing concepts and molecular approaches in the management of virulence genes in potato cyst nematodes. Annu. Rev. Phytopathol. 31:169-190.

Beasley, J. P., and J. E. Jones. 1985. The current status in the development of resistance to the reniform nematode in cotton in Louisiana. *In* Proc. Beltwide Cotton Conf., New Orleans, LA, 6-11 Jan. 1985. Natl. Cotton Counc. Am., Memphis, TN.

Bell, A. A., and A. F. Robinson. 2004. Development and characteristics of triple species hybrids used to transfer reniform nematode resistance from *Gossypium longicalyx* to *Gossypium hirsutum*. p. 422-426. *In* Proc. Beltwide Cotton Conf., San Antonio, TX. 5-9 Jan. 2004. Natl. Cotton Counc. Am., Memphis, TN.

Blasingame, D. 2006. 2005 Cotton Disease Loss Estimate. p. 155-157. *In* Proc. Beltwide Cotton Conf., San Antonio, TX. 3-6 Jan. 2006. Natl. Cotton Counc. Am., Memphis, TN.

Brown, M. S., and M. Y. Menzel. 1950. New trispecies hybrids in cotton. J. Heredity 41:291-292.

Carter, W. W. 1981. Resistance and resistant reaction of *Gossypium arboreum* to the reniform nematode, *Rotylenchulus reniformis*. *J. Nematol.* 13:368-374.

Castagnone-Sereno, P. 2002. Genetic variability of nematodes: a threat to the durability of plant resistance genes? Euphytica 124: 193-100.

Cook, C. G., L. N. Namken, and A. F. Robinson. 1997a. Registration of N220-1-91, N222-1-91, N320-2-91, and N419-1-91 nematode-resistant cotton germplasm lines. Crop Sci. 37:1029-1029.

Cook, C. G., and A. F. Robinson. 2005. Registration of RN96425, RN96527, and RN96625-1 nematode-resistant cotton germplasm lines. Crop Sci. 45:1667-1668.

Cook, C. G., A. F. Robinson, A. C, Bridges, A. E. Percival, W. B. Prince, J. M. Bradford, and J. A. Bautista. 2003. Field evaluation of cotton cultivar response to reniform nematodes. In Proc. Beltwide Cotton Conf. Natl. Cotton Counc. Am., pp. 861-862. Nashville, TN.

Cook, C. G., A. F. Robinson, and L. N. Namken. 1997b. Tolerance to *Rotylenchulus reniformis* and resistance to *Meloidogyne incognita* race 3 in high-yielding breeding lines of upland cotton. J. Nematol. 29:322-328.

Cook, R. 2004. Genetic resistance to nematodes: where is it useful? Australasian Plant Pathology 33:139-150.

Davis, R.F., S.R. Koenning, R.C. Kemerait, T.D. Cummings, and W.D. Shurley. 2003. *Rotylenchulus reniformis* management in cotton with crop rotation. J. Nematol. 35:58-64.

Dighe, N. D., A. F. Robinson, A. A. Bell, M. A. Menz, R. Cantrell, and D. M. Stelly. 2007. Tagging and mapping of upland cotton resistance to reniform nematode after interploid introgression from *Gossypium longicalyx*. Crop Sci. (submitted).

Gaur, H.S., and R.N. Perry. 1991. The biology and control of the plant parasitic nematode *Rotylenchulus reniformis*. Agricultural Zoology Reviews 4:177-212.

Gazaway, W. S., J. R. Akridge, and K. McLean. 2000. Impact of various crop rotations and various winter cover crops on reniform nematode in cotton. p. 162-163. *In* Proc. Beltwide Cotton Conf., San Antonio, TX. 4-8 Jan. 2000. Natl. Cotton Counc. Am., Memphis, TN.

Gazaway, W. S., J. R. Akridge, and R. Rodríguez-Kábana. 1998. Management of reniform nematode in cotton using various rotation schemes. p. 141-142. *In* Proc. Beltwide Cotton Conf., San Diego, CA. 5-9 Jan. 1998. Natl. Cotton Counc. Am., Memphis, TN.

Jones, J. E., J. P. Beasley, J. I. Dickson, and W. D. Caldwell. 1988. Registration of four cotton germplasm lines with resistance to reniform and root-knot nematodes. Crop Sci. 18:199-200.

Kinloch, R. A., and J. R. Rich. 2001. Management of root-knot and reniform nematodes in ultra-narrow row cotton with 1,3-dichloropropene. J. Nematol. 33:311-313.

Konan, O. N., A. D'Hont, J.-P. Baudoin, and G. Mergeai. 2007. Cytogenetics of a new trispecies hybrid in cotton: [(Gossypium hirsutum L. × G. longicalyx Hutch. & Lee]. Plant Breeding 126:176-181.

Koenning, S. R., K. R. Barker, and D. T. Bowman. 2000. Tolerance of selected cotton lines to *Rotylenchulus reniformis*. J. Nematol. 32:519-523.

Koenning, S. R., T. L. Kirkpatrick, J. L. Starr, J. A. Wrather, N. R. Walker, and J. D. Mueller. 2004. Plant parasitic nematodes attacking cotton in the United States: Old and emerging production challenges. Plant Dis. 88:100-113.

Lawrence, G. W., and K. S. McLean. 2001. Reniform nematodes. p. 42-44. *In* T. L. Kirkpatrick and C. S. Rothrock (eds.) Compendium of Cotton Diseases. APS Press, St. Paul, MN.

Lawrence, G. W., K. S. McLean, W. E. Batson, D. Miller, and J. C. Borbon. 1990. Response of *Rotylenchulus reniformis* to nematicide applications on cotton. J. Nematol. 22:707-711.

Muhammad, N., and J. E. Jones. 1990. Genetics of resistance to reniform nematode in Upland cotton. Crop Sci. 30:13-16.

Niblack, T. L., P. R. Arelli, G. R. Noel, C. H. Opperman, J. H. Orf, D. P. Schmitt, J. G. Shannon, and G. L. Tylka. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. J. Nematol. 34:279-288.

Overstreet, C., and T. L. Erwin. 2003. The use of Telone in cotton production in Louisiana. p. 277-278. *In* Proc. Beltwide Cotton Conf., Nashville, TN. 6-10 Jan. 2003. Natl. Cotton Counc. Am., Memphis, TN.

Plantard, O., and C. Porte. 2004. Population genetic structure of the sugar beet cyst nematode *Heterodera schachtii*: a gonochoristic and amphimictic species with highly inbred but weakly differentiated populations. Molecular Ecology 13:33-41.

Robbins, R. T., E. R. Shipe, L. Rakes, L. E. Jackson, E. E. Gbur, amd D. G. Dombek. 2001. Host suitability in soybean cultivars for the reniform nematode, 2000 tests. Suppl. J. Nematol. 33:314-317.

Roberts, P.A. 2002. Concepts and consequences of resistance. p. 23-42. *In* J.L. Starr, R. Cook, and J. Bridge (eds.) Plant Resistance to Parasitic Nematodes. CABI Publishing, Wallingford, UK.

Robinson, A. F. 2002. Reniform nematodes: *Rotylenchulus* species. p. 153-174. *In* J. L. Starr, R. Cook, and J. Bridge (eds.) Plant Resistance to Parasitic Nematodes. CABI Publishing, Wallingford, UK.

Robinson, A. F. 2007. Reniform in U. S. Cotton: When, where, why, and some remedies. Annu. Rev. Phytopathol. 45:11.1-11.25 (Early, online version at **http://arjournals.annualreviews.org/doi/pdf/10.1146/annurev.phyto.45.011107.143949**).

Robinson, A. F., J. R. Akridge, J. B. Bradford, C. G. Cook, W. S. Gazaway, E. C. McGawley, J. L. Starr, and L. D. Young. 2006. Suppression of *Rotylenchulus reniformis* 122 cm deep endorses resistance introgression in *Gossypium*. J. Nematol. 38:195-209.

Robinson, A. F., A. A. Bell, N. D. Dighe, M. A. Menz, R. L. Nichols, and D. M. Stelly. 2007. Introgression of resistance to nematode *Rotylenchulus reniformis* into upland cotton (*Gossypium hirsutum*) from *G. longicalyx*. Crop Sci. 47:(in press).

Robinson, A. F., D. T. Bowman, C. G. Cook, J. N. Jenkins, J. E. Jones, L. O. May, S. R. Oakley, M. J. Oliver, P. A. Roberts, M. Robinson, C. W. Smith, J. L. Starr, and J. McD. Stewart. 2001. Nematode resistance. p. 68-79. *In* T.L. Kirkpatrick and C.S. Rothrock (eds.) Compendium of Cotton Diseases. APS Press, St. Paul, MN.

Robinson, A. F., A. C. Bridges, and A. E. Percival. 2004. New sources of resistance to the reniform (*Rotylenchulus reniformis* Linford and Oliveira) and root-knot (*Meloidogyne incognita* (Kofoid & White) Chitwood) nematode in upland (*Gossypium hirsutum* L.) and sea island (*G. barbadense* L.) cotton. J. Cotton Sci. 8:191-197.

Robinson, A. F., C. G. Cook, and A. E. Percival. 1999. Resistance to *Rotylenchulus reniformis* and *Meloidogyne incognita* race 3 in the major cotton cultivars planted since 1950. Crop Sci. 39:850-858.

Robinson, A. F., and A. E. Percival. 1997. Resistance to *Meloidogyne incognita* race 3 and *Rotylenchulus reniformis* in wild accessions of *Gossypium hirsutum* and *G. barbadense* from Mexico. J. Nematol. 29:746-755.

Sciumbato, G. L., S. R. Stetina, and L. D. Young. 2005. Tolerance of popular cotton varieties to the reniform nematode. p. 150. *In* P. Duggar and D. A. Richter (ed.) Proc. Beltwide Cotton Conf. New Orleans, LA. 5–7 Jan. 2005. Natl. Cotton Counc., Memphis, TN.

Shepherd, R. L., J. C. McCarty, J. N. Jenkins, and W. L. Parrott. 1996. Registration of nine cotton germplasm lines resistant to root-knot nematode. Crop Sci. 36:820.

Starr, J. L., J. Bridge, and R. Cook. 2002. Nematode parasites of cotton and other tropical fibre crops. p. 1-22. *In* J. L. Starr, R. Cook, and J. Bridge (eds.) Plant Resistance to Parasitic Nematodes. CABI Publishing, Wallingford, UK.

Stetina, S. R., G. L. Sciumbato, J. A. Blessitt, and L. D. Young. 2006. Cotton cultivars tolerant to reniform nematode. J. Nematol. 38:294.

Stewart, J. M., and R. T. Robbins. 1995. Evaluation of Asiatic cottons for resistance to reniform nematode. p. 165-168. *In* D. M. Oosterhius (ed.) Proc. 1994 Cotton Research Meeting, Special Report 166. Arkansas Agricultural Experiment Station, Fayetteville, AR.

Stewart, J. M., and R. T. Robbins. 1996. Identification and enhancement of resistance to reniform nematode in cotton germplasm. p. 255. *In* Proc. Beltwide Cotton Conf., Nashville, TN. 9-12 Jan. 1996. Natl. Cotton Counc. Am., Memphis, TN.

Usery, S. R., K. S. Lawrence, G. W. Lawrence, and C. H. Burmester. 2005. Evaluation of cotton cultivars for resistance and tolerance to *Rotylenchulus reniformis*. Nematropica 35:121–134.

Weaver, D. B., K. S. Lawrence, and E. Van Santen. 2007. Reniform nematode resistance in upland cotton germplasm. Crop Sci 47:19-24.

Williamson, V. M. 1999. Plant nematode resistance genes. Current Opinion in Plant Biology 2:327-331.

Yik, C-P., and W. Birchfield. 1984. Resistant germplasm in *Gossypium* species and related plants to *Rotylenchulus reniformis*. J. Nematol. 16:146-153.

Ynturi, P., J. N. Jenkins, J. C. McCarty, Jr., O. A. Gutierrez, and S. Saha. 2006. Association of root-knot nematode resistance genes with simple sequence repeat markers on two chromosomes in cotton. Crop Sci. 46:2670-2674.

Young, L. D., S. R. Stietina, and W. R. Meredith, Jr. 2004. Development of cotton germplasm with resistance to reniform nematode. P. 427. In: D. A. Richter (ed.) Proc. Beltwide Cottton Conf., San Antonio, TX, 5-9 Jan. 2004. Natl. Cotton Counc., Memphis. TN.

Zimet, D., J. R. Rich, A. LaColla, and R. A. Kinloch. 1999. Economic analysis of Telone II (1,3-D) and Temik 15G (aldicarb) to manage reniform nematode (*Rotylenchulus reniformis*) in cotton. p. 111-112. *In* Proc. Beltwide Cotton Conf., Orlando, FL. 3-7 Jan. 1999. Natl. Cotton Counc. Am., Memphis, TN.