

**Host Resistance as the cornerstone for managing plant-parasitic nematodes in sustainable agroecosystems**

**Objective 1: Identification, characterization and introgression of genes for resistance and tolerance to nematodes in cotton, peanut, soybean, and major fruit and vegetable crops.**

Cotton:

Resistance to reniform nematodes (*Rotylenchulus reniformis*) from two different sources has been introgressed into upland cotton by a joint effort of USDA –ARS scientist and researchers with Texas Agrilife Research. A high level of resistance has been developed from the interspecific crosses with *G. longicalyx* and a moderate level of resistance has been developed from interspecific crosses with *G. barbadense* ‘Tx110’. This germplasm is being released and made available to other cotton breeding programs. Additionally, several new sources of resistance to the root-knot nematode (*Meloidogyne incognita*) have been identified from primitive accessions of *G. hirsutum* and are being used to expand the current limited sources of resistance to this nematode in upland cotton.

USAD-ARS scientists in Georgia have developed cotton germplasm with a high level of resistance to root-knot nematodes with yield and fiber quality better than previous resistant germplasm and equal to modern cultivars (germplasm release is in preparation). Further, they have identified a new gene for resistance to root-knot nematodes in cotton on the same chromosome as the previously identified resistance gene.

Peanut:

The peanut cultivar Tifguard was jointly released by the USDA ARS in Tifton, GA and University of Georgia. This cultivar has a high level of resistance to the peanut root-knot nematode (*Meloidogyne arenaria*) and Tomato Spotted Wilt Virus, as well as some resistance to *Cylindrocladium* black rot and southern stem rot. In a separate breeding effort, resistance to *M. arenaria* and *M. javcanica* has been introgressed into peanut germplasm with resistance to TSWV and Sclerotinia blight and also has the highly desirable high ratio of oleic to linoleic fatty acids. A release of a high yielding cultivar from this germplasm is expected in 2010 from the Texas AgriLife Research system. In collaboration with USDA ARS and University of Georgia scientists, peanut germplasm from China with a high level of resistance to the northern root-knot nematode, *Meloidogyne hapla* was identified. Nematode reproduction on the resistant germplasm was < 10% of that on the susceptible control, Georgia Green. We also identified sources of resistance to *M. arenaria* (both moderate and high levels) that appear to be different from the existing genes for resistance found in the cultivars ‘NemaTAM’ and ‘Tifguard’.

Soybean:

Soybean varieties that are submitted for inclusion in the state soybean variety trials in Mississippi were screened for their host suitability to the three major soybean nematode pathogens, the southern root-knot, the reniform and soybean cyst nematode. The southern root-knot used in this study was *Meloidogyne incognita* host race 3, the reniform nematode is *Rotylenchulus* and the soybean cyst nematode was *Heterodera glycines* race 15. Fifteen percent

of 468 cultivars screened were screened to root-knot nematodes 25.7% of 553 cultivars screened were resistant to the reniform nematode and none of the 205 cultivars were found to be resistant to the race 15 of the soybean cyst nematode nematode.

In Arkansas evaluation of the reproduction of reniform nematode (*Rotylenchulus reniformis*) on contemporary soybean varieties and breeding lines is an ongoing effort and the information from these tests are the only reports of ongoing studies of this nematode on soybean available. During the life of this project over 1,000 soybean varieties have been tested for resistance to the reniform nematode with less than 50 showing resistance at a level high enough to be effective in a cotton-soybean rotation. Data on these varieties and lines are incorporated into the Arkansas Extension Services nematode recommendations yearly.

In North Carolina homozygous RNAi soybean plants were developed from transformants to test for potential novel resistance to soybean cyst nematodes. In a separate effort 18 lines with resistance derived from PI437564 were evaluated for yield and resistance to cyst nematode field trials located in three counties. Resistance to Races 1, 3, 9 and 14 were ineffective against SCN at this location and lines with race 2 resistance had much greater yields. A soybean cultivar with resistance to races 1,2,3,5,9 and 14 was developed with resistance derived from Anand (Hartwig x Holiday).

Tolerance to reniform nematode was evaluated for 34 cotton varieties in a field study. The study was a strip-plot design treated or nontreated with Telone II at 4.5 gallons/acre. Data indicate that putative tolerance is an artifact of experimental design and perceived tolerance is actually low yield potential (Koenning and Bowman).

## **Objective 2: Development of marker assisted selection systems for more efficient introgression of multiple resistance genes into agronomically superior crop genotypes.**

### Peanut:

In cooperation with Dr. Corley Holbrook (USDA-ARS, Tifton, GA) and Dr. Peggy Ozias-Akins (Univ. of Georgia, Tifton, GA), a new PCR-based marker 197/909 was developed to identify resistance to *M. arenaria* in peanut. When used with high throughput DNA extraction, this marker will be cost effective for screening for nematode resistance in peanut breeding programs. This marker-assisted selection system is being used by several peanut breeding programs.

### Cotton:

Researchers in Georgia have identified DNA markers for a major gene for resistance to root-knot nematodes in cotton.

## **Objective 3: Deployment of resistance and tolerance to nematodes in sustainable cropping systems.**

Information on the diversity, distribution and impact of plant parasitic nematodes is an essential consideration in any plant production/protection system. Over the duration of this project, survey data for Louisiana has documented a marked reduction in the overall distribution of and damage attributable to the soybean cyst nematode, *Heterodera glycines*. Similarly, survey activities have shown a tremendous increase in the distribution of the reniform nematode,

*Rotylenchulus reniformis*. This pathogen is now widespread throughout the cotton and soybean production areas of Louisiana and all popular cultivars of both crops are susceptible to damage. Additionally, this nematode also causes significant damage to most vegetable crops, especially tomato, eggplant and cucumber. Root-knot nematodes, which include primarily *Meloidogyne incognita*, but also *M. arenaria* and *M. javanica* remain serious pests of a wide range of crops in Louisiana. Unlike that for reniform nematode, there is satisfactory resistance to root-knot nematode available in most major crops. The greatest damage from root-knot nematode, *M. incognita* in particular, results from its' interaction with root-infecting fungi in cotton.

Over the course of this project we have documented the efficacy of Agri-Terra, a new, environmentally responsible and soon-to-be registered nematicide. Evaluations of this product have been conducted under greenhouse, microplot or field conditions against all major nematode species found on cotton, soybean, sugarcane, rice, corn, peppers, tomatoes, cabbage, lettuce, cauliflower, cucumber and strawberry. On each of these 12 crops, the compound has significantly reduced nematode populations. Moreover, it has produced enhanced growth and significantly increased yields with half of the crops. In microplot and field trials where it has been compared with conventional and much more toxic nematicides such as aldicarb, phenamiphos, carbofuran and methyl bromide it has proven to be at least equally efficacious. In trials conducted during the last 2 years of this project, Agri-Terra has also proven to be an effective tool for the management of nematodes in recreational turf settings, especially golf courses.

Another line of investigation related to the chemical management of nematodes, especially reniform nematode, pursued during the tenure of this project involved the evaluation of naturally occurring, allelopathic compounds produced by three weed species endemic in cotton and soybean fields in Louisiana. Root exudates from weeds, morningglory (*Ipomoea lacunosa*), hemp sesbania (*Sesbania exaltata*), and johnsongrass (*Sorghum halepense*) inhibit reproduction of reniform nematode on both cotton and soybean by retarding the eclosion process of eggs of the nematode. This research was the subject of a Ph.D. dissertation by Dr. M.J. Pontif. Additional studies to more fully characterize exudates from roots of these weeds are in progress with reniform and root-knot nematodes.

Laboratory, greenhouse, microplot and field studies with local, regional and national isolates of reniform nematode from cotton and soybean have documented the existence of significant variation in reproduction and pathogenicity among populations. Races or "virulence pathotypes" of cyst and root-knot nematodes are commonly distinguished on the basis host range (specific plant species) or host cultivar (specific soybean cultivars) assays conducted under greenhouse or incubator conditions. Over the last two years we have tried both of these approaches, different plant species and different cotton and soybean cultivars, without satisfactory results. In other laboratories in the U.S. and abroad nematologists are attempting, also without satisfactory results to date, to differentiate among geographic isolates of reniform nematode using molecular methods. A portion of our work with this nematode over the next few years will certainly involve a continuation of these efforts to develop a set of host differentials for reniform nematode.

#### Pearl Millet:

Pearl millet is an emerging grain crop in the southern U.S. because of its drought tolerance and resistance to aflatoxins; it is also a staple food in Africa and India. Resistance to nematodes is

important to provide stability to pearl millet production and to reduce nematode populations that can damage crops grown in rotation with pearl millet. Resistance to root-knot nematodes was identified in several pearl millet accessions

#### Cotton:

Demonstrated that moderate resistance to root-knot nematodes in cotton was much more effective than previously thought at suppressing nematode levels in the field.

A spinoff project that would fit with the sustainability of cropping systems is the use of multi-temporal spectral classification of the root-knot and reniform nematode using self-organized maps.

The objective of the first part of this study was to develop a means to estimate the population numbers of specific nematodes species associated with nematode infected plants through the use of remote sensing.

The primary part of this project is to correlate hyperspectral reflectance from cotton plants infected with the reniform nematode to known reniform population levels infesting the plant in a controlled and field environment. To analyze the hyperspectral data, artificial neural networks (ANNs) utilizing self-organized maps (SOMs) are used. SOMs use pattern recognition to identify similar features. Application of SOMs using a specific MATLAB based hyperspectral toolkit (MHTK) is the analysis function of interest. Plant canopy with soil, single leaf, and plant canopy spectral measurements and corresponding reniform nematode population levels were analyzed using the MHTK with SOMs. In our analysis we used two batches of SOMs for each target. The first batch concentrated on wavelengths 451-949 (visible and NIR regions of the EMS). The second batch ran with not only wavelengths 451-949 but also 1001-1339 (NIR and SWIR). For all three targets, our classification accuracies were highest in batch one analysis. Single leaf had a classification accuracy of 94.7%, plant canopy, 100%, and plant canopy with soil 100%. Sixteen wavebands have been identified on the spectral curves for all three targets specific to the reniform nematode population levels. Thus far from our results indicates that hyperspectral data can be used to provide pertinent information relative to nematode presence on cotton.

In the second part of this project our focus is directed at the management of the reniform nematode using variable rate application technology. We first determine that a smaller sample grid provides the most precise pest distribution patterns. This ensures that actual variable rate applications occur across a range of rates. As the grid size increased to sampling size currently used in other precision agriculture applications, such as soil fertility, applications tend to resemble more conventional on-off applications, which are not variable rate. The next part was the assembly of the equipment and actual application of the nematicide. By modifying precision agriculture equipment that the producer currently is using for other applications, we were able to apply both fall and spring applications of liquid nematicides. VRT applications have resulted in higher yields compared to conventional one-rate applications and also reduced the total amounts of nematicide that were applied in the field.

#### Soybean:

A total of 89-113 commercial soybean cultivars per year were evaluated in the greenhouse and fields in Minnesota for their resistance to the soybean cyst nematode (SCN). A field plot experiment was established at Waseca in 2003 to determine the effect of different sequences of SCN-resistance sources (PI 887888, Peking, and PI437654) on dynamics of SCN population densities and their virulence phenotypes. A survey was conducted in 2007-2008 to determine distribution of the SCN and frequencies of different virulence phenotypes (HG Types) in Minnesota. The virulence of the SCN populations to the resistance-source soybean lines or commercial cultivars has increased dramatically since the last survey in 2002. Experiments were conducted in fields and the greenhouse to determine effect of cover and rotation crops on SCN population density. The study demonstrated that legumes were more effective in lowering egg population densities than monocots, Brassica species, and other dicots. The potential of liquid cultures of the nematophagous *Hirsutella rhossiliensis* and *H. minnesotensis* (Hm) in control of SCN was evaluated in the greenhouse. The fungi was highly effective in control of SCN. They appeared to be involved in suppression of SCN population in a field with a long-term monoculture of soybean. Liquid swine manure (LSM) was evaluated in the greenhouse and field for its effectiveness in control of SCN. Greenhouse experiments demonstrated that liquid swine manure (LSM) was effective in lowering SCN population density. In field, no significant effect of LSM on the SCN was observed. However application of manure can alleviate the SCN damage, and increase soybean yield.

Research to evaluate blends of SCN resistant and susceptible soybean cultivars was initiated in 2004 and completed in 2009. Delsoy 5710 was highly resistant to the race 5 population present at the Caswell research station, and Fowler and Anand were also resistant to this population. Resistant varieties did not yield more than susceptible varieties in 2008, but the highest yield was for a blend of resistant Fowler and susceptible Holladay. Lowest numbers of SCN were on resistant varieties. Fowler and Anand resistance to SCN had started to break down by the end of the experiment.

Experiments to evaluate the potential of cover crops for managing nematodes in cotton and soybean were initiated in 2008 and in 2009.

Tomato: The *Mi* gene in tomato cv. Crista has been evaluated over the past 4 years, both as a spring and autumn crop. There appears to be a high level of resistance to a mixture of root-knot nematode species that included *Meloidogyne javanica*, *M. arenaria* and *M. incognita*. and the resistance appears to be stable over the range of temperatures that plants were exposed to during each of the trials. The root-knot nematode susceptible tomato cv. Talladega was included for comparison and marketable yields were evaluated following treatment with methyl bromide, Telone C35, or nontreated. When both tomato cultivars were treated with soil fumigants there were no differences in marketable yield whereas when no fumigants were applied Crista generally produced low yields compared with Crista grown in treated plots.

Pepper: In cooperation with Dr. Judy Thies, member of regional project, the N-gene in pepper cultivars was evaluated in spring and autumn crops. N-gene appeared to be heat stable under the growing conditions the plants were exposed to in these trials. The resistance was maintained against *Meloidogyne incognita*.

## **Impact Statements:**

### Peanut

Both Tomato Spotted Wilt Virus (TSWV) and the root-knot nematode *Meloidogyne arenaria* are important pathogens of peanut in the southeastern USA. USDA and university scientists in Georgia released the first peanut cultivar, named 'Tifguard', with a high level of resistance to both *M. arenaria* and TSWV. Planting 'Tifguard' will enable growers to eliminate the use of nematicides, thus saving up to \$100 per acre.

When used with high throughput DNA extraction, the new PCR-based molecular marker 197/909 will allow for rapid, cost-effective screening for nematode resistance in peanut breeding programs which should speed the development of new cultivars with value added traits such as high oleic acid content.

### Cotton:

Root-knot nematodes cause greater damage to the US cotton crop than any other pathogen, but highly-resistant cultivars are not available for most of the US cotton production area because breeding for resistance has historically been too slow and labor intensive. Development and utilization of marker-assisted selection to breed for root-knot nematode resistance in cotton would allow companies for the first time to rapidly develop resistant cultivars. USDA and university scientists in Georgia have produced highly-resistant, high quality germplasm and DNA markers for root-knot nematode resistance that provide a foundation for high-throughput marker assisted selection programs.

### Pearl Millet

Because of its resistance to important plant-parasitic nematodes in the southern U.S., 'TifGrain 102' will be useful in rotations with peanut and cotton for nematode suppression.

### Soybean:

In the southern USA soybean and cotton are major crops and both are damaged extensively by the reniform nematode annually causing millions of dollars in yield loss. At present there are no commercial cotton varieties with reniform nematode resistance leaving either the use of nematicides or crop rotation as viable control measure. Soybean is one of the more attractive crops for rotation to reduce reniform nematode numbers to manageable levels.

From our varietal studies we have found that there is still very little resistance to the southern root-knot nematode, reniform and soybean cyst nematodes. It seems that the seed companies have devoted little work at incorporating resistance into soybean for our agricultural producers. We found that only 17.2% of the soybean varieties we examined were resistant to one or more of our test nematode populations based on (Rf) reproduction factors. This also demonstrates the need and continuation of regional research projects as the S-1046 to examine all aspect of crop resistance to help develop an end product that helps our agricultural producers.

A related project that is an off-shoot to objective 3, which is the estimation of population numbers of plant-parasitic nematodes on cotton using hyperspectral imagery data. This has resulted in a study that has developed a novel approach in the spectral classification of reniform nematode infected plants. Results have provided a high accuracy in correlating specific wavebands with in-soil nematode counts based of leaf reflectance. This technique will provide our agricultural producers with a cost efficient and reliable nematode population estimation tool that will replace the time consuming and high cost of ground sampling for nematode population levels.

A true variable rate applicator (multi-modal) was developed low volume pesticide applications. This will provide a means to accurately place the required volumes necessary to manage plant-parasitic nematodes only in problem locations in the field. This is a more environmentally friendly system of pesticide applications.

The studies in this project generated information essential for the management of the soybean cyst nematode and other plant-parasitic nematodes in Minnesota and the Midwest. The results of this project have been used for numerous extension/outreach activities such as presentations at field tours, winter crop days, SCN workshops, and growers meetings. Long-term impact of the project will be the contributions to a healthy economy and sound environment by improving SCN management, and enhancing soybean productivity.

In 2010 the first maturity group VII cultivar with resistance to races 1,2,3,5,9, and 14 of soybean cyst nematode and reniform nematode will be released. There is a desperate need for this resistance in Southern cropping systems. Blends of resistant and susceptible soybean can suppress development of resistance breaking nematode biotypes and improve soybean yield in the presence of soybean cyst nematode.

1. The root-knot nematode resistant tomato and pepper had low galling, thus appearing to be heat stable under conditions of the trials.
2. Both methyl bromide and C35 were equally effective in the suppression of root-knot nematode throughout the crop season on the root-knot nematode susceptible tomato cv. Talladega.
3. When yields were averaged across all plots treated with either methyl bromide or C35 there was no difference between yields of the root-knot nematode resistant tomato vs. the root-knot nematode susceptible tomato cultivar. When fumigated both cultivars produced significant yield increases over the nontreated plots.
4. Covering beds with VIF is more likely to result in phytotoxicity of C35 when environmental conditions are not conducive for degradation of the compound. Greater care must be taken when transplanting into bed treated with C35 when covered with VIF.
5. The performance of Christa was equal to that of Talladega in terms of marketable yield but far superior in terms of preventing root-knot nematode galling, but when untreated the yield was poor.

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