# INCREASING EFFECTIVENESS OF SOYBEAN HOST RESISTANCE USING AN IMPROVED NEMATODE IDENTIFICATION SYSTEM

Bruce Fortnum, Paula Agudelo, Sue Robinson, Mark Pullen, Trent Hale, Stephen Lewis, and Jim Frederick
Clemson University
Pee Dee Research and Education Center, 2200 Pocket Road, Florence, South Carolina 29506-9706, USA. bfrtnm@clemson.edu.

## ABSTRACT

Three root-knot nematode species predominate in the southeastern USA, namely *M. incognita*, *M. arenaria* and *M. javanica*. Traditional nematode soil assays (soil elutriation and sugar floatation) do not distinguish between root-knot nematode species, are a poor indicator of root-knot nematode soil populations and, consequently, are not very accurate in determining where root-knot nematodes may cause yield loss. Recent efforts at Clemson University using polymerase chain reaction (PCR) techniques have been successful in identifying root-knot nematode species using single second stage larvae (J2's) or eggs. The next step is to incorporate these species-specific primers into a real time PCR system. Integrating the DNA-based technique into an integrated pest management system would provide a great advancement in rapid pest detection and when integrated with precision farming technologies such as global positioning, would result in the more efficient use of host resistances. Field experiments were established to evaluate real time PCR nematode identification and compare the results to standard nematode identification methods. Three fields ranging in size from 3 – 4 hectares each were divided into 15 m grids. A soil sample was collected from each grid. Nematodes were extracted from soil by elutriation and sugar flotation. A sub sample from within each sampling grid was bioassayed for root knot species by planting a tomato cv. Rutgers into the soil, maintaining the plant in a greenhouse for 60 days and evaluating the roots for root galling, and egg mass production. Representative root samples (10%) were digested in pectinase, and adult females removed and identified with esterase phenotyping. A GIS database has been constructed for each field detailing each sampling point with soil type, J2 population, root gall index, egg mass index, and crop yield preceding the study. Soil populations of J2's were poorly correlated with egg masses produced on the tomato bioassay ($R^2$ = 0.18, $P$ = 0.001). This confirms the inaccuracy of soil J2's as a predictor of future nematode damage. *M. arenaria* and total *Meloidogyn*e spp. specific primers were developed for real time PCR. Adult female nematodes (collected from all sites) were identified to species using real time PCR and compared to identifications based on esterase phenotyping. Data suggests a real time PCR system can be used to identify *M. arenaria* populations when adult females or soil J2s are used. This rapid and accurate method should allow for site-specific planting of resistant cultivars.