Research Progress Report for 2009

PROJECT TITLE: Pathogenicity of ring nematodes: an emerging pest of blueberries (*Vaccinium* spp.)

GRANT CODE: SRSFC Research Project 2009-01

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SUMMARY

We initiated an experiment in August 2009 to test the pathogenicity of a ring nematode species, Criconemoides ornatus, on Rabbiteye blueberries (Vaccinium ashei

Reade) variety "Alapaha" under greenhouse conditions at Athens, GA and under field conditions at Byron, GA. Single one year old blueberry plants were transplanted to a plastic pot containing10 kg autoclaved sandy loam soil (pH ~ 5.0). Eight weeks after transplanting, plants were inoculated with five different inoculum levels (treatments) including 0 (water control), 10, 100, 1000 and 10,000 mixed stages of ring nematodes per plant. The nematode population in each pot was assessed 75 days after inoculation by randomly removing four 10 cm deep x 2.5 cm diam. soil cores from the root area around each plant. Individual soil cores were combined into a composite sample and nematodes were collected from 100-g soil sub-samples. Nematode population densities were expressed as numbers of nematodes/pot and their reproduction rates (Pf/Pi) were then calculated by dividing the total number of nematodes per pot (Pf = final population) by the number of nematodes added (Pi = initial population). Observations of plant vigor indicated that the ring nematode, C. ornatus was pathogenic to blueberry plants. The nematode population was increased in all the treatments but the rate of reproduction was greatest in the treatments receiving the lowest initial population level at both locations. Thus, these preliminary findings demonstrate for the first time that ring nematode, C. ornatus; can be considered as a potential pest of blueberries in Georgia.

OBJECTIVES:

1. To study the pathogenicity of two ring nematode species, *Criconemoides ornatus* and *Mesocriconema xenoplax,* on blueberries under greenhouse and field conditions.

2. To screen high yielding cultivars of rabbiteye, southern highbush and northern highbush blueberries for their reaction to pathogenic ring nematode species selected from the first objective.

JUSTIFICATION:

Blueberries, *Vaccinium* spp., are grown in more than 30 states on over 16,400 hectares in the United States. The blueberry industry in Georgia continues to grow rapidly, with substantial acreage increases on a yearly basis. However, though good sites remain for rabbiteye (*Vaccinium ashei* Reade) production, the cost associated with purchasing new land and site preparation is substantial. Due to the age of the industry in Georgia and other locations in the Southeast, many plantings are now reaching the >20

year timeframe, and producers are often deciding to replant these older sites to newer and more productive varieties, as opposed to purchasing new land. As a result, replant disorder, a general poor growth and decline associated with several pathogens to include plant-parasitic nematodes, is a likely result. Several plant-parasitic nematode species, including stubby root (*Paratricodorus* sp), spiral (*Helicotylenchus* sp), dagger (*Xiphinema* sp) and ring (*Mesocriconema* sp) nematodes, have been reported to be associated with 3 types of commercially grown blueberries in the USA (Clarke and Robbins, 1987; Converse and Ramsdall, 1982; Goheen and Braun, 1955). However, there is no data available on the effects of plant-parasitic nematodes on the growth and yield of blueberry plants. Of these four plant-parasitic nematode genera, the ring nematodes are always found in very high numbers in the blueberry root zone, but their importance as a pest is not known. Recently, Brannen (2008; unpublished data) recovered a very high population of ring nematodes (ca. 1000 nematodes per 100 cc soil) from the root zones of commercially grown blueberries at several locations in Georgia and suggested that high populations of these nematodes were possibly responsible for the poor and stunted growth of blueberry plants. Since there is no research data available on the pathogenicity and economic importance of ring nematodes on blueberries, we proposed to determine the relationship between a ring nematode species, *Criconemoides ornatus* and blueberry plant growth under both controlled greenhouse and field conditions. Thus the specific objectives of this project were:

1. To study the pathogenicity of two ring nematode species, *Criconemoides ornatus* and *Mesocriconema xenoplax,* on blueberries under greenhouse and field conditions.

2. To screen high yielding cultivars of rabbiteye, southern highbush and northern highbush blueberries for their reaction to pathogenic ring nematode species selected from the first objective.

1. Pathogenicity of *Criconemoides ornatus* on blueberries

METHODS:

Although we had proposed to test pathogenicity of two different ring nematode species, such as *Criconemoides ornatus* and *Mesocriconema xenoplax,* on blueberries in original proposal, morphometric observations showed that *C. ornatus* was the species predominantly associated with blueberry plants in South Georgia. Therefore, we initiated an experiment in August 2009 to test the pathogenecity of *C. ornatus* on Rabbiteye blueberries (*Vaccinium ashei* Reade) variety "Alapaha" at two different locations. Experiments were established under greenhouse conditions at the Department of Plant Pathology, UGA, Athens, GA (Fig. 1)

Fig. 1. Layout of pathogenicity test in plastic pots at Athens, GA location.

and under field conditions at the USDA-ARS, Southeastern Fruit & Tree Nut Res. Lab, Byron, GA (Fig 2).

Fig. 2. Layout of pathogenicity test in bucket-microplots at Byron GA location. Arrow shows an actual image of plastic pot with blueberry plant.

One year old nematode-free rooted cuttings of rabbiteye blueberries (var. Alapaha) were obtained from "Heagan Farms," a commercial nursery located near Manor, GA. Single blueberry plants were transplanted to a plastic pot (surface area 346 cm^2) filled with 10 kg autoclaved sandy loam soil (pH ~ 5.0) on May 19, 2009 at the Byron location and on June 18, 2009 at the Athens location. Established blueberry plants were then inoculated with five different inoculum levels of mixed stages of *C. ornatus* 8 and 12 weeks after transplanting at the Athens and Byron locations, respectively. Inoculum levels (treatments) included: 0 (control), 10, 100, 1000 and 10,000 nematodes per plant. Naturally infested soil with ring nematodes was collected from a well established blueberry site located at Alapaha, GA, and mixed stages of ring nematodes were collected using a centrifugation and flotation technique (Jenkins, 1964); these were then used for inoculation. Nematodes were inoculated by pipetting a suspension (10- 20 ml) into four holes around the plant in each pot. Similarly, 20 ml of water was added to each pot in the control treatment (0 inoculum level). Nematodes were inoculated on August 7 and 14, 2009 in the Athens and Byron experiments, respectively. The inoculated pots were then arranged in a randomized block design with 8 replications and held at respective locations under field and greenhouse (~ 25^{o}C) conditions. The reproduction

rates of ring nematodes on blueberry plants were recorded 75 days after inoculation (DAI). This experiment will be continued for another 5 months and during this period, additional observations on the ring nematode reproduction rates on blueberry plants will be recorded (150 and 225 DAI).

To assess nematode population levels in each pot, four 10 cm deep x 2.5 cm diam. soil cores were removed randomly from the area around each plant and a composite soil sample was prepared. Then nematodes were collected from a 100-g soil sub-sample taken from each composite sample using a centrifugation and flotation technique (Jenkins, 1964). Total numbers of ring nematodes in each sample were counted using an inverted compound microscope and their population densities were expressed as nematodes/pot (10kg of soil). The nematode reproduction rate (Pf/Pi) was calculated by dividing the total number of nematodes per pot (Pf = Final population) by the number of nematodes added (Pi = initial population). Data for reproduction rates were analyzed using General Linear Models Procedure for ANOVA (SAS Institute, 1998). Significant differences between treatments were determined using the LSD test at $P \leq 0.05$.

RESULTS AND CONCLUSIONS:

Preliminary observations revealed that the ring nematode, *C. ornatus* was pathogenic to blueberry plants. We found that the nematode population was increased in all the treatments, but the rate of reproduction was significantly greater in the treatments that received the lowest initial population level (10 nematodes/pot) 75 DAI (Table 1). Thus, these findings demonstrate for the first time that ring nematode, *C. ornatus* can be considered as a potential pest of blueberries in Georgia.

2. Reaction of rabbiteye, and southern and northern highbush blueberries to *C. ornatus.*

METHODS:

This study will be initiated in the spring of 2010 and pure cultures of *C. ornatus* that we have maintained on Bermuda grass in the greenhouse will be used for inoculation purposes. Nematode-free rooted cuttings of three high yielding cultivars each of rabbiteye (Brightwell, Climax and Powderblue), southern highbush (Bladen, O'Neal and Star) and northern highbush (Berkeley, Collins and Coville) blueberries will be obtained from commercial sources early in the spring of 2010. Since the rabbiteye blueberry variety "Alapaha" showed a susceptibility to *C. ornatus* in our pathogenicity test (see above), we will also include this variety for a comparison. The protocols described in pathogenicity tests for blueberry transplanting, nematode extraction and inoculation, and measuring plant growth parameters will be followed in this screening test. In addition, a nematode level that proved to be very detrimental to blueberry plants in the pathogenicity test will be selected to test its effect on growth parameters of different blueberry cultivars. Observations on the nematode population and plant growth parameters of all the cultivars will be recorded 75, 150 and 225 days after transplanting as described above.

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Table 1. Pathogenicity of *Criconemoides ornatus* on Rabbiteye blueberry var. Alapaha 75 DAI.

Inoculation level/pot (Initial population)	Reproduction rate (Pf/Pi)*	
	Athens	Byron
0	0.00[b]	0.00[b]
10	5.00[a]	2.50[a]
100	1.13[b]	0.75[ab]
1000	1.31[b]	0.09[ab]
10,000	0.79[b]	1.09[ab]

Pf/Pi = Final nematode population per pot (Pf = Final population) divided by initial population of nematodes added (Pi = initial population).
* Figures in the same column with same letter(s) are not significantly ($P < 0.0001$) different according to the LSD test.