Journal of Nematology 45(2):92–98. 2013.

Incidence and Pathogenicity of Plant-Parasitic Nematodes Associated with Blueberry (*Vaccinium* spp.) Replant Disease in Georgia and North Carolina

Ganpati B. Jagdale,[1] Ted Holladay,[1] P. M. Brannen,[1] W. O. Cline,[2] P. Agudelo,[3] A. P. Nyczepir,[4] J. P. Noe[1]

Abstract: Blueberry replant disease (BRD) is an emerging threat to continued blueberry (*Vaccinium* spp.) production in Georgia and North Carolina. Since high populations of ring nematode *Mesocriconema ornatum* were found to be associated with commercially grown blueberries in Georgia, we hypothesized that *M. ornatum* may be responsible for predisposing blueberry to BRD. We therefore tested the pathogenicity of *M. ornatum* on 10-wk-old Rabbiteye blueberries (*Vaccinium virgatum*) by inoculating with initial populations (Pi) of 0 (water control), 10, 100, 1,000. and 10,000 mixed stages of *M. ornatum*/pot under both greenhouse (25 ± 2°C) and field microplot conditions. Nematode soil population densities and reproduction rates were assessed 75, 150, 225, and 255, and 75, 150, 225, and 375 d after inoculation (DAI) in both the greenhouse and field experiments, respectively. Plant growth parameters were recorded in the greenhouse and field microplot experiments at 255 and 375 DAI, respectively. The highest *M. ornatum* population density occurred with the highest Pi level, at 75 and 150 DAI under both greenhouse ($P < 0.01$) and field ($P < 0.01$) conditions. However, *M. ornatum* rate of reproduction increased significantly in pots receiving the lowest Pi level of 10 nematodes/plant compared with the pots receiving Pi levels of 100, 1,000, and 10,000 nematodes 75 DAI. Plant-parasitic nematode populations were determined in commercial blueberry replant sites in Georgia and North Carolina during the 2010 growing season. *Mesocriconema ornatum* and *Dolichodorus* spp. were the predominant plant-parasitic nematodes in Georgia and North Carolina, respectively, with *M. ornatum* occurring in nearly half the blueberry fields sampled in Georgia. Other nematode genera detected in both states included *Tylenchorhynchus* spp., *Hoplolaimus* spp., *Hemicycliophora* spp., and *Xiphinema* spp. *Paratrichodorus* spp. was also found only in Georgia. In Georgia, our results indicate that blueberry is a host for *M. ornatum* and its relationship to BRD warrants further investigation.

Key words: blueberry, host-parasitic relationship, *Mesocriconema ornatum*, replant disease, ring nematode, *Vaccinium* spp.

Blueberry (*Vaccinium* spp.), is grown in more than 30 states representing more than 29,137 ha in the United States (Anonymous, 2012). The blueberry industry in Georgia and North Carolina continues to grow rapidly, with substantial acreage increases on a yearly basis. However, although good sites remain for Rabbiteye blueberries (*Vaccinium virgatum* Aiton, formerly *V. ashei* Reade) and southern highbush (*V. corymbosum* L.) production, the cost of land and site preparation is substantial, especially for southern highbush cultivars that may require added organic matter. Because of the age of the industry in Georgia and North Carolina, many plantings have been reaching the > 20-yr timeframe, and as these plantings decline in productivity, growers often replant these older sites rather than purchase new land. Also, as newer varieties with desirably horticultural traits enter the market, older varieties are often not competitive in yield or quality; therefore, older varieties are often replaced with newer varieties even prior to their natural decline. These replanted sites often exhibit poor plant growth, higher mortality, and premature decline, symptoms collectively known as Blueberry replant disease (BRD). Blueberry replant disease is considered an emerging threat to the blueberry industries in Georgia and North Carolina. In 2008, a preliminary survey of several commercial blueberry fields in Georgia revealed very high ring nematode populations (ca. 1,000 *Mesocriconema* sp. per 100-cm^3 soil) associated with the rhizosphere of blueberries exhibiting typical BRD symptoms (P. M. Brannen, Univ. Georgia, pers. com.). The ring nematode was identified as *Mesocriconema ornatum* using morphometrics. It was further demonstrated in controlled experiments that reduced *M. ornatum* populations were positively correlated with increased plant vigor in soil that was preplant fumigated with either 1, 3-dichloropropene or methyl bromide/chloropicrin as compared with the unfumigated control plots (unpub. data). Based on these preliminary results, we hypothesized that *M. ornatum* may be involved in predisposing blueberry to BRD in Georgia. However, there is currently no research data available to support this hypothesis.

Several plant-parasitic nematode genera have been associated with pathogenesis in blueberry fields throughout the United States. For example, *Paratrichodorus minor* significantly reduced root growth of small cuttings of northern highbush blueberry under greenhouse conditions (Zuckerman, 1964), whereas *P. renifer* was pathogenic to several varieties of blueberries in Oregon (Zasada et al., 2010). It is also known that *X. americanum* transmits TRSV and ToRSV to blueberry and that both viruses can cause severe damage to most blueberry cultivars (Converse and Ramsdall, 1982). Also, association of different plant-parasitic nematode species have been reported with different types of commercially grown blueberries in the United States, even though parasitism

Received for publication December 5, 2012.
[1]Department of Plant Pathology, University of Georgia, Athens, GA, 30602.
[2]Department of Plant Pathology, North Carolina State University, Horticultural Crops Research Station, Castle Hayne, NC 28429.
[3]School of Agricultural, Forest, and Environmental Sciences, 206 Long Hall, Clemson University, Clemson, SC 29634.
[4]USDA ARS, Southeastern Fruit and Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA 31008.
The authors thank Heagan Farms, Manor, GA, for supplying blueberry seedlings; Judy Cross, Charles Laster, and Merry A. Bacon for technical assistance; the Cooperative Extension Agents for their assistance in the survey; and the Georgia Blueberry Commodity Commission for funding.
E-mail: gbjagdal@uga.edu
The paper was edited by James A. LaMondia.

and pathogenicity have not been established (Goheen and Braun, 1955; Converse and Ramsdall, 1982; Clarke and Robbins, 1987). For example, in Arkansas, *X. americanum*, *P. minor*, *P. christiei*, *M. ornatum, Tylenchorynchus ewingi*, and *Helicotylenchus* spp. were associated with southern highbush blueberry (Clark et al., 1987; Clark and Robbins, 1994); and in the Pacific Northwest and Canada, *X. americanum*, *Pratylenchus* spp., and *Paratrichodorus* spp. were predominately associated with blueberries (Converse and Ramsdell, 1982; Forge et al., 2009; Zasada et al., 2010). A blueberry root knot nematode, *Meloidogyne carolinensis*, has also been described in North Carolina (Eisenbach, 1982). No information is currently available on the association of these different plant-parasitic nematodes with blueberries or their relationship with BRD in Georgia and North Carolina. Therefore, the objectives of this study were to conduct a more extensive survey to compare the incidence of plant-parasitic nematodes associated with BRD fields in Georgia and North Carolina and determine the relationship between *M. ornatum* and blueberry plant growth under greenhouse and field microplot conditions.

Materials and Methods

Source of blueberry plants: One-year-old nematode-free rooted cuttings of Rabbiteye blueberries (cv. Alapaha) were obtained from Heagan Farms near Manor, Georgia, and used in the greenhouse and field microplot pathogenicity tests.

Ring nematode source and inoculum: Naturally infested soil with ring nematodes was collected from a well-established blueberry site located in Alapaha, Georgia. The nematodes were extracted from soil using sieving and centrifugal sucrose flotation (Jenkins, 1964). This resulting nematode suspension contained a very high population of ring nematodes but only a few spiral and stubby-root nematodes. In order to obtain pure ring nematode inoculum, the nematode suspension was transferred into a modified Baermann funnel (Baermann, 1917) to separate active spiral nematodes from both the sluggish ring and stubby-root nematodes. In this technique, the bottom of a 20-ml plastic petri dish was used to support a plastic mesh on which were placed four layers of Kimwipe tissue paper and to collect active nematodes that passed through the tissue paper. Briefly, the nematode suspension was poured on the tissue paper and then the active nematodes were allowed to pass through the tissue for an hour. We found that almost all the active spiral nematodes were passed through the tissue but most of the ring and stubby-root nematodes remained on the tissue. The nematodes from the tissue paper were then resuspended in fresh water and this procedure was repeated until all the spiral nematodes were removed from the suspension. From this suspension, all the stubby-root nematodes were then hand-picked using a pick under a stereoscopic microscope. This pure ring nematode suspension was used for inoculation in the pathogenicity test. Based on morphometric and morphologic characteristics, the ring nematode was identified as *Mesocriconema ornatum*. For identification, 10 females were hand-picked for observations made in temporary water mounts with an Olympus BH2 microscope equipped with a Jenoptik camera and the software iSolution Lite (Image and Microscope Technology iSolution, Inc.). Females had elevated labial discs, bluntly rounded head, and retrorse annules with smooth margins, no anastomoses, and straight vagina. Body length averaged 415 μm (393 to 428 μm); stylet averaged 51 μm (47 to 54 μm); body annules averaged 94 (91 to 97); vulva located an average of 7 (7 to 8) annules from the terminus with distinct anterior vulval flap; and anus located an average of 6 (5 to 7) annules from terminus. Males were absent.

Occurrence of plant-parasitic nematodes associated with blueberries in Georgia and North Carolina: A systematic survey of plant-parasitic nematodes infesting commercial blueberry fields was conducted both in Georgia and North Carolina. Samples were collected in Georgia from 13 April 2010 to 14 May 2010 and from 26 October 2010 to 11 November 2010. Samples were collected from North Carolina from 24 August 2010 to 25 August 2010. In southeastern Georgia, 33 blueberry farms in 12 counties (Appling, Atkinson, Bacon, Berrien, Brantley, Clinch, Coffee, Jeff Davis, Lanier, Pierce, Ware, and Wayne) and in North Carolina, 10 blueberry farms in four counties (Bladen, Duplin, Pender, and New Hanover) were selected for the survey. At each farm, multiple samples were taken to represent different fields, cultivars, and production practices, resulting in a total of 289 and 43 survey samples from Georgia and North Carolina, respectively. Each soil sample consisted of 10 cores (2.5-cm-diam. × 30-cm deep) taken from the root zones of five consecutive plants using a soil probe. On the first survey in Georgia, a mapping system was made for each farm location, marking the exact spot for each sample. Twelve-inch garden markers were placed at the beginning and end of each sampling area. Sampling areas were generally determined by counting rows from one corner of the field and counting plants into the interior of the field. This method was used for mapping ease and for locating sampling areas during follow-up soil sampling. Usually, two to four areas would be sampled per field site depending on the field size. The mapping system was used for the purpose of follow-up sampling in Georgia. Each survey sample was placed in individual labeled plastic bags, transported back to the laboratory in coolers, and stored at 4°C until processed. Plant-parasitic nematodes were extracted from 100-cm^3 subsamples by use of sieving and the centrifugal sucrose flotation technique (Jenkins, 1964) and identified and counted using a stereomicroscope.

Pathogenicity of Mesocriconema ornatum: In August 2009, greenhouse and field microplot experiments

were initiated to evaluate the effect of *M. ornatum* on susceptibility and growth of Rabbiteye blueberry (*Vaccinium virgatum*) 'Alapha'. The greenhouse and field microplot studies were located at the University of Georgia Athens, and the USDA-ARS Southeastern Fruit and Tree Nut Research Laboratory, Byron, Georgia, respectively.

Greenhouse experiment: Blueberry seedlings were planted singly into plastic pots (25-cm-diam. × 31-cm-deep) containing ~10 kg of autoclaved sandy loam soil (83.6% sand, 10.4% silt, 4.56% clay, pH ~ 5.0) on 18 June 2009, and maintained under greenhouse conditions at 25°C ± 2°C. Eight weeks after seedling establishment (i.e., in August 2009), plants in the pots were inoculated with *M. ornatum* in a geometric series of increasing concentration (0, 10, 100, 1000, and 10,000 adults and juveniles/pot). Nematodes were inoculated by pipetting a suspension (10 to 20 ml) into four 10-cm-deep holes around the plant in each pot. Similarly, the soil in the control pots (0 inoculum level) received 20 ml of nematode-free water.

Field microplot experiment: Approximately 1-yr-old blueberry seedlings were planted singly in bucket microplots (Barker, 1985) (25-cm-diam. × 31-cm-deep) containing ~10 kg of autoclaved sandy loam soil (83.6% sand, 10.4% silt, 4.56% clay, pH ~ 5.0) on 19 May 2009. Microplots were established in a shaded area (30% shade) in the field. Twelve weeks after seedling establishment (i.e., in August 2009), plants were inoculated with *M. ornatum* in a geometric series of increasing concentration (0, 10, 100, 1,000, and 10,000 adults and juveniles/pot) as described above. Similarly, the soil in the control microplots (0 inoculum level) received 20 ml of nematode-free water.

Both the greenhouse and field microplot experiments were arranged in a completely randomized block design with eight replications. Plants were watered and fertilized as needed. Ring nematode population density in the soil (Pf) and reproduction rate were assessed 75, 150, 225, and 255 d, and 75, 150, 225, and 375 d after inoculation (DAI) for the greenhouse and field microplot experiments, respectively. Both studies were terminated 255 and 375 DAI, respectively, and the following plant growth parameters were recorded: (i) plant vigor on a 1 to 5 scale where 1 = excellent, 2 = very good, 3 = good, 4 = fair, and 5 = poor; (ii) number of branches; (iii) shoot and root lengths (cm); and (iv) fresh and dry root and shoot weights (g).

To assess nematode soil population density in each pot or microplot, four soil cores (2.5-cm-diam. × 10-cm-deep) were collected randomly at each time interval from the area around each plant and a composite soil sample was prepared. Nematodes were extracted from a 100-cm^3 soil subsample using sieving and centrifugal sucrose flotation (Jenkins, 1964). 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).

Statistical analyses: Nematode and plant growth parameter data were subjected to analysis of variance with the general linear models procedure of SAS (SAS Institute, Cary, NC). Changes in mean nematode population density and reproduction rates over time were compared using mixed repeated measures analysis and means were separated using Tukey's test at $P < 0.05$ for both the greenhouse and field microplot experiments [SAS version 7 (TSP1), SAS Institute, Cary, NC, 1998]. The differences between growth parameters [plant vigor, number of branches, shoot and root lengths (cm), and fresh and dry root and shoot weights (g)] of the nematode treated plants and the untreated (control) plants were determined using Tukey's test at $P < 0.05$ [SAS version 7 (TSP1), SAS Institute, Cary, NC, 1998].

For presentation of the survey data, the frequency of occurrence for each genus detected was calculated as the total number of samples in which the nematode genus was detected on either survey date divided by the total number of samples collected (283 samples), multiplied by 100 to convert to a percentage. For instance, a sample could have had a zero count for a given genus in the first survey, but if the genus was detected in the second survey, the site was recorded as infested. An index of abundance was calculated for each genus as the sum of nematode densities per 100-cm^3 soil divided by the total number of samples in which the nematode genus was detected on either survey date. Thus, if the genus was not detected on either sample date, the site was not used in calculation of the index of abundance. The maximum population density detected per 100-cm^3 soil for each genus was also reported. Similar statistics were calculated for the by-county data from Georgia for the data from North Carolina.

Results

Occurrence of plant-parasitic nematodes associated with blueberries in Georgia and North Carolina: The most frequently occurring plant-parasitic nematode genus detected from blueberry in southeastern Georgia was *Mesocriconema*; occurring in 55% of the samples (Table 1). Other plant-parasitic genera that have previously been reported on blueberry, such as *Dolichodorus*, *Paratrichodorus*, and *Xiphinema*, all occurred with frequencies less than 10%. *Mesocriconema* spp. was the most abundant nematode genus, with mean soil population densities of 251 and 348 *Mesocriconema* sp./100-cm^3 soil in the spring (April to May 2010) and fall (October to November 2010) samples, respectively. Comparing the data from the two sampling dates demonstrated that the abundance of *Mesocriconema* on blueberry increased an average of 39% during the 2010 growing season. Abundance of *Xiphinema* also increased during the growing season, but this genus occurred in only 2% of the samples. *Hoplolaimus* and *Tylenchorhynchus* occurred in approximately one out of every 10 samples, but the abundance

TABLE 1. Survey of frequency and abundance of plant-parasitic nematodes on blueberry in southeast Georgia on two dates.

		April–May 2010			October–November 2010		
Nematode genera	Percent frequency[a]	Abundance[b]	Standard deviation	Maximum density[c]	Abundance	Standard deviation	Maximum density
Mesocriconema	55	251	590	5,248	348	604	3,776
Hoplolaimus	11	77	183	856	75	112	568
Tylenchorhynchus	10	15	17	68	10	16	52
Hemicycliophora	7	40	42	136	29	51	196
Paratrichodorus	7	13	15	60	2	4	16
Helicotylenchus	5	24	17	52	18	60	225
Belonolaimus	3	4	7	20	7	8	24
Dolichodorus	2	19	21	60	15	25	64
Xiphinema	2	14	10	26	160	265	676
Tylenchus	1	115	116	244	32	55	96

[a] Total number of samples in which the nematode genus was detected on either survey date divided by the total number of samples collected = 283 samples, multiplied by 100 to convert to a percentage.
[b] Sum of nematode densities per 100-cm^3 soil divided by the total number of samples in which the nematode genus was detected on either survey date.
[c] Maximum nematode density detected per 100-cm^3 soil.

of these genera did not increase over the two sampling dates. *Mesocriconema* was detected in all 12 counties that were included in the Georgia survey, with a relatively high frequency of $\geq$ 50% occurrence in three-fourths of the counties sampled (Table 2). Abundance of *Mesocriconema* among counties ranged from three to 782 *Mesocriconema* sp./100-cm^3 soil in the first survey (April to May 2010) and from six to 1,212 *Mesocriconema* sp./100-cm^3 soil in the second survey (October to November 2010), with abundance of *Mesocriconema* increasing in 9 of the 12 counties during the growing season. The maximum soil population densities of *Mesocriconema* observed within individual counties ranged from 6 to 5,248/100-cm^3 soil in the first survey and from 8 to 3,776/100 cm^3 soil in the second survey, with maximum densities of *Mesocriconema* on blueberry increasing in all 12 counties throughout the 2010 growing season. In North Carolina, *Mesocriconema* sp. occurred less frequently than in Georgia, and the genus was generally less abundant. The most frequently-occurring plant-parasitic nematode genus in North Carolina was *Dolichodorus sp.*, found in 42% of the samples with a mean population density of 19 juveniles and adults/100-cm^3 soil (Table 3).

Pathogenicity of Mesocriconema ornatum on blueberry (Greenhouse): Number of *M. ornatum* remained higher ($P < 0.05$) at Pi 10,000 than with Pi treatments 10, 100, and 1,000 nematodes per/pot at 75 and 150 DAI (Fig. 1A). A similar trend was also detected at 255 DAI, even though differences among treatments were not significant (Fig. 1A). Rate of nematode reproduction increase was greatest ($P < 0.05$) at the lowest Pi level (10 *M. ornatum* per pot) than in treatments receiving Pi 100, 1,000, and 10,000 nematodes per pot at 75 DAI (Fig. 1B). Although differences in blueberry plant growth parameters as related to the presence of *M. ornatum* were not statistically significant, the average dry weights of both shoots and roots were numerically higher from untreated plants (153 and 83 g, respectively) than from those plants treated with 10,000 nematodes (145 and 76 g, respectively).

TABLE 2. Survey of frequency and abundance of *Mesocriconema* sp. on blueberry in southeast Georgia on two dates, by county.

			April–May 2010			November 2010		
County	N	Percent frequency[a]	Abundance[b]	Standard deviation	Maximum density[c]	Abundance	Standard deviation	Maximum density
Appling	32	50	216	311	1,224	229	329	1,264
Atkinson	25	36	102	242	744	64	74	204
Bacon	23	70	782	1,444	5,248	517	635	1,792
Berrien	29	59	281	292	872	321	293	864
Brantley	43	53	81	75	268	176	188	608
Clinch	28	68	446	740	2,824	701	1,023	3,776
Coffee	27	59	35	79	324	89	174	552
Jeff Davis	16	38	96	174	440	64	113	280
Lanier	8	63	14	19	44	140	256	596
Pierce	28	71	175	236	856	353	662	2,600
Ware	8	25	3	4	6	6	3	8
Wayne	8	100	342	329	952	1,212	978	2,960

[a] Total number of samples in which *Mesocriconema* sp. was detected on either survey date divided by the total number of samples collected in that county (N), multiplied by 100 to convert to a percentage.
[b] Sum of nematode densities per 100-cm^3 soil divided by the total number of samples in which the nematode genus was detected in the county on either survey date.
[c] Maximum nematode density detected per 100-cm^3 soil.

TABLE 3. Survey of frequency and abundance of plant-parasitic nematodes on blueberry in southeastern North Carolina in August 2010.

Nematode genera	Percent frequency[a]	Abundance[b]	Standard deviation	Maximum density[c]
Dolichodorus	42	19	18	66
Hemicycliophora	16	17	12	104
Mesocriconema	12	18	11	30
Tylenchorhynchus	9	17	12	30
Hoplolaimus	7	29	37	616
Xiphinema	7	25	29	58

[a] Total number of samples in which the nematode genus was detected on either survey date divided by the total number of samples collected in that county (N), multiplied by 100 to convert to a percentage.
[b] Sum of nematode densities per 100-cm^3 soil divided by the total number of samples in which the nematode genus was detected in the county on either survey date.
[c] Maximum nematode density detected per 100-cm^3 soil.

Pathogenicity of Mesocriconema ornatum on blueberry (Field microplots): Population density of *M. ornatum* was significantly greater at Pi 10,000 nematodes as compared with Pi 10, 100, and 1,000 at 75, 150, and 225 DAI (Fig. 2A). The rate of *M. ornatum* reproduction was significantly greater at the lowest Pi level (10 nematodes/pot) 375 DAI. A similar trend was observed at 75, 150, and 225 DAI, but differences among Pi treatments were not significant ($P > 0.05$) (Fig. 2B). Blueberry growth as measured by plant vigor, number of branches, shoot and root lengths, and fresh and dry root and shoot weights was not significantly affected by the presence of *M. ornatum* as compared with the uninoculated plots. However, the average dry weights of both shoots and roots were numerically higher from untreated plants (101 and 33 g, respectively) than from those plants treated with 10,000 nematodes (59 and 18 g, respectively).

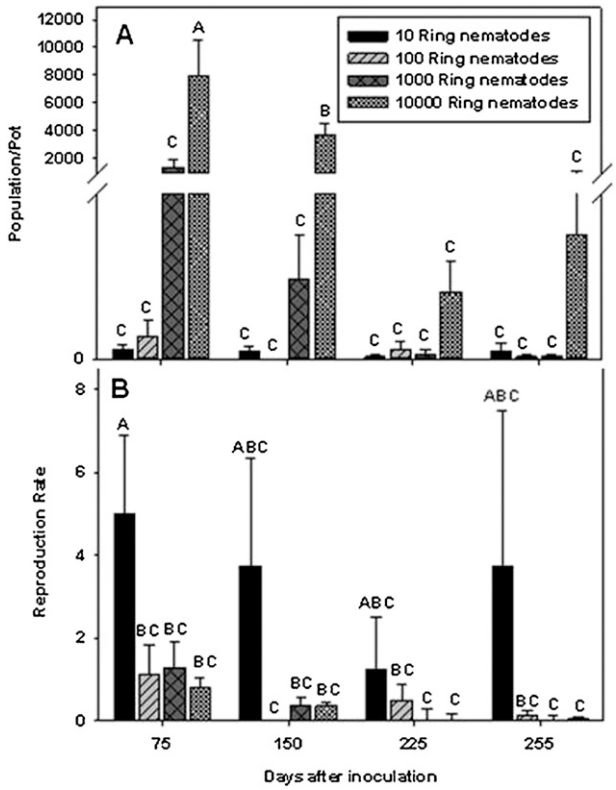

FIG. 1. Populations (A) and reproduction rate (B) of *Mesocriconema ornatum* on blueberry under greenhouse conditions at Athens, Georgia. Nematode reproduction rate (Pf/Pi), was calculated by dividing the total number of nematodes per pot (Pf = final population) by the number of nematodes added (Pi = initial population). Bars (mean ± SE) in the same and between the days after inoculation with same letter(s) are not significantly different according to mixed repeated measures analysis with means separated using Tukey's test at $P < 0.05$.

DISCUSSION

BRD is considered an emerging threat to the blueberry industries in Georgia and North Carolina and is characterized by poor plant growth and decline of the planting over time. One common biological factor present in many BRD sites in Georgia has been the ring nematode, *Mesocriconema* spp. In our 2010 Georgia blueberry survey, the high frequency (55%) and relative density of *Mesocriconema* spp. (abundance range 251 to 348 *M.* sp/100-cm^3 soil) in samples was greater than that of all other plant-parasitic genera detected. Based on morphometric and morphologic characteristics, the ring nematode most often encountered was identified as *Mesocriconema ornatum*. The occurrence of *M. ornatum* in the rhizosphere of blueberry has also been reported in Arkansas (Clark and Robbins, 1994).

In North Carolina, *Mesocriconema* sp. were also detected in 12% of the blueberry plantings sampled, but the more prominent genus was *Dolichodorus* sp. occurring in 42% of the samples collected. Since high populations of *M. ornatum* were previously found to be associated with commercially grown blueberry in Georgia, it was hypothesized that this ring nematode may be responsible for predisposing blueberry to BRD (J. P. Noe and P. M. Brannen, Univ. Georgia, pers. com.). Recently, Noe et al. (2012) reported that preplant fumigation with methyl bromide/chloropicrin, or 1, 3-dichloropropene reduced *M. ornatum* populations to nearly undetectable levels, which in turn enhanced blueberry plant vigor under commercial field conditions. These findings further suggest that *M. ornatum* may be involved with causing BRD in Georgia.

Through results obtained in our current studies, we could not confirm that *M. ornatum* is pathogenic to blueberry, because none of the plant growth parameters measured was significantly affected in the presence of this nematode. We did not observe any direct detrimental effects of nematode infection on both the shoot and root lengths, but total biomass of blueberry plants was numerically greater in untreated plants than in those treated with 10,000 nematodes. According to Schreiner et al. (2012), the ring nematode, *M. xenoplax* reduced reserves of carbohydrates, K, P, and Ca in grape roots without adversely affecting its shoot growth and total biomass. In the current study, it was observed that

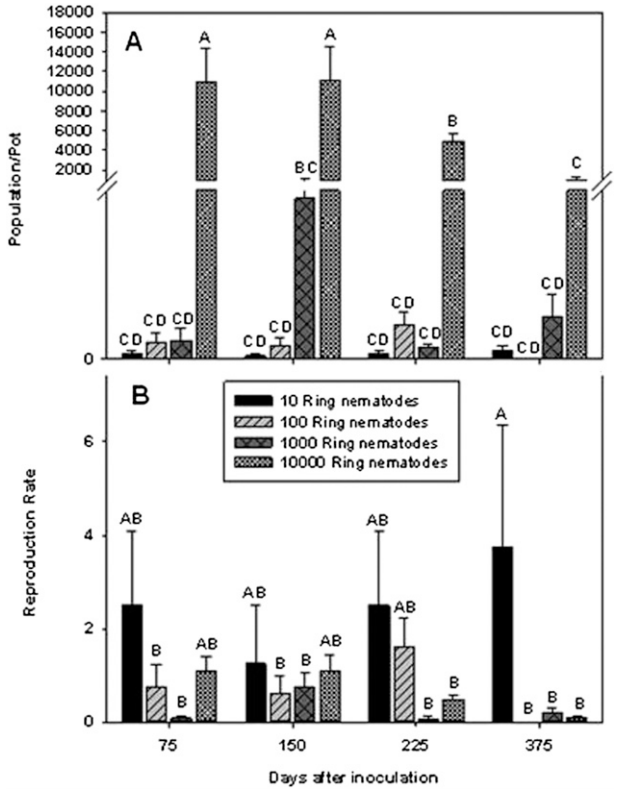

FIG. 2. Populations (A) and reproduction rate (B) of *Mesocriconema ornatum* on blueberry under field "microplot" conditions at Byron, Georgia. Nematode reproduction rate (Pf/Pi), was calculated by dividing the total number of nematodes per pot (Pf = final population) by the number of nematodes added (Pi = initial population). Bars (mean ± SE) in the same and between the days after inoculation with same letter(s) are not significantly different according to mixed repeated measures analysis with means separated using Tukey's test at $P < 0.05$.

M. ornatum readily reproduced on Rabbiteye blueberry plants grown under both greenhouse and field microplot conditions, but we were not able to collect blueberry yield data on young plants in relatively small containers. To our knowledge, this is the first experimental evidence demonstrating that blueberry is a host to *M. ornatum*. Also, *M. ornatum* populations were observed to be significantly higher on the plants that were inoculated with the lowest initial population level of Pi 10 nematodes. These results are consistent with the findings of Nyczepir et al. (1987) who reported that the reproduction rate of another ring nematode, *M. xenoplax* was inversely proportional to the Pi density levels; with significantly greater Pf density levels occurring on peach seedlings exposed to the lowest Pi level of seven nematodes. In Arkansas, *M. ornatum* was detected in seven of the 14 blueberry plantings sampled, but it was concluded that the nematode was reproducing on the grass sod middles and not the blueberry cultivars. In Florida (Ratanaworabhan and Smart, 1970) and Georgia (Nyczepir et al., 1988), *M. ornatum* reproduced on centipede grass and common bermudagrass, respectively, but not peach. Furthermore, Nyczepir et al. (1988) demonstrated that *M. ornatum* was also pathogenic to common bermudagrass, because the nematode reduced dry top weights as compared with grass grown in *M. xenoplax*-infested soil. In contrast, peach (woody perennial) was a better host to *M. xenoplax* than to *M. ornatum*.

Mesocriconema ornatum may be involved in predisposing blueberry to BRD in Georgia, but larger-scale long-term field experiments will be required to better determine the role of this nematode in the replant decline observed in infested fields. Two possible explanations for why *M. ornatum* did not significantly reduce growth parameters in the current study may be (i) the short duration time of the experiment (i.e., 375 d = 12.3 mon) and/or (ii) the absence of other microorganisms or cultural practices normally involved with BRD under field conditions. In peach, the effect of *M. xenoplax* on peach tree growth suppression was not observed until 18 mon after inoculation (Barker and Clayton, 1973). Also, the soil used in the current studies was autoclaved, which may have eliminated any other pathogenic microorganisms (fungi, bacteria, etc.) that may have been involved in causing BRD in the presence of *M. ornatum* in the field; feeding by *M. ornatum* may predispose the blueberry plant to stress and attack by other BRD-associated organisms, or vice versa.

To reiterate, we can now firmly conclude that *M. ornatum* does survive on blueberry roots in the absence of other food sources. Therefore, the ring nematode isolated from the rhizosphere of blueberries exhibiting BRD-like symptoms and identified as *M. ornatum* may be associated with a disease complex and further investigations into its specific role and association with BRD is warranted.

LITERATURE CITED

Anonymous, 2012. Noncitrus fruits and nuts: 2011 Summary. URL: http://usda01.library.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-07-06-2012.pdf.

Baermann, G. 1917. Eine einfache methode zur auffindung von anchylostomum- (Nematoden)- larven in erdproben. Geneesk. Tijdschrift Ned. Ind. 57:131–137.

Barker, K. R. 1985. Design of greenhouse and microplot experiments for evaluation of plant resistance to nematodes. Pp. 107–113 *in* B. M. Zuckerman, W. F. Mai, and M. B. Harrison, eds. Plant nematology laboratory manual University of Massachusetts Agricultural Experiment Station.

Barker, K. R., and Clayton, C. N. 1973. Nematodes attacking cultivars of peach in North Carolina. Journal of Nematology 5:265–271.

Clark, J. R., Riggs, R. D., and Moore, J. N. 1987. Plant parasitic nematodes associated with highbush blueberry in Arkansas. HortScience 22:408–409.

Clark, J. R., and Robbins, R. T. 1987. Phytoparasitic nematodes associated with three types of blueberries in Arkansas. Journal of Nematology 26:761–766.

Clark, J. R., and Robbins, R. T. 1994. Phytoparasitic nematodes associated with three types of blueberries in Arkansas. Journal of Nematology 26:761–766.

Converse, R. H., and Ramsdall, D. C. 1982. Occurrence of tomato and tobacco ringspot viruses and of dagger and other nematodes

associated with cultivated highbush blueberries in Oregon. Plant Disease 66:710–712.

Eisenback, J. D. 1982. Description of the blueberry root-knot nematode, *Meloidogyne carolinensis* n. sp. Journal of Nematology 14: 303–317.

Forge, T. A., Koch, C., Pinkerton, J. N., and Zasada, I. 2009. First report of *Paratrichodorus renifer*, a nematode parasite of highbush blueberry. Phytopathology 99:35.

Goheen, A. C., and Braun, A. J. 1955. Some parasitic nematodes associated with blueberry roots. Plant Disease Reporter 39:908.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Noe, J. P., Brannen, P. M., Holladay, W. T., and Jagdale, G. B. 2012. Preplant soil treatments to manage blueberry replant disease caused by *Mesocriconema ornatum*. Journal of Nematology 44:482 (Abstr.).

Nyczepir, A. P., Reilly, C. C., and Okie, W. R. 1987. Effect of initial population-density of *Criconemella-xenoplax* on reducing sugars, free amino-acids, and survival of peach seedlings over time. Journal of Nematology 19:296–303.

Nyczepir, A. P., Reilly, C. C., Motsinger, R. E., and Okie, W. R. 1988. Behavior, parasitism, morphology, and biochemistry of *Criconemella xenoplax* and *C. ornata* on peach. Journal of Nematology 20:40–46.

Ratanaworabhan, S., and Smart, G. C., Jr. 1970. The ring nematode, *Criconemella ornatus*, on peach and centipede Grass. Journal of Nematology 2:204–208.

SAS Institute, 1998. SAS software version 7 (TSP1). Cary, NC: SAS Institute.

Schreiner, R. P., Pinkerton, J. N., and Zasada, I. A. 2012. Delayed response to ring nematode (*Mesocriconema xenoplax*) feeding on grape roots linked to vine carbohydrate reserves and nematode feeding pressure. Soil Biology and Biochemistry 45:89–97.

Zasada, I. A., Pinkerton, J. N., and Forge, T. A. 2010. Plant-Parasitic Nematodes Associated with Highbush Blueberries (*Vaccinium corymbosum*) in the Pacific Northwest of North America. International Journal of Fruit Science 10:123–133.

Zuckerman, B. M. 1964. Studies of two nematode species associated with roots of the cultivated highbush blueberry. Plant Disease Reporter 48:170–171.