Tall Fescue 'Jesup (Max-Q)': *Meloidogyne incognita* Development in Roots and Nematotoxicity

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ABSTRACT

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumont. cv. Jesup (Max-Q)] was recently recommended as a preplant ground cover for managing plant–parasitic nematodes on peach trees [*Prunus persica* (L.) Batsch] in the southeastern United States. Jesup (Max-Q) is associated with a strain of the endosymbiotic fungus *Neotyphodium coenophialum* that does not produce ergot alkaloids that cause fescue toxicosis. To optimize use of this tall fescue for lowering populations of the root-knot nematode *Meloidogyne incognita*, greenhouse and laboratory studies were conducted on selected factors potentially contributing to suppression. Tall fescue–derived extracts and exudates were tested for nematotoxicity, and *M. incognita* life cycle development was compared between susceptible tomato (*Solanum esculentum* Mill.) and Jesup (Max-Q) roots. The highest tested root and shoot extract concentrations inhibited *M. incognita* hatch up to 46% compared with controls, and were nematotoxic to the infective second-stage juveniles (J2; up to 66% decrease in viability). Root exudates were nematotoxic to J2 (up to 27% mortality), and inhibited hatch up to 48%. Roots of susceptible tomato plants (controls) had approximately 3 to 7 times more infective J2 than tall fescue roots, 40 to 80 times more females and egg masses, >1800 times more eggs/plant, and 10 to 83 times more galls/plant. The nature of Jesup (Max-Q) suppression of *M. incognita* included low J2 penetration rate and failure of infective J2 to complete their life cycle in this cultivar. Compounds derived from Jesup (Max-Q) tall fescue decreased nematode viability, also contributing to *M. incognita* suppression by this plant.

$\mathbf{M}$ELOIDOGYNE SPP. (ROOT-KNOT nematodes; RKN) that attack young peach (*Prunus persica* (L.) Batsch) trees often cause belowground root galls, stunted growth of aboveground plant parts, early defoliation, reduced tree vigor, and occasionally tree death (Nyczepir et al., 1999; Nyczepir and Thomas, 2009). Nematode infections can consequently result in decreased yields and significant economic losses if appropriate management practices are not followed (Nyczepir and Thomas, 2009). Because of this problem, preplant fumigation in conjunction with a resistant rootstock is recommended on RKN-infested sites in the southeastern United States. However, environmental concerns about nematicide use have led to a search for alternatives to synthetic chemical control for managing these nematodes on young peach trees. Research is therefore being conducted on novel strategies for nematode suppression before orchard establishment.

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumont. = *Lolium arundinaceum* (Schreb.) Darbysh., formerly *Festuca arundinacea* Schreb.] cultivar Jesup (Max-Q), was recently recommended as a preplant ground cover for suppression of the root-lesion nematode *Pratylenchus vulnus* and two species of RKN on peach in the southeastern United States (Nyczepir and Brannen, 2012). There were multiple reasons for proposing this management strategy. Tall fescue, a forage and turf grass, is grown in many areas in the United States and can be used as a postplant ground cover to reduce soil erosion, is vigorous under poor soil conditions, withstands drought, and is resistant to various pests, including some nematodes (Ball et al., 1993; Bouton, 2002). A symbiotic relationship between tall fescue and the fungus *Neotyphodium coenophialum* [(Morgan-Jones & W. Gams) Glenn, C. W. Bacon, & Hanlin] can confer enhanced benefits on the grass, including increased competitiveness, growth, survival, and ability to survive drought (Ball et al., 1993; Bouton et al., 2001). However, some strains of *N. coenophialum* produce ergot alkaloids that cause fescue toxicosis in grazing animals (Bacon et al., 1977; Lyons et al., 1986). Jesup (Max-Q) has the advantage of being an "endophyte-friendly" tall fescue; it is associated with a strain of *N. coenophialum* that is beneficial to the plant but does not produce the ergot alkaloids (Bouton, 2002; Phillips and Aiken, 2009). When tested with the primary nematode pathogens that attack peach trees in the Southeast, Jesup (Max-Q) was a nonhost or poor host for several species

Abbreviations: ANCOVA, analysis of covariance; BPI, Bureau of Plant Industry; CI, confidence interval; DAT, days after transplanting; GLM, general linear model; GLMM, generalized linear mixed models; J2, second-stage juveniles; RKN, root-knot nematodes; SDI, sterile deionized water.

(Nyczepir and Meyer, 2010; Nyczepir, 2011). In a preliminary field trial in Georgia, Jesup (Max-Q) was as effective as Telone II fumigation in suppressing *M. incognita* populations before establishing a new peach orchard (Nyczepir, unpubl., 2011).

It is not clearly known how the presence of *N. coenophialum* in tall fescue can increase plant resistance to nematodes (Bernard et al., 1998; Elmi et al., 2000; Timper et al., 2005). One source of resistance to nematodes might be production of metabolites that are in roots or exuded into the rhizosphere (Gwinn and Bernard, 1993; Bacetty et al., 2009a, 2009b; Timper, 2009). Root-derived compounds may be toxic, repellant, or non-attractant to nematodes before infection, or might retard nematode development post entry. Activity of nematode-suppressive root compounds has been demonstrated with some tall fescue/*N. coenophialum* associations, and nematode population densities can decrease in the soil around nonhost tall fescue (West et al., 1988; Bacetty et al., 2009a). Bacetty et al. (2009b) concluded that, at least in the presence of an alkaloid-producing fungal endophyte, the effects of root extracts on nematodes supported the hypothesis that root-derived compounds were a cause of tall fescue resistance to nematodes.

Failure of the nematode to complete its life cycle may indicate that more than chemical nematotoxicity is involved. For example, the endophytic association with tall fescue can change root morphology, leading to nematode resistance (Kimmons et al., 1990; Gwinn and Bernard, 1993). It is therefore important to know what stage(s) of the nematode life cycle are inhibited to then study events that might be occurring in the plant-nematode interaction at that time.

To better understand and optimize use of Jesup (Max-Q) tall fescue for managing nematodes, two approaches were taken to investigate possible factors involved in suppression of *M. incognita* populations. First, root exudates and root and shoot extracts from Jesup (Max-Q) were tested for activity against *M. incognita* eggs and J2, including egg hatch stimulation (which could suppress nematode populations if hatch were induced in the absence of a good host), inhibition of hatch and of J2 motility, and nematotoxicity. Second, because the developmental stage at which Jesup (Max-Q) disrupts the nematode life cycle is unknown, penetration of *M. incognita* J2 in Jesup (Max Q), early development, and reproduction were quantified.

MATERIALS AND METHODS
Meloidogyne incognita Source and Inoculum

For microwell assays, *Meloidogyne incognita* (Kofoid and White) Chitwood (originally isolated in MD) was prepared similar to procedures in Meyer et al. (2006). Nematodes were grown on pepper (*Capsicum annuum* L.) cultivar PA-136 in the greenhouse, harvested egg masses immersed in 0.6% sodium hypochlorite for 1 min, and eggs were collected, rinsed, and stored overnight at 4°C. To collect J2, eggs were placed in a hatching chamber for 1 to 3 d. For nematode penetration and development studies, a population of *M. incognita* (BY-peach) originally isolated from peach in Georgia was maintained on tomato (*Solanum esculentum* Mill. cultivar Rutgers) in the greenhouse. Eggs were collected from tomato roots infected with *M. incognita* as described by Hussey and Barker (1973) and used as inoculum.

Preparation of Jesup (Max-Q) Extracts and Exudates

Tall fescue Jesup (Max-Q) seeds were planted into 15.2 cm-diam. greenhouse pots, each containing approximately 1300 cm^3 enriched loamy sand soil (16:9 sand/compost that had been steamed and air-dried; composition 82.9% sand, 5.3% silt, 11.8% clay; pH 7.3; 0.8% organic matter). Six seeds were planted in each pot; after 19 d, the seedlings were thinned to 2 per pot. Each pot received several granules of Osmocote Plus 15–9–12 (N–P–K) fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH) once every 3 wk until harvest. The plants were maintained in the greenhouse at 24 to 29°C, with natural and supplemental lighting combined for a 15- to 16-h daylength. Extracts and exudates were collected from 3-mo-old plants. The presence of the fungus, *N. coenophialum*, was not determined in any of the Jesup (Max-Q) seedlings used in these experiments, because our previous findings have shown that host susceptibility of tall fescue to *M. incognita* did not appear to be related to fungal endophyte strain (Nyczepir and Meyer, 2010). To obtain shoot and root extracts, whole plants were harvested and shoots were separated from the roots. Plant parts were washed in water and the roots and shoots dried for 8 d at 60°C. The dried plant parts were ground with a mortar and pestle (coarsely ground), and then part of each sample was ground to a powder (powdered shoots or roots) using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO). Plant material was stored at 4°C until use.

Extracts were prepared in a manner similar to that in Meyer et al. (2006). Soluble compounds were extracted by placing 10 g of plant parts in 200 mL sterile deionized water (SDI) in 500-mL Erlenmeyer flasks sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL) and foil. The flasks were placed in a refrigerated incubator shaker at 100 rpm for 24 h at 4°C. The suspensions were then filtered through eight layers of cheesecloth and the extracts centrifuged for 10 min at 3000 rpm (1157 × *g*). The supernatants were vacuum-filtered through a Whatman no. 1 filter paper (GE Healthcare Life Sciences, Piscataway, NJ), sequentially filtered through a 1.0-μm and two 0.45-μm syringe filters, and stored at −15°C until use. Before assays, the extracts were again filtered through the same sequence of syringe filters. After dilutions were prepared in SDI, each was filtered again through a 0.2-μm syringe filter.

To obtain root exudates, whole plants were harvested and the root systems submerged in 250 mL SDI in 500-mL beakers. The beakers were wrapped in aluminum foil to prevent photolysis. The plants were agitated for 24 h on a VWR Advanced Digital Shaker (VWR, Radnor, PA) at 100 rpm. For each collection time, 16 plants were placed into 8 beakers (two plants per beaker). The liquid from the beakers was decanted and centrifuged for 10 min at 11,000 rpm (15,557 × *g*), vacuum-filtered through Whatman no. 1 filter paper, and filtered through a 0.45-μm syringe filter.

For freeze-drying, 250 mL of root exudates were placed into a stainless steel metal tray, covered, and frozen at −18°C for at least 24 h. The metal tray cover was replaced with plastic wrap, 5 to 10 slits were made in the top of the plastic wrap, and the frozen tray was placed into a freeze dryer (Virtis Advantage Plus Freeze Dryer, Model ES-53; SP. Industries, Gardiner, NY). The solid product resulting from freeze-drying was placed into a

clean 4-mL glass vial with a screw cap and then into a plastic bag containing a humidity sponge. Sample recovery at the end of the cycle averaged approximately 0.65 g.

For exudate assays, a stock solution was prepared by dissolving 0.07 g of freeze-dried root exudates in 5 mL of SDI (1.40% w/v). The samples were sterile-filtered through 0.2-μm filters.

The pH of all extracts and of the water control was measured on sample portions. There was not enough exudate material for pH determinations.

Activity of Jesup (Max-Q) Root Exudates and Root and Shoot Extracts

Assays with *M. incognita* were conducted in 96-well polystyrene plates, similar to procedures in Meyer et al. (2006). Each well received 315 μL of treatment or SDI (control), and 35 μL of *M. incognita* eggs (ca. 50 eggs) or 35 μL of hatched J2 (ca. 20 J2). The extract treatments were designated as 0% (SDI control), 25, 50, 75, and 100% extracts in SDI. Wells with eggs in extracts received 500 mg L^{-1} streptomycin sulfate to prevent microbial contamination, and a similar control was included. Exudate concentrations for tests with eggs and J2 were 0.35, 0.70, 1.05, and 1.40% w/v.

Each plate was covered with a plastic adhesive sheet (SealPlate; EXCEL Scientific, Inc., CA) and placed at 26°C. In each of two or three trials, five wells were used per treatment; $n = 10$ or $n = 15$ for microwell assays ($n = 5$ for SDI rinse of J2 in shoot extracts). In assays with eggs, total numbers of J2 that hatched from eggs, and numbers of active vs. inactive J2, were counted 3, 5, and 7 d after immersion of the eggs in the treatments. This was done to determine effects on egg hatch and on J2 that had been exposed to treatments both before and after hatch. In assays with J2 from hatching chambers, numbers of active vs. inactive J2 were counted after 1 and 2 d incubation, and after 3 d with J2 in root exudates. This simulated effects on J2 that encountered the compounds only after hatching from eggs. The J2 in extracts were then placed in a water rinse for 1 d, and numbers of active vs. inactive J2 were counted again to determine if active compounds were nematotoxic or nematostatic. J2 in root exudate treatments were placed in a water rinse after 3 d in treatments.

Meloidogyne incognita Penetration and Development in Jesup (Max-Q) vs. Tomato

Penetration of tall fescue Jesup (Max-Q) and tomato Rutgers seedling roots by *M. incognita* was investigated in a greenhouse with ambient temperatures of 21 to 35°C. Single 27-d-old Jesup (Max-Q) or 59-d-old tomato seedlings were planted into individual 237-cm^3 foam cups containing 200 cm^3 sterilized sand. Seedlings were fertilized with 1.3 g Osmocote 13–13–13 (N–P–K). After 9 d, each seedling was inoculated with approximately 5000 eggs in 0.8 mL water, pipetted into two holes near the plant. The entire seedling was removed from each cup 7 d later and the root system washed. The 7-d infection period allowed synchronous nematode penetration and development. Each seedling was then transplanted into a 355-cm^3 foam cup containing 300 cm^3 sterilized sand. All seedlings were fertilized with 1.3 g Osmocote after planting. Seedlings were harvested at 7, 14, 21, and 28 d after transplanting (DAT). Root systems at 7, 14, and 21 DAT were washed and stained with acid fuchsin (Byrd et al., 1983). On each harvest

date, the root systems were evaluated for numbers of galls, infective J2 (vermiform), and swollen nematodes (all stages from swollen J2 to globose mature females; illustrated by Taylor and Sasser, 1978). At 28 DAT, root systems were washed and stained in phloxine B to determine number of egg masses (Holbrook et al., 1983). Numbers of eggs in roots were estimated by extraction with a NaOCl solution as mentioned above. The number of galls per root system and the dry root weights (dried at 70°C in aluminum foil until weight loss stopped) were also recorded.

Treatment combinations were replicated four times in a randomized complete block with a split-plot design. The whole-plot factor was the date of harvest, with plant species as the split-plot factor. The experiment was repeated once. Changes in the second test included (i) transplanting 72-d-old Jesup (Max-Q) or 31-d-old tomato seedlings, and (ii) inoculating with eggs after 6 d. Also, a third test was conducted in which the tomato and tall fescue roots were only harvested at 28 DAT and then stained with acid fuchsin to observe stage of nematode development within the roots.

Statistical Analysis

Relationships of percent egg hatch and percent active J2 with percent extract and exudate concentrations were examined by fitting Beta regression models to the data from microwell assay studies in an analysis of covariance (ANCOVA) structure, modeling compound symmetric correlation structure among repeated measurements of wells across time. These Beta ANCOVA models (i.e., generalized linear mixed models, GLMM; Stroup, 2012) were fitted using SAS PROC GLIMMIX (SAS version 9.3; SAS/STAT, 2011–2013), specifying a logit link function. Significant reduction in percent egg hatch or percent active J2, relative to percent extract or exudate concentration, was identified by statistically significant ($P \leq 0.05$), non-zero β regression slopes, on the logit-scale.

For the nematode penetration and development study, nematode data were $\log_{10}(x + 1)$ transformed and subjected to analysis of variance with the general linear model (GLM) procedure of SAS (SAS Institute, Cary, NC). Actual numerical data were used for table presentation.

For J2 mortality in extracts, a logistic regression model was fitted to replicate values for each of coarsely ground and powdered extract data to obtain LC_{50} estimates, using SAS PROC PROBIT (SAS/STAT version 9.3; SAS/STAT, 2011–2013). Confidence intervals for the LC_{50} estimates were obtained using Fieller's theorem (Finney, 1971), as implemented by specifying the INVERSECL option in the SAS PROC PROBIT MODEL statement.

RESULTS
Activity of Jesup (Max-Q) Root Exudates and Root and Shoot Extracts

The root and shoot extract pH values were 6.4 to 7.0, and 5.8 to 6.4, respectively.

Compared with streptomycin sulfate controls, hatch was not suppressed after 2 or 5 d in Jesup (Max Q) powdered root extracts (Fig. 1; Day 2 not shown). In fact, there was a slight increase in hatch with increasing extract concentration on Day 2. However, hatch was inhibited following 7 d immersion in the extracts made from powdered roots. Hatch inhibition increased
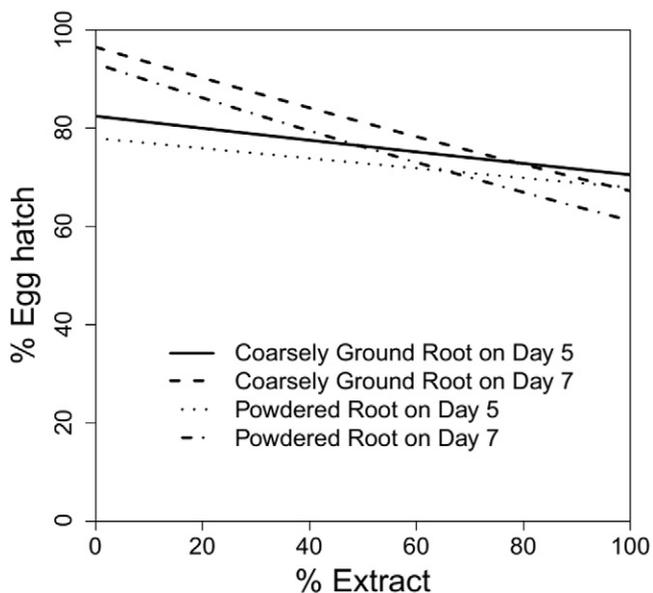
Fig. 1. Percentage *Meloidogyne incognita* hatch after 5 and 7 d incubation in water-soluble extracts from Jesup (Max-Q) tall fescue roots. The extracts were obtained from coarsely ground and powdered roots. Data are means of two trials, five wells per treatment. The 0% extract controls contained 500 mg L^{-1} streptomycin sulfate.
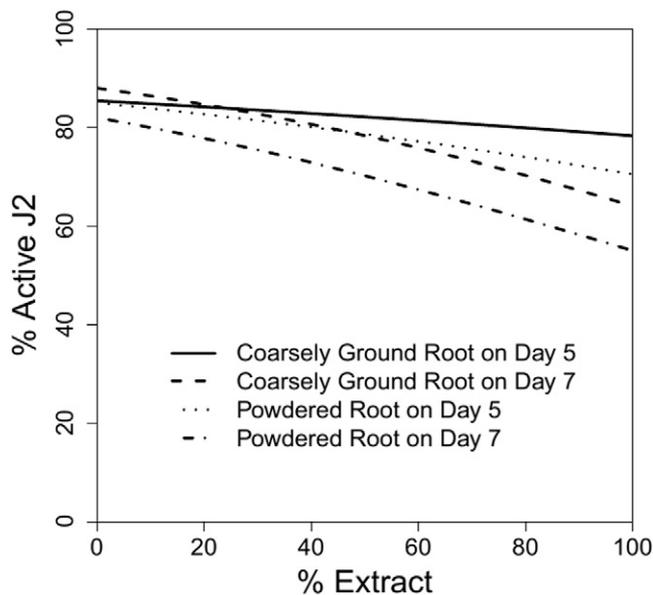

Fig. 3. Percentage of active *Meloidogyne incognita* second-stage juveniles (J2) that hatched from eggs after 5 and 7 d incubation in water-soluble extracts from Jesup (Max-Q) tall fescue roots. The extracts were obtained from coarsely ground and powdered roots. Data are means of two trials, five wells per treatment. The 0% extract controls contained 500 mg L^{-1} streptomycin sulfate.
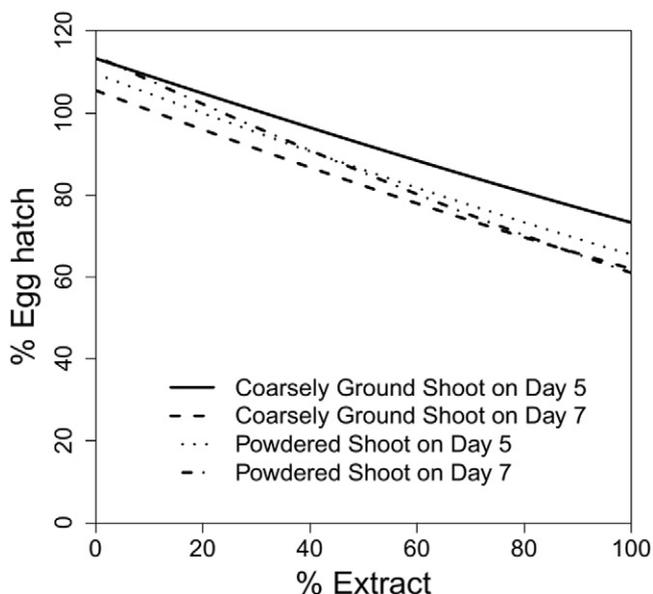

Fig. 2. Percentage *Meloidogyne incognita* hatch after 5 and 7 d incubation in water-soluble extracts from Jesup (Max-Q) tall fescue shoots. The extracts were obtained from coarsely ground and powdered shoots. Data are means of two trials, five wells per treatment. The 0% extract controls contained 500 mg L^{-1} streptomycin sulfate.

with extract concentration, with inhibition up to 34% in the 100% powdered root extract concentration compared to the streptomycin sulfate controls (Fig. 1); means and confidence intervals (CI) were 61.2% (54.7, 68.2) vs. 93.2% (85.1, 101.6), respectively. Similarly, hatch in extracts from coarsely ground roots was not inhibited after 2 or 5 d immersion, but was inhibited up to approximately 31% after 7 d; 67.2% hatch (60.5, 74.5) in 100% extract; 96.6% (88.3, 105.1) in the 0% extract (Fig. 1; Day 2 not shown). Unlike the results with powdered

roots, there was no increase in hatch after 2 d in extracts from coarsely ground roots. However, when hatch in coarsely ground vs. powdered root extracts was compared at each incubation time, there were no significant differences between the slopes of the two treatments at Day 2, at Day 5, or at Day 7.

Unlike the root extracts, shoot extracts inhibited egg hatch after 5 and 7 d incubation (Fig. 2). Extracts from both powdered and coarsely ground shoots suppressed egg hatch, and similar to root extracts, there was greater hatch suppression as extract concentration increased. In powdered shoot extract, the percent hatch inhibitions on Days 5 and 7 were up to 40 to 46%, respectively, with 65.4% (58.0, 73.5) hatch in 100% extract and 109.5% (100.5, 118.8) in 0% extract on Day 5, and 61.0% (53.8, 68.8) vs. 113.8% (104.6, 123.2) on Day 7. In coarsely ground shoot extracts, there was approximately 35 to 41% hatch inhibition on Days 5 and 7, respectively, in the 100% extract, with 73.2% (65.7, 81.3) hatch in 100% extract vs. 113.3% (104.3, 122.4) in the 0% extract on Day 5, and 62.0% (55.1, 69.4) vs. 105.5% hatch (96.6, 114.6) in 0% extract on Day 7. As with root extracts, there were no significant differences between the slopes of the powdered vs. the coarsely ground extract treatments at Day 2, at Day 5, or at Day 7.

No root extract treatment inhibited activity of J2 that had hatched from the immersed eggs by Day 2. However, by Day 5, J2 activity in the extracts from powdered and coarsely ground roots was suppressed up to 17% (in powdered root extract, 70.6% active (64.0, 76.4) in 100% extract; 85.0% active in 0% extract (81.9, 87.7) (Fig. 3). Inhibition of J2 activity by extracts from roots was greater by Day 7, with activity suppressed by as much as 27% in coarsely ground extracts to approximately 33% in powdered root extracts. On Day 7 in the 100% root extracts, mean percent active J2 were 64.1% (57.1, 70.6) and 55.0% (47.7, 62.2) in coarsely ground and powdered extracts, respectively; in
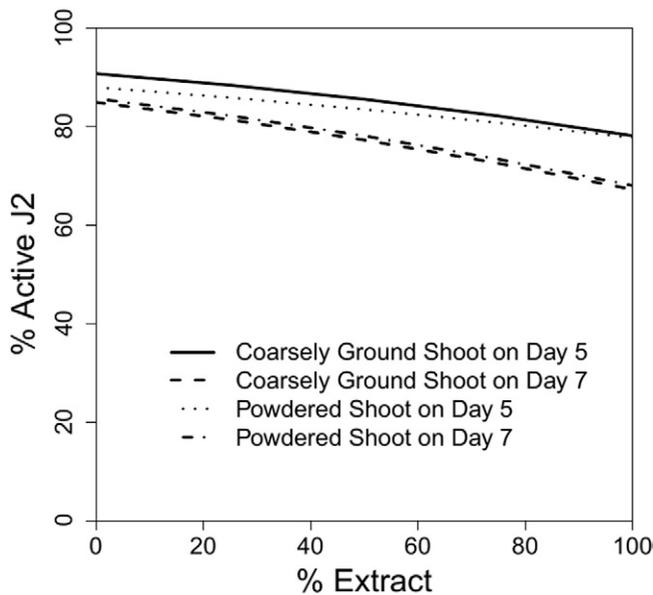
Fig. 4. Percentage of active *Meloidogyne incognita* second-stage juveniles (J2) that hatched from eggs after 5 and 7 d incubation in water-soluble extracts from Jesup (Max-Q) tall fescue shoots. The extracts were obtained from coarsely ground and powdered shoots. Data are means of two trials, five wells per treatment. The 0% extract controls contained 500 mg L^{-1} streptomycin sulfate.

Fig. 5. Percentage of active *Meloidogyne incognita* second-stage juveniles (J2) after 2 d incubation in water-soluble extracts from Jesup (Max-Q) tall fescue roots, followed by a 1-d rinse in sterile deionized water (Day 3). J2 had hatched before immersion in the root extracts. The extracts were obtained from coarsely ground and powdered roots. Data are means of three trials, five wells per treatment. The 0% extract controls were sterile deionized water.

0% extracts, corresponding means were 88.0% (85.5, 90.2) and 82.0% active J2 (78.6, 85.0).

As with root extracts, shoot extracts demonstrated activity against J2 that hatched from eggs in the wells by Days 5 and 7 (Fig. 4). On Day 5, J2 activity was suppressed by approximately 12 to 14% in powdered and coarsely ground shoot extracts, respectively. In powdered shoot extract on Day 5, there were 77.8% active J2 (73.2, 81.7) in 100% extract, and 88.0% active (85.2, 90.3) in 0% extract; results with coarsely ground shoot extracts were 78.2% (73.7, 82.1) and 90.8% (88.5, 92.7) active, respectively. By Day 7, there was greater inhibition of activity, with approximately 20 to 21% decrease in J2 activity in extracts from powdered and coarsely ground shoots, respectively. Percent active J2 in powdered shoot extracts was 68.1% (62.8, 72.9) in 100% extract and 85.7% (82.7, 88.3) in 0% extract, activity in coarsely ground shoot extracts was 67.3% (62.0, 72.1) and 85.0% (81.8, 87.6), respectively. As with egg hatch, inhibition increased with increasing concentrations of ground and powdered root and shoot extract treatments, and the slopes of the two types of treatments did not differ from each other within each incubation day.

Nematotoxicity of tall fescue root extracts was observed within 2 d after immersion of previously hatched J2, following a slight increase in J2 activity at Day 1 in extracts from coarsely ground roots. The J2 in these tests were exposed to the extracts during the entire test, whereas J2 that hatched from immersed eggs were directly exposed for different amounts of time, depending on when they emerged from the eggs. On Day 2, J2 activity was decreased up to approximately 23 and 29% in coarsely ground and powdered root extracts, respectively, compared with the water controls (Fig. 5). J2 activity at Day 2 was 53.5% (46.0, 60.9) in 100% powdered root extract and 75.5% (72.1, 78.5) in 0% extract, and 56.4% (48.9, 63.5) in 100% coarsely ground extract, vs. 73.2% J2 activity (69.6, 76.4) in
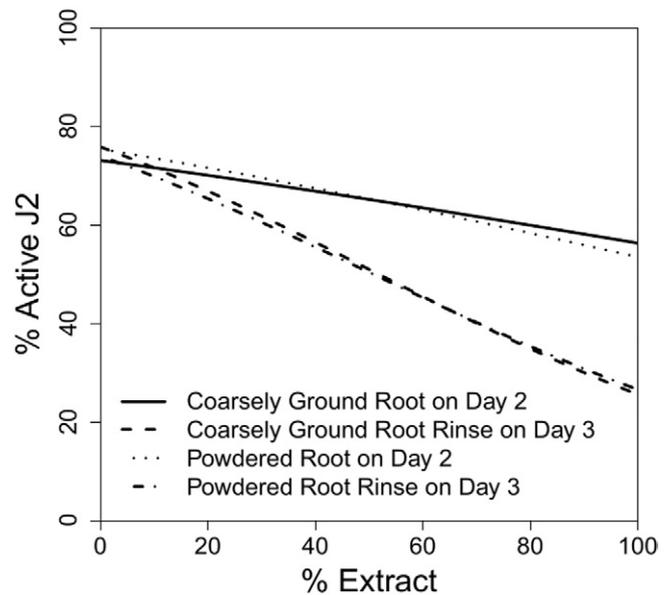
0% extract. After the 1-d water rinse (Day 3), nematotoxicity was recorded from all root extract treatments compared with the water control, with greater loss in J2 activity than at Day 2 before the water rinse. The highest percent J2 mortalities were approximately 64 to 66% (in the 100% powdered and coarsely ground root extracts, respectively). J2 activity in the Day 3 rinse was 26.6% (21.1, 33.0) in 100% powdered root extracts and 74.1% (70.6, 77.3) in 0% extracts, and in coarsely ground root extracts was 25.6% (20.3, 31.8) and 75.9% (72.5, 79.0), respectively. Root extracts were the only treatment that caused more than 50% J2 mortality at the tested concentrations. LC$_{50}$ estimates (95% confidence intervals) were: 56.3% (50.7, 62.1) and 54.1% (49.1, 59.1) for powdered and coarsely ground root extracts, respectively. Mortality increased with increasing concentrations of coarsely ground and powdered root extract treatments, and the slopes of the two types of treatments differed from each other only at Day 1.

The regression model indicted a significant decrease in percent active J2 with increasing shoot extract concentrations (Fig. 6). J2 activity in 100% vs. 0% powdered shoot extracts at Day 2 was 51.1% (38.0, 64.0) and 61.8% (48.7, 73.4), respectively, and 53.0% (39.8, 65.8) vs. 65.8% (53.0, 76.7) in coarsely ground shoot extracts. However, it should be noted that the CIs calculated for mean J2 activity in 0 and 100% shoot extracts overlapped for coarsely ground and for powdered shoot extracts at Day 2. The Day 3 count in the water rinse indicated that the shoot extracts, particularly from powdered plant material, were also nematotoxic, as there were more inactive J2 in the extracts after the water rinse. J2 viability was decreased by up to 50% in the 100% extracts from shoots. Percent J2 activity in the Day 3 water rinse at 100% vs. 0% extract was 36.6% (21.1, 55.6) and 73.4% (55.9, 85.7), respectively, for powdered shoot extracts, and 35.0% (19.8, 54.1) vs. 69.8% (51.6, 83.4), respectively, for coarsely
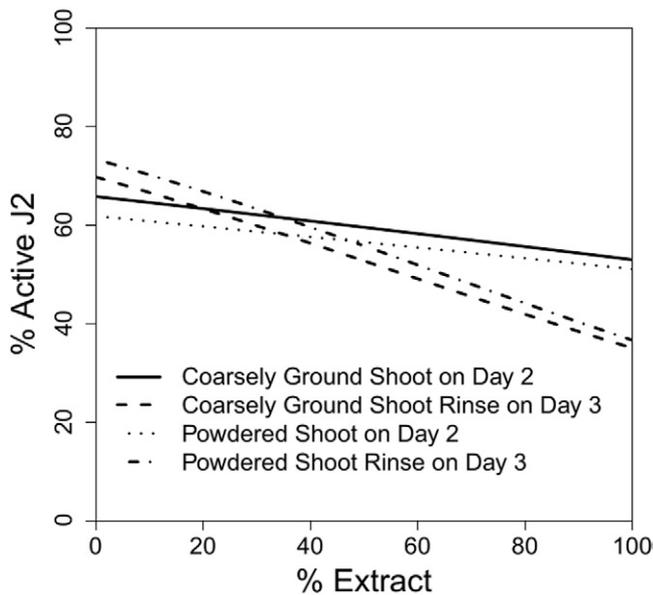
Fig. 6. Percentage of active *Meloidogyne incognita* second-stage juveniles (J2) after 2 d incubation in water-soluble extracts from Jesup (Max-Q) tall fescue shoots, followed by a 1-d rinse in sterile deionized water (Day 3). J2 had hatched before immersion in the shoot extracts. The extracts were obtained from coarsely ground and powdered shoots. Data are means of two trials, five wells per treatment; rinse (Day 3) data are from one trial. The 0% extract controls were sterile deionized water.
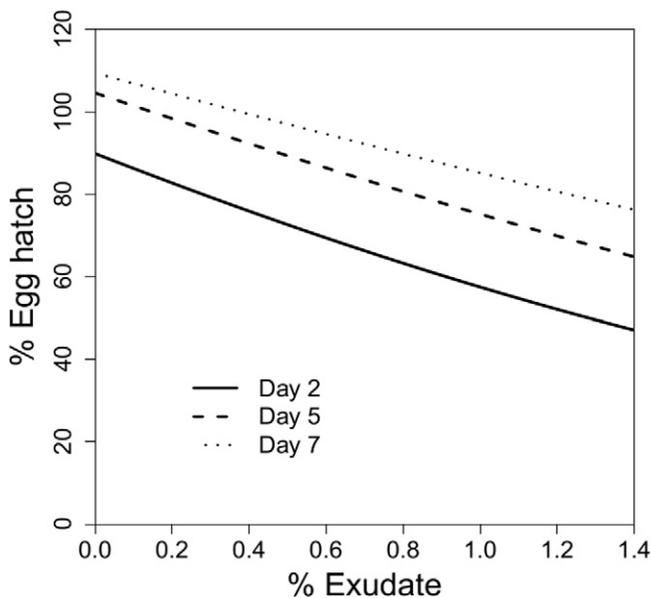

Fig. 7. Percentage of *Meloidogyne incognita* hatch after 2, 5, and 7 d incubation in root exudates from Jesup (Max-Q) tall fescue. Data are means of two trials, five wells per treatment. The 0% exudate controls were sterile deionized water.

ground shoot extracts. Again, the CIs overlapped for results with coarsely ground shoots. Slopes did not differ between the two treatments at Day 1, at Day 2, or at the Day 3 water rinse. LC_{50} estimates (95% confidence intervals) were: 65.3% (49.0, 86.1) and 57.3% (37.7, 81.7) for powdered and coarsely ground shoot extracts, respectively.

Tall fescue root exudates inhibited *M. incognita* hatch following 2, 5, and 7 d immersion in treatments (Fig. 7). Hatch
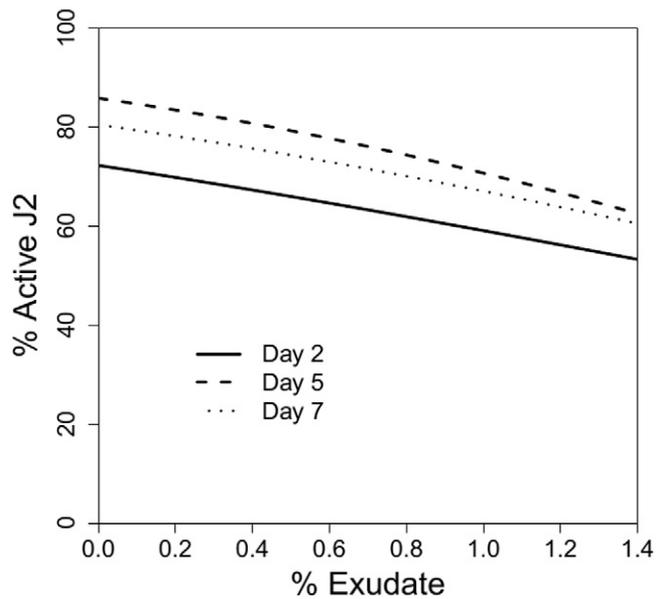

Fig. 8. Percentage of active *Meloidogyne incognita* second-stage juveniles (J2) that hatched from eggs after 2, 5, and 7 d incubation in root exudates from Jesup (Max-Q) tall fescue. Data are means of two trials, five wells per treatment. The 0% exudate controls were sterile deionized water.

was decreased by 48, 38, and 30% in the 1.4% root exudate (the highest tested concentration) at Days 2, 5, and 7, respectively. Hatch in 1.4% root exudate was 47.1% (42.1, 52.5; Day 2), 64.8% (57.2, 73.1; Day 5), and 76.2% (66.2, 87.1; Day 7); hatch in the 0% extract (water control) at those days was 89.9% (82.7, 97.3; Day 2), 104.6% (94.9, 114.6; Day 5), and 109.4% (97.6, 121.6; Day 7). Increasing exudate concentration resulted in increased egg hatch inhibition at each time.

Activity of J2 that hatched from immersed eggs was suppressed up to approximately 25 to 27% at all incubation times (Fig. 8). Percent J2 activity in 1.4% root exudates was 53.3% (49.4, 57.1; Day 2), 62.5% (59.8, 65.2; Day 5), and 60.5% (58.1, 63.0, Day 7); activity in the 0% extract at those days was 72.3% (69.4, 75.0), 85.9% (84.4, 87.3), and 80.5% (78.9, 82.1), respectively. Activity of previously hatched J2 was also suppressed by the root exudates at Days 2 and 3 (Fig. 9) by as much as 18% (1.4% root exudate). Percent J2 viability after the water rinse (Day 4) was decreased by approximately 22% in 1.4% exudate. J2 activity in 1.4% root exudate was 42.1% (39.5, 44.6; Day 2) and 39.3% (36.8, 41.9; Day 3), and percent viable in the Day 4 water rinse was 40.1 (37.5, 42.7). Percent activity in the water controls at the corresponding days was 51.2% (48.6, 53.8), 47.7% (45.1, 50.2), and 51.6% (49.0, 54.1). As with egg hatch, increasing root exudate concentration resulted in decreased J2 activity at all incubation times.

Meloidogyne incognita Penetration and Development in Jesup (Max-Q) vs. Tomato

Meloidogyne incognita J2 were found in tall fescue and tomato roots 7 DAT in both tests (Table 1), with greater ($P \leq 0.05$) numbers of J2 in tomato roots than in Jesup (Max-Q) roots in Test 2. Similar J2 root population trends were detected in Test 1, although differences were not significant ($P = 0.14$). Furthermore, on 7 DAT, unlike in tomato, several J2 in the Jesup (Max-Q) roots in Test 2 had intestines that were not completely
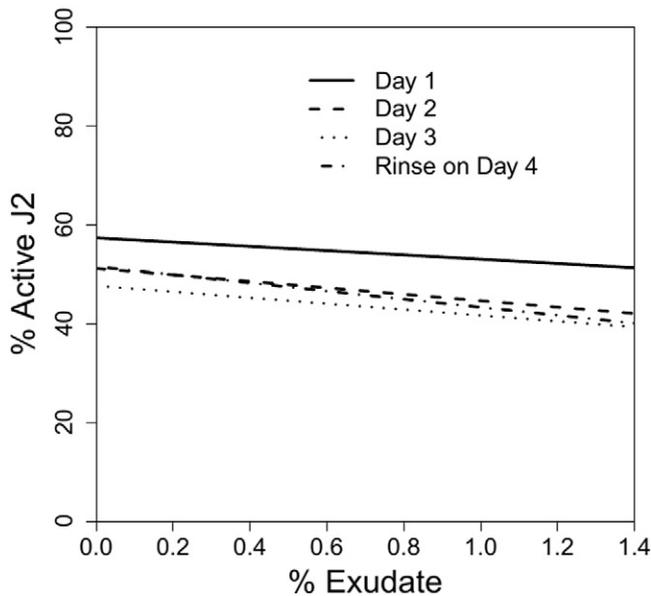
Fig. 9. Percentage of active *Meloidogyne incognita* second-stage juveniles (J2) after 1, 2, and 3 d incubation in root exudates from Jesup (Max-Q) tall fescue, followed by a 1-d rinse in sterile deionized water (Day 4). J2 had hatched before immersion in exudates. Data are means of two trials, five wells per treatment. The 0% exudate controls were sterile deionized water.

darkened (i.e., striped). The J2 stage diminished dramatically in roots of tomato and Jesup (Max-Q) by 14 DAT.

Various swollen stages of parasitic juveniles were detected in roots of tomato and Jesup (Max-Q) seedlings beginning at 14 DAT. Numbers of swollen juveniles were greater ($P \leq 0.05$) in tomato than in Jesup (Max-Q) roots on 14 DAT in both tests. On 21 and 28 DAT, numbers of late second-stage females (partially globose) and mature females (globose), respectively, were greater ($P \leq 0.05$) in tomato than in Jesup (Max-Q) roots. On 21 DAT, numbers of late second-stage females were 16 and 23 times greater in tomato roots than in Jesup (Max-Q) roots. Only one late second-stage female was detected in Jesup (Max-Q) roots in Test 1 and none in Test 2.

Egg production was observed in both tests on 28 DAT. More ($P \leq 0.05$) mature females, and numbers of egg masses per

plant, eggs per plant, and eggs per gram of root were associated with tomato roots than with Jesup (Max-Q) roots (Table 1). In Test 1 and Test 2 on 28 DAT, there were 41 and 81 times more mature females in tomato roots than in Jesup (Max-Q) roots, respectively. On Jesup (Max-Q) on this sampling date, there were only 1 or 2 females and 0 or 6 eggs (Table 1). Even though there were fewer ($P \leq 0.05$) egg masses per plant on Jesup (Max-Q) than on tomato roots on 28 DAT, egg hatch occurred from two tomato and two Jesup (Max-Q) egg masses after 8 d incubation in BPI (Bureau of Plant Industry) dishes filled with tap water.

Root galls were detected in both Jesup (Max-Q) and tomato roots on most sampling dates, except on 21 DAT when no galls were found on Jesup (Max-Q) roots (Table 1). There were always more ($P \leq 0.05$) galls on tomato than on Jesup (Max-Q) roots on all sampling dates and in both tests. In Test 3 on 28 DAT, numbers of late second-stage females or mature females were greater ($P \leq 0.05$) in tomato than in Jesup (Max-Q) roots; 18 mature females vs. 2 second-stage females, respectively. Also, as in the previous two tests, there were more ($P \leq 0.05$) root galls on tomato (73 galls) than on Jesup (Max-Q) (0 galls) roots.

DISCUSSION

Water-soluble extracts from roots and shoots of Jesup (Max-Q) tall fescue inhibited egg hatch of *M. incognita* by about a third to less than a half (31–46%) compared with hatch in controls. Root and shoot extracts were also nematotoxic to J2, with the highest concentration of root extracts resulting in >50% mortality, and the highest shoot extract concentration causing approximately 50% mortality. Particle size of material used to prepare the extracts was generally not a factor in toxicity to J2. Egg exposure of more than 2 d was required for inhibitory effects of toxic treatments.

Root exudates decreased hatch by 30 to 48%, but *M. incognita* J2 mortality was only about a fourth in the highest tested exudate concentration. It is difficult to compare activity of the exudates with activity of the extracts, since the concentrations were necessarily based on different methods of obtaining the samples. Also, phytochemicals were not identified, so it is not known whether any differences in activity between extracts and

Table 1. Penetration, early development, and reproduction of *Meloidogyne incognita* (peach isolate) in root systems of Rutgers tomato and Jesup (Max-Q) tall fescue in the greenhouse 7, 14, 21, and 28 d after transplanting (DAT). Data are means of four replicates each inoculated with 5000 *M. incognita* eggs.

| | | Nematodes/root system† | | | | Egg masses/ plant | | Eggs/plant | | Eggs/gram of root | | Galls/plant | |
| | | Vermiform‡ | | Swollen‡ | | | | | | | | | |
DAT	Plant	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
7	Tomato	38	72*	0	0	0	0	0	0	0	0	17*	4*
	Jesup (Max-Q)	12	10	0	0	0	0	0	0	0	0	4	0
14	Tomato	1	0	6*	31*	0	0	0	0	0	0	66*	118*
	Jesup (Max-Q)	0	0	0	8	0	0	0	0	0	0	1	1
21	Tomato	0	0	16*	23*	0	0	0	0	0	0	77*	198*
	Jesup (Max-Q)	1	0	1	0	0	0	0	0	0	0	0	0
28	Tomato	0	0	82*	81*	82*	81*	6,438*	11,250*	17,164*	41,926*	138*§	166*§
	Jesup (Max-Q)	0	0	2	1	2	1	0	6	0	21	13¶	2¶

* $P \leq 0.05$ on a particular date according to ANOVA. Nematode data were transformed to [$\log_{10}(x+1)$] for analysis and were back-transformed for presentation in this table.

† Egg hatch/infection period was 7 d for both tests.

‡ Developmental stages: vermiform = infective second-stage juvenile (J2; not swollen); swollen = all stages from slightly swollen parasitic J2 to mature females (globose).

§ 59 and 49% of the galls detected on tomato in Test 1 and Test 2, respectively, had mature females with associated egg masses on Day 28.

¶ 15% of the galls detected on Jesup (Max-Q) in Test 1 had mature females with associated egg masses on Day 28. Of the two galls detected in Test 2, 1 (50%) had mature females with associated egg masses on Day 28.

exudates were due to a difference in compound concentrations or chemical composition.

Endophyte infection of tall fescue can alter chemical activity of the roots (Malinowski et al., 1998). The nematotoxicity to J2 that we observed with water-soluble root extracts from Jesup (Max Q) contrasted with results from earlier studies with *P. scribneri* and tall fescue associated with different endophytes. Methanolic root extracts from E+ tall fescue Jesup (ergot alkaloid-producing endophyte; nonhost to *P. scribneri*) were nematostatic and repellant to *P. scribneri* when prepared from plants grown 45 d or more, whereas root extracts from E- tall fescue (no endophyte, susceptible plant) were attractive to the nematode (Bacetty et al., 2009a, 2009b). Several purified ergot alkaloids, identified as compounds produced in *Neotyphodium*-associated tall fescue and perennial ryegrass (*Lolium perenne* L.), were repellant to *P. scribneri*, and those that inhibited nematode motility were either nematotoxic or nematostatic (Panaccione et al., 2006; Bacetty et al., 2009a, 2009b). It is interesting to note that even though alkaloids produced in the E+ plants could be nematotoxic, root extracts from these plants were not nematicidal to *P. scribneri*. Comparisons among investigations must take into account a number of factors, including plant age, growing conditions, nematode species tested, and use of methanol vs. water for extractions. However, a clear difference is that root-derived compounds from Jesup (Max Q) were nematotoxic even though the fungus does not produce ergot alkaloids.

Whole-plant studies showed that *M. incognita* reproduction on tall fescue cultivars was not affected by the presence or absence of the endophyte, and thus by the production of ergot alkaloids (Nyczepir and Meyer, 2010). In those trials, *M. incognita* did not reproduce on Jesup (Max-Q). In greenhouse trials with endophyte-associated perennial ryegrass, removal of some or all ergot alkaloids with gene knockout strains did not alter the suppression of *P. scribneri* (Panaccione et al., 2006). This result with perennial ryegrass agrees with our finding that ergot alkaloids are not a major chemical factor in nematode suppression.

Tall fescue compounds other than ergot alkaloids have been studied for effects on nematodes. Increased production of phenolic compounds in E+ plants may be involved in plant resistance, while nematostatic polyphenols were identified from root extracts and were not associated with the presence or absence of endophyte (Malinowski et al., 1998; Bacetty et al., 2009a). There was not a clear correlation between loline production and resistance to *Pratylenchus* in tall fescue (Timper et al., 2005). At this time, the nematostatic and nematotoxic compounds in root exudates or extracts of Jesup (Max-Q) remain unidentified.

In our study, greater populations of J2 penetrated tomato (susceptible) roots than tall fescue Jesup (Max-Q) (resistant) roots. Intestinal disruption has been noted in unhealthy J2 (Habash and Al-Banna, 2011); we also detected this in several J2 in Jesup (Max-Q) roots, but not in tomato. Possible explanations for reduced *M. incognita* J2 penetration and feeding in Jesup (Max-Q) roots include allelopathy and/or plant-related chemical defenses.

There were more galls on tomato than on Jesup (Max-Q) roots. This indicates that the mechanism of resistance to *M. incognita* is different in Jesup (Max-Q) roots than in Guardian peach rootstock roots (resistant). Comparable numbers of *M. incognita* J2 were detected in Guardian and Lovell (susceptible) rootstocks at 6 DAT, and root galling was abundant on both rootstocks (Nyczepir et al., 1999). However, most of the nematodes failed to mature and reproduce in Guardian. Our results are also different from those of Bacetty et al. (2009a) and Gwinn and Bernard (1993), in which numbers of *P. scribneri* and *M. marylandi* were initially similar between E+ and E- tall fescue plants, but then declined in E+ plants. In Jesup (Max-Q), fewer J2 penetrated early in the infection process, and fewer galls were produced as compared to tomato. It appears that the primary mechanisms of resistance to *M. incognita* in Jesup (Max-Q) occur before and/or during the time of root penetration and early infection by the J2.

Not all *M. incognita* J2 were prevented from penetrating and developing into mature reproductive females in Jesup (Max-Q) roots. Our results indicate that *M. incognita* reproduction, as measured by numbers of egg masses, eggs per plant, and eggs per gram dry root, occurred in both Jesup (Max-Q) and tomato roots 28 DAT. However, nematode reproduction in Jesup (Max-Q) at 28 DAT was negligible as compared to tomato.

As mentioned above, Jesup (Max-Q) was a nonhost to *M. incognita* and there was complete nematode mortality as indicated by a lack of reproduction (Nyczepir and Meyer, 2010). This would indicate that Jesup (Max-Q), which is infected with a non-ergot alkaloid producing endophyte, may have additional non-endophyte-related suppressive effects on the nematode through the production of secondary metabolites that repel, paralyze, or kill the nematode. One explanation for the limited nematode reproduction and production of viable eggs in some egg masses on Jesup (Max-Q) roots in the current study is the length of the harvest times. In the current study, plants were harvested approximately 35 d after inoculation (ca. one life cycle), whereas in the previous study (Nyczepir and Meyer, 2010) plants were harvested approximately 87 to 123 d after inoculation (ca. 3–4 life cycles). After a longer period of time, the second generation J2 on Jesup (Max-Q) may be deterred from penetrating, establishing feeding sites, and completing the life cycle. Another explanation might simply be attrition, since the Pf/Pi ratio was low (i.e., <0.2). Fitness of *M. incognita* might also be reduced after passage through Jesup (Max-Q).

CONCLUSIONS

Interaction of plant-derived compounds and plant-parasitic nematodes is a complex issue. Amounts of chemicals, types, availability, and nematode exposure times vary with numerous parameters, which with tall fescue include endophyte status. In our studies, endophyte status in Jesup (Max-Q) tall fescue does not appear to be the primary factor involved with *M. incognita* suppression. We demonstrated that tall fescue Jesup (Max Q), grown under the conditions of our tests, can produce root and shoot compounds that inhibit *M. incognita* hatch, and that are nematostatic or nematotoxic to J2. The nematotoxic compounds in the root extracts and exudates may play a role in suppressing J2 from entering or developing in roots. Shoot-derived compounds would not be involved in inhibiting nematode root penetration, but might enhance nematode suppression if aboveground plant parts were incorporated into soil by growers. The results

presented in this article suggest that the nature of resistance in Jesup (Max-Q) to *M. incognita* included inhibition of nematode development and failure to complete the life cycle, and activity of compounds derived from Jesup (Max-Q) tall fescue that decreased nematode viability.

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