Management of Root-knot Nematode (Meloidogyne incognita) on Pittosporum tobira Under Greenhouse, Field, and On-farm Conditions in Florida

RICHARD BAIDOO, TESFAMARIAM MENGISTU, ROBERT MCSORLEY, ROBERT H. STAMPS, JANETE BRITO, AND WILLIAM T. CROW

Abstract: Root-knot nematodes are important pests of cut foliage crops in Florida. Currently, effective nematicides for control of these nematodes on cut foliage crops are lacking. Hence, research was conducted at the University of Florida to identify pesticides or biopesticides that could be used to manage these nematodes. The research comprised on-farm, field, and greenhouse trials. Nematicide treatments evaluated include commercial formulations of spirotetramat, furfural, and Purpureocillium lilacinum (=Paecilomyces lilacinus) strain 251. Treatment applications were made during the spring and fall seasons according to manufacturer’s specifications. Efficacy was evaluated based on J2/100 cm3 of soil, J2/g of root, and crop yield (kg/plot). Unlike spirotetramat, which did not demonstrate any measurable effects on Meloidogyne incognita J2 in the soil, furfural and P. lilacinum were marginally effective in reducing the population density of M. incognita on Pittosporum tobira. However, nematode reduction did not affect yield significantly. Although furfural and P. lilacinum have some potential for management of M. incognita on cut foliage crops, their use as a lone management option would likely not provide the needed level of control. Early treatment application following infestation provided greater J2 suppression compared to late application, suggesting the need for growers to avoid infested fields.

Key words: furfural, management, Meloidogyne incognita, Paecilomyces lilacinus, Purpureocillium lilacinum, pesticide, Pittosporum tobira, root-knot nematode, spirotetramat.

Root-knot nematodes (Meloidogyne spp.) are a major problem of commercial and landscape ornamentals in Florida (McGovern et al., 2002; McSorley et al., 2009). Cut foliage crops and woody ornamentals are perennials that remain in the ground for decades; hence when root-knot nematode infestation occurs after planting, population densities may build up to very high numbers over time. Eventually, the farm may become unproductive and be abandoned. Yield of cut foliage crops consists of the recent growth of stems with leaves, but infection by root-knot nematodes slows down the formation of new growth, thereby negatively impacting yield (Baidoo et al., 2014). A major cut foliage crop cultivated in Florida is P. tobira. It produces variegated foliage used in many floral arrangement designs. The yield is a collection of 6 to 7 branches from different plants with fresh leaves measuring about 45 to 50 cm length called a bunch. The number of days a bunch remains green in storage is its vase life (Stamps, 1985). Unfortunately, this plant is highly susceptible to root-knot nematodes (Crow, 2007) and the most prominent symptoms of root-knot nematode infestation on P. tobira are root galling, loss of root hairs, stunted growth, reduced yield, and susceptibility to Cercospora leaf spot caused by Cercospora pittospori (Baidoo et al., 2014).

Chemical control of nematodes is increasingly becoming difficult. Methyl bromide, which was used for preplant disinfestation, and the organophosphate nematicides fenamiphos and ethoprophos, which were used for postplant management of nematodes on cut foliage crops, have been phased out for environmental concerns (US-EPA, 2008, 2009). Because cut foliage crops are perennials, management strategies should include postplant treatments. Control of root-knot nematodes on these crops has remained an important research priority for cut foliage growers for decades. The goal of this research was to identify effective pesticides or biopesticides currently on the market that can be used for management of root-knot nematodes on P. tobira. Candidate pesticides include spirotetramat, furfural, and P. lilacinum.

Spirotetramat is a systemic insecticide labeled as Kontos (Olympic Horticultural Supply, Mainland, PA) for use on greenhouse and nursery ornamentals. It is foliarly applied with ambimobile translocation that reduces fecundity of sucking insects by inhibiting acetyl-CoA carboxylase (Nauen et al., 2008). Foliar applications of spirotetramat reduced the numbers of Pratylenchus vulnus in the rhizosphere soil around roots of walnut (Juglans spp.) and applications to grape (Vitis spp.) reduced the numbers of Xiphinema americanum, Meloidogyne spp., and Tylenchulus semipenetrans (McKenry, 2009). Spirotetramat also reduced postharvest population density of Heterodera avenae on wheat (Triticum spp.) (Smiley et al., 2011).

A furfural pesticide, Multiguard Protect EC (Agriguard, Cranford, NJ) was labeled in 2011 as a nematocide on golf course turf grasses, and also as a fungicide on ornamentals. It is suggested that the chemical affects the nematode cuticle (Fourie et al., 2014). Furfural suppressed populations of Meloidogyne arenaria, M. incognita, and Pratylenchus brachyurus on ‘Summer Crookneck’ squash (Rodriguez-Kabana et al., 1993)
and reduced population densities of Belonolaimus longicaudatus in bermuda grass putting greens (Crow and Luc, 2014).

A formulation of Purpureocillium lilacinum (=Paecilomyces lilacinus) (Luangsa-Ard et al., 2011) (MeloCon WG; Certis USA, Columbia, MD) is labeled for nematode management on ornamental plants as a soil-directed spray. Purpureocillium lilacinum is a nematode egg-parasitic fungus, also capable of parasitizing juveniles and females of root-knot nematodes. It is the most widely tested biological control agent for management of plant-parasitic nematodes (Atkins et al., 2003). Both pre-planting and at-planting applications of P. lilacinum strain 251 reduced root-knot nematode population numbers and root galling in tomato and cucumber (Schenck, 2004) and in cucumber (Kalele et al., 2010). Applications of P. lilacinum strain 251 suppressed B. longicaudatus in bermuda grass (Grow, 2013). A combination of P. lilacinum with chitin enhanced suppression of M. incognita more than using either product alone (Mittal et al., 1995).

Data on use of spirotetramat, furfural, or P. lilacinum for management of Meloidogyne spp. on P. tobira and other cut foliage crops in Florida is lacking. The general objective of this work was to provide cut foliage growers with new practical tools for managing root-knot nematode problems on this crop. The specific objectives were to determine (i) the effects of spirotetramat, furfural, or P. lilacinum on M. incognita population density over time; (ii) the effects of the above products on the yield of P. tobira over time; and (iii) the effects of the above products on the vase life of P. tobira cut foliage.

**Materials and Methods**

**Experimental trials:** The research was carried out at the University of Florida from 2012 to 2014 involving on-farm, small plot, and greenhouse trials. The on-farm trial was carried out on a grower’s farm in De Leon Springs, FL, at coordinates 29.1400°N and 81.3689°W to evaluate whether the treatments could be used to manage an existing postplanting field infestation. This site was a 2-yr-old planting of commercial P. tobira var. variegata naturally infested with M. incognita. It was previously planted with P. tobira from 1998 until 2009 and replanted in 2010 with P. tobira that subsequently grew poorly and exhibited severe root galling caused by M. incognita. The soil at the site is classified as an ‘Astatula’ fine sand single-grained, well-drained soil (>95% sand).

The small plot trial was conducted at the University of Florida’s Plant Science Research and Education Unit in Citra, FL, at coordinates 29.4117°N and 82.1100°W to evaluate nematode suppression in a new planting by early postplanting application. The soil is classified as a Candler sand (94.5% sand, 1.5% silt, and 4% clay). The field was artificially infested with the population of M. incognita originating from P. tobira growing at the on-farm trial site. Three months after field inoculation, soil samples for nematode extraction were taken from each plot to determine the initial nematode population density before planting. The field was then planted with 0.6-m-high variegated P. tobira plants from 11.5-liter containers and 2 wk later, treatment application was started.

The greenhouse trial was carried out in a shade structure (50% shade) at the University of Florida from May, 2013, to June, 2015. There were two consecutive trials, the first trial started in May, 2013, and ended in May, 2014, and the second trial from May, 2014, to June, 2015. The second trial was started in a new glasshouse at the University of Florida but the temperature in that glasshouse was too high for the plants and the nematodes, therefore, the plants were relocated to the 50% shade structure 2 mon after planting and were re-inoculated with the nematode. In both trials, P. tobira var. variegata plants were inoculated with a population of Meloidogyne spp. (that were >95% M. incognita) originating from the P. tobira farm. Meloidogyne incognita eggs were extracted from the roots of infected P. tobira plants using a 1% sodium hypochlorite solution (Clorox Company, Oakland, CA) following a modified protocol of Hussey and Barker (1973). For the first trial, 3,000 eggs were inoculated on 3-mon-old, 0.3 m-high, P. tobira plantlets, grown in 25-cm-diameter x 8-cm-deep pots containing steam-pasteurized growing medium that had been exposed to steam at 180°C for 2 h. The growing medium consisted of 2-parts masonry sand (Argos, Gainesville, FL) and 3-parts Pro-mix potting media (Premier Horticulture Inc., Quakertown, PA). For the second trial, the plants were re-inoculated with 2,000 eggs after they were relocated to make sure nematodes were present. The plantlets were raised in sterile peat soil on 25-cm² plastic tray cells.

**Table 1.** Treatment application schedule for nematicides used on an on-farm trial for evaluation of treatment programs for management of Meloidogyne incognita on Pittosporum tobira.

<table>
<thead>
<tr>
<th>Date of application</th>
<th>Nematicide</th>
<th>Spirotetramat</th>
<th>Purpureocillium lilacinum</th>
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<tbody>
<tr>
<td>March 15, 2012</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>April 6, 2012</td>
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<td>September 20, 2012</td>
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<td>October 11, 2012</td>
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<tr>
<td>October 22, 2013</td>
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Experimental design: The randomized complete block design (RCBD) was used for all the trials. The on-farm trial was a RCBD with four replications. The farm was divided into four blocks and each block had four plots. Each plot had four 18-m-long rows spaced 2.1 m apart; there were 20 plants per row. Two or three untreated guard rows separated adjacent plots and the treatments were randomized to plots within a block. The small plot and the greenhouse trials were also set up in a RCBD with 5 and 10 replications, respectively. Each plot of the small plot trial measured 2.4 m x 1.8 m with five plants per plot.

Treatment application: Pittosporum tobira undergoes a spring and a fall root flush and therefore treatment regimes were applied twice per year to best protect these newly developing roots during these periods. The on-farm, small plot, and the greenhouse trials involved three experimental treatments including P. lilacinum (MeloCon WG), furfural (Multiguard Protect), spirotetramat (Kontos), and an untreated control.

The P. lilacinum formulation was a wettable granule product containing $1 \times 10^{10}$ viable spores/gram applied at 4.5 kg/ha at each application. The formulation was mixed in water, along with a soil penetrant (LescoWet Plus; Lesco Inc., Columbus, OH) at 6.4 liters/ha to facilitate spore movement into the soil, and sprayed onto the research plots as a soil-directed spray. After application, the plots were irrigated with 6.4 mm of water to encourage spore movement into the plant root zone. Two applications were made 6 wk apart in each of the spring and fall seasons. Furfural was applied as Multiguard Protect EC (80% furfural). The maximum labeled rate of furfural is 74.8 liters/ha/application, with a maximum total of 224 liters/ha/season. Therefore, we made three applications in each of the spring and fall seasons at 74.8 liters product/ha, with 3-wk intervals between applications. For the on-farm trial, furfural was mixed with 151 liters of water and drenched evenly onto plots with the help of a pump and hose to simulate a 6.4 mm chemigation, an application method preferred by many cut foliage growers. Spirotetramat (22.4% a.i.) was tank mixed with an adjuvant (Break-Thru S240; Evonik Goldschmidt, Parsippany, NY) to increase foliar uptake at 1 liter/ha and sprayed onto plant foliage using either a tractor-mounted mist sprayer (for the on-farm trial) or a CO$_2$-powered backpack sprayer for the small plot and the greenhouse trials. Based on the recommendation of the Kontos manufacturer, two applications of 3.7 liters/ha were applied 6 wk apart each season. The treatment application

<table>
<thead>
<tr>
<th>Date of application</th>
<th>Nematicide</th>
<th>Purpureocillium lilacinum</th>
<th>Furfural</th>
<th>Spirotetramat</th>
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FIG. 1. The effects of the treatment with spirotetramat (KON), Purpureocillium lilacinum (MEL), or furfural (MUL) compared to the untreated control (UC) on the population dynamics of M. incognita J2 in the soil over time in an on-farm trial with Pittosporum tobira. Data are means of four replications. *Different from untreated according to analysis of covariance ($P \leq 0.05$).
schedule for the nematicides used in the on-farm and the small plot trials are shown in Tables 1 and 2, respectively.

**Data collection:** Soil and root samples for nematode extraction were collected 1 wk before the initial treatment application and also 2 wk after each treatment application, except that in the small plot trial, root samples for nematode extraction were taken only once at the end of the experiment. Soil and root samples were taken in February and May for spring application and September and November for fall application. For the greenhouse trial, the nematode and the plant biomass data were collected 2 wk after the final treatment applications were made.

Soil samples for nematode extraction were taken using a 1.9-cm-diameter \( \times \) 10-cm-deep cone sampler. Twelve soil cores were taken from the two center data rows per plot and mixed thoroughly, from which a subsample of 100 cm\(^3\) was taken for nematode extraction by centrifugal sugar flotation method (Jenkins, 1964). The plant-parasitic nematodes extracted were identified and enumerated. Root samples were taken using a coring device measuring 15-cm-diameter \( \times \) 7.6-cm-height (1,202 cm\(^3\)). Six root cores were taken from the two data rows per plot. The roots were manually separated from the soil by sieving, and then roots were weighed, scanned, and their length measured using WinRHIZO software (Regent Instruments, Quebec, Canada). A 10 g root subsample was taken and put onto a modified Baermann funnel (Spaull and Braithwaite, 1979) for 48 hr to extract the J2 and the number per gram (g) of root was enumerated under a compound light microscope.

Yield data from the on-farm trial was collected twice during the experiment, when it was determined to be economically worthwhile by the farm owner. Harvest was collected by the commercial farm’s staff and measured by the number of bunches per plot and total bunch weight per plot. Bunches per plot were manually counted and the weight of each bunch was recorded. Vase life was measured in April 2012, December 2012, and May 2014, for the on-farm plants. To determine the vase life, bunches were immediately immersed in water for 1 min after harvest and then stored in a cooler at 4°C to 6°C in polyethylene bags for 14 d. The stems were recut with clippers and immersed in deionized water in a 900-ml jar and placed under 24°C, 6 a.m. to 6 p.m. day length, and 14 to 16 μmol/m\(^2\) light conditions (Stamps, 1985). The number of days taken for the bunch to lose its verdant appeal when the leaves begin to drop was recorded as its vase life (Stamps, 1985).

Plant growth rate was determined for both of the small plot and the greenhouse trials based on changes in canopy volume/plant. Canopy volume was measured every 3 mon using the formula \( v = \pi (d/2)^2h \), where \( v \) = canopy volume, \( d \) = canopy diameter, and \( h \) = plant height. Roots and leaves from the greenhouse trial were collected for root galling index and biomass estimation, respectively. Roots were assigned a root-gall rating based on a scale of 0 to 10, where 0 = no galling on roots and 10 = maximum galling on roots (galls covering 100% of root surface) following Zeck (1971). The leaves from each plant were removed and placed into paper bags and oven-dried at 50°C for 5 d and leaf biomass was measured as leaf dry weight (g)/plant.

**Data analysis:** The nematode data from the on-farm and the field trials at each sampling date were subjected to analysis of covariance using the SAS 9.4 statistical software (SAS Institute, Cary, NC) with the initial nematode population density as the covariate. The
treatments (furfural, spirotetramat, and *P. lilacinum*) were compared with the untreated control and the *P* value for the comparison was used to determine treatment differences. The means of nematode numbers before and after treatment application were plotted against sampling dates to show the trend of population dynamics over the experimental period.

The effects of the experimental treatments on yield, vase life, average growth rate root galling index, root length, plant biomass, and the nematode data from the greenhouse trial were evaluated after being subjected to mixed model analysis of variance using the SAS 9.4 statistical software. The means of treatments that showed significant differences (*P* ≤ 0.05) were separated using Fisher’s least significant difference for multiple comparisons. For the greenhouse trial, the Dunnett test was used to compare the three treatments, furfural, spirotetramat, and *P. lilacinum*, to the untreated control inoculated with *Meloidogyne* spp. (UC+).

**RESULTS**

**On-farm trial:** Furfural suppressed *M. incognita* J2/100 cm$^3$ of soil compared to the untreated control on the last two sampling dates (September 2013 and November 2013), but no other treatments suppressed *M. incognita* J2 in soil. On one sampling date, spirotetramat increased the number of *M. incognita* J2/100 cm$^3$ of soil compared to the untreated (Fig. 1). Furfural and *P. lilacinum* reduced the population density of *M. incognita* J2/g of root compared to the untreated control in May 2012, whereas spirotetramat increased J2 in roots in September 2012 compared to the untreated (Fig. 2).

Yield differences were observed among the treatments. *Purpureocillium lilacinum* (MEL)-treated plots recorded the least amount of yield in terms of bunch weight at the first harvest and number of bunches per plot in both harvests (Table 3). There were no differences in root lengths among the various treatments (data not shown). Differences in vase life among the treatments were only observed in April 2012 when furfural-treated plants had significantly longer vase life compared to untreated control and *P. lilacinum*-treated plants (Table 4).

**Small plot trial:** There were significant differences among the treatments in *M. incognita* J2 population densities in the soil. In May and September 2013, furfural (MUL) and *P. lilacinum* (MEL), respectively, reduced J2 numbers in the soil compared to the untreated control (*P* ≤ 0.05). Furfural and *P. lilacinum* generally maintained numerically lower J2 numbers in the soil compared to the untreated control (UC) and spirotetramat (KON) for most of the sampling period throughout the study.

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**Fig. 3.** The effects of the treatment with spirotetramat (KON), *Purpureocillium lilacinum* (MEL), or furfural (MUL) compared to the untreated control (UC) on the population dynamics of *M. incognita* J2 in the soil over time in a small plot trial with *Pittosporum tobira*. Data are means of five replications. *Different from untreated according to analysis of covariance (P* ≤ 0.05).

**Fig. 4.** The effects of the treatment with spirotetramat (KON), *Purpureocillium lilacinum* (MEL), or furfural (MUL) compared to the untreated control (UC) on the number of *M. incognita* J2 in the roots at the end of a small plot trial with *Pittosporum tobira*. Data are means of five replications. Bars with common letters are not different based on Fisher’s least significant difference test (*P* ≤ 0.05).
periods, even though the numbers were often not statistically significant \( (P > 0.05) \) (Fig. 3). At the end of the trial, the number of \textit{M. incognita} J2 per gram of root was significantly higher in the untreated control plants compared to all other treatments, although the experimental treatments were not different from each other (Fig. 4). There were no differences among treatments \( (P > 0.05) \) in plant growth rate as determined by changes in plant volume over time (data not shown) or root gall index (Fig. 5).

**Greenhouse trial:** The experimental treatments spirotetramat (KON) and \textit{Purpureocillium lilacinum} (MEL) suppressed \( (P \leq 0.1) \) \textit{Meloidogyne} spp. J2 in the roots compared to the untreated control with nematodes (UC+) in both trials (Fig. 6). Again, KON suppressed \( (P \leq 0.05) \) \textit{Meloidogyne} spp. J2 in soil in trial 1, whereas furfural did in trial 2 compared with UC+. MEL and MUL reduced the J2 numbers in the soil compared to UC+ plants \( (P \leq 0.1) \) in the first trial (Fig. 7). The untreated control plants with nematodes had a higher galling index in trial 2, \( (P < 0.05) \) compared to KON, MEL, and MUL (Fig. 8).

**DISCUSSION**

The results from small plot and on-farm experiments suggest that both furfural (MUL) and \textit{P. lilacinum} (MEL) have the potential to reduce \textit{M. incognita} J2 population densities under field conditions. However, application of these products immediately after planting in the small plot trial provided greater J2 suppression compared to the on-farm trial with an established nematode population. These results suggest that the efficacy of furfural and \textit{P. lilacinum} against root-knot nematodes under field conditions largely depends on the time of application and the extent of nematode establishment. Early application of these products beginning at planting was a more effective postplanting management tactic than late treatment, which is consistent with previous studies where the most successful control of nematodes with these products was achieved via preplant or at-planting application (Schenck, 2004; Kalele et al., 2010).

**FIG. 5.** The effects of the treatment with spirotetramat (KON), \textit{Purpureocillium lilacinum} (MEL), or furfural (MUL) compared to the untreated controls (UC+) on the galling index of \textit{P. tobira} in a small plot trial. Galling index was based on a scale of 0 to 10, where 0 = no galling on roots and 10 = maximum galling on roots. Bars represent the mean of five replications. Bars with common letters are not different based on Fisher’s least significant difference test \( (P \leq 0.05) \).

**FIG. 6.** Effects of treatments with spirotetramat (KON), \textit{Purpureocillium lilacinum} (MEL), and furfural (MUL) compared to untreated controls inoculated with \textit{Meloidogyne} spp. (UC+) on the number of \textit{Meloidogyne} spp. J2/g of \textit{Pittosporum tobira} root in two consecutive greenhouse trials. Data are means of 10 replications. *Different from UC+ according to Dunett test \( (P \leq 0.1) \).

**FIG. 7.** Effects of treatments with spirotetramat (KON), \textit{Purpureocillium lilacinum} (MEL), and furfural (MUL) compared to untreated that was inoculated with \textit{Meloidogyne} spp. (UC+) on the number of \textit{Meloidogyne} spp. J2/100 cm\(^3\) of soil in two consecutive greenhouse trials. Data are means of 10 replications. **, *Different from UC+ according to Dunett test \( (P \leq 0.1 \text{ and } 0.05, \text{ respectively}) \).

**FIG. 8.** Effects of treatments with spirotetramat (KON), \textit{Purpureocillium lilacinum} (MEL), and furfural (MUL) compared to untreated controls inoculated with \textit{Meloidogyne} spp. (UC+) on the galling of \textit{Pittosporum tobira} induced by \textit{Meloidogyne} spp. in two consecutive greenhouse trials. Galling index was based on a scale of 0 to 10, where 0 = no galling on roots and 10 = maximum galling on roots. Data are means of 10 replications. *Different from UC+ according to Dunett test \( (P \leq 0.05) \).
Although marginally effective, neither furfural nor P. lilacinum were consistent in their activity against M. incognita, nor did either product improve the cut foliage yield or plant growth. Spirotetramat neither demonstrated any measurable effects on the J2 population density in the soil nor on cut foliage yield contrasting Smiley et al. (2011), who reported that spirotetramat reduced population density of Heterodera avenae on wheat but did not improve grain yield. Conversely, furfural improved the health of dwarf bermuda grass putting greens even when no nematode reduction was observed (Crow and Luc, 2014), suggesting myriad confounding factors leading to inconsistencies of these products under field conditions.

The lack of a relationship between J2 reduction and yield improvement may be due to the surviving J2 population still being large enough to cause economic damage. Pittosporum tobira is a perennial woody shrub with an extensive lateral root system, so when the endoparasitic nematode gets established, it is difficult to manage. Furfural is short lived in the soil and under suitable environmental and microbial conditions it is rapidly degraded, reducing its efficacy (Wierckx et al., 2011). Furfural may also dissolve Meloidogyne egg masses and stimulate rapid egg hatching leading to a transient increase in J2 numbers (Stein and Van Vuuren, 2006). The furfural concentration at application may be diluted by soil moisture, reducing its efficacy (Luc and Crow, 2013).

To conclude, furfural and P. lilacinum each has some potential for management of M. incognita on cut foliage crops. Application of these products immediately after planting in the small plot trial provided greater J2 suppression compared to the on-farm trial with an established nematode population, suggesting the need for growers to prevent nematode establishment by early treatment application. It is therefore recommended that growers avoid M. incognita-infested fields. Periodic soil sampling for nematode diagnosis and for early treatment application is necessary. However, the use of these chemicals as a lone management option would likely not provide the level of control needed to be economical in this production system. Additional management tactics in an integrated management program may be required to augment these products on the field.

**LITERATURE CITED**


