SUSCEPTIBILITY OF SEVERAL FLORICULTURE CROPS TO THREE COMMON SPECIES OF *MELOIDOGYNE* IN FLORIDA

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ABSTRACT

Kokalis-Burelle, N. and E. N. Rosskopf. 2013. Susceptibility of several floriculture crops to three common species of *Meloidogyne* in Florida. Nematropica 43:164-170.

The current and pending restriction on the use of soil fumigants and other nematicides effective in controlling nematodes in field grown floriculture crops has increased the importance of determining the relative susceptibility of these crops to important species of root-knot nematodes. Greenhouse experiments were performed to assess the susceptibility of several floriculture crops grown in Florida to the three most common species of root-knot nematode, *Meloidogyne arenaria*, *M. incognita*, and *M. javanica*. Root growth and health, as well as nematode galling and egg production were evaluated for *Celosia argentea* (cockscomb), *Delphinium elatum* (larkspur), *Antirrhinum latifolium* (snapdragon), and *Helianthus annuus* (sunflower). A susceptible host, *Solanum lycopersicum* ('Rutgers', tomato), was included in all trials for comparison. Most of the floral crops tested were highly susceptible to all three species of rootknot nematodes. *Delphinium* was not tested for susceptibility to *M. arenaria* but was consistently less susceptible to *M. incognita* and *M. javanica* than the other floral crops tested with those nematode species. Results of these greenhouse trials are consistent with observations from field trials on alternative fumigants conducted in Florida in which low levels of galling by root-knot nematodes were consistently observed on *Delphinium.*

Key words: *Antirrhinum*, *Celosi*a, cockscomb, *Delphinium*, floral crops, Florida, *Helianthus*, larkspur, *Meloidogyne* spp., root-knot nematodes, snapdragon, sunflower

RESUMEN

Kokalis-Burelle, N. y E. N. Rosskopf. 2013. Susceptibilidad de varios cultivos florales a tres especies de *Meloidogyne* frecuentes en Florida. Nematropica 43:164-170.

La restricción actual en el uso de fumigantes del suelo y otros nematicidas para el control de nematodos en cultivos florales a campo, ha mostrado la importancia de determinar la susceptibilidad de estos cultivos a las principales especies de nematodos del nudo de la raíz. En condiciones de invernáculo, se evaluó la susceptibilidad de varios cultivos florales a la agresión de *Meloidogyne arenaria*, *M. incognita* y *M. javanica* en Florida. Se tuvieron en cuenta el crecimiento de las raíces, el estado general de las plantas, así como las agallas ocasionadas por los nematodos y producción de huevos en *Celosia argentea* (cresta de gallo), *Delphinium elatum* (larkspur), *Antirrhinum latifolium* (boca de dragón) y *Helianthus annuus* (girasol). Un huésped susceptible, *Solanum lycopersicum* ('Rutgers, tomate), se incluyó en todos los ensayos para comparación. La mayoría de los cultivos considerados fueron muy susceptibles a las tres especies de nematodos. La susceptibilidad de *D. elatum* no fue evaluada para *M. arenaria*, pero fue menos susceptibles a *M. incognita* y *M. javanica* que los otros cultivos florales. Los resultados obtenidos se corresponden con las observaciones de ensayos equivalentes a campo realizados en Florida con fumigantes alternativos, en los que se observaron bajos niveles de agallas debidas a nematodos en *Delphinium*.

Palabras clave: *Celosia argéntea*, *Delphinium elatum*, *Antirrhinum latifolium*, *Helianthus annuus*, Florida, *Meloidogyne* spp, susceptibilidad.

INTRODUCTION

Production of annual flowering plants for use as cut flowers is an important agricultural industry in Florida. The floriculture industry remains highly dependent on soil fumigation for control of nematodes, other soilborne pests, and weeds. The phase-out of methyl bromide (Montreal Protocol, 1992; WMO, 2011), and increased restrictions on all chemical fumigants (USEPA, 2013), leave few nematode control options for commercial producers of cut flowers. Research on chemical alternatives for floriculture crops in Florida included early work with dimethyl disulfide (DMDS) as well as other alternative fumigants (Church *et al*., 2004; Gilreath and West, 1996; Gilreath *et al*., 2005; Kokalis-Burelle *et al*., 2006; Kokalis-Burelle *et al*., 2010a; Kokalis-Burelle *et al*., 2010b; McSorley and Wang, 2002; McSorley *et al*., 2004a; McSorley *et al*., 2004b). Due to the limitations in efficacy, and restrictions on application of these chemicals, such as buffer zone requirements, most of these control measures cannot be applied in commercial floriculture production in Florida (Rosskopf *et al*., 2005). This lack of chemical control options has necessitated a greater understanding of the susceptibility of important floriculture crops to common species of root-knot nematodes (*Meloidogyne*), which are ubiquitous in Florida. The large number and variety of floriculture crops produced in Florida makes screening for nematode susceptibility a huge undertaking, but an important one, not only to floriculture producers, but also to plant breeders and consumers. While ornamental plant breeders often focus on desirable horticultural traits for new cultivars, nematode resistance is likely to increase in importance, especially due to the lack of nematode control measures available to consumers. This research was undertaken to determine the relative susceptibility of several important floriculture crops to three prevalent species of root-knot nematode found in Florida.

MATERIALS AND METHODS

Greenhouse trials were conducted at the USDA, ARS, U.S. Horticultural Research Lab in Ft. Pierce, FL to evaluate root-knot nematode susceptibility of several floriculture crops typically produced in-ground in Florida for cut-flower markets. Nematode reproduction along with root growth and health were assessed for all floriculture crops tested following inoculation with *Meloidogyne arenaria*, *M. incognita*, or *M. javanica*.

Floral Crops Tested

Floral crops evaluated for susceptibility to two or more species of root-knot nematodes were *Celosia argentea* (Celosia 'Bombay'; Fred C. Gloeckner & Co., Inc, New York, NY), *Celosia argentea* (Celosia 'Kurume'; Vis Seed company, Inc., Arcadia, CA), *Delphinium elatum* (Larkspur 'Belladonna'; Fred Gloeckner & Co., Inc, New York, NY), *Delphinium elatum* (Larkspur 'Bellamosum'; Fred C. Gloeckner & Co., Inc, New York, NY), *Antirrhinum latifolium* (Snapdragon 'Ivory White'; Fred C. Gloeckner & Co., Inc, New York, NY), *Antirrhinum latifolium* (Snapdragon 'Potomac'; Fred C. Gloeckner & Co., Inc, New York, NY), and *Helianthus annuus* (Sunflower 'Procut Orange'; Fred C. Gloeckner & Co., Inc, New York, NY). All floral crops tested were compared to the highly susceptible host of all three nematode species, *Solanum lycopersicum* (tomato 'Rutgers', Totally Tomatoes, Randolph, WI) in all trials.

Plant Propagation

Germination and growth rates were predetermined for all flower seed used in these experiments. Seed were planted to simultaneously obtain seedlings with at least one true-leaf at the time of nematode inoculation. Seed were planted into 128 cell flats containing a mixture of washed builder's grade sand and steamed Fafard® germination mix (Conrad Fafard Inc., Agawam, MA) at a ratio of 0.4 cubic meter of Fafard mix to 91 kg sand. This mix will be referred to as soil and was used to conduct all experiments. Two 15-cm round pots were nested together with Kimwipes (Kimberly-Clark®) placed between the pots to eliminate loss of the soil during watering. Pots were then filled with approximately 1.5 L of soil. When seedlings reached the 1-2 true-leaf stage, they were transplanted into the 15-cm pots, watered daily, and fertilized once a week with 20-10-20 at 250 ppm nitrogen (J. R. Peters, Inc., Allentown, PA). Plants were separated on the greenhouse bench by 6-mm-thick polycarbonate dividers to avoid cross contamination of nematodes among pots after inoculation. Each plant was placed in a grid square which measured 30 cm × 30 cm. Each nematode species was tested in separate experiments and each experiment was repeated. Treatments in each experiment consisted of plant species and each treatment was replicated four times for each experiment. Plants were sprayed at label rates on an as-needed basis to control powdery mildew (Bayleton®, triadimefon, Bayer CropScience; Cabrio® pyraclostrobin, BASF Corp., Research Triangle Park, NC), mites (Horticultural oil, Abamectin), aphids (M-Pede, propylene glycol:potassium hydroxide, Dow AgroSciences, Indianapolis, IN), thrips and whiteflies (Safari®, Dinotefuran, N-methyl-N'-nitro-N"- [(tetrahydro-3-furanyl)methyl] guanidine, Valent U.S.A. Corp.;Talstar®, bifenthrin, FMC Corp. Philadelphia, PA).

Nematode Inoculation

Nematode inoculum was extracted from pure cultures of *M. arenaria*, *M. incognita*, and *M. javanica*, which were maintained in the USDA, ARS greenhouse in Ft. Pierce, FL on tomato (*Solanum lycopersicum*, 'Rutgers'). Nematode cultures were checked for purity

and their species identification confirmed based on their esterase phenotypes (PhastSystem,™ GE Healthcare) prior to initiation of experiments (Esbenshade and Triantaphyllou, 1985, 1990). *Meloidogyne* spp. eggs were extracted from tomato roots by cutting galled roots into 2-3-mm pieces, and placing root pieces in a 500-ml Nalgene flask containing 100 ml of a 10% NaOCl solution. Flasks were covered and placed on a wrist action shaker for 2 min. Roots and liquid were then poured through nested stainless steel sieves of 80 mesh (180 μm), 325 mesh (45 μm), and 500 mesh (25 μm) and rinsed. Eggs were collected on the 500 mesh sieve and rinsed into a 100 ml beaker. Eggs were quantified by placing one ml of the agitated water solution containing eggs onto a nematode counting slide (Chalex Corp., Issaquah, WA). The final concentration of eggs was adjusted to 1000 eggs/ml. Plants were inoculated with nematode eggs by pipetting one ml of the egg suspension into a 2-cm-deep impression in the soil mixture approximately 1.5-2.0 cm from the plant stem. Inoculation sites were covered by pressing the growing mix into place, and plants were lightly watered. All experiments were maintained in the greenhouse for eight weeks. Experiments for *M. arenaria* were conducted during the period from 24 August, 2010 – 2 November, 2010, with greenhouse temperatures ranging from daily highs of 27.7°C-28.3°C and nightly lows ranging from 24.4°C-26.1°C. Experiments for *M. javanica* were conducted during the period between 12 October, 2010 - 20 December, 2010, with greenhouse temperatures ranging from daily highs of 27.2°C-28.3°C and nightly lows between 18.3°C-24.4°C. Experiments with *M. incognita* were performed from 26 October, 2010 - 18 January, 2011, with greenhouse temperatures ranging from daily highs of 26.1°C-27.2°C and nightly lows between 18.3°C-21.1°C. The implementation and termination of all experiments was staggered 2 weeks to facilitate data collection at the end of experiments.

Plant Evaluation

At the end of all experiments, fresh root weight was recorded and roots were evaluated for galling and root condition. Root condition was used as a general indicator of root disease and was assessed using a subjective scale of 0 to 5 with 0-1 = clean/healthy roots; 1-2 = up to 25% discolored/diseased roots, 2-3 = 25% to 50% discolored/diseased roots, 3-4 = 50% to 75% discolored/diseased roots, 4-5 = 75% - 100% discolored/diseased roots. Root galling was assessed using a root gall index based on Bridge and Page (1980) using a scale of 0 to 10; with 0-1 = 0 galls to approximately 20 individual galls; 1-2 = 20-40 individual galls; 2-3 = 40-60 individual galls; 3-4 = 60-80 individual galls; 4-5 more than 100 individual galls; 5-6 = compound galls on approximately 10% of the root system; 6-7 = compound galls on approximately 20% of the roots system; 7-8 = compound galls on approximately 40% of the root system; 8-9 = compound galls on approximately 80% of the root system; 9-10 = compound galls on approximately 80-100% of the root system. Nematode juveniles were extracted from half of the root system and from 100 cm^3 soil using Baermann funnels, and counted microscopically. Nematode reproduction was calculated at the end of experiments by extracting eggs from the second ½ of the root system. Eggs were extracted from roots of all plants as described above for nematode inoculum production. The number of eggs extracted from roots at the end of experiments was used as a measure of nematode reproduction and is reported as eggs/g root.

Statistical Analysis

Six experiments were conducted using a completely randomized design with four replications for each treatment. Data were analyzed according to standard statistical procedures including SAS General Linear Models (GLM) and Least Significant Difference (LSD) procedures (SAS, Cary, NC). Results of replicated trials were subjected to Student's t-tests before being combined. Unless otherwise stated, effects and differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Meloidogyne arenaria

The numbers of *M. arenaria* juveniles isolated from both roots and soil were not statistically different among the crops tested due to high levels of variability among replications (Table 1). Nematode reproduction for *M. arenaria*, expressed as eggs/g root, was highest on tomato and lowest on *Celosia* cultivar 'Bombay'. This may have been influenced by the very low root weights, and high level of root disease (expressed as root condition) for that *Celosia* cultivar. Galling was highest on both cultivars of *Celosia*, which was comparable to galling on tomato (Table 1). *Helianthus* had the highest number of eggs/g root for *M. arenaria*, significantly higher than both *Celosia* cultivars (Table 1). However, galling was least severe on *Helianthus* compared with all other plants tested for *M. arenaria*. No seedlings of either *Delphinium* cultivar were available for testing against *M. arenaria*. Results for *Celosia* and *Antirrhinum* for *M. arenaria* egg production and galling were similar to those reported by McSorley and Frederick (1994) although different cultivars of those hosts were tested.

Meloidogyne incognita

Delphinium cultivar 'Belladonna' (the only *Delphinium* cultivar tested against *M. incognita*) supported low numbers of *M. incognita* J2 in roots and soil, and had low levels of galling on roots. These observations for galling and root-knot nematode J2 isolated from roots and soil for *Delphinium* are

Table 1. Root-knot nematode juveniles (J2) in roots and soil, nematode reproduction, plant root weight, plant root condition, and nematode gall index values for floral crop susceptibility to *Meloidogyne arenaria*.

	M. arenaria (J2/g root)	*M. arenaria* (J2/100 cm^3 soil)	Eggs/g root[w]	Root weight (g)	Root condition[x]	Gall index[y]
Solanum lycopersicum Tomato 'Rutgers'	30.8	559.9	3415 ab[z]	29.3 b	1.3 cd	6.1 a
Celosia argentea Celosia 'Bombay'	21.9	29.8	1811 b	7.3 b	2.5 ab	6.2 a
Celosia argentea Celosia 'Kurume'	20.7	82.2	1716 b	82.5 a	3.4 a	6.3 a
Antirrhinum latifolium Snapdragon 'Ivory White'	40.8	18.4	4822 ab	7.2 b	0.7 d	5.5 b
Helianthus annuus Sunflower 'Procut orange'	32.3	109.1	9367 a	9.9 b	1.9 bc	4.7 c
LSD (0.05)	NS	NS	5983	50.7	1.0	0.6

[w]Nematode eggs were extracted from one half of roots system.
[x]Root condition: 0 = clean, white roots, 5 = completely rotted and discolored roots.
[y]Gall index: 0 = no galling, 10= complete galling (Bridge and Page, 1980).
[z]Means with the same letter are not significantly different according to Fisher's protected least significant difference procedures (LSD) ($P < 0.05$).

Table 2. Root-knot nematode juveniles (J2) in roots and soil, nematode reproduction, plant root weight, plant root condition, and nematode gall index values for floral crop susceptibility to *Meloidogyne incognita*.

	M. incognita (J2/g root)	*M. incognita* (J2/100 cm^3 soil)	Eggs/g root[w]	Root weight (g)	Root condition[x]	Gall index[y]
Solanum lycopersicum Tomato 'Rutgers'	54.6 a[z]	9.9 b	6049 b	25.2 a	1.1 d	6.2 b
Celosia argentea Celosia 'Bombay'	58.6 a	14.2 b	3085 b	5.1 cd	2.8 bc	5.6 bc
Celosia argentea Celosia 'Kurume'	10.0 bc	12.8 b	1722 b	26.0 a	3.6 ab	5.4 bc
Delphinium elatum Larkspur 'Belladonna'	8.1 c	5.7 b	7248 b	2.7 cd	2.4 c	0.3 d
Antirrhinum latifolium Snapdragon 'Ivory White'	24.3 abc	0.0 b	2375 b	15.7 b	0.3 d	5.1 bc
Antirrhinum latifolium Snapdragon 'Potomac'	47.0 ab	66.6 a	3803 b	6.8 c	0.7 d	4.5 c
Helianthus annuus Sunflower 'Procut orange'	9.5 bc	1.4 b	25,578 a	0.6 d	4.0 a	9.7 a
LSD (0.05)	38.4	37.3	11,459	5.3	1.1	1.3

[w]Nematode eggs were extracted from one half of roots system.
[x]Root condition: 0 = clean, white roots, 5 = completely rotted and discolored roots.
[y]Gall index: 0 = no galling, 10= complete galling (Bridge and Page, 1980).
[z]Means with the same letter are not significantly different according to Fisher's protected least significant difference procedures (LSD) ($P < 0.05$).

Table 3. Root-knot nematode juveniles (J2) in roots and soil, nematode reproduction, plant root weight, plant root condition, and nematode gall index values for floral crop susceptibility to *Meloidogyne javanica.*

	M. javanica (J2/g root)	*M. javanica* (J2/100 cm^3 soil)	Eggs/g root[w]	Root weight (g)	Root condition[x]	Gall index[y]
Solanum lycopersicum Tomato 'Rutgers'	130.2 a[z]	156.1 ab	2700 a	18.2 a	2.1 ab	5.4 a
Celosia argentea Celosia 'Bombay'	46.2 bc	49.6 ab	2757 a	13.2 b	3.1 a	4.5 ab
Celosia argentea Celosia 'Kurume'	27.3 bc	160.2 ab	2146 a	22.9 a	3.1 a	5.0 a
Delphinium elatum Larkspur 'Belladonna'	90.3 ab	2.9 b	11,432 a	4.9 c	3.2 a	1.3 d
Delphinium elatum Larkspur 'Bellamosum'	77.5 abc	6.5 b	3339 a	4.2 c	2.3 ab	0.7 d
Antirrhinum latifolium Snapdragon 'Ivory White'	7.7 c	7.1 b	4668 a	6.4 c	2.3 ab	3.1 bc
Antirrhinum latifolium Snapdragon 'Potomac'	85.0 ab	194.2 a	992 a	7.3 c	2.3 ab	4.0 ab
Helianthus annuus Sunflower 'Procut orange'	29.0 bc	69.5 ab	8609 a	6.8 c	1.2 b	1.5 cd
LSD (0.05)	72.4	165.0	13,020	4.7	1.2	1.7

[w]Nematode eggs were extracted from one half of roots system.
[x]Root condition: 0 = clean, white roots, 5 = completely rotted and discolored roots.
[y]Gall index: 0 = no galling, 10= complete galling (Bridge and Page, 1980).
[z]Means with the same letter are not significantly different according to Fisher's protected least significant difference procedures (LSD) ($P < 0.05$).

consistent with previous observations in field trials for this crop (Rosskopf *et al*., 2010), which does not appear to be highly susceptible to *Meloidogyne* spp. In contrast, *Helianthus* cultivar 'Procut Orange' had similarly low number of *M. incognita* J2 in roots and soil, but produced extremely high levels of root galling (Table 2). Extremely low root weights for *Helianthus* were due to high levels of galling and root disease. The *Helianthus* roots that were recovered were highly galled, although few *M. incognita* J2 were recovered from roots and soil due to the advanced state of root decay. This high level of decay is illustrated by high root condition (disease) ratings. However, very high numbers of *M. incognita* eggs were recovered per gram of Helianthus root tissue. *Meloidogyne incognita* eggs isolated per gram of root were higher for *Helianthus* than all other plants tested (Table 2).

Although different cultivars of *Celosia* and *Antirrhinum* were tested in previous research by McSorley and Frederick (1994), both *Celosia* and *Antirrhinum* were found to be susceptible to *M. incognita* galling, and reportedly, had high levels of egg production. Wang and McSorley (2005) evaluated several cut flower cultivars for susceptibility to two races of *M. incognita* and found that *Antirrhinum* cultivar 'Potomac Royal' had significantly higher gall index values than *Delphinium* cultivar 'Qis White Cut'. No method to assess nematode reproduction was used in that research. Although the cultivars evaluated for each cut flower type differed from those reported here, similar levels of galling, i.e., high for *Antirrhinum* and low for *Delphinium* were reported (Table 2). Walker *et al*. (1994) tested several bedding plant cultivars for susceptibility to *M. incognita* race 3, including *Celosia*. The *Celosia* cultivars tested in those studies belonged to the species *C. plumosa*, which differs from the species tested here. Cultivars of *C. plumosa* tested were 'Apricot Brandy', 'Castle Scarlet', 'Fireglow', and 'Kimono Cream'. All the *C. plumosa* cultivars tested were highly susceptible to *M. incognita*, producing high levels of galling and infection ratings (Walker *et al*., 1994). Nematode reproduction was not assessed in those studies.

Meloidogyne javanica

The highest number of plant cultivars was available for *M. javanica* susceptibility tests, which was fortunate because information on susceptibility to *M. javanica* for floriculture crops is more limited than the other two species of nematodes tested (Walker *et al*., 1994; Wang and McSorley 2005). Two *Delphinium* cultivars,

'Belladonna' and 'Bellamosum', were tested for susceptibility to *M. javanica*. Although both *Delphinium* cultivars had numbers of *M. javanica* in roots which were similar to tomato, and among the highest numbers recorded for this species of rootknot nematode in these trials, the numbers of J2 of *M. javanica* in soil were very low for both *Delphinium* cultivars tested and were reflected in the lowest gall ratings for all crops tested for *M. javanica* (Table 3). In contrast, *Antirrhinum* cultivar 'Ivory White' had very low numbers of *M. javanica* associated with both roots and soil, yet had moderately high galling. Both cultivars of *Celosia* tested exhibited high levels of galling in response to inoculation with *M. javanica*, similar to results for *M. arenaria* and *M. incognita*. In previous research, *Celosia* was reported to be slightly less susceptible to *M. javanica* than to *M. arenaria* and *M. incognita* (McSorley and Frederick, 1994). In the experiments reported here, *M. javanica* did produce slightly less severe gall index ratings on *Celosia* than *M. arenaria* or *M. incognita*, although results were not compared statistically across nematode species (Tables 1-3).

The susceptibility of floriculture crops to species of root-knot nematodes can differ among crop cultivars and nematode species. Nematode reproduction, as measured in these studies by egg production, was not always directly correlated to root gall index values for the plant cultivars and nematode species tested. This indicates that gall rating may not be an accurate assessment of nematode susceptibility in some of these crops, and may need to be evaluated for reliability in other important crops.

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