RESEARCH NOTE/NOTA INVESTIGATIVA

ASSESSMENT OF *PYCNOSTACHYS URTICIFOLIA* SUSCEPTIBILITY TO THREE SPECIES OF *MELOIDOGYNE*

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ABSTRACT

Kokalis-Burelle, N., M. E. Hilf, and E. N. Rosskopf. 2013. Assessment of *Pycnostachys urticifolia* susceptibility to three species of *Meloidogyne*. Nematropica 43:40-43.

An exotic ornamental shrub, *Pyncostachys urticifolia*, growing in a residential planting in Orange County, Florida, was found to have root galling typical of root-knot nematodes (*Meloidogyne* spp.). Several gravid female nematodes were isolated from roots and identified as *M. arenaria* based on their esterase phenotype. Single egg mass cultures were produced and reinoculated onto *P. urticofilia* grown from seed in sterilized builder's sand in the greenhouse. The identification of subsequent egg masses was confirmed as *Meloidogyne arenaria* based on the esterase phenotype. Greenhouse experiments were then performed to assess susceptibility of *P. urticifolia* to two additional root-knot nematode species commonly found in Florida, *M. incognita* and *M. javanica*. Nematode galling and reproduction were evaluated for all three *Meloidogyne* spp. in replicated trials with *Solanum lycopersicum* (tomato cv. Rutger's) included as a susceptible control. Root galling was similar for *P. urticifolia* and *S. lycopersicum* for all three species of *Meloidogyne* tested, and the number of eggs produced per gram of root tissue was similar for *S. lycopersicum* and *P. urticifolia* for two of the three species tested, demonstrating that *P. urticifolia* is highly susceptible to *M. arenaria, M. incognita*, and *M. javanica.*

Key Words: Florida, *Meloidogyne* spp., *Pycnostachys urticifolia*, root-knot nematodes

RESUMEN

Kokalis-Burelle, N., M. E. Hilf, and E. N. Rosskopf. 2013. Evaluación de la susceptibilidad de *Pycnostachys urticifolia* a tres especies de *Meloidogyne*. Nematropica 43:40-43.

Un arbusto ornamental exótico, *Pyncostachys urticifolia*, utilizado en jardines residenciales en el condado Orange de Florida, se encontró con agallas típicas a las causadas por nematodos agalladores (*Meloidogyne* spp.). Se aislaron varias hembras con huevos de las raíces y se identificaron como *M. arenari*a basados en el fenotipo de esterasas. Se produjeron cultivos de masas de huevos individuales y se inocularon plantas de *P. urticofilia* obtenidas a partir de semilla y sembradas en el invernadero en arena estéril. La identificación de las hembras obtenidas a partir de este inóculo confirmaron a *Meloidogyne arenaria* basado en fenotipos de esterasas. Se efectuaron ensayos de invernadero para evaluar la susceptibilidad de *P. urticifolia* a otras dos especies de nematodos agalladores que se encuentran comúnmente en Florida, *M. incognita* y *M. javanica*. Se evaluó el agallamiento y la reproducción de las tres especies en ensayos replicados y usando *Solanum lycopersicum* (tomate cv. Rutger's) como control susceptible. El agallamiento en *P. urticifolia* fue similar al de *S. lycopersicum* para las tres especies de *Meloidogyne evaluadas*, y la cantidad de huevos producidos por gramo de raíz fue similar para *S. lycopersicum* y *P. urticifolia* en dos de las tres especies evaluadas, demostrando que *P. urticifolia* es altamente susceptible a *M. arenaria, M. incognita* y *M. javanica*.

Palabras clave: Florida, *Meloidogyne* spp., nematodos agalladores, *Pycnostachys urticifolia.*

For many ornamental crops imported and grown in the United States, host status to root-knot nematodes (*Meloidogyne* spp.) remains unknown. *Pycnostachys urticifolia* Hook is an aromatic perennial herb or shrub native to many countries in Africa including Kenya, Tanzania, Malawi, Mozambique, Zimababwe, and South Africa. *Pycnostachys urticifolia* is produced and sold as an ornamental plant in the U.S., but little is known about nematode and disease susceptibility of this plant.

Trials were initially undertaken to identify the species of root-knot nematode (*Meloidogyne*) causing galls on *P. urticifolia* (Annie's Annuals & Perennials, Richmond, CA) in a landscape planting in Orange County, FL. Samples were notable due to the unusual pink coloration of the gravid females in the roots (Fig. 1). Following the initial identification of the nematode species, controlled research trials were conducted to determine the susceptibility of this host to three common species of *Meloidogyne* found in Florida, M. arenaria, *M. incognita*, and *M. javanic*a, compared with the highly susceptible host to all three species, Rutger's tomato (*Solanum lycopersicum*).

Original nematode isolate identification: Gravid female root-knot nematodes were identified as *M. arenaria* from field collected *P. urticifolia* plants based on their esterase phenotype (PhastSystem,™ GE Healthcare) (Brito *et al.*, 2008). Plants were then maintained in the greenhouse for original nematode isolates and seed production.

Plant propagation for susceptibility trials: *Pycnostachys urticifolia* plants were propagated in the greenhouse from seed collected in the field. The susceptible control for all *Meloidogyne* species tested was *Solanum lycopersicum* ('Rutgers' Tomato, Totally Tomatoes, Randolph, WI). Seeds were planted into 128-cell flats containing a mixture of washed and steamed 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 will be referred to as soil and was used to conduct all experiments. Two 15-cm round pots were nested together and filled with approximately 1.5 L of soil. When seedlings reached the 1-2 true leaf stage, they were transplanted into the 1.5 L pots containing soil, watered daily, and fertilized once a week with 20-10-20 N-P-K at 250 ppm nitrogen (J. R. Peters, Inc., Allentown, PA). Plants were separated on the greenhouse bench by 6 mm thick polycarbonate dividers with each plant in a grid square measuring 30 cm × 30 cm prior to inoculation with nematodes described below. 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 whitefly (Safari®, Dinotefuran, N-methyl-N'-nitro-N"- [(tetrahydro-3-furanyl)methyl] guanidine, Valent U.S.A. Corp.;Talstar®, bifenthrin, FMC Corp. Philadelphia, PA).

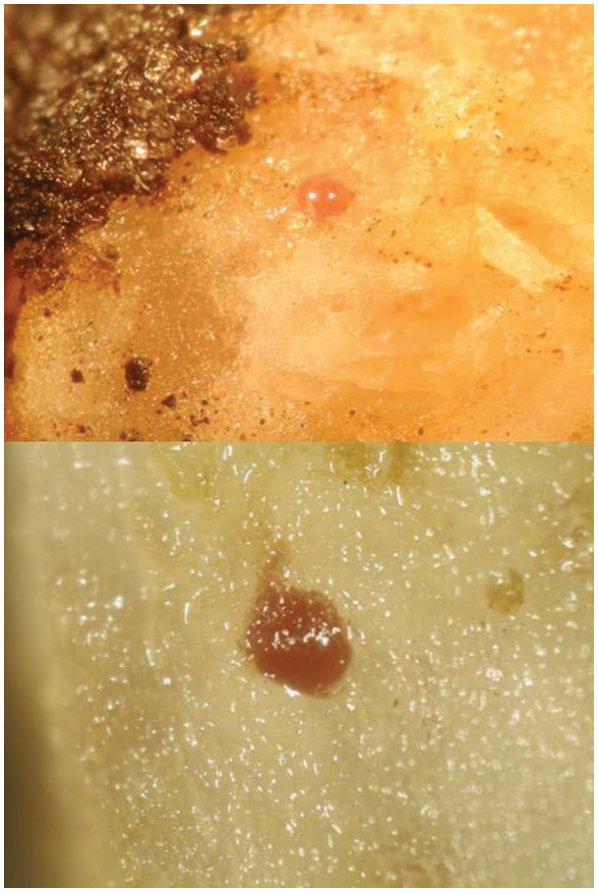

Fig. 1. Micrographs of *Meloidogyne arenaria* females exhibiting pink coloration in *Pycnostachys urticifolia* roots.

Nematode inoculation: Nematode inoculum was extracted from pure cultures of *M. arenaria, M. incognita,* and *M. javanica*, maintained in the greenhouse on tomato (*S. lycopersicum*, 'Rutgers'). *Meloidogyne* spp. eggs were extracted from tomato roots by cutting galled roots into 2-3 mm pieces, and placing root pieces in a stoppered 500 ml nalgene flask containing approximately 100 mls of a 10% commercial bleach solution. Flasks were immediately placed on a wrist action shaker for 2 min. Roots and liquid were then immediately poured through nested stainless steel sieves of 80 mesh (180 µm), 325 mesh (45 µm), and 500 mesh (25 µm) and rinsed thoroughly. Eggs were collected on the 500 mesh sieve and were counted after placing one ml of agitated solution on 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 egg suspension into a 2-cm deep impression in the soil approximately 1.5-2.0 cm from the plant stem. Inoculation sites were covered and plants were lightly watered. Experiments were maintained in the greenhouse for 8 weeks.

Plant evaluation: Eight weeks after nematode inoculation, plants were harvested and fresh root weight was recorded. 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

Table 1. Effects of three species of root-knot nematodes on root growth and root condition of *Solanum lycopersicum* and *Pycnostachys urticifolia*, and effects of host plants on nematode juvenile (J2) numbers in roots and soil, nematode gall index values, and nematode eggs/g root tissue for *Meloidogyne arenaria, M. incognita*, and *M. javanica*.

Meloidogyne arenaria						
	Root weight	J2/g root	J2/100 cm^3 soil	Gall index[y]	Root condition	Eggs/g roots
Solanum lycopersicum	29.3	30.8	559.9	6.1	1.3	3418
Pycnostachys urticifolia	73.9	4.3	39.7	5.9	2.2	2695
	**[z]	**	ns	ns	ns	ns
Meloidogyne incognita						
	Root weight	J2/g root	J2/100 cm^3 soil	Gall index	Root condition	Eggs/g roots
Solanum lycopersicum	25.2	54.6	9.9	6.2	1.1	6049
Pycnostachys urticifolia	55.7	15.1	7.1	6.4	2.6	3433
	**	**	ns	ns	**	ns
Meloidogyne javanica						
	Root weight	J2/g root	J2/100 cm^3 soil	Gall index	Root condition	Eggs/g roots
Solanum lycopersicum	18.2	130.2	311.9	5.4	2.1	2700
Pycnostachys urticifolia	45.6	17.8	195.6	5.4	2.0	1217
	**	**	ns	ns	ns	**

[y] Gall index: 0 = no galling, 10= complete galling (Bridge and Page, 1980).
[z] Means in columns are significantly different according to least significant difference procedures (LSD) ($P < 0.05$)

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. Nematodes were extracted from roots and soil using Baermann funnels, and counted microscopically. For soil, a 100 cm^3 subsample was taken from thoroughly mixed soil in each pot and placed in tissue paper. For roots, approximately 10 g of root tissue, which had been dissected into approximately 2 cm sections and mixed thoroughly, were subsampled from each root system and placed in tissue. Soil and root samples were placed in funnels containing water for 60 – 72 hrs. Nematode reproduction was quantified and expressed as the number of eggs extracted per gram of fresh root tissue at the end of the experiments. Egg extractions were performed on a 10 g subsample of roots as described above for nematode inoculum preparation.

Statistical analysis: All experiments were conducted using a completely randomized design. Data were analyzed according to standard statistical procedures including SAS General Linear Models (GLM) and Least Significant Difference (LSD) procedures (SAS, Cary, NC). Unless otherwise stated, effects and differences were considered significant at $p < 0.05$.

Effects of *Meloidogyne* spp. on plant root health assessed at the end of experiments showed that root weights were higher for *P. urticifolia* than for *S. lycopersicum* in all tests (Table 1). Nematodes isolated from roots were also significantly higher per gram of root tissue in *S. lycopersicum* than *P. urticifolia* for all three nematode species, while nematodes isolated per 100 cm^3 of soil, while numerically higher for *S. lycopersicum* for all three nematode species, did not differ statistically (Table 1). Despite significantly higher numbers of nematode J2 in *S. lycopersicum* roots compared with *P. urticifolia* roots, galling was similar for both hosts for all species of *Meloidogyne* tested (Table 1). Similarities in

gall ratings indicate comparable levels of susceptibility to root damage from all three nematode species tested for these hosts. However, because *P. urticifolia* root systems were similar in structure but significantly larger than *S. lycopersicum* roots (2:1 ratio), it is likely that the potential of *P. urticifolia* to amplify nematode populations in root debris remains high. Root condition, a measure of root disease, did not differ between *S. lycopersicum* and *P. urticifolia* for either *M. arenaria* or *M. javanica*. However, for *M. incognita*, root condition for *P. urticifolia* was significantly worse than for *S. lycopersicum* (Table 1). Nematode reproduction was assessed by quantifying the number of eggs extracted per g of root tissue at the end of experiments. The number of eggs/g root tissue for both *M. arenaria* and *M. incognita* did not differ between *S. lycopersicum* and *P. urticifolia* (Table 1). However, the number of *M. javanica* eggs was significantly higher for *S. lycopersicum* than *P. urticifolia*.

It is important to document nematode susceptibility of exotic ornamental plants being propagated and distributed within the U.S. Host status of ornamental plants to species of root-knot nematode is also useful information in the regions where these plants originate. The susceptibility and reproductive potential of these plants to important parasitic nematodes such as *Meloidogyne* spp. relative to a known susceptible host such as tomato is useful. This information can assist nurserymen in selecting material for propagation and distribution, as well as horticultural professionals and homeowners in choosing appropriate plants for their landscape needs.

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