RESEARCH/INVESTIGACIÓN

POST-PENETRATION RESPONSE OF *MELOIDOGYNE INCOGNITA* ON *CUCURBITA FOETIDISSIMA* (BUFFALO GOURD)

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ABSTRACT

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Buffalo gourd, *Cucurbita foetidissima*, is a perennial cucurbit that is commonly found in the semi-arid region of the southwestern United States and Mexico. Few studies have investigated the post-penetration response of *Meloidogyne incognita* on *Cucurbita*, and there is no information on any perennial species of *Cucurbita* such as buffalo gourd. The objectives of this study were to determine the susceptibility of buffalo gourd to *M. incognita* and evaluate the post-penetration response of *M. incognita* on buffalo gourd. Fewer second-stage juveniles (J2) penetrated the root system of buffalo gourd and horned cucumber (*Cucumis metuliferus*), which served as a resistant control, from 7 to 21 days after inoculation (DAI) compared to cucumber (*Cucumis sativus*), the susceptible control. Nematode development was delayed at 14 and 21 DAI in buffalo gourd compared to cucumber, but not as delayed as that observed on horned cucumber. Of the J2 that penetrated the root systems, fewer emerged from buffalo gourd and cucumber at 3 and 4 DAI than horned cucumber. Fecundity of the *M. incognita* females was lower on buffalo gourd and horned cucumber than cucumber. Buffalo gourd would be considered to be susceptible to *M. incognita* because of a lower percentage of J2 emigration from the root system than the resistant control and because the individuals that remained in the root developed into egg-laying females. However, although both buffalo gourd and cucumber were susceptible to *M. incognita*, buffalo gourd was a much less suitable host than cucumber.

Key words: Buffalo gourd, cucumber, *Cucumis metuliferus*, cucurbit, *Cucurbita foetidissima*, horned cucumber, *Meloidogyne incognita*, southern root-knot nematode, susceptibility.

RESUMEN

Miller, J. G., y T. R. Faske. 2015. Respuesta post-penetración de *Meloidogyne incognita* en *Cucurbita foetidissima* (calabacilla loca). Nematropica 45:178-183.

La calabacilla loca, *Cucurbita foetidissima*, es una cucurbitácea perenne que se encuentra frecuentemente en la región semi-árida del sudoeste de los Estados Unidos de América y en México. Pocos estudios han investigado la respuesta post-penetración de *Meloidogyne incognita* en *Cucurbita*, y no existe información de ninguna de las especies perennes de *Cucurbita*, como la calabacilla loca. Los objetivos de este estudio fueron determinar la susceptibilidad de la calabacilla loca a *M. incognita* y evaluar su respuesta post-penetración en la calabacilla loca. Entre los 7 y 21 días post-inoculación (dpi), pocos juveniles de segundo estado (J2) penetraron en el sistema radical de la calabacilla loca o del pepino africano o kiwano (*Cucumis metuliferus*), el cual sirvió como control resistente, en comparación con un control susceptible, pepino (*Cucumis sativus*). El desarrollo del nematodo se retrasó a los 14 y 21 dpi en la calabacilla loca, comparado con el pepino, pero no se retrasó tanto como lo observado en el pepino africano. De los J2 que habían penetrado en el sistema radical, a los 3 y 4 días tras la inoculación, emergieron menos de la calabacilla loca y del pepino, que del pepino africano. La fecundidad de las hembras de *M. incognita* fue menor en la calabacilla loca y en el pepino africano que en el pepino. La calabacilla loca podría ser considerada susceptible a *M. incognita* debido al menor porcentaje de emigración de J2 desde el sistema radical que en el control resistente y a que los individuos que permanecieron en la raíces se desarrollaron hasta hembras productoras de huevos. No obstante, aunque tanto la calabacilla loca como el pepino fueron susceptibles a *M. incognita*, la calabacilla loca fue peor hospedante que el pepino.

Palabras clave: Calabacilla loca, *Cucumis metuliferus*, cucurbitáceas, *Cucurbita foetidissima*, *Meloidogyne incognita*, nematodo formador de agallas en las raíces, pepino, pepino africano, susceptibilidad.

INTRODUCTION

The southern root-knot nematode, *Meloidogyne incognita*, is among the most important plant-pathogenic nematodes affecting cucurbit production worldwide (Thies, 1996; Sikora and Fernandez, 2005). The cucurbit or gourd family, Cucurbitaceae, is a diverse family with numerous genera and several crop species including; cucumber (*Cucumis sativus*), melon (*Cucumis melo*), pumpkin (*Cucurbita moschata*), squash (*Cucurbita pepo*), and watermelon (*Citrullus lanatus* var. *lanatus*). The infectious stage of root-knot nematodes is the second-stage juvenile (J2), which penetrates roots close to the root tip and migrates toward the vascular system to establish a feeding site that results in the formation of giant cells. Once a giant cell is initiated, the nematode becomes sedentary, enlarges, and molts through two additional juvenile stages (J3 and J4) to become an adult female. Hyperplasia of the surrounding cells leads to the formation of large fleshy galls. Egg laying females can produce hundreds of eggs during their lifespan and several life cycles can occur in a single cropping season. As result of infection, reduced nutrient and water uptake result in significant yield losses (Lamberti, 1979; Sasser, 1979; Ploeg and Phillips, 2001).

Genetic resistance would be the preferred strategy to manage root-knot nematodes in cucurbits; however, resistance to *M. incognita* is lacking. A few sources of resistance to *M. incognita* have been detected in wild-species and botanical varieties of *Cucumis* and *Citrullus*, but none in *Cucurbita* (Fassuliotis, 1970,1971; Wehner *et al*., 1991; Thies and Levi, 2007; Faske, 2013). Buffalo gourd, *Cucurbita foetidissima*, is a perennial gourd that is commonly found along fence rows and creek banks in native pastures and along the edge of production agriculture fields in the southwestern United States and Mexico. This native, wild-species of *Cucurbita* was reported to be susceptible to *M. javanica* (Rosemeyer *et al*., 1982), but its susceptibility to *M. incognita* is unknown.

Overall, cucurbit crops, such as cucumber, melon, squash, and watermelon, are susceptible to *M. incognita*; however, host suitability differs among cucurbit crops. Based on egg mass formation and nematode reproduction, squash and watermelon have a lower host suitability to *M. incognita* than melon and cucumber (Lopez-Gomez and Verdejo-Lucas, 2014). However, few studies have characterized the response of *M. incognita* infection on *Cucurbita* (Lopez-Gomez and Verdejo-Lucas, 2014; Lopez-Gomez *et al*., 2015), and none have investigated nematode response to a perennial cucurbit species such as buffalo gourd.

The objectives of this study were to evaluate the susceptibility of buffalo gourd to *M. incognita* and to evaluate the behavior of *M. incognita* J2 after entering the root system of buffalo gourd.

MATERIALS AND METHODS

Nematode culture and inoculum

Meloidogyne incognita was isolated from cotton (*Gossypium hirsutum*) and maintained in the greenhouse on tomato (*Solanum lycopersicum*) cv. "Rutgers". Eggs were collected from cultures with 0.5% NaOCl (Hussey and Barker, 1973), and J2 were collected in a hatching chamber (Vrain, 1977). Only 24-hr-old J2 or freshly collected eggs were used as inoculum.

Cucurbit species

Seeds of *C. foetidissima*, buffalo gourd, were collected along the edge of a native grass pasture in Erath Co., Texas. *Cucumis metuliferus* (PI 482452), also called African horned cucumber or horned cucumber, and cucumber, *C. sativus* 'Straight 8', were used as the resistant and susceptible control, respectively (Wehner *et al*., 1991).

Nematode penetration and maturity experiments

A greenhouse time-course experiment was used to evaluate nematode penetration and post-penetration development. Germinated seeds were planted into 85 cm^3 celled planter flats containing pasteurized sand and peat (10:1 v/v) soil mix. Seedlings were inoculated at the first-true leaf stage (2 to 3 wk after sowing) with approximately 170 J2 evenly distributed among three 2-cm deep cavities around each seedling. The experimental design was a randomized complete block design (RCBD) with four replicates for each cucurbit per sample time. The experiment was conducted twice. Root systems were harvested at 7, 14, and 21 d after inoculation (DAI) and washed free of soil. Nematodes were stained with acid fuchsin (Byrd *et al*., 1983) and classified into four stages of development; vermiform J2, sausage-shaped juveniles, female without eggs, and egg-laying females.

Nematode emigration experiments

A hydroponic system was used to determine J2 emigration from infected cucurbit roots. Each cucurbit was propagated from seed as described above into 85 cm^3 celled planter flats and inoculated at the first-true leaf stage with approximately 2,000

J2 evenly distributed among three 2-cm deep cavities around each seedling. Each cucurbit species was replicated five times in a RCBD, and the experiment was conducted twice. Roots systems were collected 2 DAI and washed free of soil. Seedlings were transferred into individual 200-ml plastic beakers filled with reverse osmosis water and fitted with a plastic tube attached to a small air-pump. Air was pumped through the water to provide enough oxygen to keep roots healthy. Second-stage juveniles that emigrated from the roots were collected daily from 3 to 6 DAI and counted using a stereoscope.

Nematode reproduction and fecundity experiments

Nematode reproduction and fecundity were evaluated in a greenhouse pot experiment. Germinated seeds were planted in 1,025 cm^3 standard pots containing a pasteurized sand and peat (10:1 v/v) soil mix. Seedlings were inoculated at the second-true leaf stage, 3 to 4 wk after seeding, with approximately 4,000 eggs distributed among three 2-cm deep cavities per seedling. The experimental design was a RCBD with four replications for each cucurbit species, and the experiment was conducted twice. Experiments were maintained in a greenhouse where ambient temperature ranged from 23 to 32°C. Roots were sampled 7 wk after inoculation and washed free of soil. Five single egg masses were collected at random from each root system and treated with 1.0% NaOCl to extract eggs. Root

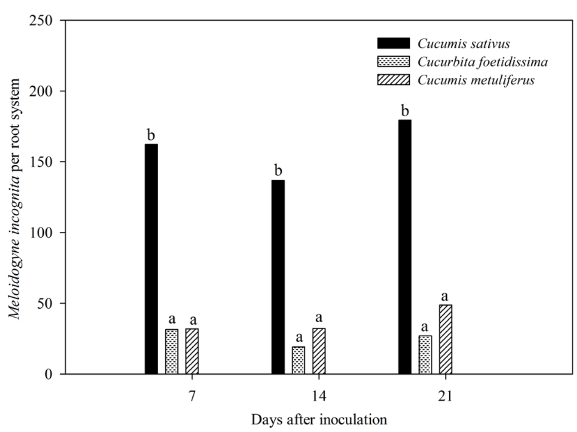

Fig. 1. Number of *Meloidogyne incognita* that penetrated the roots system of *Cucumis sativus* (Straight 8), *Cucurbita foetidissima* (buffalo gourd), and *Cucumis metuliferus* (PI 482452). The susceptible control was *C. sativus* and resistant control was *C. metuliferus*. Initial population density of *M. incognita* was 200 J2/100 cm^3 soil. Different letters over bars indicate significant differences at $\alpha = 0.05$ according to Tukey's HSD test.

systems were weighed, cut into 1-cm pieces, and treated with 1.0% NaOCl to extract eggs as indicated earlier. A reproduction factor (Rf) was calculated by dividing the final eggs per root system (Pf) by the initial inoculation density (Pi).

Statistical analysis

All data were transformed [ln (x + 1)] to normalize for statistical analysis and non-transformed data are reported. Results were similar between the two repetitions of the experiments, and data were combined for final analysis. Data were subjected to analysis of variance and mean separations by Tukey's honestly significant difference test, while data from the post-penetration development experiment were subject to chi square analysis using SPSS 19.0 (SPSS Inc., Chicago, IL).

RESULTS

Penetration and subsequent nematode development was affected by cucurbit species. Significantly more ($P \leq 0.05$) *M. incognita* J2 found infection sites at 7, 14, and 21 DAI on the root systems of *C. sativus* than *C. foetidissima* and *C. metuliferus* (Fig. 1). Nematode development was delayed ($P \leq 0.05$) at 14 and 21 DAI on *C. foetidissima* compared to *C. sativus*, but not to the same magnitude as that of *C. metuliferus* (Fig. 2). Nematode development was delayed ($P \leq 0.05$) at each sample time on *C. metuliferus* compared to *C. sativus* and *C. foetidissima*. No egg-laying females were observed at 21 DAI on *C. metuliferus* whereas 9.0% and 14.0% of the nematodes present were egg-laying females on *C. foetidissima* and *C. sativus*, respectively.

Emigration of J2 from the root system was observed at all sample times on each cucurbit species with 36% ($P \leq 0.05$) emerging at 3 DAI across species (Fig. 3). Of the J2 penetrating the root system, more ($P \leq 0.05$) emerged at 3 and 4 DAI from *C. metuliferus* than *C. sativus* and *C. foetidissima*. Further, 94% of J2 penetrating the root system of *C. metuliferus* emerged within 6 DAI whereas 71% and 27% emerged from roots of *C. foetidissima* and *C. sativus*, respectively.

Reproduction of *M. incognita* was higher ($P \leq 0.05$) on *C. sativus* and *C. foetidissima* than *C. metuliferus* (Fig. 4). Nematode fecundity on *C. foetidissima* and *C. metuliferus* averaged 312 eggs/egg mass, which was lower ($P \leq 0.05$) than on *C. sativus*, which averaged 631 eggs/egg mass.

DISCUSSION

These data support the susceptibility of buffalo

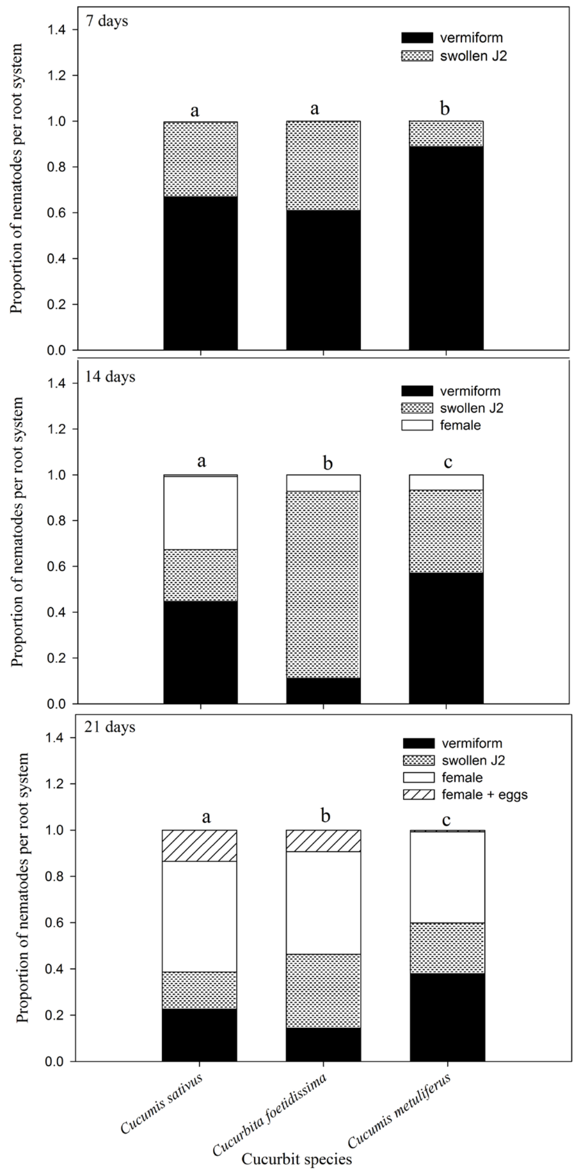

Fig. 2. Post-infection development of *Meloidogyne incognita* at 7, 14 and 21 d after inoculation within the root system of *Cucumis sativus* (Straight 8), *Cucurbita foetidissima* (buffalo gourd), and *Cucumis metuliferus* (PI 482452). The susceptible control was *C. sativus* and resistant control was *C. metuliferus*. Initial population density of *M. incognita* was 200 J2/100 cm^3 soil. Different letters over bars indicate significant differences at $\alpha = 0.05$ according to chi-square analysis applied in pairs of species.

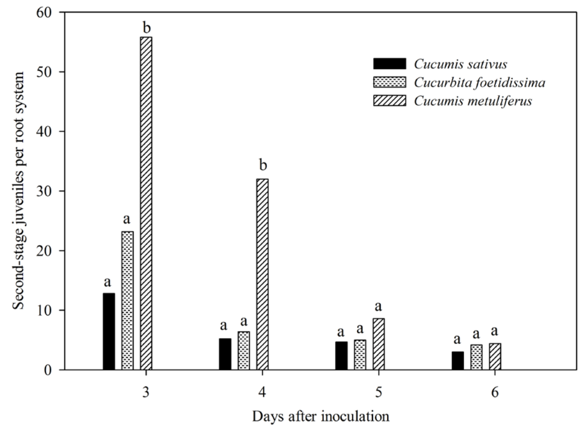

Fig. 3. Number of *Meloidogyne incognita* second-stage juveniles (J2) that emerged from the root system of *Cucumis sativus* (Straight 8), *Cucurbita foetidissima* (buffalo gourd), and *Cucumis metuliferus* (PI 482452). The susceptible control was *C. sativus* and resistant control was *C. metuliferus*. Initial population density of *M. incognita* was 2,400 J2/100 cm^3 soil. Different letters over bars indicate significant differences at $\alpha = 0.05$ according to Tukey's HSD test.

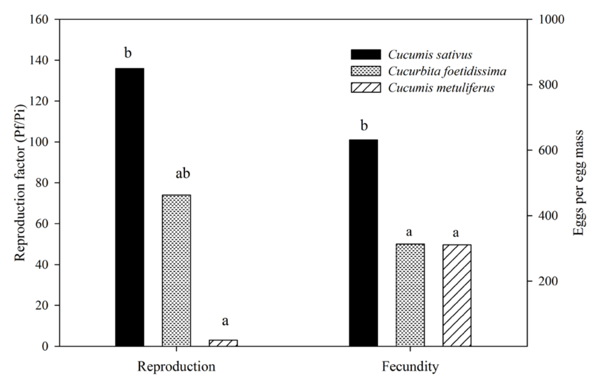

Fig. 4. Reproduction and fecundity of *Meloidogyne incognita* on *Cucumis sativus* (Straight 8), *Cucurbita foetidissima* (buffalo gourd), and *Cucumis metuliferus* (PI 482452). The susceptible control was *C. sativus* and resistant control was *C. metuliferus*. Each species was harvested 7 wk after inoculation with 400 eggs/100 cm^3 soil. Different letters over bars indicate significant differences at $\alpha = 0.05$ according to Tukey's HSD test.

gourd, a perennial species of *Cucurbita*, to *M. incognita*. Fewer nematodes entered the root system of buffalo gourd than cucumber, which differs from other studies where penetration of *M. incognita* was similar between squash and cucumber (Lopez-Gomez and Verdejo-Lucas, 2014). Buffalo gourd has a tuberous root system, with fewer lateral roots on the developing seedlings for nematode infection compared to the tap root system of squash or cucumber. Alternately, of the J2 that penetrated the root system of buffalo gourd, 24% fewer emerged compared to the resistant host, *C. metuliferus*, which had a similar number of infectious sites. This is consistent with other reports where fewer *Meloidogyne* J2 emerged from the root system of a susceptible than resistant host (Herman *et al*., 1991; Bendezu and Starr, 2003; Faske, 2013).

Post-infection development of *M. incognita* at 7 DAI was similar between buffalo gourd and cucumber; however, at 14 DAI nematode development on buffalo gourd lagged behind that of cucumber and at 21 DAI contributed to 30% fewer egg-laying females than those on cucumber. These results suggest an effect on the post-penetration development of *M. incognita* in buffalo gourd. Saponions have been reported to have some nematicidal activity on *M. incognita* and saponions have been reported in buffalo gourd (Dubois *et al*., 1988; Meher *et al*., 1988), but the role of saponions was not investigated in this study.

Although both cucumber and buffalo gourd were susceptible hosts for *M. incognita*, buffalo gourd was a less suitable host than cucumber. Statistically, nematode reproduction was similar between buffalo gourd and cucumber, but the Rf value for buffalo gourd was 46% lower than that of cucumber. Factors that contributed to the lower Rf value included: fewer J2 entering the roots system, a higher percentage (50%) of J2 leaving the root system, delayed nematode development, and reduced female fecundity (50% fewer eggs/egg-laying females). Similarly, differences in Rf value (96%) were reported between several cultivars of squash and cucumber (Lopez-Gomez *et al.*, 2015). These data suggest similar host suitability of perennial and annual species of *Cucurbita* compared to cucumber against *M. incognita*.

Overall, the susceptibility of buffalo gourd to *M. incognita* was associated with fewer J2 emerging from the root system and those that remained in the root developed into egg-laying females. Susceptibility did not appear to be due to an increase in fecundity among egg-laying females. Given that buffalo gourd is a perennial gourd it could act as a reservoir for root-knot and other nematode species in the semi-arid region of the southwestern United States and Mexico.

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