RESEARCH/INVESTIGACIÓN

REPRODUCTION AND INVASION DYNAMICS OF *GLOBODERA PALLIDA* AND *MELOIDOGYNE* SPP. IN *SOLANUM SISYMBRIIFOLIUM*

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ABSTRACT

Baker, H. V., I. A. Zasada, C. Gleason, and L. M. Dandurand. 2023. Reproduction and invasion dynamics of *Globodera pallida* and *Meloidogyne* spp. in *Solanum sisymbriifolium*. Nematropica 53:70-81.

Plant-parasitic nematodes cause economic damage to several agricultural crops in the Pacific Northwest of the United States. Of particular concern are the root-knot nematodes, *M. hapla* in potato, vegetables, and wine grape production, and *M. chitwoodi* in potato production. In addition, the limited distribution of the potato cyst nematode, *Globodera pallida*, in Idaho is a major concern given its quarantine status. *Solanum sisymbriifolium* has been proposed as a strategy to manage plant-parasitic nematodes. The goal of this research was to evaluate the reproduction and invasion of *M. hapla*, multiple races of *M. chitwoodi*, *M. incognita,* and *G. pallida* on *S. sisymbriifolium*. Reproduction of these plant-parasitic nematodes was minimal or nonexistent on *S. sisymbriifolium*, with few or no eggs recovered. In invasion assays, *M. chitwoodi* did not invade *S. sisymbriifolium* roots seven days post inoculation. In the same short-term assay, *M. hapla* was able to invade *S. sisymbriifolium* roots (5.3% to 5.9% *M. hapla* second-stage juveniles in roots compared to tomato) but did not develop. Similar to *M. hapla*, over a 10-wk period*, G. pallida* invaded *S. sisymbriifolium* roots, but rarely developed to a third-stage juvenile and never to female. The resistance of *S. sisymbriifolium* to *M. hapla*, multiple races of *M. chitwoodi*, *M. incognita*, and *G. pallida* indicates there is the opportunity for widespread deployment of this plant species as a trap crop to control important plant-parasitic nematodes.

Key words: M. hapla, M. chitwoodi, M. incognita, roots, resistance

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RESUMEN

Baker, H. V., I. A. Zasada, C. Gleason, and L. M. Dandurand. 2023. Dinámica de reproducción e invasión de *Globodera pallida* y *Meloidogyne* spp. en *Solanum sisymbriifolium*. Nematropica 53:70-81.

Los nematodos parásitos de plantas causan daño económico a varios cultivos agrícolas en el Noroeste del Pacífico de los Estados Unidos. De particular preocupación son los nematodos agalladores de raíces, *M. hapla* en la producción de papa, hortalizas y vid, y *M. chitwoodi* en la producción de papa. Además, la distribución limitada del nematodo del quiste de la papa, *Globodera pallida*, en Idaho es una preocupación importante dado su estado de cuarentenario. *Solanum sisymbriifolium* se ha propuesto como una estrategia para manejar nematodos parásitos de plantas. El objetivo de esta investigación fue evaluar la reproducción e invasión de *M. hapla*, múltiples razas de *M. chitwoodi*, *M. incognita* y *G. pallida* en *S. sisymbriifolium*. La reproducción de estos nematodos parásitos de plantas fue mínima o inexistente en *S. sisymbriifolium*, con pocos o ningún huevo recuperado. En los ensayos de invasión, *M. chitwoodi* no invadió las raíces de *S. sisymbriifolium* siete días después de la inoculación. En el mismo ensayo a corto plazo, *M. hapla* fue capaz de invadir las raíces de *S. sisymbriifolium* (5,3% a 5,9% de juveniles en segundo estado de *M. hapla* en raíces en comparación con tomate) pero no se desarrolló. Similar a *M. hapla*, durante un periodo de 10 semanas, *G. pallida* invadió las raíces de *S. sisymbriifolium*, pero rara vez se desarrolló en un juvenil en tercer estado y nunca a una hembra. La resistencia de *S. sisymbriifolium* a *M. hapla*, múltiples razas de *M. chitwoodi*, *M. incognita* y *G. pallida* indica que hay una oportunidad de un despliegue generalizado de esta especie de planta como cultivo trampa para el manejo de importantes nematodos parásitos de plantas.

Palabras clave: M. hapla, *M. chitwoodi*, *M. incognita*, raíces, resistencia

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INTRODUCTION

In the Pacific Northwest (PNW) there are several plant-parasitic nematodes that limit the production of important agricultural crops such as potato, *Solanum tuberosum*, and wine grapes, *Vitis vinifera* (Zasada *et al*., 2018). *Meloidogyne* spp. are parasites of a diversity of crops in the region. Important to the production of potato is *Meloidogyne chitwoodi*. There is no tolerance for *M. chitwoodi* in seed potato in international export markets, and because it is a quarantine pathogen in certain export markets, its presence can result in the rejection of an entire shipment (King and Taberna, 2013). At least three races of *M. chitwoodi* exist in the United States; race 1 was identified first in the Columbia Basin of the PNW, race 2 is typically found when potatoes are grown in rotation with alfalfa (*Medicago sativa*), and Roza is a resistance-breaking race (Mojtahedi *et al*., 1988; Mojtahedi *et al*., 2007). In wine grapes, *M. hapla* can reduce the productivity of vines (East *et al*., 2021); this nematode is also a parasite of potato and vegetables (Zasada *et al*., 2018). The impact of this nematode on the production of potato is not as severe as that of *M. chitwoodi*, and it appears that this nematode is not as widespread in the region as *M. chitwoodi* (Zasada *et al*., 2019). Another plant-parasitic nematode that is of importance in the region, but not widespread, is *Globodera pallida*. This nematode is found on less than 1% of the potato acreage in the PNW (Dandurand *et al*., 2019b), where it is limited to a small region in Idaho. However, because of the potential for this nematode to reduce potato yield, strict quarantine regulations are in place to prevent *G. pallida* from spreading. For all of these plant-parasitic nematodes, the primary strategy for management is the use of fumigant and/or nonfumigant nematicides (Dandurand *et al*., 2019b; Zasada *et al*., 2018). There is a need for additional strategies to manage these plant-parasitic nematodes and contribute to an integrated nematode management approach in PNW crops.

One such strategy may be the trap crop *Solanum sisymbriifolium*, which has been demonstrated to reduce population densities of *G. pallida* and *G. rostochiensis* (Dandurand *et al*., 2019a; Mhatre *et al*., 2021; Timmermans, 2005). *Solanum sisymbriifolium* is native to South America. This plant was shown to be nearly as effective as potato at inducing egg hatching but was resistant to subsequent development and reproduction of both *G. pallida* and *G. rostochiensis* (Scholte and Vos, 2000; Scholte, 2000; Kooliyottil *et al*., 2016). Under greenhouse conditions, *S. sisymbriifolium*, unlike the non-host barley, effectively reduced *G. pallida* populations by 99% in a subsequent potato crop, even when initial nematode populations were high (Dandurand and Knudsen, 2016). The effect of *S. sisymbriifolium* on *Meloidogyne* spp. has been reported to be more variable. Scholte and Vos (2000) reported that while the roots were readily invaded by *M. hapla* second-stage juveniles (J2), the invasion resulted in only minor swelling or small galls, and no nematode maturation or egg production was observed. Dias *et al*. (2012) evaluated multiple cultivars of *S. sisymbriifolium* against *M. chitwoodi*, *M. arenaria*, *M. hapla*, *M. hispanica*, and *M. javanica*; the host status of the *S. sisymbriifolium* cultivars varied among the *Meloidogyne* spp. Variability in host resistance of *S. sisymbriifolium* cultivars to *Meloidogyne* spp. was also observed for *M. arenaria*, *M. incognita*, *M. haplanaria*, *M. javanica*, and *M. enterolobii* (Hajihassani *et al*., 2020). The results of these studies collectively indicate that further characterization of *S. sisymbriifolium* accessions is needed to effectively understand its resistance response with different species, and even within populations of plant-parasitic nematodes. The goals of this research were to: 1) determine the host status of *S. sisymbriifolium* to *M. hapla*, *M.*

chitwoodi (several races), *M. incognita*, and *G. pallida*, and 2) characterize the invasion dynamics of *S. sisymbriifolium* by *M. chitwoodi*, *M. hapla*, and *G. pallida*.

MATERIALS AND METHODS

The accession of *S. sysimbriifolium* (PI 381291) used in all experiments was obtained from Chuck Brown (USDA-ARS, Prosser, WA). The selection of the accession had reduced spines and was demonstrated to induce hatch of *G. pallida* (L.-M. Dandurand, personal communication). Reduced spines is a desirable trait due to many factors, most importantly to protect humans and equipment from injury and damage, respectively, in the field.

Reproduction dynamics of Meloidogyne *spp. on* S. sisymbrifolium

Reproduction experiments were conducted using cultures maintained at USDA-ARS in Prosser, WA or Corvallis, OR. The *M. hapla* population was originally collected from potato in Prosser, WA. Several populations of *M. chitwoodi* were evaluated: 1) *M. chitwoodi* WAMcRoza (Mojtahedi *et al*., 2007), a population of race 1 originally collected in Prosser, WA, distinguished by its ability to reproduce on roots of potato plants that have resistance to race 1 conferred by RMc1(blb), 2) WAMc1 (Pinkerton *et al*., 1987), a population representative of race 1 originally collected in Prosser, WA, 3) WAMc27 (Santo and Pinkerton, 1985), a population representative of race 2 originally collected in Prosser, WA, and 4) CAMc2 originally collected in Tulelake, CA. *Meloidogyne incognita* originally collected from grape in Parlier, CA and was also included in the experiment. All *Meloidogyne* spp. were maintained on tomato (*Solanum lycopersicum*) 'Rutgers' at both locations as previously described (Wram and Zasada, 2020). Eggs were extracted from the roots by initially removing soil from the roots and shaking in a solution of 0.6% NaClO for 3 min at 300 RPM. The bleached roots were further rinsed with water over nested 250- and 25-µm sieves to remove debris and collect rinsed eggs. Eggs were enumerated using an inverted microscope and stored in water at 4°C.

Tomato 'Rutgers' was included as a susceptible control for all *Meloidogyne* spp. populations evaluated. Seeds were planted in standard plastic 6-pack containers containing soilless media to germinate. When seedlings were approximately 3 to 4 wk old, they were transplanted into 15 cm round clay pots containing approximately 1 liter of soil. In Corvallis, a 1:1 steam pasteurized sand and Willamette loam mix was used. At transplanting, plants were watered with 9N-45P-15K fertilizer (The Scotts Company, Marysville, OH) at recommended concentration. In Prosser, a sterilized mixture of 75% sand and 25% soil with 2.0 g Osmocote 14N-14P-14K Flower and Vegetable Smart-Release Plant Food (The Scotts Company, Marysville, OH) per liter of sand-soil mixture was used.

Plants were inoculated 5 days after transplanting by pipetting 2,000 *Meloidogyne* spp. eggs in a total volume of 3 ml per plant into 3 holes approximately 2.5 cm deep into the soil near the base of each plant. Holes were covered with soil, and the plant was lightly watered for the next 48 to 72 hr. Plants were grown in a greenhouse under long-day conditions, 16-hr photo period, with 23/18°C day/night temperatures. In Corvallis, plants were fertilized twice each week with 20N-20P-20K fertilizer (The Scotts Company, Marysville, OH) and in Prosser, the Osmocote 14N-14P-14K previously mentioned was used. Each *Meloidogyne* spp./plant combination was replicated four (Corvallis) or six (Prosser) times. Plants were blocked by *Meloidogyne* spp. to avoid cross contamination between plants. Within each block, tomato or *S. sisymbriifolium* were arranged in a randomized design. After 55 days, plants were destructively harvested. The aboveground portion of the plant was removed and discarded. The contents of the pot were emptied onto a tray and roots removed from soil. The roots were rinsed in water and then eggs were extracted and quantified as described above. For each *Meloidogyne* spp./plant combination, the Reproduction Factor (RF) was calculated as final egg density (Pf)/initial egg density (Pi). Within each *Meloidogyne* spp., RF data from tomato and *S. siymbriifolium* were analyzed by nonparametric Kruskal–Wallis Test with difference significant at $P < 0.05$ using JPM vs. 9.1 (SAS Institute, Cary, NC).

Invasion dynamics of Meloidogyne *spp. on* S. sisymbrifolium

Invasion dynamics experiments were conducted at Washington State University

(Pullman, WA) using *M. hapla* (VW9) originally collected from Davis, CA and *M. chitwoodi* (WAMc1) originally collected in Prosser, WA. Both *Meloidogyne* spp. were maintained on tomato 'Rutgers' and extracted from roots as described above. Eggs were further concentrated by sucrose gradient centrifugation. For this step, one volume of a 70% sucrose solution was added to an equal volume of egg suspension and mixed well. Approximately 1 ml DI water was layered on top of the sucrose-egg mixture. Centrifugation of the samples was completed at 1,400 rpm for 3 min. Following the sucrose flotation, the eggs were collected from the top water layer using a transfer pipette and rinsed of remaining sucrose solution. The extracted eggs were placed in a hatching chamber (Zhang and Gleason, 2021) and incubated in the dark for three days at room temperature when hatched *M. hapla* or *M. chitwoodi* J2 were collected. The suspension was adjusted to deliver approximately 500 J2 in 500 µl water or 1,000 J2 in 1000 µl water.

Solanum sisymbriifolium and tomato as a susceptible control were used in the experiment. *Solanum sisymbriifolium* seeds were allowed to imbibe in 50 ml DI water for 24 hr in the dark before sterilization with 0.6% NaClO for 10 min. Tomato seeds were sterilized using a 1.2% NaClO solution for 20 min without imbibing. The seeds were rinsed a minimum of three times with DI water after sterilization to ensure all traces of NaClO were removed. Seeds were allowed to air dry for 1 hr at room temperature. Experiments were conducted in a growth chamber (Conviron, Pembina, ND) maintained at 25°C with a 16-hr photoperiod. Surface-sterilized seeds were planted in building sand (Lane Mountain Company, Valley, WA) in 3.8 x 21 cm cone-tainers (Hummert International, Earth City, MO). Osmocote 14N-14P-14K was added to the surface of the sand before seeds were planted. Once the plants reached the 4- to 6-leaf stage, which occurred around 4 to 5 wk after planting, they were inoculated by pipetting 500 or 1,000 *Meloidogyne* spp. J2 per plant in 500 or 1,000 µl of DI water, respectively, into 2 holes that were created by inserting a 1-ml pipette tip approximately 2 cm into the sand. Plants were not watered for 48 hr following inoculation. A minimum of five replicates for *S. sisymbriifolium* and three replicates for tomato were used for each low inoculum (500 J2) experiment. Low inoculum experiments were conducted three times for *M. hapla* and two times for *M. chitwoodi* race 1. A minimum of seven replicates for *S. sisymbriifolium* and tomato were used for each high inoculum (1,000 J2) experiment. High inoculum experiments were repeated three times for both *M. hapla* and *M. chitwoodi* race 1. Replicates were arranged in a completely randomized design.

Seven days after inoculation, plants were destructively harvested by removing and discarding the aboveground portion of the plant before the cone-tainers were inverted to remove the roots from the sand. The roots were rinsed in tap water and stained with acid fuchsin to target nematodes within the roots. (Byrd *et al*., 1983). Roots were rinsed following staining to remove excess stain then subsequently placed in 50 ml tubes with acidified glycerol and stored at 4˚C. The number of galls (defined as an area of the root where swelling had occurred and at least one nematode was present) was determined using a stereo microscope (Discovery.V8; Zeiss, Oberkochen, Germany) and the number of stained *Meloidogyne* spp. J2 within the roots was determined using a compound microscope (Axio Observer.A1; Zeiss, Oberkochen, Germany). The average number of galls on the susceptible tomato for each experiment was used for normalization of the *S. sisymbriifolium* data by using the average amount of galling as the maximum possibility of the plant roots. The percentage galling of the *S. sisymbriifolium* roots was then calculated using this normalized value. The number of *Meloidogyne* spp. J2 per plant was normalized for each experiment following the same method. An unpaired t-test with Welch's correction was used to determine if there were significant differences in the level of galling or infection between the hosts. All analyses were performed using Graphpad Prism v9.2.0 (GraphPad Software, San Diego, CA).

Reproduction and invasion dynamics of Globodera pallida *on* S. sisymbrifolium

The host status experiments with *G. pallida* were conducted in an USDA-APHIS approved facility at the University of Idaho (Moscow, ID). Cysts were initially obtained from infested fields in Shelly, ID; the identity of *G. pallida* was confirmed by morphological and molecular methods (Skantar *et al*., 2007). The nematode was cultured on susceptible potato 'Désirée' under greenhouse conditions with a day temperature of 18°C and night temperature of 10°C at a 16:8-hr light: dark period (Dandurand *et al*., 2019a). After 16 wk, cysts were extracted using a USDA-type semi-automated elutriator (USDA-APHIS, 2009) and picked by hand under a stereomicroscope (Leica Microsystems, Wetzlar, Germany). All cysts were incubated at 4°C for a minimum of 16 wk before experimental use. Cysts were placed in pouches (2.54 cm^2) made of wear-resistant nylon mesh (248.92 µm opening; McMaster-Carr, Elmhusrt, IL) and were hydrated for three days prior to use. To estimate the number of eggs in cysts, 10 cysts were crushed with a rubber stopper, eggs were washed into a container, adjusted to a desired volume, and eggs/ml were determined using an inverted microscope (Leica Microsystems).

Solanum sisymbriifolium seeds were grown in plastic pots for 4 wk in a greenhouse as described above before transplanting into pots, with one plant per pot. Potato 'Désirée' was grown from tissue culture plantlets in standard media (Murashige and Skoog, 1962) for 4 wk before being transplanted into pots. All plants were maintained at even moisture by watering with 75 ml water twice daily and fertilized with Jack's Classic 20N-20P-20K all-purpose fertilizer (J. R. Peters Inc., Allentown, PA) three-times-per-week at the recommended rate.

Experiments were conducted under greenhouse conditions as described above. Air-dried Prosser fine sandy loam soil was sieved through a mesh (5 mm opening) and mixed with sand (Lane Mountain Company, Valley, WA) resulting in a 2:1 soil: sand mixture (56% sand, 35% silt, 8% clay, pH 7.0). The soil mix was autoclaved twice (at the interval of 24-hr) at 121°C for 90 min before use. Experiments were conducted in 15 cm Terra cotta clay pots (The Home Depot, Atlanta, GA) containing 1.5 kg soil mix. Cysts in bags were placed directly under the roots of transplants to achieve an initial nematode density of 5 eggs/g soil. Treatments (potato or *S. sisymbriifolium*) were replicated four or five times depending on the trial (two trials were conducted), and pots were arranged in a randomized complete design. Plants were watered daily for the duration of the bioassay and fertilized using 20N-20P-20K all-purpose fertilizer three times per week at the recommended rate. After 12 or 16 wk, the host assay was terminated. Cysts were extracted from soil and the number of cysts/pot were determined.

The invasion dynamics of *G. pallida* was evaluated under greenhouse conditions at the University of Idaho. Plants of *S. sisymbriifolium* or potato 'Désirée' were produced as described above for the reproduction experiment with *G. pallida*. The effect of *S. sisymbriifolium* or potato on development of *G. pallida* life-stages in roots was determined as follows: 4-wk-old plants were inoculated at a rate of 5 eggs/g soil as described above for the host assay. Plants were destructively sampled at 2, 4, 6, 8, or 10 wk post planting. At each sampling time, plants were removed from the pots, tops were discarded, and roots were rinsed with water to remove soil, then stained as described for the *Meloidogyne* spp. invasion assays. At each sampling interval post planting, the life stages of the nematode J2, third stage juvenile (J3), male or female in the entire root system were enumerated by using a stereomicroscope (M80, Leica Microsystems, Deerfield, IL) Four replicates of each treatment (potato or *S. sisymbriifolium*) were arranged in a randomized complete block design and the experiment was conducted two times. The repeated experiment followed the same protocol, but five replicates were used.

Data were analyzed by ANOVA using the General Linear Model statement in Statistical Analysis Software (SAS), SAS Institute Inc., Cary, NC. To meet ANOVA assumptions, a square root transformation was used to ensure a normal distribution and constant variation of the data. Statistically significant differences ($P \leq 0.05$) among treatments were computed by least significant difference test (LSD) once ANOVA indicated a significant F-value.

RESULTS

Reproduction and invasion dynamics of Meloidogyne spp. *on* S. sisymbrifolium

Solanum sisymbriifolium was not a host for any of the *Meloidogyne* spp./races evaluated in the greenhouse experiments (Table 1). All species of *Meloidogyne* reproduced on tomato with RF values ranging from 7 to 108, indicating the experiments were successful.

In the invasion dynamics experiments, it was further demonstrated that *S. sisymbriifolium* was not a host for *M. hapla* or *M. chitwoodi*. No galling of *S. sisymbriifolium* roots was observed at 7 days post inoculation (DPI) with 500 J2 of *M. hapla* and

Table 1. Reproduction of *Meloidogyne* spp. on *Solanum sisymbriifolium* and tomato (*Solanum lycopersicum*) 'Rutgers'.

Meloidogyne spp. and race	*Solanum sisymbriifolium* Reproduction Factor (RF)[y]	Tomato
M. chitwoodi CAMc2	0.0 (±0.0)[z]	38.6 (±16.0)
M. chitwoodi WAMc1	0.0 (±0.0)	33.5 (±21.2)
M. chitwoodi WAMc27	0.0 (±0.0)	26.2 (±9.7)
M. chitwoodi WAMcRoza	0.0 (±0.01)	14.3 (±7.2)
M. hapla	0.1 (±0.1)	99. 5 (±14.5)
M. incognita	0.14 (±0.2)	92.0 (±26.8)

[y] RF is the reproduction factor = final population density/initial population density.

[z] Values are the mean of 10 observations ± standard error.

M. chitwoodi race 1 (Figs. 1A and 1C, respectively). *Meloidogyne hapla* J2 were observed in the *S. sisymbriifolium* roots but at significantly lower levels compared to tomato (Fig. 1B). An average of 5.3% *M. hapla* J2 were observed in the *S. sisymbriifolium* roots at 7 DPI relative to tomato. The *M. hapla* J2 within the tomato roots were fatter and sausage-shaped indicating the successful development of the infective nematode in a susceptible host (Fig. 2A). The *M. hapla* J2 within the *S. sisymbriifolium* roots were thinner indicating development of the nematode to J3 or J4 did not initiate (Fig. 2B). No *M. chitwoodi* J2 were observed in the *S. sisymbriifolium* roots at 7 DPI (Fig. 1D). Increasing the inoculum density to 1,000 J2 did result in an increase in the number of *M. hapla* observed in the roots, with a mean of 15.9% root galling and 5.9% *M. hapla* J2 in *S. sisymbriifolium* compared to tomato (Fig. 3A and 3B, respectively). Inoculation density did not alter the level of infection on *S. sisymbriifolium* roots by *M. chitwoodi* (Fig. 3C and 3D).

Reproduction and invasion dynamics of Globodera pallida *on* S. sisymbriifolium

Although *G. pallida* successfully reproduced on potato (RF = 32 to 34), no reproduction occurred on *S. sisymbriifolium*, indicating that this plant was not a host for *G. pallida*. Although a few *G. pallida* J2 invaded *S. sisymbriifolium* roots at 2- or 4-wk post inoculation, the number of *G. pallida* J2 in *S. sisymbriifolium* roots was 79 to 82% less than those found in potato. In the first experiment, the greatest number of *G. pallida* J2 were observed after 4 wk in both potato and *S. sisymbriifolium*, but when repeated, a greater number of *G. pallida* J2 were present in roots at 2 wk post inoculation (Table 2). Only a few *G. pallida* J3 were observed in *S. sisymbriifolium*, and males were observed in one experiment but not in the other. However, females were not observed in *S. sisymbriifolium* at either time point in either experiment providing further evidence that *S. sisymbriifolium* is not a host for *G. pallida* (Table 2).

DISCUSSION

Evaluation of reproduction and invasion of *Meloidogyne* spp. and *G. pallida* compared to known hosts tomato and potato, respectively, demonstrated that *S. sisymbriifolium* is resistant to these nematodes. Unlike susceptible tomato or potato, if *S. sisymbriifolium* was employed in a field infested with any of these nematodes, potentially as a trap crop, the nematodes would not be able to increase their population size (Dandurand *et al*., 2019a). Therefore, *S. sisymbriifolium* could be a useful tool in terms of nematode management or as a source of nematode resistance.

Previous work has shown variability in the response of *S. sisymbriifolium* to plant-parasitic nematodes, which could perhaps be attributed to the cultivar being tested. For example, several different species of root-knot nematodes (*M. arenaria*, *M. chitwoodi*, *M. hapla*, *M. hispanica*, and *M. javanica*) were evaluated for their ability to reproduce on four cultivars/lines of *S. sisymbriifolium* available in Europe ('Domino', 'Sharp', 'Sis 4004', and 'Pion') (Dias *et al*., 2012). The cultivars varied in their ability to support *Meloidogyne* spp. reproduction. *Solanum sisymbriifolium* 'Pion' was susceptible to *M. arenaria*, *M. hapla*, *M. hispanica,* and *M. javanica*, while the others varied in their reactions to *Meloidogyne* spp. All cultivars were resistant to *M.*

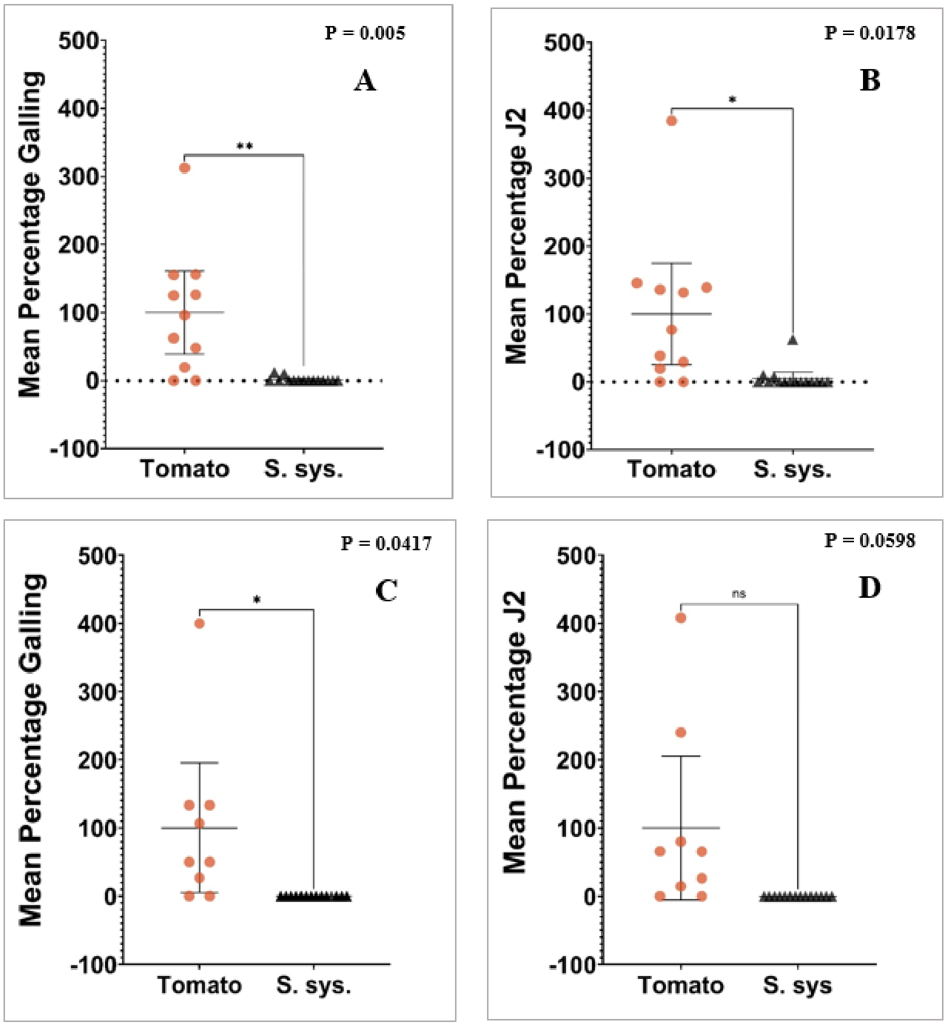

Figure 1. Infection of *Solanum sisymbriifolium* and tomato ‘Rutgers’ by *Meloidogyne hapla* and *M. chitwoodi* 7 days post inoculation at an inoculation density of 500 second-stage juveniles (J2) per plant. (A) *M. hapla* number of galls on roots [N = 11 for tomato (circles), 15 for *S. sisymbriifolium* (S. sys, triangles)], (B) *M. hapla* number of J2 in roots (N = 11 for tomato, 15 for *S. sisymbriifolium*), (C) *M. chitwoodi* number of galls on roots (N = 9 for tomato, 15 for *S. sisymbriifolium*), and (D) *M. chitwoodi* number of J2 in roots (N = 9 for tomato, 15 for *S. sisymbriifolium*). Gall and *Meloidogyne* spp. J2 counts were normalized by dividing the number of galls or J2 per *S. sisymbriifolium* plant by the average number of galls or J2 present in tomato roots. These values were then used for calculation of mean percentage. Bars represent the mean percentage with 95% confidence interval. Asterisks indicate level of significance between the plant types (* = $P < 0.05$; ** $P < 0.005$; ns = $P > 0.05$).

chitwoodi. Interestingly, a recent report found a different *S. sisymbriifolium* cultivar ‘Sis 6001’ was resistant to *M. chitwoodi* (Perpétuo *et al*., 2021), suggesting that resistance to this nematode is widespread in *S. sisymbriifolium* cultivars. Hajihassani *et al*. (2020) tested the *S. sisymbriifolium* line ‘PI 381291’, which is the same line evaluated in this study, and found that it was resistant to *M. arenaria*, *M. incognita*, *M. haplanaria,* and three isolates of *M. enterolobii*, while it was susceptible to *M. javanica*. The host status of other *S. sisymbriifolium* cultivars (‘Quattro’, ‘Diamond’, and ‘White Star’) were variable in their ability to support reproduction of these *Meloidogyne* spp.

In terms of cyst nematode resistance, Dias *et*

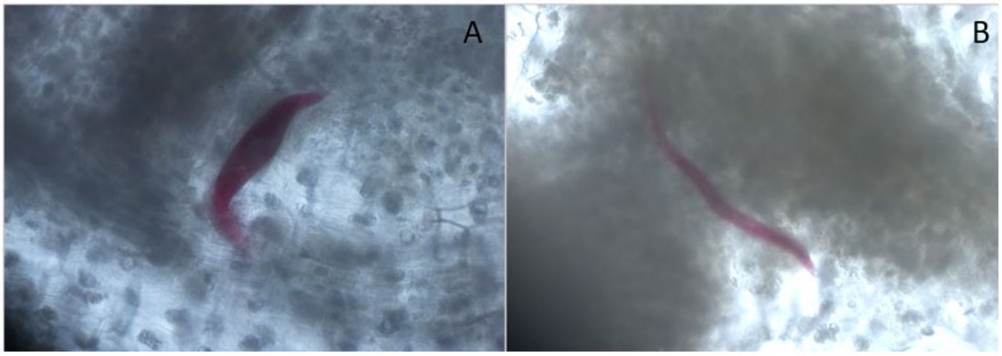

Figure 2. *Meloidogyne hapla* second-stage juveniles stained with acid fuchsin on (A) tomato 'Rutgers' and (B) *Solanum sisymbriifolium* roots at 7 days post inoculation. Images were captured at 20x magnification.

al. (2017) evaluated the host status of *S. sisymbriifolium* cultivars/lines 'Melody', 'Pion', 'Sharp', 'Sis 4004', and 'Sis 6001' against *G. pallida* and *G. rostochiensis* and found all to be resistant to these nematodes. A similar result for *Globodera* spp. was reported by Mhatre *et al*. (2021) in South Africa using a locally adapted selection of *S. sisymbriifolium*. These data are consistent with our results in which *S. sisymbriifolium* line 'PI 381291' was resistant to *G. pallida*. Overall, the data demonstrate that *S. sisymbriifolium* is resistant to several species of plant-parasitic nematodes; however, the level of resistance can vary depending upon the *S. sisymbriifolium* cultivar and nematode species/population.

Invasion studies were conducted to understand whether the early interaction of *M. hapla*, *M. chitwoodi*, and *G. pallida* with *S. sisymbriifolium* was attributed to resistance. The invasion assay for *G. pallida* spanned from 2 to 10 wk, and females were not observed in roots at any time point. These results are similar to those of Mhatre *et al*. (2021) where no *G. pallida* development was observed on roots 60 days after planting. Also similar to our findings, no galls were reported on *S. sisymbriifolium* 'PI 381291' 10 wk after inoculation with *M. hapla* (Scholte and Vos, 2000). In a study with *M. chitwoodi* (Perpétuo *et al*., 2021), nematodes were found in *S. sisymbriifolium* 'Sis 6001' roots 70 days after inoculation, however, only J2 or adult males were present. We did not observe *M. chitwoodi* entering the roots of *S. sisymbriifolium* at 7 DAI at either initial inoculum density evaluated. We cannot rule out that the *M. chitwoodi* J2 would have entered the roots of *S. sisymbriifolium* after 7 DAI. Despite *M. chitwoodi* containing races that have different host ranges and virulence on a resistant potato breeding line, *S. sisymbriifolium* was resistant to all races, indicating that *S. sisymbriifolium* could be used to control different races of *M. chitwoodi*. Additionally, *M. incognita* was not able to reproduce on *S. sisymbriifolium* in this study, highlighting that *S. sisymbriifolium* has broad spectrum *Meloidogyne* spp. resistance. Root tissues of *S. sisymbriifolium* were stained for histopathological study and differences were observed among varieties/lines (Hajihassani *et al*., 2020). In regard to *S. sisymbriifolium* 'PI 381291', limited hypertrophy was observed in the roots suggesting that *M. enterolobii*, *M. arenaria*, *M. incognita*, and *M. haplanaria* J2 that entered roots failed to establish a feeding site. While our study focused on other *Meloidogyne* spp., we did not observe swelling of *M. hapla* J2 in roots 7 days after invasion as compared to the swelling observed in tomato.

It has been hypothesized that nematodes capable of entering *S. sisymbriifolium* roots may leave or die once they have determined the roots to be an uninhabitable space (Perpétuo *et al*., 2021). The induction of resistance before the nematodes can enter the roots is termed passive resistance. The lack of *M. chitwoodi* J2 within *S. sisymbriifolium* roots seen in this study indicates that the nematode is either unable to enter the roots or are leaving very quickly after entering. The results of the current study indicate that passive resistance may be occurring with *M. chitwoodi* and possibly, but to a lesser extent, during *M. hapla* and *G. pallida* infection.

The evaluation of different initial population densities of *M. hapla* and *M. chitwoodi* on invasion indicated that the resistance exhibited by *S. sisymbriifolium* was impacted by inoculum density. This is supported by previous research where the impact of *S. sisymbriifolium* on *M. hapla* was evaluated in fields with moderate and severe infestations (Scholte and Vos, 2000). In the field with a moderate infestation there was significantly lower root galling on an indicator plant, lettuce (*Lactuca* sp.), following *S. sisymbriifolium* compared to galling on an indicator plant following

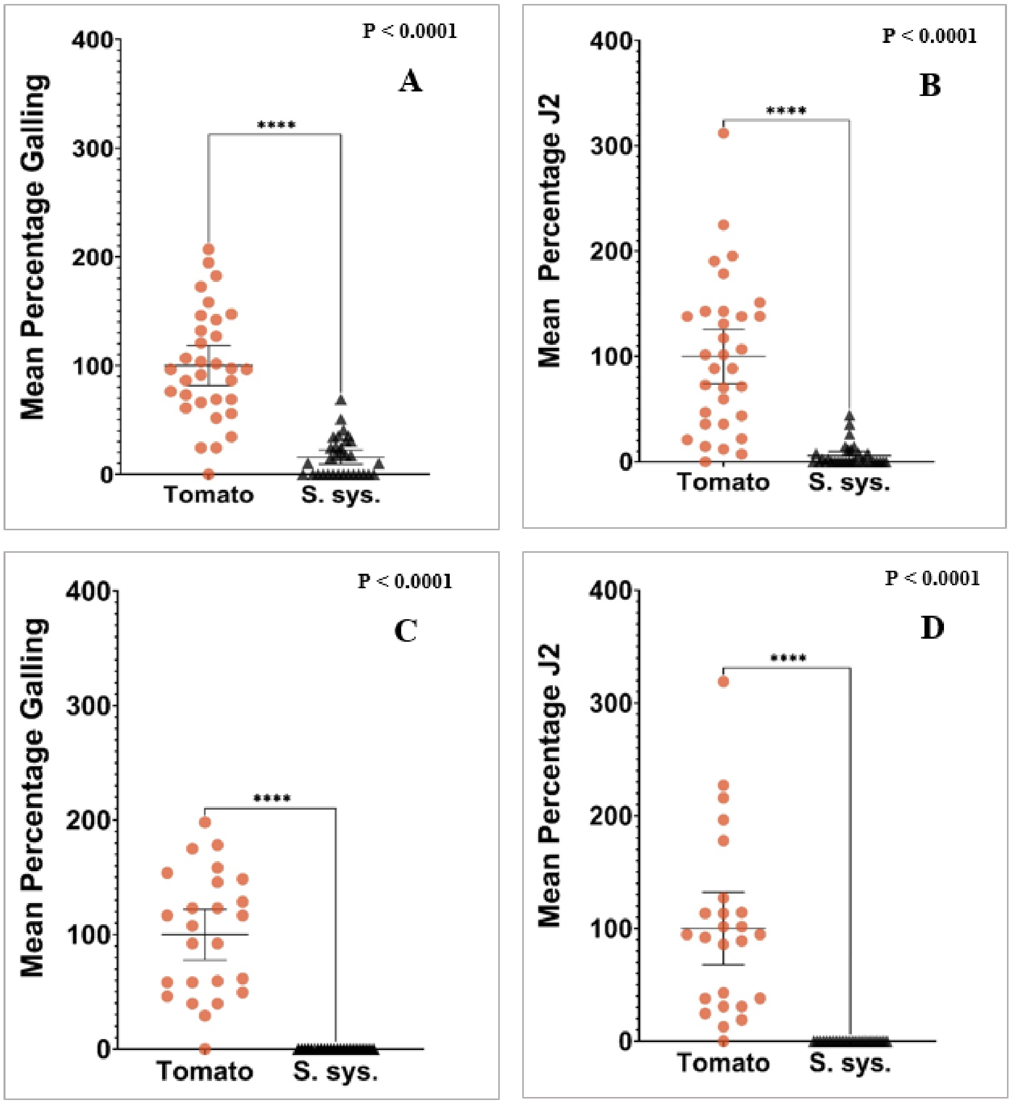

Figure 3. Infection of *Solanum sisymbriifolium* and tomato 'Rutgers' by *Meloidogyne hapla* and *M. chitwoodi* 7 days post inoculation at an inoculation density of 1,000 second-stage juveniles (J2) per plant. (A) *M. hapla* number of galls on roots [N = 32 for tomato (circles) and *S. sisymbriifolium* (S. sys, triangles)], (B) *M. hapla* number of J2 in roots (N = 32 for tomato and *S. sisymbriifolium*), (C) *M. chitwoodi* number of galls on roots (N = 25 for tomato, 24 for *S. sisymbriifolium*), and (D) *M. chitwoodi* number of J2 in roots (N = 25 for tomato, 24 for *S. sisymbriifolium*). Gall and *Meloidogyne* spp. J2 counts were normalized by dividing the number of galls or J2 per *S. sisymbriifolium* plant by the average number of galls or J2 present in tomato roots These values were then used for calculation of mean percentage. Bars represent the percentage mean with 95% confidence interval. Asterisks indicate significant between the plant types (**** = $P < 0.0001$).

Table 2. Development of *Globodera pallida* into second- (J2) or third- (J3) stage juveniles, males, or females on potato or *Solanum sisymbriifolium* over a 10-week period.

Trial 1[y]

	Potato				*S. sisymbriifolium*			
	Number of nematodes in roots							
Time (weeks)	J2	J3	Male	Female	J2	J3	Male	Female
2	1 a	0 a	0 a	0 a	1 a	0 a	0 a	0 a
4	148 c	39 b	0 a	0 a	27 a	0 a	0 a	0 a
6	71 b	75 c	127 c	43 c	2 a	1 a	0 a	0 a
8	11 a	48 b	8 b	17 bc	1 a	0 a	0 a	0 a
10	11 a	27 b	18 b	13 b	2 a	0 a	0 a	0 a

Trial 2[y]

	Potato				*S. sisymbriifolium*			
	Number of nematodes in roots							
Time (weeks)	J2	J3	Male	Female	J2	J3	Male	Female
2	61 a	2 a	0 a	0 a	49 a	0.6 a	0 a	0 a
4	38 a	48 b	34 b	21 c	8 a	5 a	3 a	0 a
6	82 a	6 a	23 b	6 b	2 b	0 a	3 a	0 a
8	5 b	5 a	8 a	4 ab	2 b	0 a	0 a	0 a
10	84 a	6 a	1 a	1 ab	4 a	3 a	0 a	0 a

[y] Values are the mean of four (Trial 1) and five (Trial 2) replicates.

[z] Values followed by different letters within each row are significantly different by least significant difference test ($P < 0.05$).

potato. In the field with a severe *M. hapla* infestation there was also a reduction in root galling on an indicator plant after *S. sisymbriifolium* treatment, but it was less pronounced. Galling was never observed on the roots of *S. sisymbriifolium*. The characterization of the resistance response by *S. sisymbriifolium* to *M. chitwoodi* has been more reliable with resistance being identified in several studies (Dias *et al*., 2012; Perpétuo *et al*., 2021).

The mechanism of nematode suppression by *S. sisymbriifolium* has been explored. *Pratylenchus goodeyi* was exposed to leaf and root extracts of *S. sisymbriifolium*, and effects on nematode mobility and mortality were observed (Pestana *et al*., 2014). These effects were attributed to the presence of alkaloids, flavonoids, and saponins commonly found in members of the family Solanaceae (Cai *et al*., 2010). Exudates of *S. sisymbriifolium* cultivars have been evaluated for their effects on hatching of *Meloidogyne* spp. (Dias *et al*., 2012). For *M. hapla* and *M. chitwoodi,* the exudates ranged from having no to minimal effect on egg hatch. Responses varied among *S. sisymbriifolium* cultivars and *Meloidogyne* spp. For example, *M. hapla* egg hatch inhibition was observed after treatments of exudates from several *S. sisymbriifolium* cultivars were tested while *M. chitwoodi* egg hatch inhibition was only observed when the eggs were treated with exudates from *S. sisymbriifolium* 'Sharp'. Additionally, recent research with *G. pallida* indicated that nematode stress tolerance and detoxification genes were expressed early after exposure to *S. sisymbriifolium* exudates, suggesting that the nematodes were trying to counteract the toxins they are encountering (Kooliyottil *et al*., 2019; Kud *et al*., 2022).

These controlled studies demonstrated the potential of *S. sisymbriifolium* to be deployed as a management practice for plant-parasitic nematodes in the PNW. However, additional information is needed to determine how *S. sisymbriifolium* might be best deployed in a field setting. *Solanum sisymbriifolium* is considered an invasive weed in Idaho, but not in Washington or Oregon. In Idaho, information is needed on how to prevent *S. sisymbriifolium* escaping from a field. The logistics of seeding rate, planting date, and incorporation timing are needed to maximize the benefits of *S. sisymbriifolium* for plant-parasitic nematode management in the region.

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