

WEED HOSTS OF ROOT-KNOT NEMATODES IN TOMATO FIELDS

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Received: May 14, 2011

Accepted: January 9, 2012

Abstract: Root-knot nematodes (*Meloidogyne* spp.) are one of the three most economically damaging genera of plant parasitic nematodes on horticultural and field crops. Root-knot nematodes are distributed worldwide, and are obligate parasites of the roots of thousands of plant species. All major field crops, vegetable crops, turf, ornamentals, legumes and weeds are susceptible to one or more of the root-knot species. In this study, nineteen weed species were found to be hosts for *Meloidogyne incognita*, *M. javanica*, *M. arenaria* race 2, and *M. hapla* in tomato fields in Khorasan Province, Iran. Egg mass production and galling differed ($p < 0.05$) among these weed species: *Amaranthus blitoides*, *Portulaca oleracea*, *Polygonum aviculare*, *Convolvulus arvensis*, *Cyperus rotundus*, *Plantago lanceolatum*, *Rumex acetosa*, *Solanum nigrum*, *Datura stramonium*, *Acroptilon repens*, *Alcea rosa*, *Alhaji camelorum*, *Chenopodium album*, *Echinochloa crusgalli*, *Hibiscus trionum*, *Kochia scoparia*, *Malva rotundifolia*, *Setaria viridis*, *Lactuca serriola*. The species *P. oleracea*, *A. blitoides*, *S. nigrum*, *P. lanceolatum*, *Ch. album*, and *C. arvensis* are major threats to the natural ecosystem in the Iranian province of Khorasan. *A. blitoides* collected from tomato fields was a good host for 4 *Meloidogyne* species. *C. arvensis*, as an important weed, was a distinguished appropriate host for *M. hapla*, *M. incognita*, *M. javanica*. *S. nigrum* and *Ch. album* were good hosts for *M. hapla*, *M. javanica*, *M. incognita* race 1, and *M. arenaria* race 3. In this survey, we reported *E. crusgalli* as a new host of *M. javanica* and *C. rotundus* was a good host for *M. arenaria* and *M. incognita*. *S. nigrum* was also reported as a new host of *M. hapla*. *R. acetosella* was reported as a host of *M. arenaria*. *M. incognita* was recently described as a new species infecting *D. stramonium* worldwide.

Key words: *Lycopersicon esculentum*, *Meloidogyne* spp., weeds

INTRODUCTION

Weeds are major constraints in agricultural production. They compete with crop plants for water, soil nutrients, and light and also interfere with distribution water and efficient fertilizer application. Weeds compete for resources with crop plants, particularly in the early stages of growth. They can reduce crop yields more than 70%. Due to their presence before, during, and after a crop cycle, weeds serve as reservoirs for plant pathogens and nematodes. Damage in future crops may be caused by the pathogens and nematodes. The problem of weed hosting plant parasitic nematodes is particularly severe in the subtropical and tropical environments where weeds grow year round. Previous research has shown many common agricultural weeds to be excellent hosts of plant parasitic nematodes. The root knot nematodes, *Meloidogyne* spp. mainly due to the wide host range, is known to exceed 3,000 wild and cultivated plant species. The nematodes cause greater yield losses and can affect crop productions by reducing the potential benefit of crop rotation. Galling on plant roots usually indicates nematode reproduction on a weed or crop plant. Generally the

greater the galling, the greater the nematode reproduction on the plant. There are 226 weed species that have been reported in Florida, USA. The Fabaceae family are the major species and then the Asteraceae family are the families which threaten the natural ecosystem in Florida, USA (Rich *et al.* 2006). Eighteen weed species were found to be hosts for *Meloidogyne hapla* (Dabey and Jenser 1990). Also, 32 species were found to be hosts for *Meloidogyne arenaria* and *M. incognita*, in South Carolina, USA. Among weed species, the family Chenopodiaceae and Euphorbiaceae were good hosts of *M. arenaria*, and the family Polygonaceae and Portulacaceae were moderate hosts. The Compositae and Convolvulaceae and Graminae and Solanaceae families were poor hosts for *M. arenaria* (Tedfort and Fortnum 1988). A survey was done of *Meloidogyne* species in vegetable fields in the Khorasan province of Iran, through a differential host experiment using the six standard test plants. The survey revealed the presence of race 2 *M. incognita* and *M. arenaria* (Ahmadiyan Yazdi and Akhyani 2004).

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MATERIALS AND METHODS

Nineteen plant species were evaluated for relative host status of *Meloidogyne* spp. in greenhouse trails. The stock nematode culture used in the experiments was derived from a single egg mass of *M. hapla*, *M. incognita* race 1, *M. javanica*, and *M. arenaria* that originated from infected feeder roots of weed species in tomato fields. The stock culture was established by placing an egg mass of the nematodes beneath the root system of a cv. Rutgers tomato (*Lycopersicon esculentum*) seedling. Inoculum for experiments was obtained by extracting eggs and second stage juveniles (j2) from 2 month old tomato plant cultures using 1% sodium hypochlorite solution (Hussey and Barker 1973).

A reproductive rating (R) was determined by dividing the average egg mass index for tomato, which is a host of four nematode species. A suboptimal initial population (pi) of root-knot nematodes (50% of the pi used in a differential host test) was chosen to separate weed species that were better hosts than tomato. Based on R values, hosts were rated as follows: $R > 1$ = good host, $0.5 < R \leq 1.0$ = moderate host, $0.1 < R \leq 0.5$ = poor host, and $R \leq 0.1$ = non host.

Differential hosts test

The response of the four species *Meloidogyne* population to differential hosts was evaluated in a glasshouse at $25 \pm 3^\circ\text{C}$ (Taylor and Sasser 1978). The differential host-set included cotton (*Gossypium hirsutum* cv. Delta pine 16), Peanut (*Arachis hypogaea* cv. Florunner), Pepper (*Capsicum annuum* cv. Early California wonder), Tobacco (*Nicotiana tabacum* cv. NC 95), Watermelon (*Citrullus vulgaris* cv. Charleston grey) and tomato cv. Rutgers. Seedlings which were 20-days-old, of the differential host plants, were transplanted (one per pot) into 700 ml clay pots filled with autoclaved sandy soil. Two days later, individual seedling were inoculated by adding 10 ml of a suspension containing 10,000 eggs and j2s of the populations of *M. hapla*, *M. javanica*, *M. incognita*, and *M. arenaria*. The control plants received the same amount of distilled water. Plants were maintained in a glasshouse adjusted to $25 \pm 3^\circ\text{C}$. There were four plants for each host. Fifty days after inoculation, plants were uprooted and their roots gently washed free of adhering soil with tap water for 20 min, and stained with phloxine B (Dickson and Struble 1965). The numbers of egg masses were counted and root gall severity (RGS) was assessed on a 0–5 scale: 0 = no galls; 1 = 1–2; 2 = 3–10; 3 = 11–30; 4 = 31–100; 5 = 100 galls (Barker *et al.* 1985).

Galled roots naturally infected from four species of *Meloidogyne* which then infected nineteen of the sampled plant species, and galled roots from plants artificially infected in the inoculum during the density plant growth experiment (see below), were selected for histopathological studies. Roots were gently washed to free adhering soil and debris, and individual galls were selected together with healthy roots. These root tissues were fixed in formaldehyde chromo acetic solution for 48 h, dehydrated in

a tertiary butyl alcohol series (40-70-85-90-100%), and embedded in paraffin. The paraffin had a 58°C melting point. Embedded tissues were sectioned, and placed on glass slides. The tissues were stained with safranin and fast-green, then mounted permanently in 40% xylene solution of a polymethacrylic ester, examined microscopically, and photographed (Hartman and Sasser 1985).

RESULTS

Nineteen weed species common in the province of Khorasan were hosts of root-knot nematodes. Among the recognized weed species in Khorasan tomato fields, *Rumex acetosa* collected only from Birjand was considered a good host for *M. arenaria* race 2. *Polygonum aviculare* in Birjand showed infection from *M. incognita* race 1, *M. arenaria* race 2, and *M. javanica*. *Cyperus rotundus* in Sarakhs, was a good host for *M. hapla*, and *M. incognita*. *Amaranthus blitoides* collected from several different fields (Mashhad, Kashmar, Tabas, Khaf, Birjand, Sarakhs) was a good host for 4 species of *Meloidogyne*. *M. javanica* and *M. incognita* were isolated from *P. lanceolatum* in Birjand and Mashhad. In tomato fields in Mashhad, *Chenopodium album*, *Portulaca oleraceae*, *Plantago lanceolaum*, *Solanum nigrum* supported moderate galling and small to large galls. While *Setaria viridis*, *Datura stramonium* and *Malva rotundifolia* were highly infected and showed gall sizes which ranged from small to medium (Table 1).

M. hapla is an important vegetable crop pest and is a major limiting factor in temperate regions. Moderate reproduction of *M. hapla* was supported by *S. nigrum*, *Ch. album* and *C. arvensis*, while *P. oleracea*, *A. blitoides*, and *P. lanceolatum* sustained a low reproduction of this nematode. Roots of *A. blitoides*, *Ch. album* and *C. arvensis* exhibited no distinct galling caused by *M. hapla* but low and moderate populations of the nematode were supported on the three weed species. Since *M. javanica* multiplied on all the weed species, controlling the weeds, especially *S. nigrum*, *Ch. album*, *P. aviculare*, *Hibiscus trionum*, *D. stramonium*, and *C. arvensis* was very important for preventing spread of *M. javanica* onto vegetable and agronomic crops. In the province of Khorasan, *S. nigrum*, *P. lanceolatum*, and *Ch. album* were moderate hosts of *M. arenaria* race 2.

Inoculation of the *M. incognita* race 1 population onto differential hosts indicated that it was unable to parasitize cotton, peanut, and tobacco, but was able to reproduce on tomato, watermelon and pepper. *M. javanica* was unable to parasitize cotton, pepper, and peanut but was able to reproduce on tomato, tobacco, and watermelon. *M. hapla* was unable to parasitize watermelon and cotton, but was able to reproduce on tobacco, pepper, tomato, and peanut. *M. arenaria* was unable to parasitize cotton, pepper, or peanut, but was able to parasitize tobacco, watermelon, and tomato. Therefore this population was identified as race 2 *M. arenaria*. Differences ($p \leq 0.01$) in egg mass production and galling by *M. hapla*, *M. javanica*, *M. incognita*, and *M. arenaria* were observed for the tested weeds (Table 1).

Table 1. Plants commonly found in tomato fields in the Khorasan province, which were noted as suitable hosts of *Meloidogyne* spp.

Scientific name of weed	Common name of weed	Family	Location	Reproductive rating [R] ¹	Egg mass index ²	Root gall index ³
<i>Portulaca oleracea</i>	Common purslane	Portulacaceae	Birjand, Tabas, Mashad, Kashmar, Khaf, Gonabad	<i>M. javanica</i> , <i>M. incognita</i> , <i>M. arenaria</i> , <i>M. hapla</i>	<i>M. javanica</i> , <i>M. incognita</i> , <i>M. arenaria</i> , <i>M. hapla</i>	<i>M. javanica</i> , <i>M. incognita</i> , <i>M. arenaria</i> , <i>M. hapla</i>
				0.5,0.8,0.1,0.3	2.2,2.2,0,0	7.3,3.8,0.1,0.1
<i>Amaranthus blitoides</i>	Prostrate pigweed	Amaranthaceae	Mashad, Kashmar, Khaf, Birjand, Tabas, Sarakhs	0.7, 0.6, 0.3, 0.2	2.3, 2.8, 1.0, 0.4	1.0, 0.4, 0, 0
<i>Solanum nigrum</i>	Black nightshade	Solanaceae	Mashad, Fariman, Kashmar, Sabzevar, Gonabad, Birjand, Sarakhs, Dargaz, Tabas	1.0, 1.0, 0.6, 0.8	2.9, 3.8, 1.6, 1.9	2.4, 2.4, 0.4, 0.2
<i>Plantago lanceolatum</i>	Ribwort	Plantaginaceae	Birjand, Sarakhs, Ferdos, Mashd	0.2, 0.1, 0.1, 0.1	4.0, 0.2, 0.5, 0.1	0.9, 0.6, 0.4, 0.2
<i>Chenopodium album</i>	Common lambs - quarters	Chenopodiaceae	Birjand, Sarakhs, Dargaz, Tabas, Mashad, Kashmar, Sabzevar, Khaf	1.3, 0.9, 1.2, 0.8	2.2, 2.2, 0, 0	0.5, 0.2, 0.8, 0
<i>Convolvulus arvensis</i>	Field bindweed	Convolvulaceae	Sabzevar, Gonabad	0.8, 0.6, 0.1, 0.4	2.5, 1.6, 0.3, 0.1	1.3, 0.5, 0.2, 0
<i>Cyperus rotundus</i>	Purple nutsedge	Cyperaceae	Sarakhs	0, 0.5, 0.4, 0	0, 1.3, 1.3, 0	0, 0.1, 0.1, 0
<i>Polygonum aviculare</i>	Prostrate knotweed	Polygonaceae	Birjand	1.0, 0, 0, 0	2.9, 0, 0, 0	2.4, 0, 0, 0
<i>Lactuca serriola</i>	Prickly lettuce	Compositae	Nehbandan	0.2, 0.8, 0, 0	0.5, 0.6, 0, 0	0.8, 0, 0, 0
<i>Setaria viridis</i>	Green foxtail	Graminae	Mashad	0.2, 0.4, 0, 0	0.5, 1.3, 0, 0	0, 0.1, 0, 0
<i>Rumex acetosella</i>	Red sorrel	Polygonaceae	Birjand	0, 0, 0.8, 0	0, 0, 2.2, 0	0, 0, 0.9, 0
<i>Datura stramonium</i>	Jimson weed	Solanaceae	Mashad	0, 0.9, 0, 0	0, 2.7, 0, 0	0, 0.4, 0, 0
<i>Acroptilon repens</i>	Russian knapweed	Compositae	Mashad, Khaf	0.6, 0, 0, 0	0.2, 0, 0, 0	0.4, 0, 0, 0
<i>Alcea rosa</i>	Mallow	Malvaceae	Dargaz	0.2, 0, 0, 0	0.7, 0, 0, 0	0.1, 0, 0, 0
<i>Echinochla crus-galli</i>	Barnyard grass	Graminae	Mashad, Birjand	0.1, 0, 0, 0	0.3, 0, 0, 0	0.1, 0, 0, 0
<i>Hibiscus trionum</i>	Pod	Malvaceae	Mashhad	0.1, 0, 0, 0	0.1, 0, 0, 0	0.1, 0, 0, 0
<i>Kochia scoparia</i>	Kochia	Chenopodiaceae	Sabzevar	0.1, 0, 0, 0	0.5, 0, 0, 0	0, 0, 0, 0
<i>Malva rotundifolia</i>	Common mallow	Malvaceae	Mashad, Khaf	0.6, 0, 0, 0	1.6, 0, 0, 0	0.1, 0, 0, 0
<i>Alhaji camelorum</i>	Camels thorn	Leguminosae	Sarakhs	1.4, 0, 0, 0	4.0, 0, 0, 0	7.3, 0, 0, 0

¹ reproductive rating for each weed species was determined by dividing the egg-mass index of each weed species by the average egg mass index for tomato

² egg mass index: 0 = no egg-masses, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 = > 100 egg mass

³ root gall index based on a 0–10 scale: 0 = no root galling and 10 = 100% of the root surface galled

DISCUSSION

The genus *Meloidogyne* includes more than 60 species, with some species having several races. Four *Meloidogyne* species (*M. javanica*, *M. arenaria*, *M. incognita*, *M. hapla*) are major pests worldwide. The host range of root-knot nematodes is very extensive including monocotyledonous and dicotyledonous herbaceous and woody plants. Many common broad-leaved weeds are able to host root-knot nematodes, maintaining levels of *Meloidogyne* spp. in fallow ground. Pigweed (*P. oleracea*), also known as Purslane, is a cosmopolitan weed. A heavy infestation of Pigweed occurred in a summer fallowed bed on an or-

ganic tomato farm in the Khorasan province. *S. viridis* has been reported as a host in tomato Mashhad fields (Akhyani *et al.* 1986) and also recognized as a reservoir of *M. incognita* and *M. javanica*. *C. arvensis* was collected from several fields (Sabzevar and Gonabad) and considered a good host for *M. hapla*, *M. incognita*, *M. javanica* and *M. arenaria*. *Kochia scoparia*, *E. crusgalli*, *A. repens*, *L. serriola*, *Alcea rosa*, *Alhaji camelorum*, *D. stramonium*, *H. trionum*, and *Malva rotundifolia* were good host for *M. incognita* and *M. javanica* which were observed in some tomato fields. In greenhouse tests, high populations of *M. hapla* on *Bidens cernua*, *B. frondosa*, *B. vulgate*, *Matricaria*

matricarioides, *Pastinaca sativa*, *P. scabrum*, *Sium suave* and *Thlaspi arvense* were found (Belair and Benoit 1996).

Ambrosia artemisiifolia, *E. crus-galli*, *S. viridis* and *Taraxacum officinale* were also reported as good hosts of *M. hapla* (Doucet *et al.* 2000). In greenhouse tests, 34 species of weeds in 32 genera belonging to 19 botanical families were found to be good hosts of *M. incognita* (Gowda *et al.* 1995). *M. incognita* was associated with major weeds in Florida, USA, especially, *Cyperus dactylon*, *Richardia scabra* and *Solanum americanum* (Myers *et al.* 2004). *Emilia sonchifolia* is a new host of *M. incognita* in India and it has been suggested that *M. incognita* was the major nematode infesting crops and weeds in that country (Velasquezvalle 2001).

Amaranthus hybridus, *Bidens pilosa*, *Digitaria horizontalis*, *Euphorbia heterophylla*, *Sida rhombifolia* and *S. americanum* served as reservoir hosts for *M. javanica*. Since this nematode multiplied on all the weed species, control of weeds especially *A. hybridus*, *E. heterophylla* and *S. americanum*, was of high importance for preventing multiplication of *M. javanica*. Other weeds reported as hosts of *M. javanica* are *Gutenbergia cordifolia* in Kenya (Desaeger and Rao 2000), *Melilotus alba* in Argentina (Lorenzo *et al.* 2002), *P. oleracea* in Australia (Walker *et al.* 2002), and *Sesbania aculeate* in India (Khan and Murmu 2004).

In weed host status studies done in North Carolina, USA, *Ch. album*, *E. maculata* and *Vicia villosa* were described as good hosts while *A. hybridus*, *A. palmeri*, *A. artemisiifolia*, *Ipomoea hederacea* var. *integriuscula*, *Setaria lutescens*, *Side spinosa*, *P. oleracea*, *Rumex acetosella* and *R. crispus* were moderate hosts of *M. arenaria* race2 (Tedford and Fortnum 1988).

A. spinosus, *Ipomoea triloba*, *Jacquemontia tamnifolia*, *Macroptilium lathyroides*, *P. oleracea* and *Physalis angulata* have been identified as weed hosts of *M. arenaria* in Florida. Other weeds reported as hosts of *M. arenaria* include *Abutilon theophrasti*, *Amaranthus retroflexus*, *A. spinosus*, *Cnidioscolus stimulosus* and *Phytolacca Americana* (Kaur and Rich 2007). From the results of our research, it can be concluded that *E. crus-galli* acts as a new host of *M. javanica* in the Mashad and Birjand regions. *C. rotundus* was a good host of *M. arenaria* and *M. incognita* (Sarakhs). *S. nigrum* has been reported as a new host of *M. hapla* (Dargaz and Tabas). *R. acetosella* was reported as a host of *M. arenaria* (Birjand). *M. incognita* was recently described as a new species in the world infecting *D. stramonium*.

This research is the first Iranian record noting *Meloidogyne* spp. on weeds in tomato farms, and indicates that these weeds should be regarded as an alternative host in the development of management systems for *Meloidogyne* spp. in tomato production. To conclude, weeds present in agricultural fields anytime during the year require carefully documented use of herbicides and proper cultivation to be used on tomato plants in order to provide reasonable control of the weeds. Effective rotation systems for nematode management are also needed. Weed management both within and after the normal cropping cycle is an often overlooked yet critical component of nematode management systems. Results indicated that weeds are a major reservoir of root-knot nematodes and should be considered as factors affecting the success of

integrated nematode management programs. Controlling the weeds would be a good initial step in reducing root-knot nematode populations.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Tavakoli, Dr. Hajjyan, and Dr. Eskandari for their technical assistance.

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