

## HOST SUITABILITY OF TWELVE COMMON WEEDS TO *PRATYLENCHUS PENETRANS* AND *MELOIDOGYNE HAPLA* IN POTATO FIELDS OF BULGARIA

H. SAMALIEV\* and Sht. KALINOVA  
Agrarian University, BG - 4000 Plovdiv, Bulgaria

### Abstract

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Twelve weeds commonly found in commercial potato fields in Bulgaria were evaluated in a pot trial for their host suitability to *Pratylenchus penetrans* and *Meloidogyne hapla*. *Apepa spica-venti* (L.) P.B., *Elytrigia repens* (L.) Nevski, *Cirsium arvense* (L.) Scop., *Chenopodium album* L. and *Solanum nigrum* L. were hosts of *P. penetrans* with multiplication rates of 1.5, 1.6, 1.7, 1.9 and 2.0, respectively. The multiplication factor of *P. penetrans* on *Echinochloa crus-galli* L. was similar to the one recorded on *Nicotiana tabacum* L., the susceptible control. *Sorghum halepense* (L.) Pers. was non-hosts as no specimen of the target nematode was found in the roots. *Amaranthus retroflexus* L. is considered a poor host with low population densities in the root. In fallow pots after 14 weeks, only 7.47% of the population of *P. penetrans* was still alive. *Chenopodium album* L. and *Cynodon dactylon* (L.) Pers. were not hosts for *M. hapla* as the end of the experiment there were no significant differences between the population in the fallow pots and in these weeds and no egg masses and eggs were observed on these weeds. *Cirsium arvense* (L.) Scop., *Echinochloa crus-galli* L., *Lamium amplexicaule* L. and *Portulaca oleracea* L. with few egg mass producing per root plant, but maintaining significantly higher population densities in the soil than were recorded in the fallow pots and is considered a poor host. *Convolvulus arvensis* L. and *Solanum nigrum* L. were hosts of the *M. hapla* with reproduction factors of 1.81 and 5.50, respectively. Fourteen weeks after inoculation of fallow pots, only 2.93% of the population of *M. hapla* was still alive.

**Key words:** Host range, natural decline *Pratylenchus penetrans*, potato fields, weeds, *Meloidogyne hapla*, reproduction factor

### Introduction

Root-knot nematodes, *Meloidogyne* spp., and lesion nematodes, *Pratylenchus* spp., are major constraints in most potato (*Solanum tuberosum* L.) cropping systems in worldwide causing both yield reductions and quality loss (Sasser and Freckman, 1987; Samaliev and Stoyanov, 2007). In Bulgaria *Meloidogyne hapla* Chitwood and *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven are distributed in the main potato growing regions as *M. hapla* is more common spe-

cies in potato fields than *P. penetrans* (Samaliev, 2011). In the different areas population densities of *M. hapla* above the damage threshold of 50 nematodes per 100 g soil (Markova and Samaliev, 2011) are common. For *P. penetrans* above the damage threshold of 80 nematodes per 100 g soil is limited in few locations in Plovdiv and Pazardjik potato regions (Samaliev, 2011, 2011a). Traditionally, the control of root-knot nematodes and lesion nematodes commonly involves the use of crop rotations with non-host crops, the application of chemicals, the use of resistant varieties, fallow and organic amend-

\*E-mail: h.y.samaliev@abv.bg

ments (Samaliev and Stoyanov, 2007). The basic principle in these management strategies is to decrease the population densities of the target nematode so that densities are reduced to below damage thresholds when the next host crop is grown. This scheme can be seriously thwarted when survival of these nematodes, or even increased densities, during fallow periods and crop rotations with resistant cultivars occurs as a result of the presence of weeds acting as hosts. Many weed hosts of both plant-parasitic nematodes have been reported (Townshend and Davidson, 1960; Stoyanov, 1980a; Edwards and Jones, 1984; Samaliev, 1997; Caswell-Chen et al., 1995; Thies, 1995; Bélair and Benoit, 1996; Luc et al., 2005; Kutwayo and Been, 2006; Samaliev and Stoyanov, 2007; Bélair et al., 2007; Rich et al., 2009). In Bulgaria host status of *P. penetrans* and *M. hapla* for many common weeds in potato growing region is unknown.

In this study, twelve weeds species that are considered a significant problem to potato crop and fallows were examined to determine their role in maintenance or multiplication of *P. penetrans* and *M. hapla* in the Plovdiv and the Pazardjik potato growing region.

## Materials and Methods

Mature seed heads from grass and dicotyledonous weeds commonly prevalent in potato fields in the Plovdiv and the Pazardjik potato growing region (Kalinova and Hristoskov, 2011) were collected in summer-autumn 2007 and 2008. After collection, seed heads were stored in paper bags at room temperature. Each weed species was collected from numerous sites in order to represent the inherent variability within these grass and dicotyledonous weeds. Weed species tested were: *Amaranthus retroflexus* L., *Sorghum halepense* (L.) Pers., *Cirsium arvense* (L.) Scop., *Chenopodium album* L., *Echinochloa crusgalli* L., *Elytrigia repens* (L.) Nevski, *Apepa spica-venti* (L.) P.B., *Solanum nigrum* L., *Cirsium arvense* (L.) Scop., *Convolvulus arvensis* L., *Cynodon dactylon* (L.) Pers., *Lamium amplexicaule* L. and *Portulaca oleracea* L. (Table 1). Tobacco (*Nicotiana tabacum* L.) cv. Burley 1000 and tomato (*Lycopersicon esculentum* Mill.) cv. Tiny Tim were used as the susceptible controls for *P. penetrans* and *M. hapla*, respectively.

*Pratylenchus penetrans* (location Dragor) was obtained from a pure culture of this species reared on tobacco cv. Burley 1000 at 20-25°C in the glasshouse. Nematode adults and larvae were recovered from roots following a mist chamber extraction for 14 days at 22°C (Seinhorst, 1950) and were used until day ten after storing at 5°C. *Meloidogyne hapla* (location Sitovo) was obtained from cultures derived from single egg masses maintained on tomato plants cv. Velocity) in a greenhouse at 20-25°C. Eggs of *M. hapla* were extracted from roots by using the sodium hypochlorite method (Hussey and Barker, 1973), second stage juveniles (J<sub>2</sub>) were obtained from these eggs by the Baermann pan method (Townshend, 1963) and were used until day ten after storing at 5°C.

The pot trial was carried out in the Agrarian University - Plovdiv 2009. The experimental design was a randomized complete block design. Plastic pots (16 cm/d, 1.5-liter volume) were filled with 1500 g sandy loam

**Table 1**  
Scientific names and phenophase at harvest of 12 common weeds in potato fields of the Plovdiv and the Pazardjik growing regions

Family, plant species	Harvest	
	phenophase	Weeks
Amaranthaceae <i>Amaranthus retroflexus</i> L.	mature seeds	12
Asteraceae <i>Cirsium arvense</i> (L.) Scop.	mature seeds	10
Chenopodiaceae <i>Chenopodium album</i> L.	mature seeds	12
Convolvulaceae <i>Convolvulus arvensis</i> L.	mature seeds	10
Lamiaceae <i>Lamium amplexicaule</i> L.	mature seeds	12
Poaceae <i>Apepa spica-venti</i> (L.) P.B.	mature seeds	14
<i>Cynodon dactylon</i> (L.) Pers	mature seeds	10
<i>Echinochloa crus-galli</i> L.	mature seeds	12
<i>Elytrigia repens</i> (L.) Nevski	mature seeds	10
<i>Sorghum halepense</i> (L.) Pers.	mature seeds	12
Portulacaceae <i>Portulaca oleracea</i> L.	mature seeds	14
Solanaceae <i>Solanum nigrum</i> L.	mature seeds	12

(77.4% sand) soil. The soil had been steam sterilised and after three weeks aeration period pots were inoculated with *P. penetrans* at a density of 1 nematode/g soil (1500 nematodes/pot) and *M. hapla* at 4 J<sub>2</sub>/g soil (6000 J<sub>2</sub>/pot). The nematodes were injected into the soil with 4 mL suspension/pot in six holes using pipette which was inserted into the soil almost to the bottom of the pot. In the *first experiment* (with plants) seeds were sown immediately after nematode inoculation. Initially, seeds of each weed species were sown in each pot in order to compensate for a germination rate of approximately 82% for weeds. After germination, only one seedling was selected for further development. Each treatment was replicated six times. In the *second experiment* (fallow) 4 series of six pots inoculated with each of the nematode species the plants were not planted. Each of the pots were placed in a plastic tray to avoid cross-contamination, watered in necessity and maintained in a growth room at 20°C ± 2°C with a photoperiod of 16 h day and 8 h night at ~75% humidity. Pots were watered with water as needed. Phostrogen liquid fertiliser (N:P:K-14:4.4:22.5) at 0.5 g / L was watered weekly onto soil in each pot (75/ mL pot), beginning 4 weeks after planting.

At the phenophase in mature seeds of each weed species (from 10 to 14 weeks, Table 1) and controls (tobacco and tomato - 14 weeks after planting) final records from the experiments were taken. For pots inoculated with *P. penetrans*, soil and root samples from each pot were collected to determine the number of nematodes. Soil nematode density was estimated by processing two 100 g subsamples for each pot as described above. The entire root system in each pot was washed under running tap water, damp dried, weighed and placed in a misting chamber for a 14 days extraction period, as described above. After the extraction period, roots were oven-dried (65°C) for 18 h and weighed. Nematodes were quantified and expressed as numbers per 100 g soil, numbers per g dry root system weight and numbers per pot. For each plant, a reproduction factor (Rf = Pf/Pi) was calculated, where Pf = final population (total number of nematodes from soil and roots for each pot) and Pi = initial number of nematodes inoculated in soil per pot.

For pots inoculated with *M. hapla*, at termination of each treatment, root systems were removed from the

pots and carefully washed with tap water to remove soil, damp dried and weighed. Individual root systems were stained with Bromothymol blue (Stoyanov, 1980) and rated for nematode reproduction with an egg mass number and for root galling with a linear scale of 0 - 5, where, 0 = no galling and 5 = 100% of roots galled (Hussey and Janssen, 2002). Eggs were extracted from each root system by the NaOCl method (Hussey and Barker, 1973), and then the roots were processed by Coolen's method to extract the remaining eggs and swollen stages of the nematode. Soil nematode density was estimated by processing two 100 g subsamples for each pot by Coolen's method (Coolen, 1979) and at the end nematode reproduction factor (Rf = final population/initial population) was calculated.

The host suitability of weeds for *P. penetrans* and *M. hapla* was determined by comparing the nematode multiplication rate (Pf/Pi) of each weed species to tobacco cv. Burley 1000 and tomato cv. Tiny Tim, respectively: host - Rf (Pf/Pi) > 1, non-host - no specimen of the target nematode has not been found in the roots.

In the *second experiment* (with fallow), the nematode density was recorded two times: First series, one week after inoculation to assess for nematode survival/recovery of both nematode species and next series, at each breakdown date the phenophase mature seeds of each weed species (see Table 1), to check natural decline levels. Soil nematode density was estimated by processing two 100 g subsamples for each pot by the Baermann pan method.

**Statistical analysis.** Data were analyzed by analysis of variance, using procedures of the SPSS-12 programme, significance being determined at  $P_{0.05}$ .

## Results and Discussion

### *Pratylenchus penetrans*

In the experiment with plants pots, the 8 species of weeds evaluated in this study belonged to 6 families. Weeds differed significantly for *P. penetrans* reproduction. *Nicotiana tabacum* cv. Burley 1000 was included as a positive control and showed the highest reproduction level ( $P_{0.05}$ , Table 2).

*Cirsium arvense*, *Ch. album*, *Ech. crus-galli*, *E. repens*, *A. spica-venti* and *S. nigrum* were hosts of *P. penetrans*

(reproduction level > 1). The multiplication of the nematode on these of the six weeds was lower than in the susceptible control (*Nicotiana tabacum* cv. Burley 1000). The nematode load per g of dry root was highest in *Ech. crus-galli* but there were no differences between the other 5 hosts. This study presents the first report of *A. spica-venti* as a host for *P. penetrans*. In relation to weeds *Cirsium arvense*, *Ch. album*, *E. repens* and *S. nigrum* our results confirm earlier work done by Townshend and Davidson (1960) and Be'lair et al. (2007), and this with *Ech. crusgalli* done by Kutuwayo and Been (2006).

*Sorghum halepense* and *A. retroflexus* were with reproduction factors <1. With *S. halepense* no specimen of the target nematode was found in the roots and is considered a non-host. *Amaranthus retroflexus* L. is considered a poor host with low population densities in the root. Low numbers of *P. penetrans* per 100 g soil and

high numbers per g dry root of host weeds were obtained in this experiment. Counts of *P. penetrans* from only the soil, as used in most of the contemporary extraction methods, will underestimate population densities.

In the experiment with fallow pots the nematodes of *P. penetrans* declined with time and at every sampling date there was a significant change in population density and 14 weeks after inoculation, only 7.47% of the initial population density of *P. penetrans* was alive ( $P_{0.05}$ , Table 3). Kutuwayo and Been (2006) reported that after 16 weeks only 1.2% of the initial population of *P. penetrans* was still alive. Townshend (1984) reports survival of *P. penetrans* in moist soils for 13 weeks at -4°C and for 9 weeks at 40-70°C. According to date of the same author in an anhydrobiotic condition *P. penetrans* could survive for 110 weeks provided the moisture loss is gradual. The period of persistence depends on many

**Table 2**  
**Number of *Pratylenchus penetrans* in the root, the soil and nematode multiplication (Pf/Pi) on the selected weeds at harvest (phenophase mature seeds) and a susceptible tobacco control after inoculation with 1500 nematodes per pot at planting**

Plant species	Number of nematodes <sup>2</sup>		Pf/Pi <sup>4</sup>
	per 100 g soil	per g dry root	
<i>Amaranthus retroflexus</i>	45.6±3.6 c <sup>3</sup>	319±72.8 c	0.85±0.36 c
<i>Sorghum halepense</i>	17.0±2.4 d	0.0	0.17±0.02 c
<i>Cirsium arvense</i>	20.2±12.1 cd	559±111.2 b	1.7±0.21 b
<i>Chenopodium album</i>	61.2±17.5 b	585±171.1 b	1.9±0.14 b
<i>Echinochloa crus-galli</i>	112.6±13.6 a	1206±324.4 a	2.4±0.33 ab
<i>Elytrigia repens</i>	49.5±9.4 c	612±188.2 b	1.6±0.37 b
<sup>1</sup> <i>Apepa spica-venti</i>	79.0±10.1 b	669±154.2 b	1.5±0.26 b
<i>Solanum nigrum</i>	34.2±5.1 c	728±198.6 b	2.0±0.42 b
<i>Nicotiana tabacum</i> cv. Burley 1000 (Control)	88.4±9.5 ab	837±256.4 b	4.9±4.70 a

<sup>1</sup>New host to *M. hapla*; <sup>2</sup>Values are actual means ± SE - statistical analysis is based on means of transformed (log10[x+1]) date; <sup>3</sup>Means in the same column followed by the same letter are not significantly different at  $P_{0.05}$  according to Duncan's Multiple Range Test; <sup>4</sup>Pf/Pi (reproduction factor) = initial population inoculated/final population at harvest.

**Table 3**  
**Natural decline of *Pratylenchus penetrans* and *Meloidogyne hapla* in fallow pots after soil inoculation with 1500 and 6000 nematodes per pot, respectively**

Nematode species	Mean population density of nematodes per pot, weeks after soil inoculation <sup>1</sup>			
	1	10	12	14
<i>P. penetrans</i>	426±112 a <sup>2</sup>	238±79 b	163±32 c	112±15 d
<i>M. hapla</i>	3455±406 a	1221±212 b	798±116 c	176±31 d

<sup>1</sup>Values are actual means ± SE - statistical analysis is based on means of transformed (log10[x+1]) date; <sup>2</sup>Means in the same row followed by the same letter are not significantly different at  $P_{0.05}$  according to Duncan's Multiple Range Test.

factors, including soil conditions (moisture, temperature), including the age of the nematodes and their food and should be investigated.

### *Meloidogyne hapla*

The results show that *in the experiment* under fallow conditions the population of *M. hapla*, similar to that of *P. penetrans*, declined with time and at every sampling date there was a significant change in population density. In the end of the experiment the (14 week after inoculation) only 2.93% of the initial density of the parasite was still alive ( $P_{0.05}$ , Table 3).

At the *experiment* with plants pots, *M. hapla* were included 8 species of weeds belonging to 7 families. Weeds differed significantly for *M. hapla* reproduction. The control *Lycopersicon esculentum* cv. Tiny Tim showed high reproduction level ( $P_{0.05}$ , Table 4).

*Convolvulus arvensis* and *S. nigrum* were hosts of the *M. hapla* with reproduction factors of 1.81 and 5.50, respectively. None of them had a multiplication rate comparable to the susceptible control. Both egg mass and egg production were higher in the control but with *S. nigrum* as egg mass so and egg production was significantly more than *C. arvensis*. The host status of *S.*

*nigrum* to *M. hapla* contradicts the findings of Stoyanov (1980a) who recorded the weed as a poor host. As *M. hapla* is a one of general pest of the Solanaceae in Bulgaria (Samaliev and Stoyanov, 2008; Samaliev, 2011a), the likelihood of multiplication on *S. nigrum*, which belongs to the same family, is high and the results obtained in this experiment mark *S. nigrum* as a host. This experiment is a first report of *C. arvensis* as a host of *M. hapla*. Considering that this weed can have one and partial second generations per year under temperate climates, its impact on nematode multiplication can be significantly greater than as ascertained in this experiment. The same applies to *S. nigrum* which can complete a similar number of generations.

*Cirsium arvense*, *Ech. crus-galli*, *L. amplexicaule* and *P. oleracea* sustained low galling and supported low reproduction, and they could be considered a poor hosts. However, at harvest these weeds had a significantly higher population density in the weeds pots than in the fallow pots. Therefore, these weeds can play a role in maintaining nematode.

*Cynodon dactylon* and *Ch. album* were not hosts for *M. hapla*. At the times of their phenophase mature seeds, there were no significant differences between the

**Table 4**

**Gall index, numbers of egg masses and eggs per root, number of second-stage juveniles (J2) in soil per pot and nematode multiplication of *Meloidogyne hapla* on selected weeds at harvest (phenophase mature seeds) and a tomato control after inoculation with 6000 J<sub>2</sub>/pot at planting**

Plant species	Gall index <sup>2</sup>	Mans number of nematodes <sup>3</sup>			Pf/Pi <sup>5</sup>
		number egg mases / root	number of eggs / root	number of J <sub>2</sub> in soil / pot	
<i>Cirsium arvense</i>	1.0±0.15c <sup>4</sup>	2.1±3.5 c	186±163 d	1160±291 d	0.22±0.10 e
<i>Chenopodium album</i>	0.0	0.0	0.0	112±68 e	0.0
<sup>1</sup> <i>Convolvulus arvensis</i>	1.8±0.1 b	59±19 b	1412±115 c	9420±1560 c	1.81±0.35 c
<i>Echinochloa crus-galli</i>	1.0±0.0 c	18±8.4 c	406±105 d	3260±501 c	0.61±0.14 d
<i>Cynodon dactylon</i>	0.0	0.0	0.0	52±32 e	0.0
<i>Lamium amplexicaule</i>	1.2±0.1 c	11±7.0 c	508±111 d	2125±312 c	0.44±0.14 d
<i>Portulaca oleracea</i>	1.2±0.0 c	14±8.2 c	706±112.4 d	4411±690 c	0.85±0.15 d
<i>Solanum nigrum</i>	2.6±1.85 a	80.2±18.2 b	6908.0±341 b	25980±2144 b	5.5±0.90 b
<i>Lycopersicon esculentum</i> cv. Tiny Tim (control)	3.2±0.39 a	327.0±59.8 a	59415±13520 a	48922±14380 a	18.1±4.60 a

<sup>1</sup>New host to *M. hapla*; <sup>2</sup>Gall index: 0 = no galls, 1 = 1-10% of root system galled, 2 = 11-35% galled, 3 = 36-65% galled, 4 = 66-90% galled and 5 = 91-100% galled (Hussey and Janssen, 2002); <sup>3</sup>Values are actual means ± SE statistical analysis is based on log transformed (log<sub>10</sub>[x+1]) date; <sup>4</sup>Means in the same column followed by the same letter are not significantly different at  $P_{0.05}$  according to Duncan's Multiple Range Test; <sup>5</sup>Pf/Pi (reproduction factor) = initial population inoculated/final population at harvest.

population in the fallow pots and in these weeds. Also, no egg masses and eggs were observed on these weeds. *Chenopodium album* has been reported previously as a host of *M. hapla* (Dabaj and Jenser, 1990) on the basis of gall formation and egg masses. This could be due to differences in botanical cultivars and varieties. There could be differences in host range among varieties necessitating identification of the weed to varietal level and their reaction to *M. hapla* infection. *Meloidogyne hapla* has also different races (Sasser, 1979) which may react differently to this weed.

## Conclusion

The successful infection of most common weed species in the Plovdiv and the Pazadjik potato growing regions in Bulgaria by *P. penetrans* and *M. hapla* stresses the importance for adequate weed management programs for potato crop. Weeds serving as reservoirs of both parasites inoculum not only threaten susceptible crop cultivars but resistance - breaking strains may develop when weeds maintain these nematodes in monoculture growing of potatoes where resistant cultivars are grown for several years (Samaliev and Stoyanov, 2007). Results further indicate that knowledge of a weed infestation in a given field and its potential for harboring plant-parasitic nematodes such as root-knot (*Meloidogyne* spp.) and root lesion (*Pratylenchus* spp.) is beneficial to an integrated pest management program.

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