ABILITY OF EIGHT COMMON WEEDS IN POTATO FIELDS OF BULGARIA TO HOST THE ROOT LESION NEMATODES *PRATYLENCHUS PENETRANS AND P. NEGLECTUS*

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ABSTRACT

Eight weed species commonly found in commercial potato fields in Bulgaria were assessed as hosts of the root lesion nematodes *Pratylenchus penetrans and P. neglectus,* under greenhouse conditions. Based on comparison of the reproductive factor (final population/initial population, or *Pf/Pi*) weeds were classified as non-hosts, poor hosts or good hosts. *Apepa spica-venti* (L) P.B., *Elytrigia repens* (L.) Nevski, *Cirsium arvense* (L.) Scop., *Chenopodium album* L., *Solanum nigrum* L. and *Echinochloa crus-galli* L were good hosts of *P. penetrans* with multiplication rates of 1.2 to 2.4. *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. *Solanum nigrum* and *Elytrigia repens* were good hosts of *P. neglectus* (multiplication rates of 1.4 to 2.2). *Sorghum halepense*, *A. spica-venti*, *E. repens* and *Ch. album* were poor host with low population densities in the root. In fallow pots after 14 weeks, only 8.2 and 6.8% of the population of *P. penetrans* and *P. neglectus, respectively,* was still alive.

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

Introduction

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 & Freckman, 1987, Vanstone et al. 1998, Taylor et al. 1999, Samaliev and Stoyanov, 2008). In Bulgaria *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven and *Pratylenchus neglectus* (Rensch) Filipjev Schuurmans & Stekhoven are distributed in the main potato growing regions as is *P. penetrans* more common species in potato fields than *P. neglectus* (Samaliev and Stoyanov, 2008). In the areas Plovdiv and Pazardjik, population densities of *P. penetrans* above the damage threshold of 80 nematodes per 100 g soil (Samaliev, 2011, 2011a) are common. For *P. neglectus* above the damage threshold of 90 nematodes per 100 g soil is limited in few locations in Pazardjik potato regions (Samaliev, 2011). Traditionally, the control of 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 amendments (Samaliev and Stoyanov, 2008). 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 & Davidson, 1960; Caswell-Chen et al., 1995; Thies, 1995; Luc et al., 2005; Kutywayo and Been, 2006, Samaliev and Stoyanov, 2008; Bélair et al., 2007). In Bulgaria host status of *P. penetrans* and *P. neglectus* 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 *P. neglectus* in the Plovdiv and the Pazardjik potato growing region.

Materials and methods

Mature seed heads from grass and dycotyledonous weeds commonly prevalent in potato fields in the Pazardjik and the Plovdiv potato growing region (Kalinova and Hristoskov, 2011) were collected in summer-autumn 2007. 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., *Apepa spica-venti* (L) P.B., *Cirsium arvense* (L.) Scop.,*Chenopodium album* L., *Echinochloa crus-galli* L., *Elytrigia repens* (L.) Nevski, *Solanum nigrum* L. *Sorghum halepense* (L.) Pers., (Table 1). Tobacco (*Nicotiana tabacum L.)* cv. Burley 1000 and oats (*Avena sativa* L.) cv. Monida were used as the susceptible controls for *P. penetrans* and *P. neglectus*, respectively.

Table 1. Scientific names and phenophase at harvest of 8 common weeds in potato fields of the Plovdiv and the Pazardjik growing regions, and tobacco/oats control

Family, plant species	Harvest	
	phenophase	Weeks
Amaranthaceae	mature seeds	12
Amaranthus retroflexus L.		
Asteraceae	mature seeds	10
Cirsium arvense (L.) Scop.		
Chenopodiaceae	mature seeds	12
Chenopodium album L.		
Poaceae	mature seeds	14
Apepa spica-venti(L) P.B.		
Echinochloa crus-galli L.	mature seeds	12
Elytrigia repens (L.) Nevski	mature seeds	10
Sorghum halepense (L.) Pers.	mature seeds	12
Solanaceae	mature seeds	12
Solanum nigrum L.		
Nicotiana tabacum L. cv. Burley 1000 (Control)	mature seeds	14
Avena sativa L. cv. Monida (Control)	mature seeds	16

Pratylenchus penetrans (location Dragor) and *P. neglectus* (location Sitovo) 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^{o}C.

The pot trial was carried out in the Agrarian University - Plovdiv 2012. 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 (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 (adults and juveniles) / 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 84% 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 24°C ± 3°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 wheat - 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.

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 (Table 1). 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).

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[2]		Pf/Pi[4]
	per 100 g soil	per g dry root	
Amaranthus retroflexus	45.6±3.6 c[3]	319±72.8 c	0.85±0.36 c
Sorghum halepense	17.0±2.4 d	0.0	0.17±0.02 c
Cirsium arvense	20.2±12.1cd	559±111.2 b	1.7±0.21 b
Chenopodium album	61.2±17.5 b	585±171.1 b	1.9±0.14 b
Echinochloa crus-galli	112.6±13.6 a	1206±324.4 a	2.4±0.33 ab
Elytrigia repens	49.5±9.4 c	612±188.2 b	1.6±0.37 b
[1]Apepa spica-venti	79.0±10.1 b	669±154.2 b	1.5±0.26 b
Solanum nigrum	34.2±5.1 c	728±198.6 b	2.0±0.42 b
Nicotiana tabacum cv. Burley 1000 (Control)	88.4±9.5 ab	837±256.4 b	4.9±4.7 a

[1]New host to *P. penetrans* ; [2]Values are actual means ± SE - statistical analysis is based on means of transformed (log10[x+1]) date; [3]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; [4]Pf/Pi (reproduction factor) = initial population inoculated/final population at harvest.

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), Be´lair et al. (2007) and Samaliev and Kalinova, 2011, and this with *Ech. crusgalli* done by Kutywayo and Been (2006).

Sorghum halepense and *A. retroflexus* were with reproduction factors <1. With *Sorghum halepense* (L.) Pers. 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.

Pratylenchus neglectus: Six weed species did not host *P. neglectus* (*Pf/Pi* = 0.17– 0.84), with nematode multiplication and nematodes per plant significantly ($P_{0.05}$) less than the control oats cv. Monida (Table 3).

Table 3. Number of *Pratylenchus neglectus* 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[2]		Pf/Pi[4]
	per 100 g soil	per g dry root	
Amaranthus retroflexus	16.6±4.1 d[3]	411±81.0 d	0.44±0.9 d
Sorghum halepense	19.0±1.9 d	0.0	0.19±0.01 e
Cirsium arvense	26.3±12.1d	319±99.3 d	0.48±0.17 d
Chenopodium album	60.1±19.5 c	362±123.2 d	0.86±0.16 d
Echinochloa crus-galli	22.6±10.1 d	159±114.2 d	0.33±0.13 d
[1]Elytrigia repens	96.6±12.3 b	794±289.4 b	1.4±0.19 c
Apepa spica-venti	53.5±10.1 c	311±191.2 d	0.74±0.28 d
Solanum nigrum	84.1±5.1 b	611±178.4 bc	2.2±0.24 ab
Avena sativa cv. Monida	119.3±8.2 a	1119±256.4 a	3.6±3.0 a

[1]New host to *P. neglectus* ; [2]Values are actual means ± SE - statistical analysis is based on means of transformed (log10[x+1]) date; [3]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; [4]Pf/Pi (reproduction factor) = initial population inoculated/final population at harvest.

Apepa spica-venti was hosts (*Pf/Pi* = 1.4).Nematode multiplication and nematodes per plant in this weed species was significantly less ($P_{0.05}$) than the control. This study presents the first report of *A. spica-venti* as a host for *P. neglectus*.

Solanum nigrum (*Pf/Pi* = 2.2) was classified a good host of *P. neglectus*, with nematode multiplication and numbers per plant significantly ($P_{0.05}$) the same as in oats cv. Monida. But the nematode load per g of dry root was higher in *Ech. crus-galli* than *S. nigrum*.

In the experiment with fallow pots the nematodes of *P. penetrans* and *P. neglectus* declined with time and at every sampling date there was a significant change in population density and 14 weeks after inoculation, only 8.2 and 6.8% of the initial population density of *P. penetrans* and *P. neglectus* were alive ($P_{0.05}$, Table 4). Kutywayo 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 factors, including soil conditions (moisture, temperature), including the age of the nematodes and their food and should be investigated.

Table 4. Natural decline of *Pratylenchus penetrans* and *Pratylenchus neglectus* in fallow pots after soil inoculation with 1500 nematodes per pot

Nematode species	Mean population density of nematodes per pot, weeks after soil inoculation			
	1	10	12	14
P. penetrans	426 a*	242 b	172 c	123 d
M. hapla	508 a	269 b	165 c	102 d

*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.

Conclusion

The successful infection of most common weed species in the Plovdiv and the Pazadjik potato growing regions in Bulgaria by *P. penetrans* and *P. neglectus* 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, 2008). Results further indicate that knowledge of a weed infestation in a given field and its potential for harboring plant-parasitic nematodes such as root lesions (Pratylenchus spp.) is beneficial to an integrated pest management program.

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