Host Range and Growth of Stem and Bulb Nematode (*Ditylenchus dipsaci*) Populations Isolated from Garlic and Chicory

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Abstract

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Host range and growth of two *Ditylenchus dipsaci* (Stem and Bulb Nematode) populations on onion, garlic, leek, spinach and chicory were compared. Inoculation was conducted by placing a droplet of 1.5% CMC suspension containing nematodes between the first leaves. The life cycle of the parasite was completed in variants of the original host plants, i.e. salad chicory was infected with the chicory population, and garlic and onion with the garlic population. Survival of the nematodes on garlic and spinach inoculated with the chicory population, and on chicory inoculated with the garlic population was determined. Nematodes of the chicory population did not survive on onion and leek, nor did the garlic population survive on spinach and leek. Nematode infection did not affect the dry weight of aboveground parts of plants, although a reduction would probably occur if the experiments had lasted longer. Symptoms of the infections were apparent in chicory inoculated with the chicory population. Nematodes in the substrate in which the plants were grown were found only in those variants with plants in which the life cycle was completed.

Keywords: *Ditylenchus dipsaci*; vegetable; host range

The Stem and Bulb Nematode, *Ditylenchus dipsaci* Kühn (Filipjev), belongs to the major pests of several vegetable crops. Considerable losses are repeatedly reported from cultures of garlic, onion (Aftalion & Cohn 1990), carrot (Greco *et al*. 2002), field bean (Kornobis 1994) and chicory (Urek *et al*. 1998). From other crops, lucerne, clover and peas are frequently attacked; the next important hosts are rye, oat, sugar beet and also ornamental plants like hyacinth, tulip and narcissus (Janssen 1994). *Ditylenchus dipsaci* is listed in registers of quarantine organisms of the EU, EPPO and the Czech Republic. Its host range is extremely wide and encompasses about 400 (Brzeski 1998), 450 (Hooper 1972) or 500 (Esquibet *et al*. 1998) plant species.

The key problem that complicates effective management of this nematode is the existence of morphologically mostly indistinguishable populations with different feed preferences. The taxonomic position of these populations (often labelled as "host races") still remains unclear, although differences in chromosome number ($2n$ = 12–60; Brzeski 1998) were found. Particularly the status of the giant race, which differs also morphologically, is still discussed (Esquibet *et al*. 2003).

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Because the main control method of this nematode is to design and adhere to an appropriate crop rotation system, which includes periods when host plants unsuitable for stem and bulb nematode reproduction are grown on the infected field. It is, therefore, necessary to recognise which race occurs on the infected plot and its feeding preferences. The classical technique for discrimination of races are biological experiments, conducted in pots or using tissue cultures and there is also an effort for molecular characterisation of different races (Palmer *et al*. 1991; Wendtová *et al*. 1993; Esquibet *et al*. 1998; Esquibet *et al*. 2003). Another problem to effectively manage *D. dipsaci* is its ability to survive for a long period in an anabiotic stage (Wharton & Marshall 2002).

The purpose of this study was to get specifications of the host range of a *D. dipsaci* population isolated from chicory, and compare the findings with results from a garlic population.

MATERIAL AND METHODS

Preparation of inocula and inoculation were performed as described by Plowright *et al*. (2002), methods that were originally designed to test lucerne and bean varieties for their reaction to *Ditylenchus dipsaci*. Two *D. dipsaci* populations were used in the experiments; the first was isolated from chicory originally from Slovenia and three times reproduced on the same host plant in a greenhouse. The second population was obtained from heavily infested cloves of garlic grown in the area of the village Blatnice pod Svatým Antonínkem (eastern part of the Czech Republic); this population was not reproduced before the inoculation. A modified Baermann's funnel method was used for nematode extraction. The acquired nematode suspension was allowed to settle in a glass tube, the supernatant was repeatedly siphoned off and the tube refilled with clean water. Then the nematodes were transferred to a 1.5% solution of carboxymethyl cellulose (CMC) and stored in a glass tube at 4°C. The final dilution of the nematode suspension was approximately 50 specimens per 20 µl.

Fifteen seeds of onion, garlic, leek, chicory or spinach were sown to each pot, filled with sterilised pot substrate. Each plant species was planted in five replicates. Plants were grown at natural photoperiod in an air-conditioned greenhouse for 2 weeks at a constant temperature of 20°C and then the five weakest plants were removed. Forty plants were used for inoculation and the remaining 10 as control.

Nematodes were inoculated on to seedlings in a small drop of suspension placed between the first leaves. Sample droplets were taken at the start and end of inoculation to ensure homogeneity of the inoculum. The suspension was blended regularly during inoculation to prevent settling of the nematodes at the bottom of the glass tube. Air humidity was raised for 24 h after inoculation using automatic misting. Plants were re-inoculated in the same manner after 48 h to prevent escapes from inoculation. After that the plants were grown for 12 weeks and symptoms of infection were scored during that time. The aboveground parts of the plants were then harvested, one half was cut into 4–5 cm pieces and dried for 60 min at 150°C and weighed to evaluate the dry aboveground biomass of individual experimental variants. The other half of the harvested plants were cut into 1 cm pieces and extracted using a modified Baermann's funnel method. Presence of the nematodes in the acquired suspension was determined under a stereomicroscope.

RESULTS AND DISCUSSION

After 12 weeks a progressing life cycle of the parasite (i.e. presence of a population with reproduction, containing juvenile development stages) was detected in the original host plants salad chicory inoculated with chicory population and garlic and onion inoculated with the garlic one. This indicates that the method used for inoculation was correct and environmental conditions during the trials were optimal for development of the nematodes. Individual adult *Ditylenchus dipsaci* nematodes (without establishing a reproducing population) survived on garlic and spinach inoculated with the chicory population, and on chicory inoculated with the garlic population. Nematodes of the chicory population did not survive on onion; specimens of the garlic population did not survive on spinach. Leek did not support the garlic nor the chicory population (Table 1). Statistic evaluation of dry matter weight (ANOVA) revealed no significant difference between infected plants and healthy control. Even in samples with heavy infestation and completed life cycle of the nematode the plants had the same weight as the control. Merely in the case of onion statistically considerable dif-

Table 1. Host range of the two tested *Ditylenchus dipsaci* populations

	Onion	Garlic	Leek	Spinach	Chicory
Population from chicory	0	1	0	1	2
Population from garlic	2	2	0	0	1

0 – no nematodes survived; 1 – only single adult individuals survived; 2 – life cycle of the parasite completed

Table 2. Average dry mass of the experimental plants (mg)

	Population from garlic	Population from chicory	Control	*P**
Onion	246.95	185.72	610.11	1.42697E-07
Garlic	1095.61	1467.08	1220.2	0.331148277
Leek	317.49	312.67	317.96	0.996149761
Spinach	1172.28	866.36	799.05	0.199370147
Chicory	1963.44	2075.71	1859.4	0.970353228

*results of ANOVA at $P < 0.05$

ference was detected between the trial variants and control ($P < 0.05$; Table 2), but after further examination the difference was not detected mutually between the trial variants. Nematodes of the chicory population died out on onion, therefore the revealed difference could not be ascribed to nematode infection and the factor which caused this finding is unclear. Symptoms of the infection were scored on chicory inoculated with the chicory population. Only single nematodes were found in the substrate the plants were grown in; in all cases they were adults and occurred only in variants with a complete life cycle (Table 3).

Populations of *D. dipsaci* isolated from onion or garlic are not distinguished in agricultural practice and are mostly marked as "Onion and Garlic race" although the existence of populations attacking only onion and not garlic is known (Aftalion & Cohn 1990). The nematodes isolated from garlic and used in this experiment showed no difference in reproduction on onion and garlic, but did not survive in leek, this finding was not with the kinship of these species expected. This result corresponds with the previously reported diversity of host range of *D. dipsaci* populations attacking onion bulbs – e.g. separate races infecting tulip and narcissus are commonly divided (Janssen 1994).

The host range of the population that attacks chicory is not yet well known, but according to the present study we can consider spinach and garlic as host plants of this population. These crops will probably not be damaged at an economically important level; however, nematodes of the chicory population could persist in garlic and onion to the next growing seasons.

No impact of nematode infection on dry matter weight of aboveground parts of the tested plants was detected. This finding is interesting since there was a relatively considerable intensity of infection on the original host plants (especially on chicory) (Table 2). A significant decrease of the weight of infested plants would occur most likely if the experiments had lasted longer. A qualitative damage was observed only in the variant of chicory inoculated with the chicory population – it was thus confirmed that nematode infection may not

Table 3. Occurrence of nematodes in the substrate the plants were grown in

	Population from	
	chicory	garlic
Onion	-	+
Garlic	-	+
Leek	–	–
Spinach	–	–
Chicory	+	-

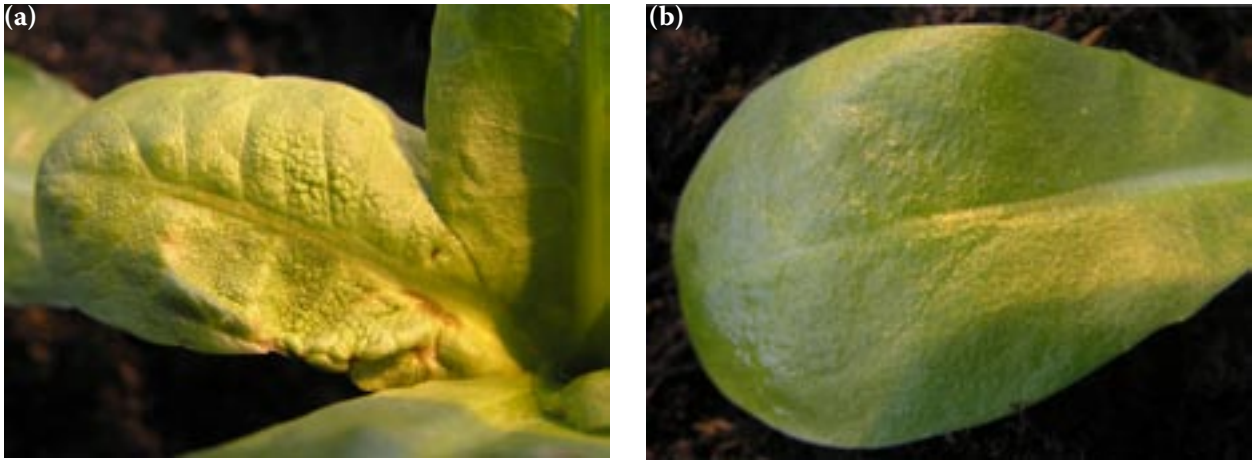

Figure 1. (a) Leaf of salad chicory infested by *Ditylenchus dipsaci* nematode; (b) healthy control

manifest itself outwardly. Symptoms probably develop only during very heavy infection while in a case where nematodes are just surviving in plants without intensive reproduction the infestation would not be noticeable. Symptoms on chicory were observed after 4 weeks and consisted of tiny distortions and deformation of the young leaves (Figure 1). The occurrence of only single adult individuals in the substrate the test plants were grown in shows that at a low level of infestation the majority of nematodes rather stays in the host plant and does not migrate into the soil.

The question of the existence of morphologically indistinguishable *D. dipsaci* populations differing only in their host plant preferences has been discussed by many authors for a long time, but without achieving a generally accepted answer. Even uniformity in terminology has not been reached yet – e.g. Brzeski (1998) uses the term pathotype for his populations, while the traditional term "race" is used more frequently (Viglierchio 1971; Esquibet *et al.* 2003). For the purposes of this study the more general term "population" was used since the *D. dipsaci* population which infects chicory was not defined as a separate race. The situation is further complicated by the fact that field populations of *D. dipsaci* could be a mixture of races. After all, plants can be infected with more than one race at the same time, e.g. onion, potatoes, sugar beet and especially bean are hosts of many populations of different races (Whitehead *et al.* 1987). Thus, the question of terminology still has to be solved. Naming simply after one host is maybe too general, but if we would like to be precise we have to test the whole host range and name the population after the most susceptible host, which is practically impossible. Still, a more accurate description of the population used in experiments or found on an infested field should be done. In spite of all uncertainty there is no doubt that differences among races (or populations) of the nematode *Ditylenchus dipsaci* exist. The results obtained in this study are consistent with this fact, because even with the low number of tested host plants differences between the host ranges of the two examined populations were revealed.

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Souhrn

Douda O. (2005): **Hostitelský okruh a nárůst populací háďátka zhoubného (*Ditylenchus dipsaci*) izolovaných z česneku a čekanky**. Plant Protect. Sci., **41**: 104–108.

Byl srovnáván hostitelský okruh a nárůst dvou populací háďátka zhoubného (*Ditylenchus dipsaci*) na cibuli, česneku, pórku, špenátu a čekance. Inokulace byla prováděna umístěním kapky suspenze háďátek v 1,5% CMC mezi první listy rostlin. Životní cyklus parazita proběhl ve variantách původních hostitelských rostlin, tj. salátové čekanky inokulované čekankovou populací a česneku i cibule inokulovaných česnekovou populací. Přežívání jednotlivých háďátek bylo zjištěno na česneku a špenátu inokulovaných čekankovou populací a na čekance inokulované populací česnekovou. Na cibuli a pórku háďátka čekankové populace nepřežila vůbec stejně jako v případě česnekové populace na špenátu a pórku. Vliv napadení háďátky na hmotnost sušiny nadzemní části testovaných rostlin nebyl zjištěn, redukce by pravděpodobně nastala, pokud by pokusy trvaly delší dobu. Kvalitativní poškození bylo pozorováno u salátové čekanky inokulované čekankovou populací. V substrátu byla háďátka zjištěna ve variantách s normálním populačním růstem.

Klíčová slova: *Ditylenchus dipsaci*; zelenina; hostitelský okruh

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