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Effect of host plant and saprophytic soil fungi on population size of *Globodera rostochiensis*. (Woll.) Behrens

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Summary. We studied the possible effects of host plants (potato, *cv.* Mila, and tomato, *cv.* Ika) and saprophytic fungi (*Stachybotrys chartarum* and *Penicillium frequentans*) on population size of the golden nematode, *Globodera rostochiensis*. Coexistence of *G. rostochiensis* and the saprophytic fungi resulted in a significant reduction of the nematode population (reduced numbers of cysts, eggs, and juveniles). Also, differences were found between potato and tomato plants in relation to their suitability as host organisms for the nematodes. It was found that population density of the nematodes in soil with potatoes was many times greater than that in soil with tomatoes. The study has demonstrated that both the nematode host plant and saprophytic soil fungi significantly influence population density of *G. rostochiensis*.

Key words: *Globodera rostochiensis*, interaction, potato, saprophytic soil microorganisms, tomato.

The effect of soil fungi on populations of the golden potato cyst nematode, *Globodera rostochiensis*, represents an interesting subject that might contribute to capacity of biological plant protection (Kerry, 1980; Rössner, 1987). The fundamental strategy of plant protection consists in maximal utilisation of natural processes that take place in the agrobiocenosis along with maintenance of ecosystem homeostasis (Miętkiewski *et al.*, 1992). Soil microecosystems, the habitats for phytophagous nematodes and their host plants, are dynamically formed by microorganisms present. However, neither the role of saprophytic fungi, which excrete metabolites antagonistic or competitive against *Globodera rostochiensis* into the rhizosphere of plants, nor the possibility (probability) that the fungi affect the physiology of host plant have been fully explained.

A variety of methods, including agrotechnical and agrochemical techniques, have been developed to control populations of *G. rostochiensis*. Particularly promising are the results of research on natural antagonists of the pests that coexist with the nematode in the rhizosphere of a host plant (Morgan-Jones & Rodriguez-Kabana, 1986; Wronkowska & Janowicz, 1988; Zawiślak & Rzeszutek, 1996). Obligatory fungal phytopathogens, which are also parasitic to *G. rostochiensis* cysts (Kerry, 1984, 1988; Goswami & Rumpenhorst, 1978; Wilcox & Tribe, 1974), are of little use as a supporting element in the biological control of the golden nematode. Thus, saprophytic microorganisms that display nematoxic and nematicidal properties become more promising for practical application.

The aim of this study was to investigate the effect of a host plant (potato and tomato) and commonly found saprophytic soil fungi (*Penicillium frequentans* and *Stachybotrys chartarum*) on the population size of *Globodera rostochiensis*.

MATERIAL AND METHODS

Pot experiments were carried out on potato and tomato (cultivars Mila and Ika, respectively,-both susceptible to *G. rostochiensis*) during three growing seasons in 1999-2001. The substrate had been prepared at the start of the study and comprised leaf compost, peat, and sand (3:2:1). The soil was placed in 15 dm^3 pots and used throughout the experiment.

At the onset of each season, healthy potato tubers of similar size were disinfected in 0.4% formalin solution for 20 min, and were thereafter left to germinate. Tomato seedlings were grown in glasshouse until the 4 leaf stage. One potato tuber or one tomato seedling was planted in each pot, and then the pots were placed outside the glasshouse (natural conditions). No chemical protecting agents were applied to the plants.

At the start of each growing season, a 21-day-old inoculum of one of the saprophytic fungi, *Penicillium frequentans* (Westling) or *Stachybotrys chartarum* (Ehrenb. ex Link) Hughesi, was placed together with potato tubers or tomato seedlings. Single-spore strains of the fungus with PDA nutrient were placed in a pot on four 10-cm diameter disks. In the controls, four 10-cm diameter disks of sterile PDA nutrient were placed in each pot.

In the first year of observation, *G. rostochiensis* cysts, superficially disinfected in ethyl alcohol and triple-rinsed with sterile water, were placed in soil together with potato tubers and tomato plants. Initial density (P_i) was 10 cysts in 100 g of soil, which corresponded to 19 eggs and juveniles per g of soil. In subsequent years, the density of cysts, eggs, and juveniles was examined directly upon completion of potato and tomato growth. The final density (P_f) of cysts, eggs, and juveniles of the previous year represented the initial density (P_i) of the subsequent year.

In the experiment with potato and tomato plants, the substrate was inoculated with *Globodera rostochiensis*; *G. rostochiensis* + *P. frequentans*; *G. rostochiensis* + *S. chartarum*. Controls consisted of substrate free of *G. rostochiensis* or saprophytic fungi + potato/tomato plant.

Each treatment was carried out in 4 replications, where a single plant in a pot represented a replicate.

Upon the completion of each growing season (the last ten days of September), nematode densities were determined in each treatment. Cyst number in 100 g of soil and number of eggs and juveniles in 1 g of soil were analysed to determine the population density index (P_f/P_i), i.e. the ratio of final number of cysts to their number at the start of a growing season. Cyst, eggs, and juvenile densities were determined using the method of Seinhorst & Den Ouden (1966). Soil samples for analysis were collected after completion of plant growth. An amount of 0.5 kg of soil (thoroughly mixed in each pot before sampling) was collected randomly from each pot using soil sampling tube; then 4 subsamples of 100 g (4 replications) from each soil sample were taken for analysis. The fecundity of *G. rostochiensis* females was expressed as a number of eggs and juveniles per one cyst. The fecundity was evaluated based on an analysis of 30 cysts from each treatment (Brzeski, 1998; Brzeski et al., 1976).

The results were analysed for each year using one-way ANOVA. Significance of differences between means of treatments was estimated with the Neumann-Keuls test, at the significance level of $\alpha = 0.05$.

RESULTS

A significant restrictive effect of *P. frequentans* and *S. chartarum* on *G. rostochiensis* was observed both on potato and tomato plants. It was also demonstrated that potato is the better host plant for the nematode than the tomato, both in soil with or without the fungi (Tables 1-4).

In soil free from the fungi, a particularly dynamic increase in the number of *G. rostochiensis* cysts was observed after the first season of potato growth. During the growing season of 1999, cyst density on potato plants increased more than 14-fold (Table 1), while on tomato plants the dynamics were less intensive (Table 2). In years 2000-2001, the number of *G. rostochiensis* cysts on potato plants slightly increased (Table 1), while remaining unchanged on tomato plants (Table 2). A lower cyst number was recorded on tomato plants compared to the respective treatments in the potato experiment in each year and each treatment of experiments (Tables 1 and 2); this also applies to the numbers of eggs and juveniles of *G. rostochiensis* (Tables 3 and 4).

An analysis of *G. rostochiensis* female fecundity demonstrates that host plants modify reproduction of the nematode.

A significant effect of the saprophytic fungi on the reduction of golden nematode offspring in the second and third growing seasons was recorded in the potato experiment (Table 5). Such tendency of reducing the fecundity of females in the presence of saprophytic fungi was not (statistically) demonstrated for tomato, although it was observed

Table 1. Effect of saprophytic soil fungi on the number of *Globodera rostochiensis* cysts in soil of potato culture.

Treatment	Cyst number in 100 g of soil Year			
	1999 (spring) P_i	1999 (autumn) P_f	2000 (autumn) P_f	2001 (autumn) P_f
Globodera rostochiensis	10	146.2 b	432.1 b	447.3 b
G. rostochiensis + *Penicillium frequentans*	10	61.5 a	181.6 a	262.2 a
G. rostochiensis + *Stachybotrys chartarum*	10	84.2 a	256.5 a	395.4 b

Data in columns followed by the same letter do not differ significantly.
Final value (Pf) recorded in autumn constitutes next spring initial value (Pi).

Table 2. Effect of saprophytic soil fungi on the number of *Globodera rostochiensis* cysts in soil of tomato culture.

Treatment	Cyst number in 100 g of soil Year			
	1999 (spring) P_i	1999 (autumn) P_f	2000 (autumn) P_f	2001 (autumn) P_f
Globodera rostochiensis	10	85.0 c	84.3 b	78.5 a
G. rostochiensis + *Penicillium frequentans*	10	75.5 b	53.3 a	79.3 a
G. rostochiensis + *Stachybotrys chartarum*	10	49.8 a	60.5 a	70.0 a

Data in columns followed by the same letter do not differ significantly.
Final value (Pf) recorded in autumn constitutes next spring initial value (Pi).

Table 3. Effect of saprophytic soil fungi on population density of *Globodera rostochiensis* (number of eggs and juveniles per 1 g of soil) in potato culture.

Treatment	Population density Year			
	1999 (spring) P_i	1999 (autumn) P_f	2000 (autumn) P_f	2001 (autumn)P_f
Globodera rostochiensis	19	163.3 b	769.3 b	400.8 b
G. rostochiensis + *Penicillium frequentans*	19	74.0 a	141.3 a	176.7 a
G. rostochiensis + *Stachybotrys chartarum*	19	78.9 a	225.7 a	262.4 a

Data in columns followed by the same letter do not differ significantly.
Final value (Pf) recorded in autumn constitutes next spring initial value (Pi).

Table 4. Effect of saprophytic soil fungi on population density of *Globodera rostochiensis* (number of eggs and juveniles per 1 g of soil) in tomato culture.

Treatment	Population density Year			
	1999 (spring) P_i	1999 (autumn) P_f	2000 (autumn) P_f	2001 (autumn) P_f
Globodera rostochiensis	19	86.0 b	37.0 c	46.5 b
G. rostochiensis + *Penicillium frequentans*	19	76.3 ab	32.3 c	24.8 a
G. rostochiensis + *Stachybotrys chartarum*	19	60.8 a	29.0 bc	21.8 a

Data in columns followed by the same letter do not differ significantly.
Final value (Pf) recorded in autumn constitutes next spring initial value (Pi).

in the first (1999) and the last (2001) year of the study (Table 6). In the last year of the experiment (2001), the fecundity of *G. rostochiensis* females in all treatments of potato experiment was nearly twice that of the respective treatments of tomato experiment (Tables 5 and 6).

Table 5. Effect of saprophytic soil fungi on fecundity (number of eggs and juveniles per 1 cyst) of *Globodera rostochiensis* females in potato culture.

Treatment	Fecundity of females Year		
	1999	2000	2001
Globodera rostochiensis	111.7 a	199.6 b	88.2 b
G. rostochiensis + *Penicillium frequentans*	120.7 a	63.4 a	65.9 a
G. rostochiensis + *Stachybotrys chartarum*	93.7 a	75.4 a	65.9 a

Data in columns followed by the same letter do not differ significantly.

Table 6. Effect of saprophytic soil fungi on fecundity (number of eggs and juveniles per 1 cyst) of *Globodera rostochiensis* females in tomato culture.

Treatment	Fecundity of females Year		
	1999	2000	2001
Globodera rostochiensis	111.7 a	199.6 b	88.2 b
G. rostochiensis + *Penicillium frequentans*	120.7 a	63.4 a	65.9 a
G. rostochiensis + *Stachybotrys chartarum*	93.7 a	75.4 a	65.9 a

Data in columns followed by the same letter do not differ significantly.

Moreover, the population growth index (Pf/Pi) was many-fold higher (particularly in the first year) on potato plants than on tomato ones (Tables 7 and 8).

DISCUSSION

It has been commonly known that *G. rostochiensis* belongs to the most dangerous pests of potato, and is particularly difficult to control (Seinhorst, 1982; Decker, 1969). The results of this work show that increased population density of *G. rostochiensis* is accompanied by reduced reproduction capacity of the nematode. In field conditions, we may expect faster population growth rate of the nematodes if their initial numbers were low (Barker & Olthof, 1976; Seinhorst, 1982; Rzeszutek et al., 1987; Lutomirska, 1995; Marshall, 1989). It should be stressed that the number of cysts can gradually decrease only if there are no host plants. In the presented experiment, the golden nematode showed restricted ability to develop, even in soil without the fungi (Tables 1 and 2). This results mainly from the fact that potato is grown in a monoculture, which, according to Tribe (1977), adversely affects health and fecundity of cysts due to antagonistic soil organisms. By contrast, monoculture in pots hampers root system development and leads to chronic deficits of nutrients necessary for the plants (Trudgill & Cotes, 1983; Evans & Trudgill, 1978; Haverkort *et al.*, 1994). This impedes the growth of the plant's above-ground parts (Fasan & Haverkort, 1991; Smit & Vamerali, 1998). The degradation of female reproductive ability observed in our studies may be treated either as an effect of shortening root life (Evans *et al.*, 1975; Trudgill, 1987) or a "host-saving" strategy of the parasite (Haverkort & Trudgill, 1995). In pot experiments, very high density of the nematodes in soil represents a remarkable factor of its population collapse.

Table 7. Effect of saprophytic soil fungi on population density (number of eggs and juveniles per 1 g of soil) index (Pf/Pi) in potato culture.

Treatment	Population density index (Pf/Pi) Year		
	1999	2000	2001
Globodera rostochiensis	8.6	4.7	0.5
G. rostochiensis + *Penicillium frequentans*	3.9	1.9	1.2
G. rostochiensis + *Stachybotrys chartarum*	4.1	2.9	1.2

The fact that a number of authors had demonstrated experimentally a destructive effect of saprophytic fungal excretions on embryogenesis and morphology of invasive second-stage juveniles (J2) indicated the direct antagonistic effect of saprophytic soil fungal metabolites on different stages of nematodes inhabiting the soil (Moreau & Trique, 1966; Mazurkiewicz-Zapałowicz *et al.*, 1999; Chen *et al.*, 2000). The pot experiments on potato presented in this work confirmed the

susceptibility of *G. rostochiensis* populations to the tested fungi, *P. frequentans* and *S. chartarum*. The interaction between the fungi and *G. rostochiensis* resulted in significant reduction of cysts, eggs, and juveniles densities in soil (Tables 1-4) and reduced reproductive capacity of the pest (Tables 5 and 6) and depended on the host plant species. The host plant, and particularly its physiology, directly influenced the density of *G. rostochiensis*. It was shown that potato serves as better host plant for golden nematode than tomato and *G. rostochiensis* were many-fold more numerous in potato experiments compared with tomato ones (Tables 1-4). Presumably, the fact that potato plants had the better developed root system was the favourable factor for the infective J2 to penetrate. Host plant physiology can also be an important factor affecting development of the phytophagous nematode. This suggestion has been confirmed by recent studies (Robinson *et al.*, 1985) which demonstrated a significant effect of a potato cultivar on the vitality of invasive J2 of *G. rostochiensis*. Unexplained specific physiological properties of potato modify lipid management of the nematode juveniles, which has a direct impact on the vitality of invasive juveniles and, finally, on entire ontogenesis of the golden nematode and the population size (Holtz *et al.*, 1999).

Table 8. Effect of saprophytic soil fungi on population density (no. of eggs and juveniles per 1 g soil) index (Pf/Pi) in tomato culture.

Treatment	Population density index (Pf/Pi) Year		
	1999	2000	2001
Globodera rostochiensis	4.5	0.4	1.3
G. rostochiensis + *Penicillium frequentans*	4.0	0.4	0.8
G. rostochiensis + *Stachybotrys chartarum*	3.2	0.5	0.8

As previously demonstrated, soil saprophytic fungi may stimulate potato resistance to infections (Mazurkiewicz-Zapałowicz, 2002). Similar positive responses of some other cultivated plants to the interaction of root system with saprophytic fungi were reported by Schroth & Hancock (1982) and Linderman (1991). The nature of these effects is still little understood; probably, the saprophytic microorganisms improving the condition and healthiness of the plants enhance their immunity, while the immunological status of plants may depend on the biochemical activity of the micro-organisms.

The presented studies confirmed the considerable role of the host plant in the formation of nematode population. It was also demonstrated that saprophytic fungi commonly found in soil (*P. frequentans* and *S. chartarum*) can constitute an important biological factor controlling population density of *G. rostochiensis*.

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Janowicz K., Mazurkiewicz-Zapałowicz K., Kaup G., Kuźna-Grygiel W. Воздействие растения-хозяина и сапробиотических почвенных грибов на численность популяции *Globodera rostochiensis* (Woll.) Behrens.

Резюме. Было исследовано возможное воздействие растений-хозяев (картофеля сорта Mila и томата сорта Ika) и сапробиотических почвенных грибов (*Stachybotrys chartarum* и *Penicillium frequentans*) на размер популяций нематоды *Globodera rostochiensis*. Внесение сапробиотических грибов приводило к существенному подавлению популяции нематод *G. rostochiensis* (снижение числа цист, яиц и личинок). Также наблюдали различия между картофелем и томатами как растениями-хозяевами, пригодными для развития нематод. Плотность популяции нематод под картофелем была в несколько раз выше, чем плотность популяции этих же нематод в почве под томатами. Исследование продемонстрировало, что как вид растения хозяина, так и присутствие почвенных сапробиотических грибов существенно влияет на плотность популяции *G. rostochiensis*.