The persistence of *Phasmarhabditis hermaphrodita* (Rhabditida: Rhabditidae) in different substrates

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Summary. The effect of different substrates on persistence of the facultative slug parasitic nematode *Phasmarhabditis hermaphrodita* was studied. Four different substrates (sand, leaf compost, organic horticultural substrate and garden soil) were inoculated with *P. hermaphrodita* strain B1, isolated in central Bohemia, and the persistence of this nematode was observed for 8 months. A new model of nematode trap was proposed to isolate nematodes from the substrates. We found that *P. hermaphrodita* was able to persist in slightly wet sand for at least 5 months. The density of dauer larvae decreased markedly in sand. The persistence in compost was very low, probably due to the presence of large amounts of antagonistic organisms. Organic horticultural substrate and garden soil provided the best conditions for persistence of *P. hermaphrodita*. Nematodes were able to persist in these substrates in very high densities for more than 8 months. We demonstrated that *P. hermaphrodita* preferably persisted in organic soil or habitats with a high content of organic matter and readily reacted to the presence of the trap.

Key words: nematode isolation, slug parasitic nematodes, survival, trap.

The slug parasitic nematode *Phasmarhabditis hermaphrodita* is a bacteriophagous nematode. It does not live in a close association with only one species of bacteria as do entomopathogenic nematodes (EPNs), but is able to feed on many bacterial species that are common in a habitat (Wilson *et al.*, 1995). This nematode is capable of killing many slug and snail species, such as *Deroceras reticulatum*. The dauer larvae infect slugs in the area beneath the mantle surrounding the shell, causing a disease with characteristic symptoms, particularly swelling of the mantle. Infection leads to the death of the slug, usually between 7 and 21 days afterwards. New dauer larvae emerge from the slug cadaver, spread into the soil and look for new hosts (Wilson *et al.*, 1993).

Survival of entomopathogenic nematodes in soil is strongly influenced by many factors such as predation, desiccation or starvation. Indirect evidence for lethal effects of nematode antagonists is seen in the fact that infective juveniles survived longer in sterilised than in non-sterilised media (Ishibashi & Kondo, 1986, 1987; Timper *et al.*, 1991). Starvation of nematodes is correlated with the level of lipid reserves that decline during storage (Patel *et al.*, 1997) and is probably one of the main causes of nematode mortality (Fitters, 1999; Qui & Bedding, 2000). The level of lipid reserves is also dependent on the movement activity of entomopathogenic nematodes and soil moisture. Nematodes are more active in the moist than in dry soil, and more active nematodes utilise lipid reserves quicker, which is the reason for the better survival of EPNs in soil with lower water content (Hass *et al.*, 2002). On the other hand, desiccation (extremely low water content) is very important factor that negatively influences a survival of EPNs (Grant & Villani, 2003; Preisser *et al.*, 2005).

Phasmarhabditis hermaphrodita is a facultative parasite that is able to live and reproduce in many rich organic substrates (Rae *et al.*, 2009), *e.g.*, leaf litter (MacMillan *et al.*, 2009) or compost (Nermuť, unpublished). So far, there is no data on the persistence of *P. hermaphrodita* in different substrates though this information can help to understand the biology of this species. Our data shows the ability of *P. hermaphrodita* to exploit different substrates (sand, soil, horticultural substrate and leaf compost) and helps to understand where and how this nematode can survive. This is the first study demonstrating the effect of different habitats on persistence of *P. hermaphrodita*.

MATERIALS AND METHODS

For the experiment, the B1 strain of *P. hermaphrodita* was used. This strain was isolated in central Bohemia and kept in laboratory culture having reared on freeze-killed slugs *Deroceras reticulatum*. Sand and horticultural substrates were bought at a hobby market whereas compost and garden soils were obtained from the author's private garden. Substrates were not sterilised prior to the experiment.

Persistence of *P. hermaphrodita* was assessed in 10 l plastic pots with a lid. Each pot was filled with one of the four substrates: sand (grain 1.3 mm in diam.), leaf compost, organic horticultural substrate, garden soil and inoculated with 14 000 dauer larvae. Each experiment included four replicates and a control (no nematodes added). Pots were kept at 15°C in dark and substrates were maintained slightly wet.

A new model of the nematode trap used in the experiment was made from the plastic 1.5 ml test tube (Eppendorf). The tube bottom was cut off and replaced with a sieve (holes 1 mm in diam.). Twelve additional holes 1 mm in diam. were made on sides of the tube. Sand (size of particles 1.3 mm) was placed in the trap and 100 µl of attractant was pipetted on it. The attractant was made from homogenised and sterilised (autoclaved at 121°C for 15 min) bodies of grey garden slugs *Deroceras reticulatum* (1 g of sterilised slug body + 2 ml of tap water) that were collected in the garden of Institute of Entomology in České Budějovice in the Czech Republic.

The presence of *P. hermaphrodita* was checked with the use of the traps. During the first 5 months of the experiment, nematode isolation was performed monthly while the last isolation was performed 3 months after the 5th one, i.e. after 8 months from the start of the experiment. Traps were placed into the substrate of each pot and were kept there for 48 h. Then traps were removed and placed in Petri dishes lined with moist filter paper and incubated at 15°C in dark for next 72 h. After this, the sand was rinsed with a small amount of tap water, centrifuged and the supernatant was poured on the counting dish. *Phasmarhabditis* females, that are relatively easily distinguished based on morphology (Hooper *et al*., 1999, Andrássy, 1983), were collected and counted under the stereomicroscope.

Statistical analyses were performed using two way factorial ANOVA and Tukey HSD test (Statistica program, version 7, StatSoft Inc.). Logarithmic transformation was necessary to meet the homogeneity of variance.

RESULTS

Results of analyses of variance confirm that both time ($F = 2.74$, $df = 5$, $P = 0.025$) and substrate ($F = 44.90$, $df = 3$, $P = 0.001$) have a significant influence on the persistence of *P. hermaphrodita* in different substrates. According to the data gained from the experiment it seems that sand and leaf compost are not suitable substrates for the persistence of *P. hermaphrodita*. The number of isolated nematodes in sand decreased with time and at the last isolation (after 8 months) there were no *Phasmarhabditis* females. Leaf compost also provided poor conditions for the persistence of *P. hermaprodita*. No *Phasmarhabditis* females were recorded after 5 months of the experiment, but during the last isolation after 8 months a single *Phasmarhabditis* female was found. Other substrates, i. e. horticultural substrate and garden soil, provided the better conditions for persistence of *P. hermaphrodita*. The number of isolated *Phasmarhabditis* females was very high in comparison with that from leaf compost and sand (Fig. 1). There was no significant difference between garden soil and horticultural substrate ($P = 0.999$) and between sand and leaf compost ($P = 0.440$), but the number of isolated *Phasmarhabditis* females in both garden soil and horticultural substrate was significantly higher than in sand (both $P < 0.001$) and leaf compost (both $P < 0.001$). No *P. hermaphrodita* was isolated in the control pots. Other nematodes identified as diplogasterids and rhabditids were also found in the traps.

DISCUSSION

Survival of nematodes, including EPNs, is dependent on many factors, *e.g.*, starvation, predation and soil moisture (Ishibashi & Kondo, 1986; Fitters, 1999, Haas *et al.*, 2002). The advantage for *P. hermaphrodita* is that it is a facultative parasite (Rae *et al.*, 2007, 2009) that lives on many organic materials (MacMillan *et al.*, 2009) and does not need a live host as required by EPNs and other obligatory parasites. We found that *P. hermaphrodita* was unable to survive longer than 8 months in slightly wet sand. The probable reason is that this substrate does not provide any host or other organic substrate for continued existence. Nematodes are active in the moist sand, starve, utilise their lipid reserves and die (Hass *et al.*, 2002).

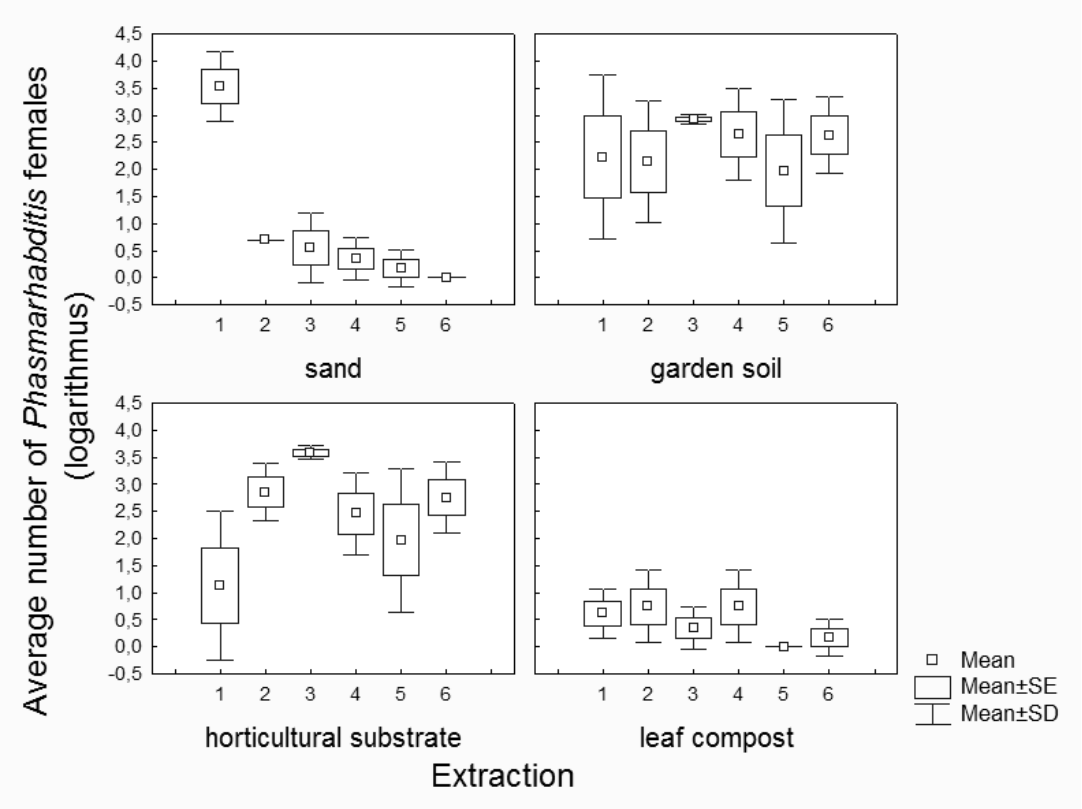

Fig. 1: Average number of *Phasmarhabditis hermaphrodita* females isolated from different substrates during 8 months (isolation periods 1–6).

The opposite situation should occur in the leaf compost where *P. hermaphrodita* was able to grow and reproduce (Nermut', unpublished). Similar results were observed by MacMillan *et al.* (2009) who found that *P. hermaphrodita* reproduced in the leaf litter. Thus, it was expected that *P. hermaphroditas*urvive and persist in the leaf compost. However, in the present study the number of isolated nematodes in the leaf compost was very low during the whole period of our experiment compared with other substrates as a single *Phasmarhabditis* female was isolated from four pots at the end of the experiment. We suppose that *P. hermaphrodita* was overcome by predators and other antagonistic organisms such as mites, springtails or fungi that were numerous in this leaf compost and strongly affected the survival of nematodes (Strong, 2002; Wilson & Gaugler, 2004). The low number of isolated *Phasmarhabditis* females in the leaf compost could also be caused by the poor aeration of the substrate. The leaf compost used in the experiment was the more compact substance with the lower aeration compared to the horticultural substrate and garden soil which could be the reason for the higher mortality of nematodes.

Our results show that both garden soil and horticultural substrates were suitable habitats for *P. hermaphrodita*. Nematodes were able to persist in these substrates for the whole period of our experiment or at least for 8 months in high densities. The horticultural substrate used was similar to the leaf compost or other organic soils but the absence of predators and other antagonistic organisms was beneficial for *P. hermaphrodita*. The study confirmed the ability of *P. hermaphrodita* to reproduce on substrates with the high content of organic matter as it was shown with the leaf litter by MacMillan *et al.*, 2009.

Thus, the persistence of *P. hermaphrodita* is better in substrates with the higher content of organic matter but lacking increased numbers of antagonists. The new method for *P. hermaphrodita* isolation is proposed.

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J. Nermuť. Устойчивость рабдитид *Phasmarhabditis hermaphrodita* к различным субстратам.

Резюме. Исследовано воздействие различных субстратов на выживание нематод *Phasmarhabditis hermaphrodita* – факультативных паразитов наземных моллюсков. Нематод *P. hermaphrodita* штамм B1, изолированных в центральной Богемии, вносили в 4 различных субстрата (песок, листовой компост, органический субстрат для цветоводства и садовую почву). Наблюдения проводили в течение 8 месяцев. Было показано, что *P. hermaphrodita* способны выживать в слегка увлажненном песке в течение как минимум 5 месяцев. При этом численность дауэр-личинок в песке значительно сокращалась. Выживаемость к компосте была низкой, предположительно из-за обилия организмов-антагонистов. Органический субстрат для цветоводства и садовая почва представляли наилучшие условия для выживания *P. hermaphrodita*. Нематоды сохранялись в этих субстратах при высокой численности в течение более 8 месяцев. Было показано, что *P. hermaphrodita* выживает наилучшим образом в органических субстратах, активно реагируя на предлагаемые им искусственные приманки.