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Growth and reproduction of *Aphelenchus avenae*, *Aphelenchoides saprophilus* and *Aphelenchoides besseyi* in mono- and mixed cultures cultivated on the fungus *Alternaria tenuis*

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Summary. The biocontrol potential of *Aphelenchoides saprophilus* and *Aphelenchus avenae* has been studied for the control of plant pathogenic fungi since the 1970s. In conditions of accelerating climate change, it is not clear how these nematodes will interact with the parasitic nematode *Aphelenchoides besseyi*, which may rapidly increase in numbers under higher temperature regimes. To prevent harvest losses, we need a complex model that will help to predict the behaviour of each species of nematodes and their mode of interaction. This paper investigates the interactions between the fungivorous nematodes, *Aphelenchus avenae* and *Aphelenchoides saprophilus*, and the plant-parasitic nematode *A. besseyi*. Competitive interactions between the nematodes resulted in decreased numbers of nematodes when they were grown in mixed cultures. *Aphelenchus avenae* was more competitetive than *A. besseyi* and *A. saprophilus* at 15-25°C and 30°C. At 5°C, only *A. saprophilus* increased in numbers. The experiments demonstrated that temperature, reproductive rates and intrinsic characteristics influenced the competitive interactions between the three nematode species.

Keywords: fungivorous nematodes, interspecific competition, plant-parasitic nematodes, temperature regimes.

The fungivorous nematodes *Aphelenchus* and *Aphelenchoides* are the most common genera in soil (Freckman & Caswell, 1985; Yeates *et al.*, 1993; Zolda, 2006; Monokrousos *et al.*, 2021). *Aphelenchus avenae* Bastian, 1865 and *Aphelenchoides* spp. have significant potential as biocontrol agents against plant pathogens and could be used for large-scale field control (Lootsma & Scholte, 1997; Ishibashi *et al.*, 2000; Hasna *et al.*, 2007; Shchukovskaya *et al.*, 2014). *Aphelenchoides saprophilus* can be found in the root systems of clover, even under snow cover, where it successfully develops and reproduces by feeding on soil fungi (Shesteperov, 1985). This species also feeds on mycorrhizal fungi and can influence mycorrhizal development and affect competition between the species of soil fungi (Ruess & Dighton, 1996). *Aphelenchoides besseyi* Christie, 1942 is a plant-parasitic nematode that can cause devastating plant damage and crop losses (Fortuner & Orton Williams, 1975; Rahman & Miah, 1989; Ni *et al.*, 2020; ISC, 2021). *Aphelenchoides besseyi* can be found in soils (Terry, 1972; Nguyen-thi, 1982) and it can also feed on fungi (Jamali *et al.*, 2008). Knowledge of the interspecific interactions between nematodes and the effects of the factors influencing these interactions are important for the prediction of nematode abundance and resistance of plants to parasitic nematodes, and for application of biological and chemical methods of control. Interspecific interactions between nematodes may be complicated due to complex multispecies nematode communities inhabiting any substrate, such as soil, plant tissues or organic materials (compost, mushroom spawn, rotting root, *etc.*) where more than 30 species of nematodes of different ecological groups can be present (Yeates, 1979; Yeates & Bongers, 1999). The occurrence of more than one species in a restricted area where many species use common sources of food can result in several types of interactions. However, little is known about density-dependent population

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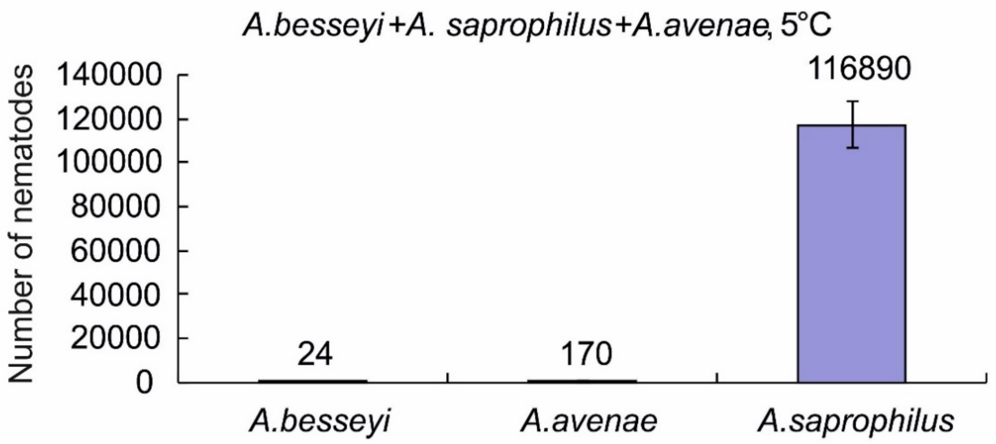

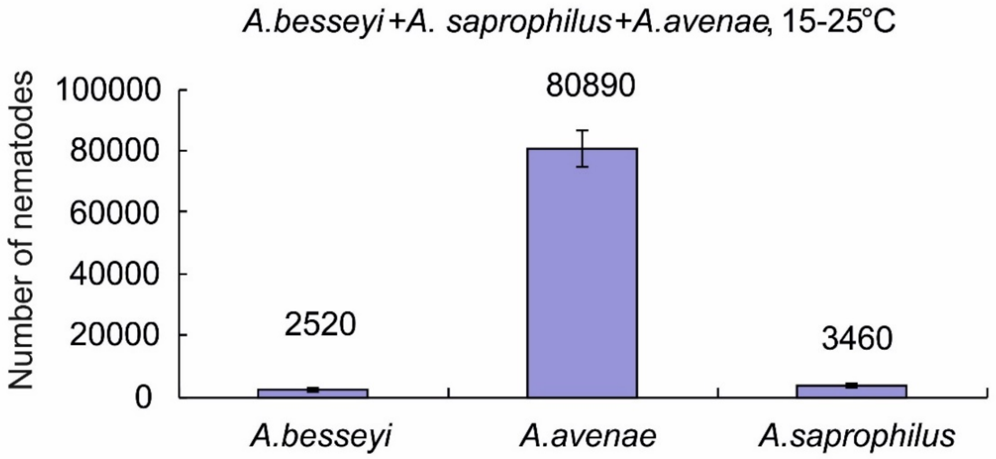

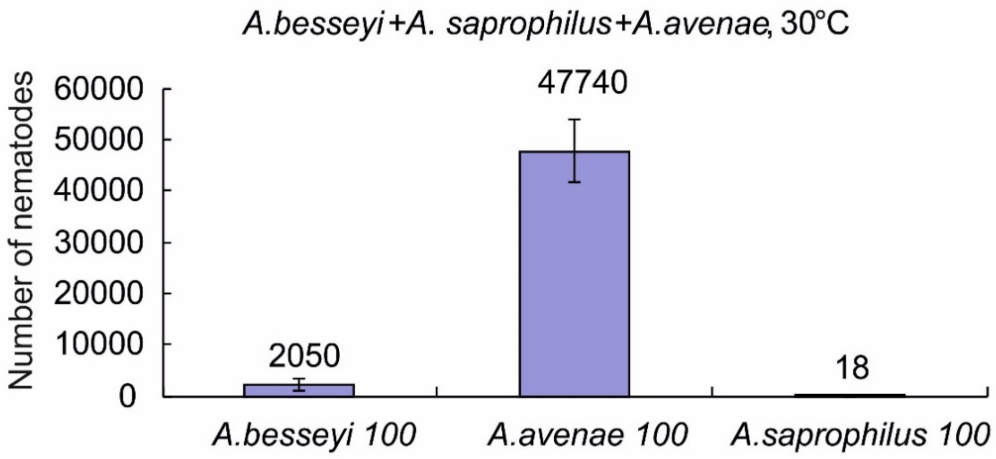

Fig. 2. Mean number ± SD of of *Aphelenchus avenae*, *Aphelenchoides saprophilus* and *Aphelenchoides besseyi* at 5°C, 15-25°C and 30°C in mixed culture from initial concentration (100 individuals). Each bar is the mean of five replications (n = 5).

Table 4. Population structure (%) in mono- and mixed culture of *Aphelenchus avenae* and *Aphelenchoides saprophilus* at 15-25°C from initial concentration (100 individuals).

Variant	Species checked	Population structure (%)			
		Females	**Males**	**Juveniles**	**F/M**
monoculture					
A. besseyi 100 ind.	*A. besseyi*	57 ± 20 (35)[1]	3 ± 1 (33)	40 ± 16 (40)	22 ± 12^{b}(54)
A. avenae 100 ind.	*A. avenae*	15 ± 14 (93)	–	85 ± 4 (5)	–
A. saprophilus 100 ind.	*A. saprophilus*	15 ± 5 (33)	3 ± 2 (67)	82 ± 25 (30)	4.6 ± 2^{a}(43)
mixed culture					
A. besseyi 100 ind. + *A. saprophilus* 100 ind. + *A. avenae* 100 ind.	*A. besseyi* (100 ind.)	60 ± 22 (37)	14 ± 5 (36)	26 ± 9 (35)	6.8 ± 4.7^{a}(69)
	A. avenae (100 ind.)	20 ± 5 (25)	–	80 ± 13 (16)	–
	A. saprophilus (100 ind.)	44 ± 29 (66)	20 ± 5 (25)	36 ± 19 (53)	3.5 ± 1.3^{a} (37)

[1]Mean number ± SD (coefficient of variation, %), n = 5; means with different letters are significantly different at $P < 0.05$. F/M is a ratio of females to males calculated on absolute number of nematodes.

Table 5. Population structure (%) in mono- and mixed culture of *Aphelenchus avenae* and *Aphelenchoides saprophilus* at 30°C from initial concentration (100 individuals).

Variant	Species checked	Population structure (%)			
		Females	**Males**	**Juveniles**	**F/M**
A. besseyi 100 ind.	*A. besseyi*	54 ± 41 (76)[1]	32 ± 22 (69)	14 ± 10 (71)	1.74 ± 0.6^{a}(34)
A. avenae 100 ind.	*A. avenae*	13 ± 6 (46)	–	87 ± 47 (54)	–
A. saprophilus 100 ind.	*A. saprophilus*	80 ± 9 (11)	0	20 ± 3 (15)	–
A. besseyi 100 ind. + *A. saprophilus* 100 ind. + *A. avenae* 100 ind.	*A. besseyi* (100 ind.)	70 ± 54 (77)	23 ± 9 (39)	7 ± 5 (71)	3.3 ± 1.3^{b}(39)
	A. avenae (100 ind.)	41 ± 4 (10)	–	59 ± 11 (19)	–
	A. saprophilus (100 ind.)	88 ± 7 (8)	0	12 ± 3 (25)	–

[1]Mean number ± SD (coefficient of variation, %), n = 5; means with different letters are significantly different at $P < 0.05$. F/M is a ratio of females to males calculated on absolute number of nematodes.

(2001). In our study, the largest numbers of negative interspecific interactions were recorded under the 15-25°C regime. All species of nematodes developed well in the monoculture. A significant decrease of nematode numbers in mixed cultures was a consequence of the competition for fungal food resources.

There is an overall trend for nematode populations to increase the proportion of males when they are subjected to some form of stress including temperature and crowding (Yeates, 1987). Our results have shown that in mixed cultures the proportion of *A. besseyi* and *A. saprophilus* males increased from 3% to 14-20% at 15-25°C. The temperature regime of 15-25°C was favourable for all the nematode species and we suggest that interspecific competition between nematodes is a major cause of the increase in the number of males in the populations. The analysis of the population structure of competitors is important because the species with the highest rate of reproduction will have a competitive advantage (Odum, 1971).

Aphelenchus avenae showed the best competitive capacity compared with the other nematode species at 15-25°C and 30°C. This species can be found in different structures and in the rhizosphere of higher plants in various combinations with fungivorous and other trophic groups of nematodes (Ishibashi *et al.*, 2000; Taher *et al.*, 2017; Javed & Khan, 2021). *Aphelenchus avenae* has a set of adaptations for the occupation of different ecological niches and resistance to interspecific competition. It consumes a variety of fungal species and is characterised by high fecundity (more than 200 eggs at a time, laying eggs without feeding), parthenogenesis and survival of desiccation (Kostuk, 1971; Turlygina & Chizhov, 1971; Huang *et al.*, 1972; Ishibashi *et al.*, 2000, Okada & Ferris, 2001; Chen *et al.*, 2017; Javed & Khan, 2021). All these features enable the species rapidly to achieve high numbers under favourable conditions. Moreover, this species can attack other nematodes (Decker, 1962; Zhang *et al.*, 2020). In our experiments, population numbers of *A. besseyi* and *A. saprophilus* at 15-25°C in the cultures with *A. avenae* decreased drastically, particularly the numbers of juveniles. We speculate that the stylet-bearing nematode *A. avenae* may have predated the eggs and/or slow-moving juveniles. Haraguchi & Yoshiga (2020) also demonstrated the suppression of plant parasitic nematode *Ditylenchus destructor* by *A. avenae* at 20°C on nutrient medium and in soil.

At 5°C, *A. saprophilus* exhibited a higher reproductive rate than *A. besseyi* and *A. avenae*. This growth at low temperatures is consistent with *A. saprophilus* being a soil-living species. The previous experiments showed that this species developed and reproduced under snow cover and reached 10,000 individuals (25 g roots)$^{-1}$ (Shesteperov, 1985).

Aphelenchoides besseyi and *A. avenae* did not reproduce and compete for food at 5°C; *A. saprophilus* did not compete at 30°C, which can be explained by its inability to develop rapidly at high temperatures. Although in monoculture the population numbers of this species were the highest at 5°C and 15-25°C, in the presence of other species of nematodes it was unable to compete and its population numbers fell dramatically at 15-25°C.

As a parasite of higher plants, *A. besseyi* was unable to compete with the fungivorous nematodes *A. avenae* and *A. saprophilus* because of its slower growth rate. In all treatments other than at 30°C where *A. saprophilus* could not develop, the numbers of *A. besseyi* were considerably lower than those of the other nematode species. This suggests that in the course of its evolution *A. besseyi* became parasite of higher plants and lost its competitive ability as mycophagous organism. Negative effects of temperature on the growth and development of *A. besseyi* at 5°C may be used to develop a biotechnological approach for suppressing this plant parasite.

The awareness of temperature dependent interaction effects as documented in the present study is essential if we are to understand the patterns of nematode population dynamics, to predict and assess the plant's resistance to parasitic nematodes and to develop the strategy of biological and chemical control of pathogenic microorganisms in conditions of rapid climate change. The chosen model takes account of biological characteristics of nematodes under different temperature regimes using the data obtained in the course of experiments. The study has demonstrated that population dynamics of the investigated species of nematodes are related to temperature and there are temperature optimums for each of the species, with clearly visible patterns of highly competitive interactions between them. These patterns are characterised by changing sex ratios dependent on temperature regimes: unfavourable conditions caused increases in the number of males in the population.

The results of these experiments may contribute to a better understanding of the mechanisms of competition between different species of nematodes. The observed patterns of population growth of rare species of nematodes in plants and soil have led us to suppose that dominant species prevail in competitive interactions with other species owing to their competitive capacity in these conditions but without the elimination of rare species. Under environmental conditions unfavourable for the dominant species, they are displaced by the species possessing ecological attributes that enable them to reproduce and survive in this biotope. Permanent changes in environmental conditions and original biological properties of nematodes may allow co-existence of different nematodes in natural ecosystems.

The results of the present study are particularly relevant to the production of rice; further research is needed to examine the behaviour of nematodes living in other terrestrial ecosystems.

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А.А. Шестеперов и В.Д. Мигунова. Рост и размножение *Aphelenchus avenae*, *Aphelenchoides saprophilus* и *Aphelenchoides besseyi* в чистых и смешанных культурах при культививировании на грибе *Alternaria tenuis*.

Резюме. *Aphelenchoides saprophilus* и *Aphelenchus avenae* используются для биологической борьбы с патогенными грибами растений с 1970-х годов. При изменении климатических условий не совсем очевидно, как эти нематоды будут взаимодействовать с фитопаразитом *Aphelenchoides besseyi*, который может быстро увеличивать свою численность при высоких температурах. Для предотвращения потерь урожая необходима модель, которая поможет предсказать поведение каждого вида нематод при их взаимодействии. В статье исследуются взаимодействия между грибоядными нематодами *Aphelenchus avenae* и *Aphelenchoides saprophilus* а также фитопаразитической нематодой *A. besseyi*. Конкурентные взаимодействия между нематодами привели к снижению численности животных при их совместном содержании в смешанных культурах. *A. avenae* оказался более конкурентноспособным, чем *A. besseyi* и *A. saprophilus* в интервалах 15-25°C и 30°C. При 5°C только *A. saprophilus* значительно увеличил свою численность. Эксперименты показали, что температура, репродуктивный потенциал, а также биологические характеристики вида влияют на конкурентные взаимодействия между тремя видами нематод.