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INFLUENCE OF THE PREVIOUS CROP ON THE ANHYDROBIOTIC ABILITY OF *PRATYLENCHUS THORNEI* AND *MERLINIUS BREVIDENS*

by

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Summary. The influence of the previous crop on the anhydrobiotic survival of *Pratylenchus thornei* and *Merlinius brevidens* was investigated in a pot experiment with three different cultivars of wheat, sunflower, opium poppy and dwarf chickling as host crops. Nematodes were extracted from the soil after six months of plant growth and three months later, after a slow dehydration of soil and storage in dry conditions. A clear influence of the previous crop on the ability to survive the dry season was observed for both nematode species, since survival was significantly greater when nematodes were cultured previously on a good host crop, such as wheat cvs Yecora and Cajeme for *P. thornei* and wheat cvs Yecora, Cajeme and Donpedro or sunflower for *M. brevidens*. Densities of anhydrobiotic nematodes were also monitored in the field, for seven consecutive fortnights, after harvest of wheat cv. Yecora. Percentages of anhydrobiotic nematodes were always greater for *P. thornei* than for *M. brevidens* at every fortnight sampling and under the same soil conditions, indicating a greater anhydrobiotic ability of *P. thornei*.

Many species of nematodes are able to enter in a survival stage of reduced metabolism (quiescence) or apparently suspended metabolism (cryptobiosis) in response to some environmental stresses. If the cause of the stress is dehydration, this particular stage is called anhydrobiosis (Womersley *et al.*, 1998). A correlation between anhydrobiotic ability and habitat conditions has been reported for *Anguina tritici*, *Meloidogyne incognita*, *Pratylenchus scribneri* and *Tylenchulus semipenetrans* (Tsai and Van Gundy, 1989) and it has been stated that the ability to enter anhydrobiosis depends on the levels of stress occurring in the natural habitat of the nematode (Womersley *et al.*, 1998).

In the dry cultivated fields of southern Spain, nematode populations are subjected to long periods of drought during the summer, but with-

stand this environment by entering an anhydrobiotic stage (Tobar *et al.*, 1995a).

Some research has been done on survival strategies of nematodes inhabiting dry or semi-arid regions (Egunjobi and Bolaji, 1979; Glazer and Orion, 1983; Swanepoel *et al.*, 1987; Mani, 1999) but little is known about the ability to enter anhydrobiosis of many species present in the Mediterranean area (Tobar *et al.*, 1996) and no data are available about the effect of the previous host crop on their survival ability. The objectives of the present work were to monitor the entrance into anhydrobiosis of *Pratylenchus thornei* and *Merlinius brevidens* during a Mediterranean dry summer season and to establish the effects of the quality of the previous crop on the dry season survival.

Material and methods

A vertic sandy loam soil, infested with *P. thornei* Sher et Allen and *M. brevidens* (Allen) Siddiqi was taken from a dry-cultivated field in South Spain, sieved through a 2.5 mm mesh to retain crop residues and thoroughly mixed before filling 132 pots of 12.5 cm diameter and 2 l capacity. The soil was sampled in dry conditions (soil moisture 3.7%) in October 1992, after three months of dry fallow following the harvest of the previous crop of wheat cv. Yecora.

Twelve pots were used to determine recovery times needed for anhydrobiotic and active nematodes. Six pots were watered regularly to field capacity to maintain wet conditions (soil moisture around 13.7%) and the other six pots were kept in the dry sampling conditions (3.7% soil moisture). Two weeks later, the soil from each pot was mixed again and two 100 cm³ subsamples were processed by differential sedimentation in water in an Oostenbrink elutriator (Oostenbrink, 1960), sieving the supernatant through four 53 µm pore sieves and washing off the retained nematodes into a beaker, followed by active migration of the nematodes through a cottonwool filter to clean tap water. Owing to the delay in migration time for anhydrobiotic nematodes, due to rehydration and revival of nematodes before activity is resumed (Barret, 1991), migrations were allowed for 15 hours and five additional periods of 24 hours, up to 135 hours (Tobar *et al.*, 1995a). After each period, the filter was washed off into a Petri dish, collected nematodes were counted, and the filter was then placed in a fresh volume of tap water to reactivate the remaining anhydrobiotic nematodes.

Hundred and twenty plastic pots, filled with soil infested with *P. thornei* and *M. brevidens*, were planted to common wheat (*Triticum aestivum vulgare* L. Vill. cv. Yecora and cv. Cajeme), English-durum wheat (*T. turgidum turgidum durum* L. Desf. cv. Donpedro), sunflower (*Helianthus annuus* L. cv. Sungro 386), opium poppy

(*Papaver somniferum* L. cv. Nigrum) and dwarf chickling (*Lathyrus cicera* L.). The plants were grown for six months and then the plant shoots were cut at soil level and removed; nematodes were recovered from ten pots of each crop. The soil from each pot was thoroughly mixed and two 100 cm³ subsamples were processed as previously described. The roots from each pot were cut into 2 cm fragments and processed in a mistifier for 14 days to recover the endoparasitic stages of *P. thornei* (Seinhorst, 1950); population densities within the roots were added to soil densities to calculate total population densities after each crop.

The other ten pots from each crop were left undisturbed for three months, allowing the soil to dry out slowly at a temperature range of 26-33 °C, and then the nematodes were recovered.

Survival percentages after three months of drought were calculated in relation to average densities at the time of harvest. Percentages were arcsine transformed and subjected to analysis of variance. When F values were significant, means were compared by the HSD Tukey test.

Soil nematodes were sampled fortnightly in a field plot (24 m x 4 m), following harvest of the host crop (wheat cv. Yecora) in early July 1992, until the first autumn rains in early October 1992, resulting in seven sampling dates. At each sampling date, four composite soil samples were collected, by taking 24 cores of 3.8 cm diameter and 20 cm depth.

Soil moisture was calculated by weighing, before and after drying, four soil subsamples in an oven at 65 °C.

Nematodes were extracted from the soil by the previously mentioned techniques. Those recovered from the second to the sixth migration times (15-135 h), were considered to be in an anhydrobiotic stage and their densities transformed into percentages of the total population as above.

Data were arcsine transformed and analysed by ANOVA. When F values were significant, means were compared by the HSD Tukey test.

Results and discussion

In wet soil and for both nematode species, more than 95% of the population was recovered in the first 15 hours of migration (Table I), indicating that when the soil remains humid (13.7%) most of nematodes are in an active stage and can be readily recovered. Reductions in percentage of recovery during this 15 h period, when the soil is dry, seem to be caused by the "lag phase" (Barret, 1991) or time needed to rehydrate and reactivate nematodes. It is, therefore, possible to estimate the number of anhydrobiotes in these populations by counting the number of individuals that take more than 15 h to migrate through the cottonwool filter to tap water, as in the method described by Tobar *et al.* (1995a).

Survival percentages after three months of drought are shown in Fig. 1. Maximum survival rates, were associated with wheat cv. Yecora for *P. thornei*, (74.8%) and wheat cv. Cajeme for *M. brevidens* (66.7%). Wheat cvs Yecora and Cajeme have been cited as good hosts of *P. thornei* and *M. brevidens*, conversely, opium poppy cv. Nigrum and dwarf chickling are poor hosts for both nematodes and wheat cv. Donpedro and sunflower cv. Sungro 386 are good hosts of *M. brevidens* but poor hosts of *P. thornei*. (Tobar *et al.*, 1995b). Our results agree with these reports in the way that populations obtained from good hosts had a greater survival than those associated with poor hosts.

Most of the studies on anhydrobiotic survival in fallow soils have been done after rearing the nematode populations on a good host (Egunjoi and Bolaji, 1979; Mani, 1999). No research has been done on dry fallow survival of nematode populations after the culture of a non or poor host crop. The fact that populations maintained under a good host have a greater survival in dry conditions can be explained in terms of their greater ability to enter into anhydrobiosis, probably owing to their nutritional status. It has been shown that desiccation-activation cycles consume a large amount of the food reserves of nematodes (Glazer and Orion, 1983), thus if these nutritional reserves are not available due to starvation, many nematodes would not be able to enter into anhydrobiosis and eventually die. However, the mechanisms that may be associated with this phenomenon are still unknown.

Soil moisture, expressed as percentage of the soil weight and percentage of anhydrobiotes at the seven sampling dates, during the summer dry season, are shown in Fig. 2.

Regarding the percentage of anhydrobiotes recovered at each sampling date, significant differences between the two nematode species were observed only at the third sampling date, after five weeks of drought, when 75.7% of the *P. thornei* population was in an anhydrobiosis vs a 49.1% of *M. brevidens*.

Tobar *et al.* (1996) compared the recovery time for populations of *P. thornei* and *M. brev-*

TABLE I - Percentage of the nematode population recovered at six consecutive times from wet and dry soils.

Migration time (hours)	<i>Pratylenchus thornei</i>		<i>Merlinius brevidens</i>	
	Wet soil	Dry soil	Wet soil	Dry soil
15	96.0	3.5	98.4	13.6
39	2.4	61.9	0.8	60.3
63	0.8	25.6	0.3	17.4
87	0.4	5.5	0.3	5.2
111	0.3	2.3	0.2	2.6
135	0.1	1.3	0.1	1.4

idens from wet and dry soils, and observed that the patterns of emergence from anhydrobiosis were similar between both species for wet soils and extended time dry soils, but when the soil was dry for only a short period of time, the patterns were different, since *M. brevidens* populations were recovered faster than *P. thornei* ones. They suggested that *M. brevidens* needed a longer time in dry soil than *P. thornei* to reach the same stage of deep anhydrobiosis. In our experiment, we have timed this phenomenon and observed that a 75% of the population of *P. thornei* was in anhydrobiosis five weeks after harvest, but this percentage was not reached by the *M. brevidens* population until almost seven weeks after harvest. In addition, the percentages of anhydrobiotes in the population were always smaller for *M. brevidens* than for *P. thornei* during the whole sampling period and in the same soil conditions. Therefore, it seems that in Southern Spain, *P. thornei* populations

have greater ability to enter into anhydrobiosis than *M. brevidens*.

It has been demonstrated that under natural conditions *P. thornei* remain within senescing host roots and can be recovered from them after the summer season (Egunjobi and Bolaji, 1979; Tobar *et al.*, 1995a). Nematodes within the roots would have an additional physical barrier to slow evaporative water loss and thus have an advantage over those nematodes unable to penetrate into the roots, such as *M. brevidens*. Nevertheless, this differential anhydrobiotic ability could be due also to various other factors, as nutritional status of the nematode population as a consequence of the previous host quality or metabolic differences between the two species in the process of achieving anhydrobiosis. Further research on physiological and behavioural adaptations of these two species to control drying is needed to clarify this phenomenon.

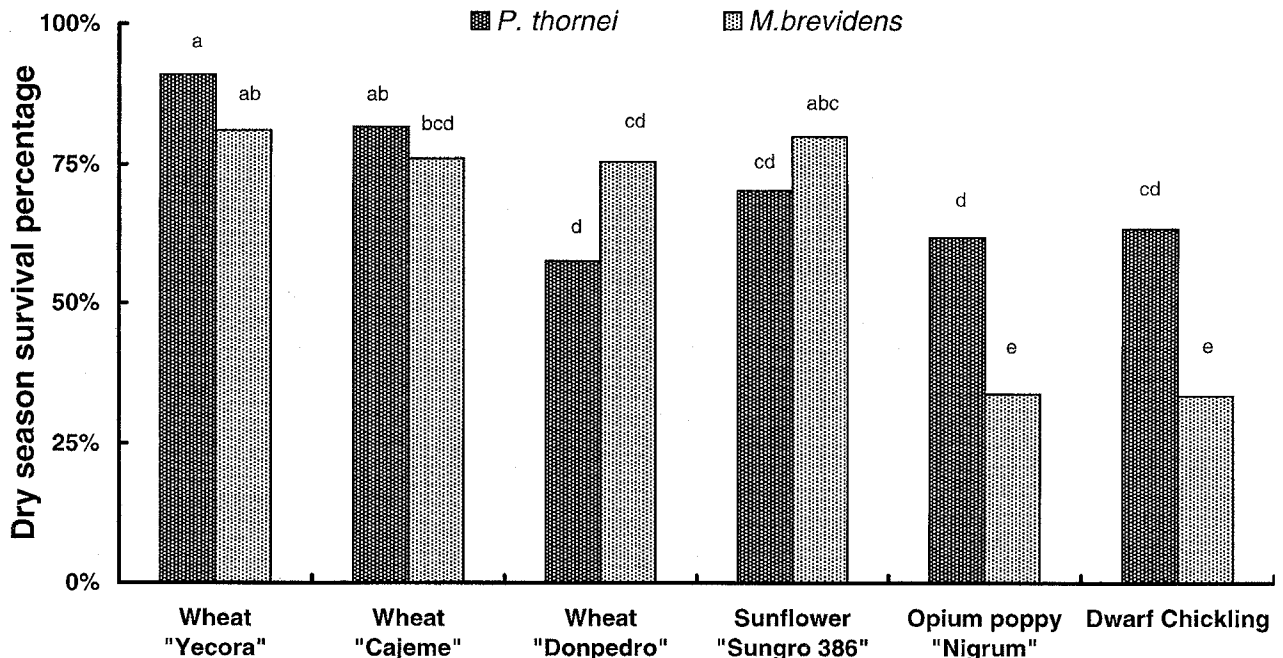


Fig. 1 - Percentage of nematodes that survived three months in dry soil in pots without a host. Bars with the same letter at the top are not significantly different.

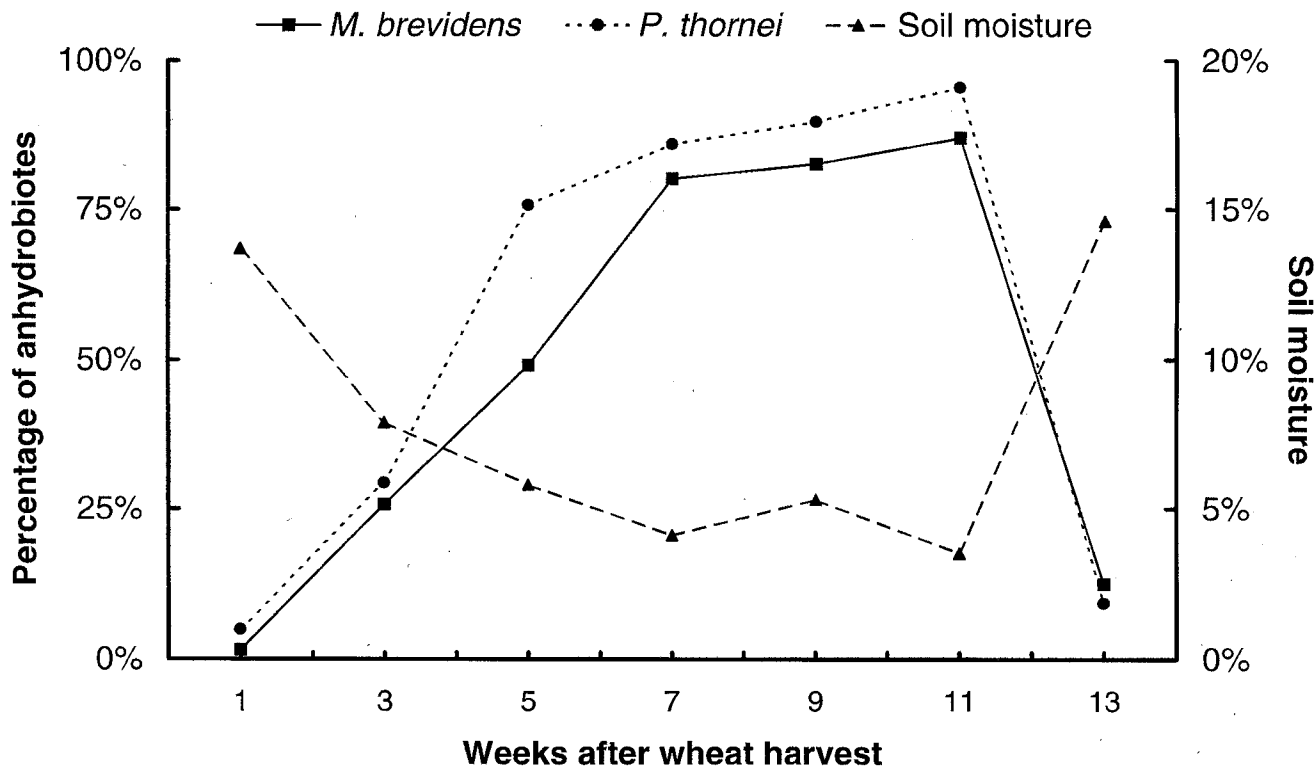


Fig. 2 - Percentage of anhydrobiotes and soil moisture at seven sampling dates during the dry fallow summer season in a field plot in southern Spain.

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