

## Post-Anhydrobiotic Viability of *Pratylenchus thornei* and *Merlinius brevidens*

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After natural desiccation in the field, anhydrobiotic populations of *Pratylenchus thornei* Sher & Allen and *Merlinius brevidens* (Allen) Sidiqqi were treated in three different ways: some were kept dry to maintain nematodes in the anhydrobiotic stage; some were rehydrated and then allowed to dry again to induce a new desiccation cycle; and some were rehydrated and maintained as such, to reactivate and keep nematodes active. *P. thornei* populations from the dry treatment had a greater survival rate than the two other treatments. Culturing of the nematodes under wheat revealed that the three treatments did not alter the ability of *P. thornei* to penetrate the roots, but nematodes given two cycles of desiccation had a greater rate of reproduction than those exposed to one desiccation cycle and kept in dry soil for a longer period (dry treatment). Survival of *M. brevidens* rehydrated and retained in the active state was lower than that of nematodes undergoing the two other treatments.

KEY WORDS: Anhydrobiosis; *Pratylenchus thornei*; *Merlinius brevidens*; root lesion nematode; survival; viability.

### INTRODUCTION

Yield losses suffered by cultivars of wheat in the Mediterranean semi-arid climate, apparently caused by nematodes, could not be explained by the low densities of active *Pratylenchus thornei* Sher & Allen or *Merlinius brevidens* (Allen) Sidiqqi detected after summer and before planting. Nevertheless, the high densities of anhydrobiotic nematodes found in those fields after the dry summer season led us to consider the possibility that reactivated nematodes were infective and could cause damage to wheat.

This study was carried out to test the survival and viability of anhydrobiotic populations of *P. thornei* and *M. brevidens* after a summer drought, comparing their survival with those in the same soil but subjected to simulation of occasional rain, which commonly occurs in the field, followed by an additional desiccation, before the autumn rainy season. The same soil kept wet throughout served as control.

According to the literature, the ability of rehydrated *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven to penetrate host roots (infectivity) and reproduce within (reproductivity) was not affected by anhydrobiosis (11). Similarly, populations of *P. thornei* that had survived 7–8 months of summer drought in the field returned to full activity at the beginning of the rainy season (3), but individuals reactivated from artificially induced desiccation were apparently devoid of reserve materials; only 3% of the population survived three cycles of desiccation and reactivation, although reactivated nematodes multiplied twice as much as fresh nematodes (3).

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## MATERIALS AND METHODS

A sandy loam vertic soil sample was taken after the dry summer season and before the first autumn rains from a non-irrigated area at Ecija (southern Spain). The previous crop had been wheat cv. 'Gallareta'. The soil was mixed thoroughly and four subsamples of 100 cm<sup>3</sup> were taken to estimate the initial nematode densities.

The rest of the soil, approximately 30 dm<sup>3</sup>, was distributed into three 40 × 40 × 10 cm containers and treated as follows. One container was kept dry (2.50–2.13% water, as percentage of the soil weight) with the nematodes resting in their anhydrobiotic forms (dry treatment); the second was watered to field capacity, and then allowed to become dry again (soil moisture: 5.01% after 14 days, 2.90% after 28 days and 2.21% after 42 days of storage) to induce a new reactivation–desiccation cycle (wet–dry treatment); and the third was watered regularly (soil moisture: 15.18–17.80%) to reactivate nematodes and keep them active (wet treatment).

After 75 days of treatment, a period similar to that which nematodes withstand in the field between the end of summer and sowing, the soil of each container was homogenized again and 24 subsamples of 100 cm<sup>3</sup> were taken from each. At that time (75 days after the sampling in the field), eight subsamples (of the 24 from each treatment) were diluted in water and the nematodes were extracted, to estimate survival after the summer drought and following the three different treatments. Another eight subsamples were transferred into small pots and planted with 10-cm-tall seedlings of wheat cv. 'Yecora' (four per pot), and the remaining eight used as soil-only controls. After 25 additional days, approximately the time of one life cycle of *P. thornei* (100 days after soil sampling), soil and roots from four pots under wheat and from four controls were processed to estimate the infectivity (penetration of reactivated *P. thornei* into the roots), before the appearance of second stage juveniles (J2). After another 25 days (125 days after soil sampling), soil from the four remaining pots under wheat and the four remaining controls was processed to estimate nematode reproductivity, before senescence of the plant cultures. Spare pots were used to determine the two time limits.

Extraction of nematodes was done by differential sedimentation in water in an Oostenbrink elutriator (4), sieving the supernatant through four 53- $\mu$ m pore sieves and washing off nematodes and debris retained into a beaker, followed by active migration of the nematodes through a cottonwool filter to clean tap water (9). Migration through the filter was allowed for 15 h and five additional periods of 24 h each, up to 135 h. After each period, the filter was washed off into a petri dish, the collected nematodes were counted, and the filter was relocated in a fresh volume of tap water to reactivate the remaining anhydrobiotic nematodes (10).

The roots present in each of the replicates planted to wheat were cut into small pieces and processed for 14 days in a mist chamber, to recover the endoparasitic stages. The estimated nematode densities were added to those from the corresponding soil. Nematode densities were transformed into logarithms and percentages were angular transformed and analyzed by analysis of variance and the LSD test.

The identification of living promorphs was done using a stereomicroscope. For specific determination, using a compound microscope, nematodes were killed by applying gentle heat, fixed in 3–5% formalin, and processed and mounted in dehydrated glycerine (7).

## RESULTS AND DISCUSSION

Densities of *P. thornei* and *M. brevidens* obtained after every treatment at the three different times are shown in Figures 1 and 2, respectively. Densities of *P. thornei* in the remaining roots of the previous crop, wheat cv. Gallareta, were always lower than 0.5 per 100 cm<sup>3</sup> soil, and have not been included in the study.

### *P. thornei* survival

Dry-treatment *P. thornei* densities at 75 days after sampling were significantly greater ( $P \leq 0.05$ ) than those after wet–dry and wet treatments (Figs. 1a, 1b). Tsay and Van Gundy (12) observed that *Tylenchulus semipenetrans* Cobb juveniles, kept in anhydrobiosis for 48 days, had their intestines full of reserves, but when maintained in distilled water they became depleted, indicating a reduction of metabolism during anhydrobiosis. In addition, prolonged activity of the population in the absence of a host expended nematode reserves and led to a decrease in densities, due to starvation, as occurred during these 75 days in the wet treatment and partially so in the wet–dry treatment. Therefore, the higher percentage of survival after the dry treatment seems to have been due to the longer anhydrobiotic resting period, as nematodes were reactivated nearly  $2\frac{1}{2}$  months later than those in the two other treatments in which starvation occurred.

The low survival rate after the wet–dry treatment (Fig. 1a) also agrees with the results obtained by Glazer and Orion (3) in *P. thornei* after two consecutive cycles of desiccation–activation. They suggested that desiccation–activation cycles consume a large amount of reserves and result in a decrease in the survival percentage of *P. thornei*. Dehydration/rehydration cycles have also been reported to be detrimental to other nematodes, such as *Ditylenchus dipsaci* (Kühn) Filipjev (5) and *Anguina tritici* (Steinbuch) Filipjev (13,14).

Controls without host (Fig. 1b) showed a gradual decline of the *P. thornei* population throughout the entire experiment. Densities were greater 75 days after sampling than at 100 and 125 days after ( $P \leq 0.05$ ) in all three treatments. This indicates that nematodes, when kept beyond a certain time in wet soil without a host, expend their reserves and lose their capability to migrate through the filter used in the extraction procedure, owing to starvation. The pattern was different under a host crop (Fig. 1a): after 100 days, densities were greater under a host than without a host, as a consequence of nematodes feeding on host roots and therefore maintaining their food reserves, energy levels and motility.

### *P. thornei* infectivity

Despite the fact that *P. thornei* populations recovered at 100 days after sampling were larger after the dry treatment than after the two other treatments, a comparative analysis (Kruskal-Wallis test) of the percentage of active nematodes at 75 days that had penetrated roots at 100 days did not uncover any significant difference. Therefore, densities after dry treatment seem to be maintained at a high level as a consequence of the greater survival in the population, and not from any differences in infectivity among the three treatments (Fig. 1b). Neither was infectivity of *P. penetrans* affected by anhydrobiosis (11), although the population was exposed to only one desiccation–reactivation cycle. In our populations it seems that infectivity is not affected even by two cycles of desiccation–reactivation.

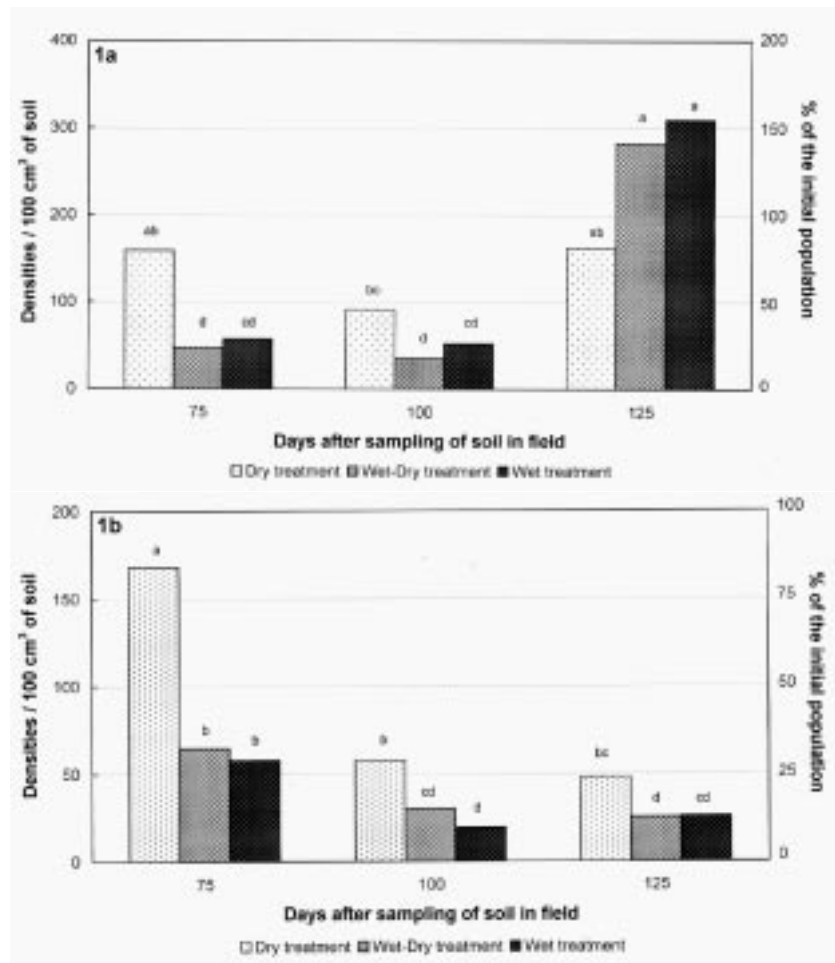


Fig. 1. *Pratylenchus thornei* densities recovered after three different watering treatments, 75, 100 and 125 days after soil sampling. Percentage of the initial population found in soil. Data are means of four replicates. Columns labeled with a common letter do not differ significantly according to LSD test ( $P \leq 0.05$ ). 1a, top: culturing under host (wheat); 1b, bottom: soil storage without host.

#### *P. thornei* reproductivity

In the presence of a host (Fig. 1a), densities at 125 days were greater than at 75 and 100 days in the wet–dry and wet treatments, but no significant differences were observed among the times after the dry treatment (Fig. 1a). In all three treatments, J2 appeared in the population after 125 days, but no J2 were found after 75 and 100 days (Table 1). *P. thornei* clearly maintains reproductive fitness following anhydrobiosis and wet–dry or wet treatments, as shown by the increase in their densities and the appearance of J2 in the population (Table 1). In the case of the dry treatment there were no differences among the three times.

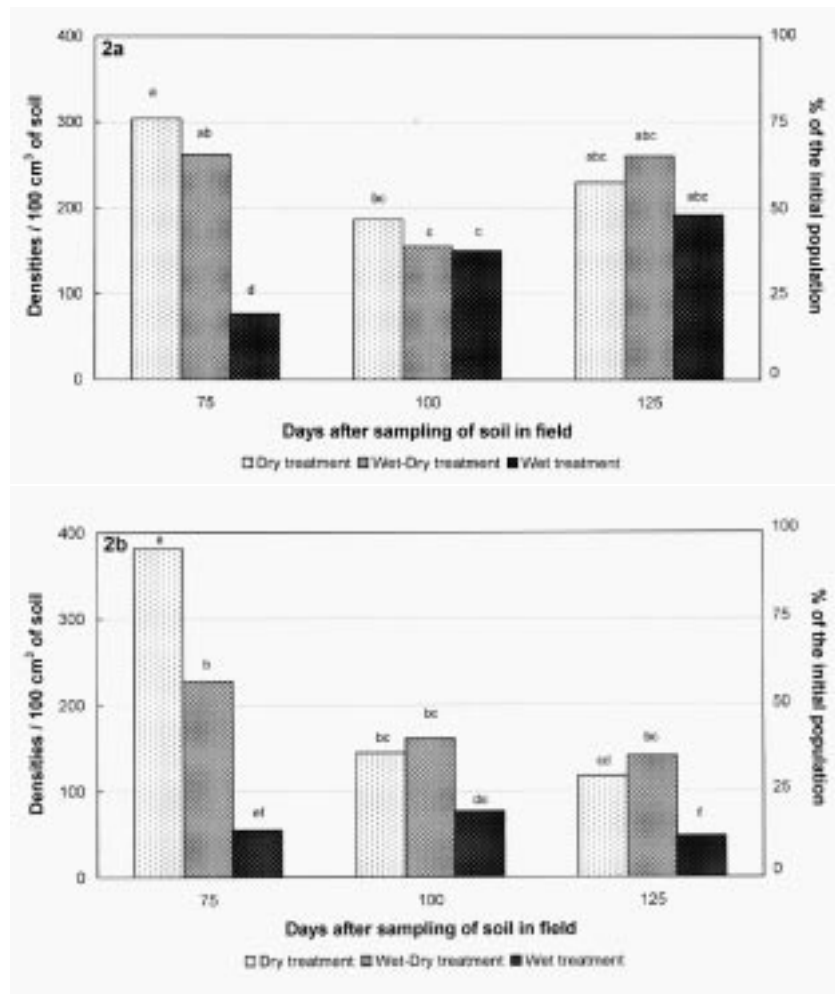


Fig. 2. *Merlinius brevidens* densities recovered after three different watering treatments, 75, 100 and 125 days after soil sampling. Percentage of the initial population found in soil. Data are means of four replicates. Columns labeled with a common letter do not differ significantly according to LSD test ( $P \leq 0.05$ ). 2a, top: culturing under host (wheat); 2b, bottom: soil storage without host.

The effect of anhydrobiosis on reproductivity seems to depend on the nematode species studied and the duration of anhydrobiosis. Reproductivity in *P. penetrans* (11) and *Pratylenchus zae* Graham (8) was not affected by anhydrobiosis. On the other hand, multiplication rates of anhydrobiotic *Scutellonema cavenessi* Sher were higher than those of fresh ones (1), and reactivated *P. thornei* multiplied twice as much as fresh nematodes (3). In our populations, multiplication rates ( $P_{125} / P_{100inroots}$ ), as determined by individuals within the roots at 100 days, were 2.4 for dry treatment, 13.5 for wet-dry and 7.5 for wet, but only the differences between the dry and wet-dry treatments

TABLE 1. Proportion (%) of the different stages of *Pratylenchus thornei* found in soil and roots at different times and treatments, with and without host

Stage and location	With host									
	Treatment:	Dry			Wet-Dry			Wet		
	Days:	75	100	125	75	100	125	75	100	125
Females in soil		44.7	20.4	10.4	39.6	18.9	2.8	63.8	9.3	2.6
J3-J4 in soil		55.3	20.4	8.0	60.4	18.9	2.1	36.2	20.4	5.5
Females within roots		–	33.3	8.6	–	35.1	4.6	–	20.4	3.9
J3- J4 within roots		–	25.8	53.4	–	27.0	46.5	–	50.0	70.4
J2 within roots		–	–	19.6	–	–	44.0	–	–	17.7
	Without host									
	Treatment:	Dry			Wet-Dry			Wet		
	Days:	75	100	125	75	100	125	75	100	125
Females in soil		43.5	62.1	56.3	39.1	76.7	46.1	50.0	57.9	69.2
J3-J4 in soil		56.5	37.9	43.7	60.9	23.3	53.9	50.0	42.1	30.8

were significant. Nematodes desiccated and reactivated twice showed a higher rate of reproductivity than nematodes kept in dry soil. These results are similar to those published by Glazer and Orion (3) and may be explained by the fact that individuals capable of surviving more cycles of desiccation–reactivation are the strongest or best nourished of the initial population. Furthermore, the multiplication rate of nematodes in the wet treatment (only one desiccation–reactivation cycle) was three times that of nematodes from the dry treatment. Baujard and Martiny (1) found that multiplication rates of *S. cavenessi* under anhydrobiosis for 90–180 days were higher than those under anhydrobiosis for 30–60 or 210–240 days. Prolonged anhydrobiosis could thus have a depressive effect on reproductivity of reactivated nematodes. Demeure (2) noted that females of *S. cavenessi* rehydrated after 30 months in dry soil were incapable of laying eggs. Immature females of *Rotylenchulus reniformis* Linford and Oliveira, reactivated after 180 and 450 days, infected and reproduced at lower rates than fresh nematodes (6).

It may be concluded that *P. thornei* retains its infectivity and reproductivity after surviving the summer in an anhydrobiotic stage. It therefore ensures its existence and survival in dry soils, thus enabling it to parasitize the succeeding crops.

#### *M. brevidens*

*M. brevidens* densities at 75 days, after wet treatment were significantly lower than after wet–dry and dry treatments (Figs. 2a, 2b). However, at 100 days under wet treatment, densities were already higher than after 75 days. It seems that *M. brevidens* did not have enough time to reproduce significantly during the 125 days of the experiment, since no significant differences were found among densities at the three times, except for the wet treatment, in which densities recovered at 75 days were very low. As with *P. thornei*, the wet treatment seems to expend more nematode energy than the two other treatments during the first 75 days, as the population recovered in the wet treatment was significantly lower than in the two other treatments, due to the effect of starvation.

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