Studies on Anhydrobiosis of Pratylenchus thornei[1]

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Abstract: Large populations of *Pratylenchus thornei,* a winter pest of cereals, legumes, and potatoes in the northern Negev region of Israel, survive 7-8 months of summer drought and return to full activity at the beginning of the rainy season. To demonstrate that it survives the summer in an anhydrobiotic state, all developmental stages of *P. thornei* were exposed to gradually reduced relative humidity (RH) using glycerin water solutions. At 97.7% RH the nematodes were coiled and able to survive exposure to 0% RH. About 40% of artificially desiccated nematodes could be reactivated by gradually increasing the humidity to the final water environment. Desiccated nematodes could withstand temperatures up to 40 C. Reactivated individuals showed intestines apparently devoid of reserve materials. Only 3% survived three cycles of desiccation and reactivation. *P. thornei* reactivated after anhydrobiosis multiplied twice as much within *Vicia sativa* roots as did fresh nematodes.

Journal of Nematology 15(3):333-338. 1983.

Anhydrobiosis as a means of survival for free-living and plant parasitic nematodes during the dry season is a well-known phenomenon reviewed by Van Gundy (16), Evans and Perry (5), Crowe (1), and Demuere and Freckman (3). Nematodes in this physiological state are highly resistant to extreme environmental factors (3,7,8,16).

Pratylenchus thornei Sher and Allen, parasitizes cereal crops, legumes, and potatoes in the semi-arid northern Negev region in Israel (11). The nematode, however, is active only during the rainy season, from December to April. During the 7 months of summer drought no hosts grow; soil temperature rises and relative humidity drops in the upper layers of soil. A preliminary survey showed that despite these unfavorable conditions *P. thornei* numbers do not decline significantly (11). This suggests that *P. thornei* survives the dry season in an anhydrobiotic state and is reactivated by the following winter rains. This possibility was tested experimentally, and the effects of desiccation on survival and the morphology of *P. thornei* are reported.

MATERIALS AND METHODS

Stock cultures of *P. thornei* were maintained in pots in greenhouse on *Vicia sativa* at a constant temperature of 20 ± 1 C. Nematodes were extracted from heavily infested roots using the Seinhorst's mistifier technique (13).

Inducing anhydrobiosis: Fresh nematodes obtained from the mistifier were transfered from the suspension to 25-mm Millipore filter discs (0.65-μm pore), 1,600 ± 100 adults and larvae per disc, by means of a suction flask. Each disc served as a replicate in the anhydrobiosis experiment described below.

Anhydrobiosis was evaluated according to two criteria: 1. The survival rate of desiccated nematodes after exposure to 0% relative humidity (RH) for 24 h. The RH was maintained by phosphorous pentoxide in closed chamber (15). The number of surviving individuals was determined by counting the active nematodes following hydration. 2. The percentage of nematodes present in coiled form in the population. The optimal RH required to induce complete anhydrobiosis was determined by exposing samples of *P. thornei* on Millipore filter discs to five levels of RH for various periods of time according to Table 1.

Each treatment was replicated eight times. Following each treatment, six of these replicates were placed in water for rehydration and the nematodes both in stretched and coiled forms were counted. Twenty-four

Table 1. The period of time (hours) *Pratylenchus thornei* populations were exposed to various relative humidity levels.

	Relative humidity				
Treatments	100	98.8	97.7	96	93
1	96				
2	24	96			
3	24	24	96		
4	24	24	24	96	
5	24	24	24	24	96

Received for publication 24 May 1982.

[1] Contribution from the Agricultural Research Organization, No. 284-E, 1981 series.

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hours later the active nematodes were counted in each replicate (Fig. 1). Two more replicates were exposed to 0% RH for 24 hours and then gradually rehydrated by exposing them to 97.7, 100% RH for 24 hours each before placing in water.

Effect on morphology: Individuals at all stages of the anhydrobiosis process were examined under a light microscope. Fixation and slide preparation were done according to Seinhorst's glycerol-methanol method (14). Naturally desiccated *P. thornei* obtained from dry field soil by a sugar floation technique (9) were also observed. Desiccated nematodes were mounted on stubs, coated with gold, and observed with a Cambridge 180 scanning electron microscope (SEM) at 15 kV at 3,000 magnification.

Effects of external factors: 1. Exposure of the nematodes to various temperatures—0, 10, 20, 30, 40, and 60 C—for 24 h after they were desiccated at 22 C. To avoid drastic changes in the RH, the dehydrated nematodes were placed on Millipore filters in closed chambers at 97.7% RH. 2. Observations of the percent of survival following four cycles of dehydration/rehydration. In both experiments, each treatment consisted of six replicates. The state of anhydrobiosis was evaluated by the survival of the nematode at 0% RH and the percentage in the coiled form.

Effect on pathogenicity: The effect of anhydrobiosis on the pathogenicity of *P. thornei* was investigated under greenhouse conditions. Ten *V. sitiva* plants were each inoculated with 4,000 ± 100 fresh or laboratory induced anhydrobiotic *P. thornei.* Control plants were not inoculated. Five plants of each treatment were harvested at 24 and 48 days after inoculation, and the fresh root weights and the number of *P. thornei* within the roots were determined.

RESULTS

Inducing anhydrobiosis: The effect of the various RH's on the anhydrobiosis of *P. thornei* is shown in Fig. 1. The highest percentage of reactivated nematodes (coiled individuals as well as survival percentage) after exposure for 24 h to 0% RH was obtained by desiccating *P. thornei* at 97.7% RH. The percentage of coiled nematodes in desiccation treatments No. 2–5 was higher than the percentage of final surviving individuals (Fig. 1).

Effects on morphology: Laboratory dessicated nematodes and individuals extracted from dry soil were found in coiled

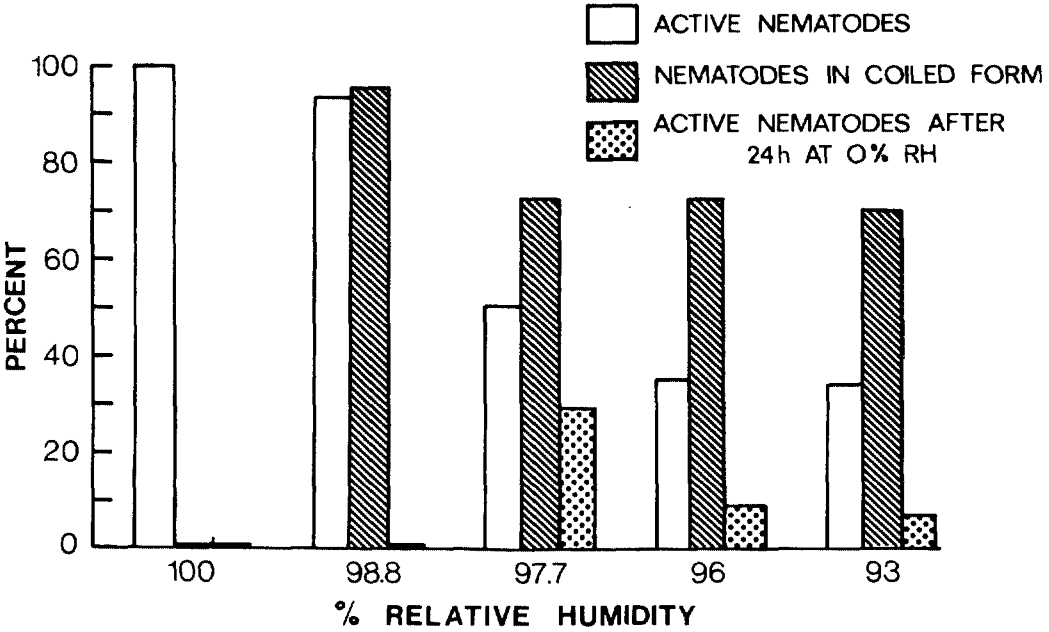

Fig. 1. The effect of various relative humidity (RH) regimes on the survival and coiling of *Pratylenchus thornei* populations.

form, but individuals extracted from *V. sativa* roots were found in stretch form. In the desiccated individuals the somatic muscles (Fig. 2) are severely deformed and the body wall is shrunken (Fig. 3). After rehydration vacuoles were observed in the intestines.

Effects of external factors: 1. The effect of various temperatures on the survival of *P. thornei* is demonstrated in Figure 4. As the temperature at which desiccation is induced is increased, there is a decrease in the percent survival. The highest (63%) was at the lowest temperature (0 C). All the active nematodes in the control treatments were dead after exposure to temperatures above 0 C.

2. Four cycles of desiccation and rehydration successively reduced the level of survival (Fig. 5), so that after the fourth cycle no nematode survived.

Effect on pathogenicity: Numbers of *P. thornei* in *V. sativa* roots infected by anhydrobiotic nematodes were almost twice as high as in roots infected by fresh nematodes (Fig. 6). Despite poor growth of *V. sativa* and low numbers of nematodes, results of several replications were consistent.

DISCUSSION

The optimal RH for inducing anhydrobiosis was 97.7%. This corresponds to the approximate RH in the soil of the wilting point (10). The process of anhydrobiosis is probably accomplished in nature during this humidity rate which occurs in the late spring. Lower RH is beyond the natural edaphic conditions at late spring or early summer and probably reduces the efficiency of inducing anhydrobiosis in the nematodes. Thus, the rest of the experiments in this study were carried out by inducing anhydrobiosis at 97.7% RH.

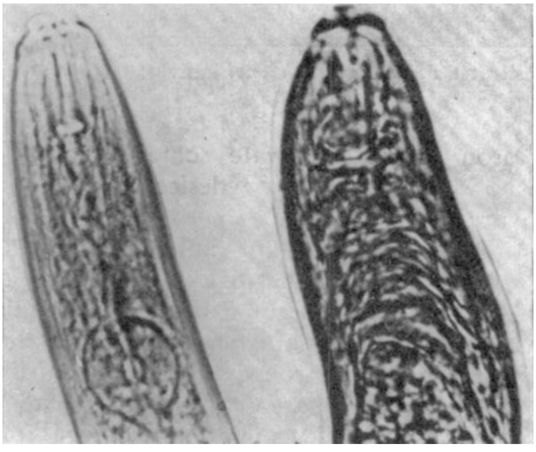

Fig. 2. Anterior region of *Pratylenchus thornei;* right—anhydrobiotic, left—active (× 1,000).

Anhydrobiotic *P. thornei* obtained under both experimental and natural conditions are morphologically similar to other anhydrobiotic, plant parasitic, and mycophagous nematodes such as *Aphelenchus avenae, Scutellonema bracyurum,* and *S. cavenessi* (2). Thus it appears that coiling is the general form of protection for the anhydrobiotic nematode. Coiling provides minimal exposure to the external environment (3). Although the cuticle does not change during anhydrobiosis, changes occur in the nematode's somatic muscles; this may be due to desiccation of the pseudocelomic fluids.

Although differences in survival were found between developmental stages of *A. avenae* (5) and *Ditylenchus dipsaci* (12), no differences were found in the survival ability of *P. thornei* larvae and adults. Because of the morphological similarity between the nematode in natural dry soil and the artificially desiccated nematodes and the ability of artificially desiccated nematodes to survive at 0% RH, we believe that the experimental procedure of gradual desiccation is similar to the procedure which occurs in nature in the spring.

The large vacuoles present in the intestine of rehydrated *P. thornei* may be an indication of the lack of storage materials. This condition resembles that of starving nematodes. Since such vacuoles are not found in fresh or in anhydrobiotic individuals, one may conclude that during rehydration the nematodes consume large amounts of reserve materials. This may explain why at all RH levels there is a higher percentage of coiled nematodes than of surviving nematodes. Perhaps the coiled individuals that did not survive depleted their energy sources before complete rehydration. Van Gundy (16), Freckman (7), and Crowe (1) noted that anhydrobiosis induces higher resistance to unfavorable environmental conditions. Anhydrobiotic *P. thornei* survived temperatures up to 40 ± 2 C. This should enable them to sur-

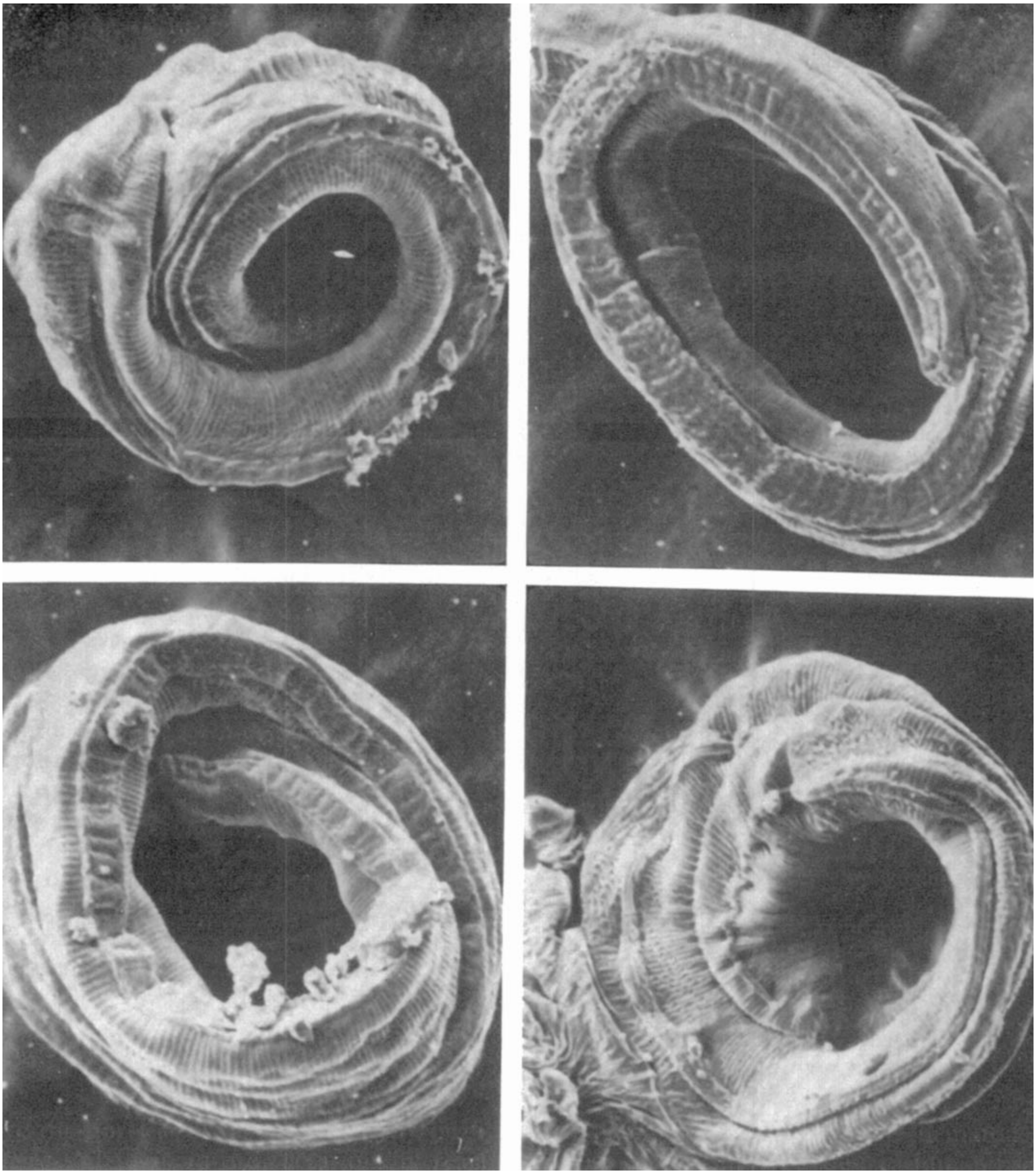

Fig. 3. Scanning electron micrograph of artificially desiccated *Pratylenchus thornei* individuals (× 3,000).

vive the hot season in the northern Negev, where soil temperatures occasionally reach 40 C. A similar pattern was found by Fassuliotis (6), who tested the survival of desiccated *Hoplolaimus columbus* in a range of 0–80 C.

Progressive reduction in survival of *P. thornei* with successive desiccation/rehydration cycles agrees with the results obtained by Womersley (18) on *Anguina tritici*. This phenomenon might have a practical application; irrigation of dry fields in the hot season might reactivate some nematodes who would not survive redesiccation and rehydration.

Infectivity rates of anhydrobiotic *P. thornei* was twice that of the fresh population. This might occur if most of the individuals surviving anhydrobiosis were the physiologically strongest of the initial population. Demeure (2) noted that *Scutellonema cavenessi* females, rehydrated following storage for 30 months in dry soil, are incapable of laying eggs. Also, Webster (17) noted that a population of *D. dipsaci* following anhydrobiosis was less infective than fresh nematodes.

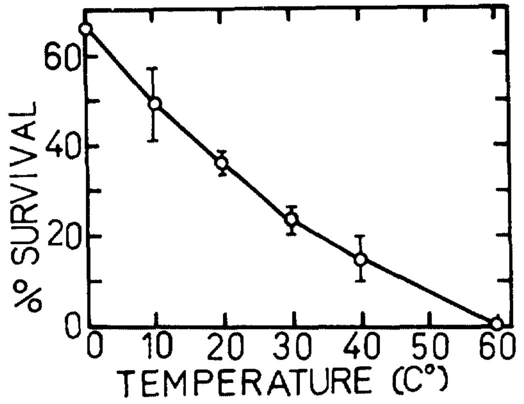

Fig. 4. The survival percentage of anhydrobiotic *Pratylenchus thornei* exposed to various temperatures during desiccation.

The present work provides evidence supporting the assumption that *P. thornei* is native to the semi-arid zones in parts of the Middle East (11); it also indicates that the nematode survives the arid period by anhydrobiosis.

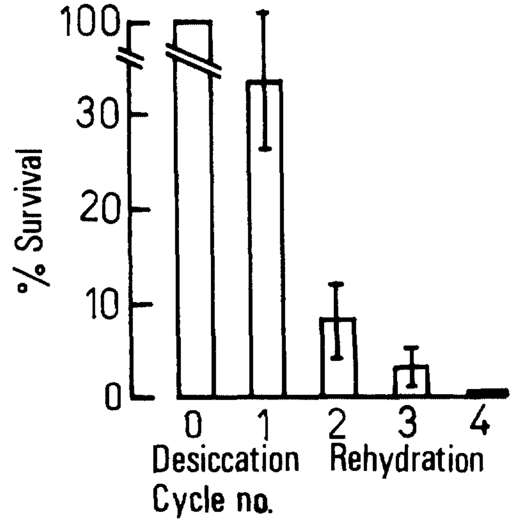

Fig. 5. The effect of up to four cycles of desiccation/rehydration on the survival of *Pratylenchus thornei*.

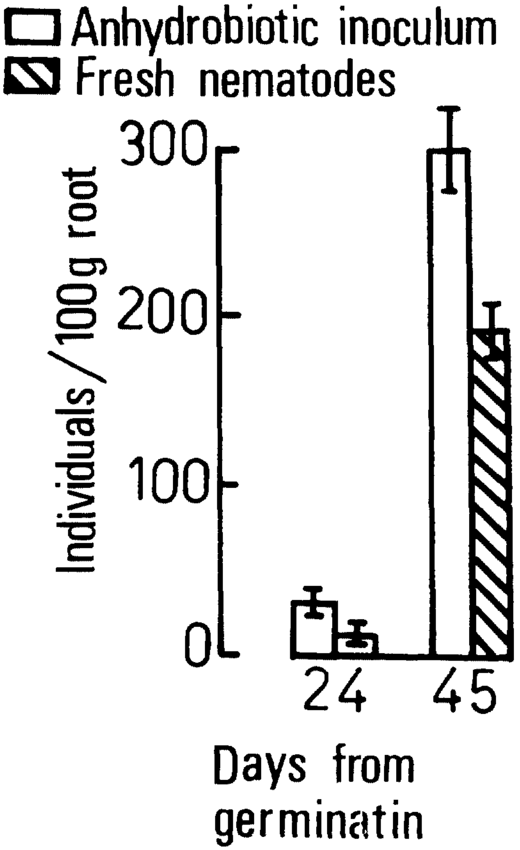

Fig. 6. Infectivity of fresh and rehydrated anhydrobiotic *Pratylenchus thornei* on *Vicia sativa*.

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