Journal of Nematology 16(3):282–289. 1984.

Anhydrobiosis in *Pratylenchus penetrans*[1]

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Abstract: Anhydrobiotic survival of *Pratylenchus penetrans* was compared in several soil moisture regimes. Bodies of anhydrobiotic nematodes were coiled. In slow-dried soils, Vineland silt loam (VSL) and Fox loamy sand (FLS), 70 and 58% of the total *P. penetrans* populations were anhydrobiotic when soil moistures reached ca. 3% and water potential 15 kPa or greater. Coiling began at a much lower water potential in FLS than in VSL. In fast-dried soils, only 31 and 22% of the *P. penetrans* populations in the same two soil types had entered the anhydrobiotic state at comparable moistures. In the above soils, 76–96% of the *P. penetrans* were alive immediately after entering the anhydrobiotic state. In slow-dried VSL, some nematodes (1%) survived 770 days. In the other soils, all anhydrobiotic nematodes were dead after 438 days. Anhydrobiosis increased the ability of nematodes to survive subzero temperatures, but it did not increase their ability to survive temperatures above 40 C. Infectivity and reproductivity of rehydrated *P. penetrans* were not affected by anhydrobiosis.

Key words: soil moisture, water potential, nematode extraction efficiencies, anhydrobiotes, survival, infectivity, reproductivity.

Experimental soils at the Vineland Research Station are often air dried by spreading them on greenhouse benches to destroy *Pratylenchus penetrans* Cobb without destroying microflora. Such soils are used in laboratory experiments and in the field to restore the microflora in crop loss assessment experiments (8,9). Air-dried soils are assessed for nematodes before being used experimentally, and *P. penetrans* are recovered when the soil is spread too deeply on a bench to allow rapid drying. Recent studies suggest that *P. penetrans* may survive soil drying in a dry state (5,7,10, 16,17). This paper presents data on anhydrobiosis in *P. penetrans* in two Ontario soils and the effects of anhydrobiosis on infectivity, reproductivity, and the ability to survive extreme temperatures. Methods for extracting normal and anhydrobiotic nematodes are compared.

Received for publication 11 October 1983.

[1] The author thanks Mrs. Diane Beaulieu-Aruvee for her excellent technical assistance.

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MATERIALS AND METHODS

Anhydrobiosis in *P. penetrans* was studied in two soil types, a Vineland silt loam (VSL: 61% sand, 28% silt, and 11% clay)

TABLE 1. Efficiencies of three methods for the extraction of *Pratylenchus penetrans* from a Vineland silt loam and a Fox loamy sand.

Soil type	Nematode stages*	Number of *P. penetrans*/50 g soil			$LSD_{10\%}$
		Sieving	Flotation	Pan	
Vineland silt loam	A + J_4	25	295	990	128
	J_3 + J_2	5	75	560	74
	Total	30	370	1,550	108
Fox loamy sand	A + J_4	15	60	220	24
	J_3 + J_2	0	10	170	32
	Total	15	70	390	48

* A = adult; J_2, J_3, J_4 = second-, third-, fourth-stage juveniles.

and a Fox loamy sand (FLS: 85% sand, 10% silt, and 5% clay). Both soils were placed in greenhouse groundbeds and planted with sweet corn (*Zea mays* L. cv. Earlivee) to maintain *P. penetrans* populations.

Extraction methods: The efficiencies of three nematode extraction methods were compared: the Cobb decanting and sieving method (12), a simple centrifugal sugar-flotation method (4), and the pan method (13). On five separate occasions, nematodes were extracted from eight 50-g samples of infested VSL and FLS by each extraction technique. Developmental stages were grouped and counted as either adults plus J_4, or J_3 plus J_2, and data were subjected to an analysis of variance. Based on the results obtained, as modified by Freckman et al. (7), the sugar-flotation method for fixed anhydrobiotic nematodes and pan method for active nematodes were selected for the anhydrobiotic studies.

Anhydrobiotic nematodes were killed and extracted as follows. Soil was randomly sampled in 50-g aliquots. Each soil sample was poured into 200 ml of 4% formaldehyde solution at 40 C in a 250-ml centrifuge bottle, shaken, and centrifuged for 5 minutes at 1,100 *g*. The supernatant was discarded, and the pellet was resuspended in 200 ml of sucrose solution (specific gravity 1.18–1.20) and centrifuged as before. Nematodes were recovered from the sugar solution by pouring the final supernatant three times through a 38-μm-pore sieve and back-flushing the sieve with water after each passage. Nematodes were then concentrated for counting with a micropore filter (8 μm). Coiled nematodes were assumed to be anhydrobiotic based on the work of Freckman et al. (7). Living nematodes were extracted from an additional 50-g sample by the pan method for 7 days. The number of nematodes extracted was used to estimate the total number of live nematodes, hydrobiotic and anhydrobiotic, at the time of sampling. Representative anhydrobiotes of *P. penetrans* were mounted on slides and photographed using a compound microscope.

Long-term anhydrobiotic survival in fast- and slow-dried VSL and FLS soils: Infested soils from the ground beds were screened and mixed in a mechanical shaker to remove root debris and to distribute the nematodes evenly. To fast dry the two soils at ca. 27 C day and 22 C night, 100 kg of VSL and FLS were spread to a depth of 1.3 cm on a greenhouse bench; to slow dry the soils, 100 kg of each soil was spread to a depth of 9 cm. The four soil masses were covered with paper. The soil moisture characteristic and the particle size distribution of the two soils were determined by the Department of Land Resource Science, Ontario Agriculture College, University of Guelph, Guelph, Ontario.

Sampling was at 0 days, then at 3-day intervals for the first 24 days, and finally at increasingly longer intervals over the next 2 years. At each sampling interval, twenty 50-g samples were taken from each soil mass: eight for anhydrobiotic and eight for active nematode extractions and four to measure percentage of soil moisture.

Survival of temperature extremes by anhydrobiotic P. penetrans: To examine cold survival, 10 kg of infested VSL, slow dried for 44 days, was placed in an untied plastic bag, preconditioned for 7 days at 2 C and stored at −4 C. An equal weight of moist infested VSL from a greenhouse groundbed was stored in the same manner. The two soils were sampled at 0, 7, and 14 days and at

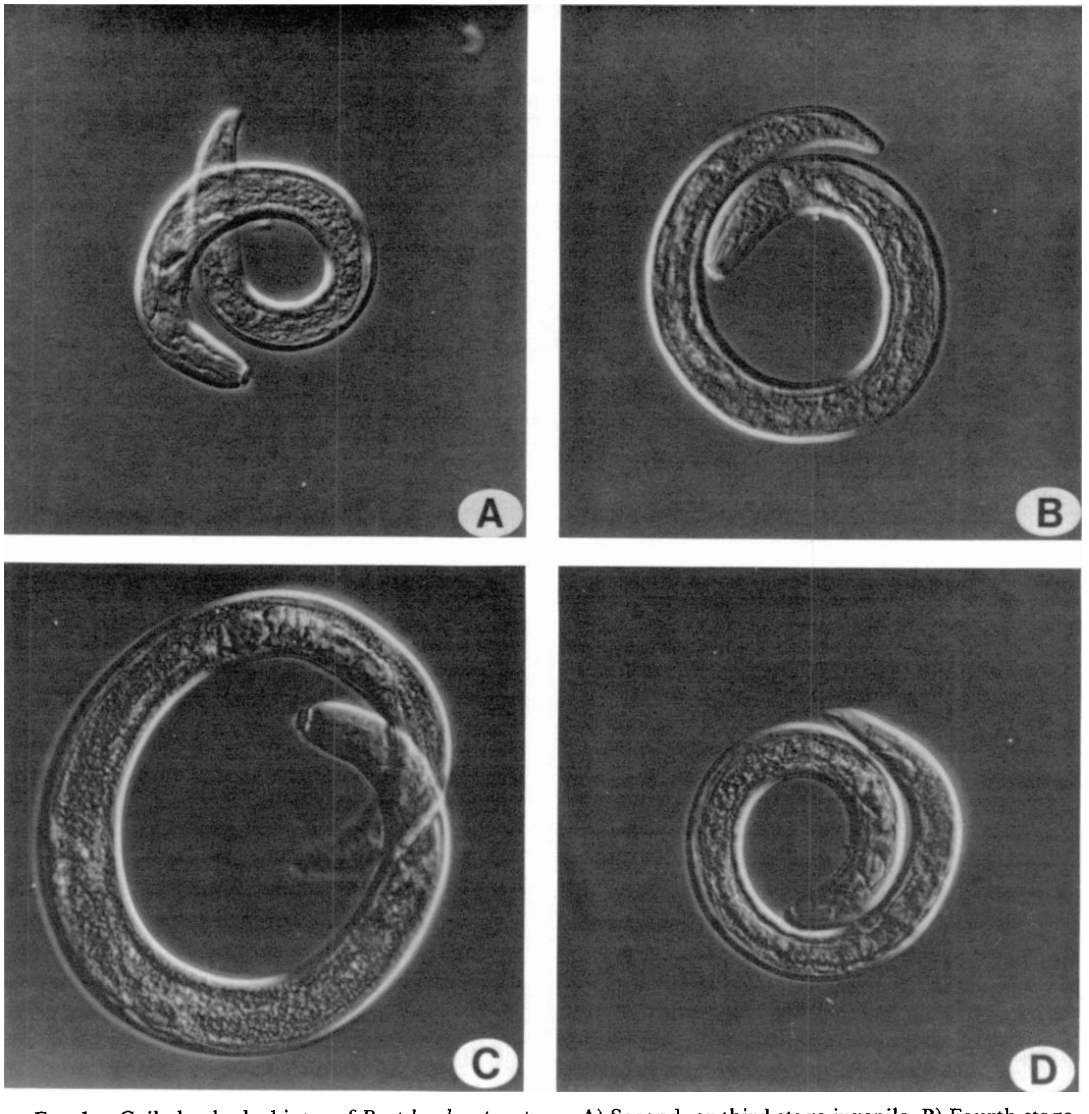

FIG. 1. Coiled anhydrobiotes of *Pratylenchus penetrans.* A) Second- or third-stage juvenile. B) Fourth-stage juvenile. C) Female. D) Male.

greater intervals thereafter. Surviving nematodes were extracted from eight 50-g samples from each soil mass by the pan method. High temperature survival by anhydrobiotic *P. penetrans* was examined by storing 10 kg each of moist infested FLS and 58-day-old slow-dried FLS at 40, 55, and 70 C. Surviving nematodes were extracted by the pan method from eight 50-g samples from each soil mass. Sampling, upon initiating the experiments, was at 0 days, then at 7-day intervals for 2 weeks, and finally at longer intervals over 546 days.

Infectivity after anhydrobiosis: Nematodes were extracted by the pan method from slow-dried VSL (64 days) that contained only anhydrobiotes. Using aseptic procedures, alfalfa seedlings were placed on water agar (five seedlings per plate), a drop of water was placed by each root, a female was transferred into each drop, and the roots were covered with silica sand. Eight plates were prepared. Eight more plates were prepared with females from moist soil that had not been dried. Inoculated seedlings were incubated 72 hours at 22 C, and the roots were stained in cottonblue (2,000 ppm) lactophenol stain to detect nematodes.

Reproductivity after anhydrobiosis: Slow-

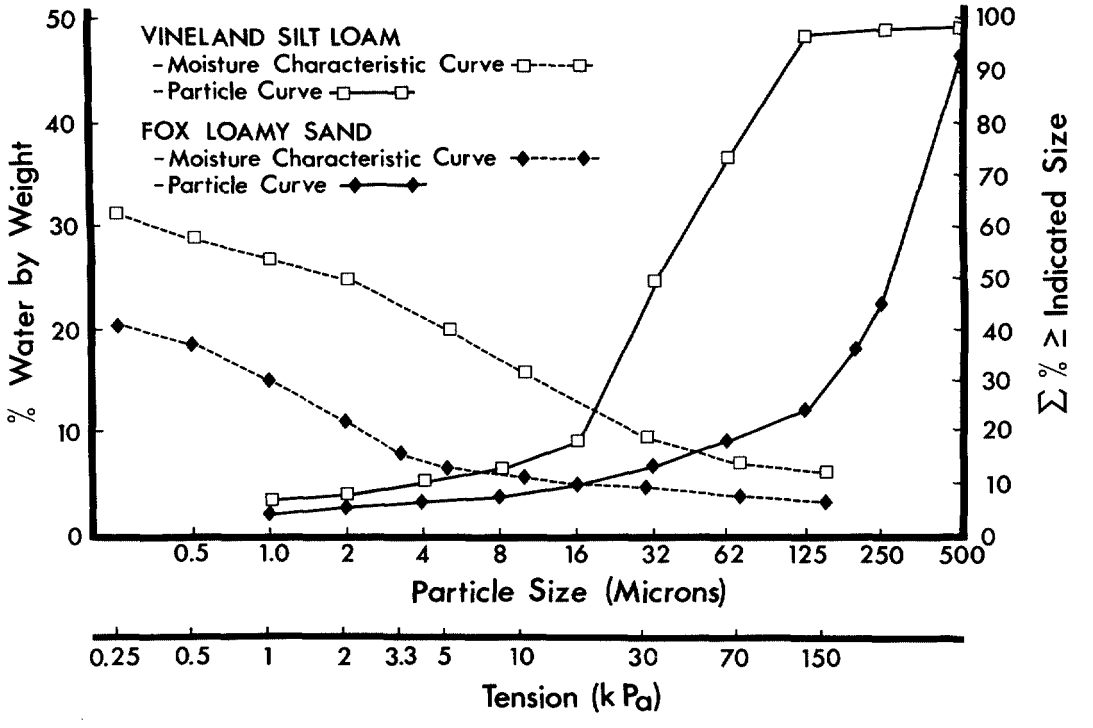

FIG. 2. Moisture characteristic and particle distribution curves for Fox loamy sand and Vineland silt loam.

dried VSL (207 days) was placed in twenty-four 12-cm-d clay pots, planted with 5-week old celery transplants, and watered. An equal number of controls was prepared with a moist VSL that had not been dried. The pots were randomized in pairs on a greenhouse bench at 20–25 C. Four pairs of pots (control and treatment) were selected at random at regular intervals during 21 weeks, the roots were excised and washed, and the nematodes were extracted for 2 weeks in Baermann pans and counted.

RESULTS

Extraction methods: The efficiency of the pan method for the extraction of *P. penetrans* from VSL and FLS, was respectively, 50 and 15 times greater than that of the Cobb decanting and sieving method and 5 times greater than that of the sugar-flotation method (Table 1). With repeated tests a correction factor of 3 × was obtained to compare *P. penetrans* extracted by sugar flotation with those extracted by pan.

Long-term anhydrobiotic survival: The coiled configuration was noted in all post-hatching stages of *P. penetrans* fixed in air-dried soil (Fig. 1). The protrusion of the male tail from the coil and the smaller size of the coil formed by juveniles made the identification of these stages possible (Fig. 1). The degree of coiling ranged from a single ring coil to a 2¼ ring coil (Fig. 1). Highly contorted configurations were uncommon. Nematodes fixed in a moist soil were found rarely in a coiled state but often in open Cs, nearly closed Cs, and contorted configurations suggesting displacement by soil particles.

The particle size analyses are shown as cumulative curves (Fig. 2). The curves are similar, but in VSL 80% of the particles range from 15 to 125 μm, whereas in FLS 70% range from 125 to 800 μm. The moisture characteristic curve of VSL is greater than that of FLS. Water potentials were determined from these curves.

At day 0 in the slow-dried and fast-dried VSL ca. 3% of the *P. penetrans* were coiled, and in the slow-dried and fast-dried FLS ca. 13% were coiled (Figs. 3, 4). The moisture content of the four sets of soils was 11.5%, with a water potential of 1.9 kPa for FLS and 19 kPa for VSL.

Twenty-five percent of the *P. penetrans* population was anhydrobiotic in the slow-

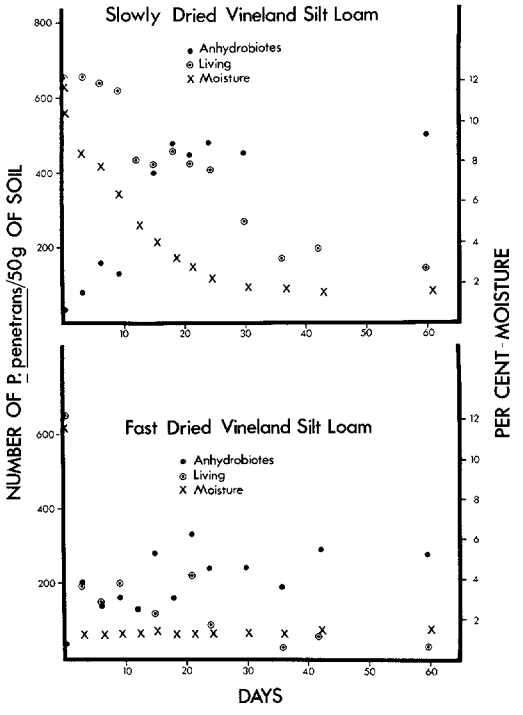

FIG. 3. Numbers of *Pratylenchus penetrans* extracted at various intervals during a 60-day period from slow- and fast-dried Vineland silt loam and percentage of soil moisture at each extraction. The extraction methods yielded estimates of total living nematodes and total coiled nematodes or anhydrobiotes, dead and alive, at each extraction interval.

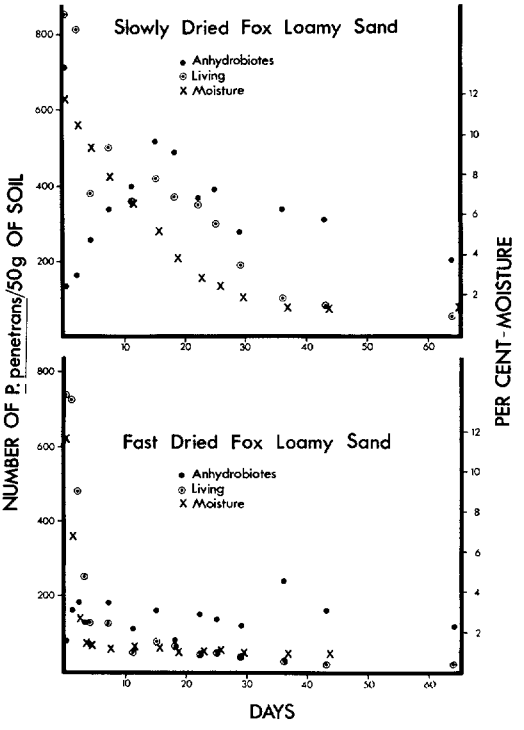

FIG. 4. Numbers of *Pratylenchus penetrans* extracted at various intervals during a 60-day period from slow- and fast-dried Fox loamy sand and percentage of soil moisture at each extraction. The extraction methods yielded estimates of total living nematodes and total coiled nematodes or anhydrobiotes, dead and alive, at each extraction interval.

dried VSL at day 6 when the soil moisture was 7.9% and the water potential was 70 kPa. The entire population was not anhydrobiotic until day 18 when the soil moisture was 3.3% and the water potential was greater than 150 kPa (Fig. 3). At this time the anhydrobiotic population represented 74% of the original population. By day 60 only 31% of the anhydrobiotic population was alive and soil moisture was 1.5% (water potential WP 150 kPa). In the slow-dried FLS the entire *P. penetrans* population was anhydrobiotic by day 15 when soil moisture was 5.2% (Fig. 4) and the water potential was 25 kPa. The anhydrobiotic population represented 61% of the original population. Thirty percent of the population was anhydrobiotic as early as day 4 when the soil moisture was 9.3% and the water potential was 2.5 kPa. By day 64 only 25% of the anhydrobiotic population was alive, only 6% of the original population.

In the fast-dried VSL the *P. penetrans* population was fully anhydrobiotic by day 3 when soil moisture was 1.2% (WP 15 kPa) (Fig. 3). The anhydrobiotic population represented 31% of the original population, only half of that of the slow-dried VSL. By day 60 only 11% of the anhydrobiotic population was still alive. In the fast-dried FLS the entire *P. penetrans* population was anhydrobiotic by day 4 (Fig. 4) when soil moisture was 1.3% (WP 15 kPa). The anhydrobiotic population represented only 22% of the original population, less than half of that of the slow-dried FLS. By day 64 seventeen percent of the anhydrobiotic population was alive, representing only 3% of the original population.

After day 64 the number of anhydrobiotes of *P. penetrans* remaining alive declined rapidly (Table 2). Death was more rapid in fast-dried than in the slow-dried soils. By day 365 all nematodes in the fast-dried soils were dead. In slow-dried VSL

TABLE 2. Long-term survival of *Pratylenchus penetrans* in slow- and fast-dried Vineland silt loam (VSL) and Fox loamy sand (FLS).

	Number of surviving *P. penetrans*/50 g soil			
	VSL		FLS	
Day	Slow-dried	Fast-dried	Slow-dried	Fast-dried
0	650	650	850	730
4	660	190	380	130
24	480	90	300	50
62	140	30	50	20
104	56	5	45	5
156	9	2	27	3
260	4	0	17	4
365	5	0	15	0
556	1	0	2	0
770	1	0	0	0
$LSD_{10\%}$	53	45	53	43

living anhydrobiotic nematodes were found at day 770. All post-egg stages of *P. penetrans* were capable of long-term anhydrobiotic survival.

Survival of temperature extremes by anhydrobiotic P. penetrans: When slow-dried VSL was stored at −4 C for 546 days, the average number of viable anhydrobiotic *P. penetrans* (170 nematodes/50 g soil) remained constant (Table 3). However, *P. penetrans* in a moist VSL stored at −4 C declined from 750 nematodes/50 g soil to 0 nematodes in 101 days (Table 3). At 40 C both anhydrobiotic *P. penetrans* in slow-dried FLS and hydrated *P. penetrans* in moist FLS gradually declined in number and all were dead within 101 days (Table 3). At higher temperatures (55 and 70 C), nematodes died in less than 7 days.

Infectivity and reproductivity after anhydrobiosis: The infectivity of *P. penetrans* was not affected by anhydrobiosis. Equal numbers of females (73%) from slow-dried FLS and moist FLS penetrated the roots of alfalfa seedlings. Reproductivity of *P. penetrans* also was not affected by anhydrobiosis (Table 4). In slow-dried VSL that had been moistened and planted to celery, the number of nematodes in the roots increased from 1 nematode/g fresh root weight at day 7 to 800 nematodes/g fresh root weight at day 147. During the same period *P. penetrans* in moist VSL that had never been allowed to dry similarly increased from 6 to 770 nematodes/g fresh root weight.

TABLE 3. Cold and heat survival of *Pratylenchus penetrans* as active nematodes in moist Vineland silt loam (VSL) and Fox loamy sand (FLS) and as anhydrobiotes in slow-dried VSL and FLS.

	Number of *P. penetrans*/50 g soil			
	Cold survival (−4 C)		Heat survival (40–70 C)	
Day	Moist VSL*	Slow-dried VSL†	Moist FLS‡	Slow-dried FLS§
0	750	175	520	70
7	500	160	20	15
14	150	180	2	10
35	20	190	1	20
56	10	100		
63			1	10
91	4	170		
101	0	240	0	0
119	0	240	0	0
546	0	160		
$LSD_{10\%}$	50	ns	42	25

* Moisture: 25% at day 0, 1.7% at day 546.
† Moisture: 1.3% at day 0, 1.3% at day 546.
‡ Moisture: 11.6% at day 0, 5.3% at day 142.
§ Moisture: 1.4% at day 0, 0.5% at day 142.

DISCUSSION

Many anhydrobiotic nematodes are characterized by coiled configuration (6,7). Active stages of *P. penetrans* became coiled as soils dehydrated. Eggs also may have become anhydrobiotic, but many eggs passed through the 38-μm-pore sieve used in the flotation extraction and were lost. Demeure et al. (16) found that 80–96% of the nematodes in soil containing *Acrobeloides* sp., *Aphelenchus avenae*, and *Scutellonema brachyurum* became coiled and anhydrobiotic when soil moisture was depleted to 6–9 monomolecular layers of water. At this point, the moisture contents of the two soils they used were in the 1.8–3.4% range. The soils used in my study were physically similar to those used by Demeure et al., and nematodes responded to soil drying similarly, provided the soil was dried slowly. When moisture content was 5.2 and 3.3% in the slow-dried Vineland silt loam and the Fox loamy sand, 74 and 61% of the *P. penetrans* were coiled anhydrobiotes. By comparison, in the fast-dried VSL and FLS only 31 and 22% of the *P. penetrans* were anhydrobiotic. The difference in the sizes of the anhydrobiotic populations resulting from differences in the rates of soil drying suggests that the rate of nematode dehy-

TABLE 4. Reproduction of *Pratylenchus penetrans* in celery: moist Vineland silt loam (VSL) contained active nematodes and the slow-dried VSL contained anhydrobiotes which were reactivated upon moistening at planting.

Day	Number of *P. penetrans* per g fresh root	
	Moist VSL	Slow-dried VSL
0	0	0
7	6	1
35	23	16
91	150	160
119	675	510
147	770	800
$LSD_{10\%}$	165	155

dration is an important factor in the induction of anhydrobiosis in *P. penetrans*. Crowe and Madin (2) have shown that slow dehydration can be necessary for induction of anhydrobiosis of *Aphelenchus avenae*.

Low revival of anhydrobiotics of *P. penetrans* was found in fast-dried soils. This low revival may be the result of the rapid dehydration of the nematodes (2). In slow-dried soils revival of anhydrobiotics was greater and may be the result of slower dehydration of the nematodes (2).

The rate of desiccation also seems to affect long-term survival. Where the loss of soil moisture was rapid, anhydrobiotes survived less than 450 days, whereas in the slow-dried soils, particularly in the Vineland silt loam, anhydrobiotes survived more than 2 years.

Pratylenchus penetrans in the anhydrobiotic state survived freezing temperatures but active *P. penetrans* did not. Although southern Ontario soils are moist in autumn, possibly micro-pockets exist where the water potential is sufficiently great to cause coiling of the nematode and anhydrobiosis, particularly in loamy sands. Thus *P. penetrans* could overwinter in the soil proper as well as in roots.

Earlier experiments proved that *P. penetrans* caused more damage to tobacco grown on Fox loamy sand in Ontario when springs were cool and wet, as opposed to warm and dry (14,15). Less damage to tobacco in warm dry springs may be due, in part, to anhydrobiosis in *P. penetrans*. In Fox loamy sand, which is composed primarily of large particles, relatively low water potentials induce coiling and anhydrobiosis. Thus the destructive level of the *P. penetrans* population is reduced.

Pratylenchus penetrans can be considered only a moderately successful anhydrobiote compared to *Anguina tritici* and *Ditylenchus dipsaci* (5). Rates of soil and nematode dehydration are critical factors affecting the development of anhydrobiosis in *P. penetrans* and its ability to survive long periods without moisture. Its ability to survive freezing as an anhydrobiote is likely due to the loss of body water. Loss of moisture reduces ice needle formation that is so destructive to cells (11). Infectivity and reproductivity are not affected by anhydrobiosis. It is not known if repeated wetting and drying has deleterious effects on *P. penetrans*.

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