Factors Influencing Survival of *Ditylenchus dipsaci* (Kühn, 1857) in Soil

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Abstract: *Ditylenchus dipsaci* larvae survived in soil without a host plant for at least 242 days when held at 15 C and 21 C. Larvae held at 15 C remained infective for 212 days. Moisture levels within both clayey and sandy soils did not appreciably affect recovery of larvae. Active nematodes recovered from soil are not necessarily infective. Temperatures of −12, 0 and 4 C had little adverse effect on larvae in infected leaf tissues in soil. Larvae in soil exposed to 0 C for short periods of time were not affected adversely. Recovery of larvae from sandy soil by Baermann funnels was significantly better at 24 C than at 4 C. Differences in recovery from clay soil were not significant at these temperatures.
Key Words: Survival, Soil type, Soil moisture, Soil temperature, *Ditylenchus dipsaci.*

Ditylenchus dipsaci (Kühn) Filipjev infects many plant species and produces a variety of symptoms depending on the host and the type of tissue involved. It is a major pest on alfalfa, garlic and onion in California. Understanding of its dependence upon soil temperature, moisture and texture is basic to design of effective control measures.

Seinhorst reported *D. dipsaci* reproduced more rapidly in heavier soils during cold, wet periods (3) and that during the winter populations declined more rapidly in sandy than in clayey soils (4). Lewis and Mai (1) determined that adults and pre-adult larvae which overwinter best declined rapidly when frozen and thawed repeatedly. Sayre and Mountain (2) found *D. dipsaci* larvae were not active and infective at 21 C and reported optimum survival in soil at 0 C with moisture at permanent wilting point.

The present investigations were undertaken to determine the effects of controlled soil temperature, soil moisture and soil type on the survival of *D. dipsaci* larvae.

Materials and Methods

Source of Nematodes: The nematode inoculum used in all experiments was obtained from leaves of garlic bulbs infected with *D. dipsaci* which were selected from a commercial garlic field.

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Temperature Effect on Baermann Funnel Extraction of Larvae: Since the Baermann funnel was used exclusively for the recovery of *D. dipsaci* larvae, its efficiency was tested at room temperature (24 C) and at the normal refrigerator temperature of approximately 4 C.

Five replications of clay soil and a sandy soil, of 50 g each, were placed on funnels and incubated at 4 and 24 C. Nematode suspensions containing 2,000 larvae were pipetted onto the surface of the soil and incubated for 3 days before counts were made of the recovered larvae.

Influence of High Temperature, Soil Type and Soil Moisture on the Survival of Larvae in Soil: Two soil types were used: (i) Dublin clay loam (mEq 23.7 per cent) from a garlic-growing area near Gilroy, California and, (ii) a sandy soil (mEq 12.3 per cent) from the Sacramento, California area. The soils were screened through a 3-mm screen, autoclaved and air-dried. Four hundred grams of each soil type were placed in each of four polyethylene bags (7.5 × 15 × 10 cm) which were sealed and placed in 2-liter crocks immersed in controlled temperature water baths. Ten crocks of each soil type were placed at 15, 21, 26 and 32 C and covered with a heavy foil lid perforated to allow exchange of air. After temperature equilibration, 5,000 fourth-stage larvae (obtained from garlic bulb leaves on Baermann funnels) were added to

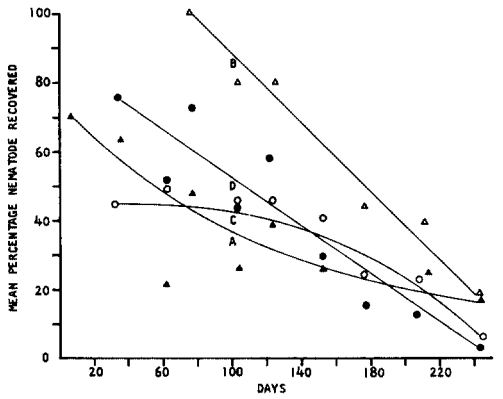

FIG. 1. Nematode recovery with a soil temperature of 15 C. **A.** Clay soil—17.8 per cent moisture; **B.** Clay soil—5.9 per cent moisture; **C.** Sandy soil—9.2 per cent moisture; **D.** Sandy soil—2.5 per cent moisture.

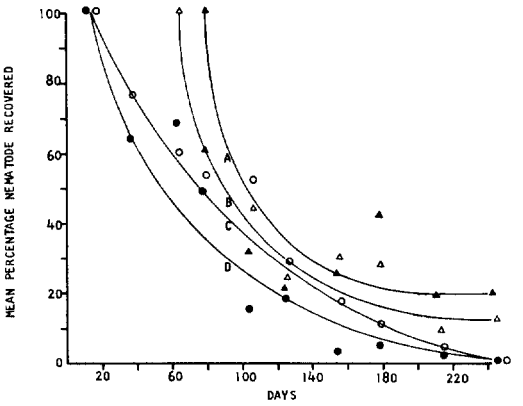

FIG. 2. Nematode recovery with a soil temperature of 21 C. **A.** Clay soil—17.8 per cent moisture; **B.** Clay soil—5.9 per cent moisture; **C.** Sandy soil—9.2 per cent moisture; **D.** Sandy soil—2.5 per cent moisture.

each bag of soil along with sufficient water to bring soil moisture to either 25 or 75% of the moisture equivalent. The soil was mixed in the bag by kneading and shaking to insure even distribution of the larvae and moisture.

Four replications of 50 g of soil were removed from the four bags at intervals and placed on Baermann funnels at 24 C. Larval counts were obtained 3 days later. Portions of the soil samples were used to check infectivity of the larvae by planting onion seeds (var. Yellow Globe). The onions were grown for 21 days at 21 C after which the seedlings were examined under the dissecting microscope for the presence of larvae.

EFFECT OF LOW TEMPERATURE ON LARVAE IN GARLIC LEAF TISSUES IN SOIL: Finely ground dry leaf tissues (120 g) obtained from *D. dipsaci*-infested garlic were blended with 11 liters each of the sand and clay soils described earlier. The garlic leaves were ground in a household food grinder. After blending, the soil was divided into 100-g portions which were placed in 144 polyethylene bags (5 × 7.5 × 12.5 cm). Sufficient water was added to each sample so one half had a moisture percentage of 75% of the moisture equivalent and the other half 25%. The samples were segregated into groups of four replications of soil type and soil moistures. The bags were then placed in refrigerators with temperatures of −12, 0, and 4 C. Groups of samples were removed after exposures of 7, 28 and 56 days, Baermann-extracted at room temperature for 3 days, and the recovered larvae counted.

RESULTS

INFLUENCE OF TEMPERATURE ON EFFICIENCY OF BAERMANN FUNNEL EXTRACTION OF LARVAE: Recovery of larvae at 24 C in both clay and sand was higher than at 4 C. However, the differences between these temperatures in extractions from sandy soils were significant at the 5 per cent level.

INFLUENCE OF HIGH TEMPERATURE, SOIL TYPE, AND SOIL MOISTURE ON SURVIVAL OF LARVAE IN SOIL: To determine the percentage of larvae recoverable immediately following infestation, four replications of each soil were placed on Baermann funnels. The sandy soil yielded 229 ± 32 larvae per 50 g and the clay soil yielded 190 ± 27 larvae; 50 and 32%, respectively, of the number introduced.

Recovery of larvae stored at 15 C (Fig. 1)

TABLE 1. Average exposure period (days) in soil resulting in loss of infectivity of larvae of *Ditylenchus dipsaci*.

Soil type	% Moisture	15 C	21 C	26 C	32 C
Clay	17.8	130–155	60–80	45–60	10–30
	5.9	212 +	30–45	30–60	30–45
Sand	9.2	212 +	90–115	60–80	60–80
	2.5	212 +	130–155	60–80	60–80

declined gradually during 242 days. There were no apparent differences within the clay or sandy soils at the two moisture levels. The ability of larvae to infect onions after exposure to 15 C (Table 1) remained high. Larvae remained infective for 212 days, except in the clay soil (17.8% moisture) where ability to infect onions was variable after an 80-day exposure period and no infections resulted after 155 days.

Recovery of larvae from 21 C storage decreases more rapidly than at 15 C after 125 days (Fig. 2). Infectivity also declined more quickly at the higher temperature and in clay soil than in sandy soil.

Unfortunately the temperature control mechanism on the bath failed after 80 days terminating the 26 C treatment. However, results to this point indicated that infectivity of the larvae in sand at that temperature may have continued over a longer period. Larvae from clay soil did not infect onions after 60 days (Table 1).

Storage in soil at 32 C was unfavorable for survival of larvae (Fig. 3). After 63 days the larval populations dropped abruptly and after 105 days none were recovered. The onion seedlings grown in infested soil (onions grown for 21 days at 21 C) held at 32 C for 60 days were infected only when grown in the sandy soil. The larvae in the clay soil were unable to infect onion seedlings after 45 days.

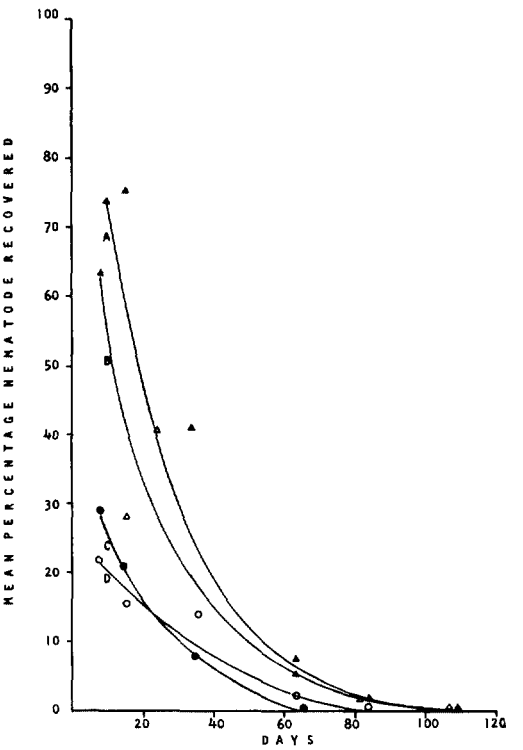

FIG. 3. Nematode recovery with a soil temperature of 32 C. **A.** Clay soil—17.8 per cent moisture; **B.** Clay soil—5.9 per cent moisture; **C.** Sandy soil—9.2 per cent moisture; **D.** Sandy soil—2.5 per cent moisture.

TABLE 2. Average number of *Ditylenchus dipsaci* larvae recovered from 100 g of soil containing infected garlic leaf tissues and subjected to low temperatures for 56 days.

Soil type	% Moisture	−12 C	0 C	4 C
Clay	17.8	31 ± 14	37 ± 6	49 ± 6
	5.9	93 ± 14	67 ± 29	160 ± 99
Sand	9.2	50 ± 19	90 ± 40	26 ± 15
	2.5	33 ± 14	31 ± 21	30 ± 5

EFFECT OF LOW TEMPERATURE ON LARVAE IN GARLIC LEAF TISSUES IN SOIL: There is an apparent tendency of the populations in all series to decline progressively at the three exposure times. Baermann recovery of larvae varied more in the sandy soil than in the clay soil. Data (Table 2) indicate larval recovery from clay soil was consistently higher in the lower moisture series at all temperatures.

Discussion

Experiments such as the preceding which are carried out over relatively long periods of time, and which deal with living organisms, will naturally effect changes in these organisms. The lowering of population levels of an organism with time is expected, especially when no host is available and when the organism is an obligate parasite. From data obtained here, 15 C is the optimum for larvae of *D. dipsaci* to survive in soil and retain their ability to infect onion. The loss of infectivity of the larvae at 21 C although fair numbers were still being recovered from the soil is interesting. If we assume that at higher temperatures the nematodes are more active it is possible that they are using more stored food in the process, thereby reducing infectivity. The clay soil with high moisture had some effect since larvae were not infective after 60 days. Larvae in sandy soil (2.5% moisture) remained infective longer. The possibility exists that under the stress of the drier conditions existing in the sand, larvae may have entered a temporary resting stage. A temperature of 32 C was noticeably detrimental to larvae.

Fourth stage larvae can become inactive and better able to survive adverse environmental conditions. This faculty greatly enhances survival. The optimum conditions for survival stated by Sayre and Mountain (2) do not conflict with results obtained from experiments reported in this study. They infested soil with dried plant tissues, not active larvae, as was the procedure in these studies. Their optimum conditions were temperatures of 0 C or lower and soil moisture near the permanent wilting point. It would appear that under these conditions, dormant larvae in plant tissues will remain dormant but alive while the active larvae will die over a long period of time.

Movement and infectivity of nematodes are not always correlated. It was demonstrated in this study that larvae may move about actively when recovered from soil, but are incapable of infecting a host.

According to Seinhorst (3) the activity of *D. dipsaci* is greater in clay soil than in sandy soil. This study shows that after 242 days larval recovery was greatest from the clay soil. There is less agreement on the role of temperature affecting recovery of this nematode by means of Baermann funnels. He reported that 5 C is more favorable than 20 C. The purpose of our experiments was to determine the relative activity of the nematode at different temperatures, and not necessarily the maximum numerical recovery.

From the experiments reported here, it is apparent that below freezing temperatures have little effect on *D. dipsaci* in dry garlic tissue. The survival of this nematode in areas of freezing winter temperatures is possible as long as host plants are available during the growing season.

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