Effect of Acclimation Temperature on Infection of Alfalfa by Ditylenchus dipsaci[1]

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Abstract: Ditylenchus dipsaci showed an affinity, in relation to infection, for the temp at which it had been acclimated. The optimum infective temp was also correlated with field temp when collections were made during different seasons and from climatically different geographical areas. Nematode developmental stage did not influence infectivity. *Key Words:* alfalfa stem nematode, *Medicago sativa,* seasonal temp, geographical temp.

Temperature has long been considered a primary factor in nematode activity and nematode-host relationships. Bird and Wallace (1) found that *Meloidogyne hapla* Chitwood had thermal optima of 25, 20, and 25-30 C for hatching, mobility, and maturation, respectively; but *M. javanica* (Treub) Chitwood showed optimum temp of 30, 25, and 25-30 C for the same stages.

Wallace (7) stated that *Ditylenchus dipsaci* (Kühn) Filipjev showed a temp preference of 10 C when introduced into a temp gradient, but its greatest mobility occurred at 15-20 C. However, Croll (3), using a temp gradient, found that *D. dipsaci* in narcissus bulbs showed a preference for the temp at which the nematodes had been stored for the preceding 30 days.

In the Intermountain Region of the USA, the alfalfa stem nematode has been found in alfalfa crown bud tissue (*Medicago sativa* L.) under the snow during the winter months at near-freezing temp and in alfalfa stem tissue during the summer when temp approach 30 C. In veiw of Croll's findings (3), the study reported here was made to determine whether seedling infection by *D. dipsaci* is affected by the temp to which the nematode has previously been exposed or at which reproduction occurred.

MATERIALS AND METHODS

Six-month-old 'Ranger' alfalfa plants were inoculated with a suspension of *D. dipsaci,* collected from previously inoculated Ranger alfalfa plants, and grown in growth chambers at 15, 20, and 25 C, and in a greenhouse chamber at 22 ± 2 C. After 60 days of reproduction, nematodes were extracted from the plants, at the same temp at which they were reproduced, with Baermann funnels placed in the growth chambers and greenhouse chamber. Ranger alfalfa seeds, used in all experiments in this study, were germinated on filter paper in petri dishes at a laboratory temp of 23 ± 3 C. After 48 h, when alfalfa seed radicles were 3-5 mm long, seeds were planted in 10-cm diam plastic pots of Provo sand and inoculated with 50 *D. dipsaci* (mixed stages) per seed. Treatments were arranged so nematode progeny from each reproductive temp (15, 20, and 25 C) were introduced onto seeds grown in growth chambers at 15, 20, 25, and 30 C. Each treatment consisted of five pots per replicate, four seeds per pot. After 14 days, seedlings were stained and infection determined (5).

A comparable experiment, using like numbers of replicates and inoculum concns, was conducted by growing infected Ranger alfalfa plants for 60 days at 15 and 25 C, harvesting the nematode, and inoculating germinated seeds planted in 10-cm diam plastic pots. Treatments in this experiment were also designed so that nematodes from each reproductive temp (15 and 25 C) were placed on seeds grown at 15 and 25 C. In this test, however, inoculations were made with second- and third-stage larvae, fourth-stage larvae, and adult males and non-gravid adult females. The criterion of Yuksel (8) was used for separating developmental stages. After 7 and 14 days, seedlings were stained to determine infection.

Another experiment consisted of inoculating germinating Ranger alfalfa seeds with a population of nematodes collected at three seasons of the year from a Logan, Utah alfalfa nursery.

1) Winter—crown buds and stems from beneath the snow at 1 C.

2) Spring—infected stems at a diurnal temp of 1-12 C.

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TABLE 1. Effect of the temp at which *Ditylenchus dipsaci* reproduced on the subsequent infection of 'Ranger' alfalfa seedlings by their progeny.

Reproductive temp (C)[a]	Infective temp (C)[b]							
	Infection rate[c]				% Plants infected			
	15	20	25	30	15	20	25	30
15	31	25	23	8	100	88	77	64
20	21	33	20	9	100	100	74	60
25	17	21	26	12	87	92	96	73
22 ± 2	18	29	24	16	91	100	100	86
LSD ($P = 0.05$)	4				7			

[a] Ranger alfalfa plants inoculated with *D. dipsaci* and grown in growth chambers at 15, 20, and 25 C, and in a greenhouse chamber at 22 ± 2 C, for 60 days.
[b] Plant infection determined 14 days after inoculation.
[c] Nematodes/infected plant.

TABLE 2. Effect of the temp at which *Ditylenchus dipsaci* was reproduced on infection of 'Ranger' alfalfa by different nematode stages.

Reproductive temperature and nematode stage[a]	Nematodes/infected plant at indicated day after inoculation[b]			
	7 days		14 days	
	15 C	25 C	15 C	25 C
15 C				
Adults	22	14	46	34
4th Stage	23	16	37	21
2nd-3rd Stage	19	15	15	23
25 C				
Adults	12	24	36	56
4th Stage	14	26	20	39
2nd-3rd Stage	10	23	19	33
LSD ($P = 0.05$)	5		7	

[a] Ranger alfalfa plants inoculated with *D. dipsaci* and grown in growth chambers at 15 and 25 C for 60 days.
[b] Germinated seed (3-5-mm radicle), inoculated with 50 nematodes.

TABLE 3. Effect of field-collection temp on infection of 'Ranger' alfalfa seedlings by *Ditylenchus dipsaci*.

Diurnal temp[a]	Nematodes/infected plant at indicated day after inoculation[b]			
	7 days		14 days	
	10 C	20 C	10 C	20 C
Winter (1 C)	11	8	18	16
Spring (1-12 C)	23	16	37	27
Summer (13-32 C)	13	24	27	34
LSD ($P = 0.05$)	4			

[a] *Ditylenchus dipsaci* extracted from seasonally collected alfalfa tissue at Logan, Utah.
[b] Germinated seed (3- to 5-mm radicle), inoculated with 50 nematodes.

3) Summer—blackened stem stubble at a diurnal temp of 13-32 C.

Nematodes were extracted in Baermann funnels over a 2-h period at 1, 5, and 20 C for the winter, spring, and summer collections, respectively. Germinated seeds were inoculated with 50 nematodes (mixed stages) per plant and grown at 10 and 20 C. Although no attempt was made to separate developmental stages, nematodes from the summer collections were mainly fourth-stage larvae. Seedlings, 20 per treatment, were harvested after 7 and 14 days, stained, and nematode infection determined.

A final experiment consisted of using nematodes collected from alfalfa during the first part of April, from the Logan, Utah alfalfa nursery and from a field in St. George, Utah. Diurnal temp were 2-17 C at Logan and 6-27 C at St. George. Both nematode populations were stored at about 5 C before inoculation; and nematodes of both the Logan and St. George populations were extracted at 10 and 20 C, respectively, within 24 h after collection. Germinated Ranger alfalfa seeds were inoculated and grown at 10, 20, and 30 C; 50 nematodes were used per seed and each treatment was replicated 20 times. After 7 days, the seedlings were stained, and extent of infection was determined by direct counts.

RESULTS

Ditylenchus dipsaci became acclimated to the temp at which the nematode was introduced onto alfalfa seeds and at which reproduction occurred. There was a direct correlation between the acclimation temp and the temp at which the greatest nematode

infection occurred (Table 1). There was also a direct correlation between the percent of alfalfa seedlings infected and the acclimation temp. The correlation of infection of alfalfa by *D. dipsaci* with the acclimated greenhouse temp of 22 ± 2 C was not as great as with the more constant temp in the growth chambers, but compared closely to optimum infective temp at 20 and 25 C.

Similar results were obtained when germinated seeds were inoculated with different larval stages. Obviously, the developmental stage had no effect on the ability of the nematode to infect alfalfa (Table 2). However, after 14 days more nematodes were found in alfalfa inoculated with adults than with the larval stages. This apparently resulted from some reproduction occurring in adult-infected alfalfa tissue within 14 days. Although non-gravid females were used as a source of inoculum, some apparently had mated.

Infective rates were different for nematodes collected from the field during winter, spring, and summer. Nematodes were more infective at the temp more nearly the same as the average field-collection temp (Table 3). Similar results were seen when nematodes collected from climatically different areas were used. There were 28, 20, and 9 nematodes per infected seedling at temp of 10, 20, and 30 C, respectively, from a Logan, Utah, field collection made when the diurnal temp ranged 2-17 C. This compared to 22, 29, and 14 nematodes per infected seedling at temp of 10, 20, and 30 C, respectively, from a St. George field collection made when the diurnal temp ranged 6-27 C [LSD (P = 0.05) = 4].

DISCUSSION

The preferred infective temp of *D. dipsaci* is correlated with the acclimation temp of the nematode. This agrees with Croll's findings (3) and may be a result of the "eccritic temperature," since Croll found that the storage temp was the preferred temp of the nematode. If the storage temp is the "comfort index temp," it can be assumed nematodes will be more active at that temp and that this will directly affect plant infection. The ability of *D. dipsaci* to adapt to different temp in relation to infection helps explain the ability of this nematode to infect and parasitize alfalfa over a wide temp range. (As previously stated, all stages of this nematode occur in alfalfa tissue at temp from just above freezing to near 30 C, if proper moisture levels exist and succulent tissues are available). However, the possibility of the existence of an indirect acclimation cannot be ignored. The plant grown at a temp other than that from which the nematode was obtained might act as a less desirable environment for the nematode, and affect its susceptibility to nematode attack.

The effect of the acclimation temp on temp preference and infection may explain differences in infection temp preference that have been reported for this nematode such as 21 C for onions, *Allium cepa* L. (6); 15 C for oats, *Avena sativa* L. (2); and 20 C for alfalfa (4). Although differences in races and hosts cannot be ignored, the acclimation of the nematode to temp may be more important than previously supposed.

Continued studies on the effect of environmental conditions on host-parasite relationships may show a closer relationship between nematode populations and races where significant differences are known to exist.

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