

# AGE EFFECT OF ONION PLANTS ON *Ditylenchus dipsaci* REPRODUCTION UNDER CONTROLLED TEMPERATURE <sup>1</sup>

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## SUMMARY

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The effect of age of onion seedlings on *Ditylenchus dipsaci* reproduction (teasel race) was verified under 20°C controlled temperature room during 4 weeks. Differences were found between 4 and 7 day-old onion seedlings in the reproduction of *D. dipsaci* in all periods of observation. Significant difference however was shown only on the 4th week after inoculation, in which the number of nematodes was higher in 4-day-old seedlings than in 7-day-old plants. The population compositions were quantified and each different proportion was shown. The number of young juveniles was higher for 7-day-old seedlings during the 2nd week while the 4 day-old seedlings showed bigger number of J3-J2 in the 4th week.

Key words: onion, *Allium cepa*, *Ditylenchus dipsaci*, reproduction, age effect.

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## RESUMO

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Neste estudo, observou-se a reprodução de *Ditylenchus dipsaci* raça teasel sobre duas diferentes idades de plântulas de cebola (quatro e sete dias após a germinação), durante quatro semanas sob 20°C e umidade de 70-90%. Diferenças significativas entre essas duas idades foram encontradas somente na 4ª semana da inoculação. Os diferentes estádios de *D.dipsaci* apresentaram variações de acordo com a semana de observação. O maior número de larvas do 2º e 3º estádios, para plantas inoculadas ao 7º dia após a germinação, foi encontrado na 2ª semana, enquanto para plantas com 4 dias, o número de larvas (2º e 3º estádios) foi maior na 4ª semana.

Palavras-chave: cebola, *Allium cepa*, *Ditylenchus dipsaci*, reprodução, efeito da idade.

## INTRODUCTION

The *D. dipsaci* life-cycle and host plant are synchronized by seasonal factors, allowing juveniles to rehydrate in soil as the host plant germinates or resumes its growth. This may explain the ability of *D.dipsaci* to infect and parasitize plants over a wide temperature range, if proper moisture levels exist and succulent host tissues are available (Griffin, 1974). The life cycle of *D.dipsaci* took 19 to 23 days on onion plants at 15°C (Yuksel, 1960). Sayre & Mountain (1962) reported that 21°C is the optimum temperature for this species of parasite on onion plants, but Tenente & Evans (1992) found 20°C to be the best temperature for *D. dipsaci* teasel race.

This work was done therefore to investigate the effect of the plant age on the invasion and reproduction of *D.dipsaci* in onion seedlings.

## MATERIAL AND METHODS

The teasel biological race of *D. dipsaci* was revived from dried tissue of previously infected courgette (Hooper & Cowland, 1987) by the modified-tray technique (Whitehead & Hemming, 1965). The modification consisted in using small trays (53x21x5cm) lined with one layer of plastic mesh (2mm aperture) supporting Kiwwepe paper. Distilled water added in sufficient quantity to wet the tissue samples was removed daily to another tray, water was added again and left for the next 24 hours. Water from the first tray was passed through a 25µm sieve. Contents of the sieve were quickly and carefully transferred into a beaker and the number of nematodes of each stage counted under a dissecting microscope. This procedure was repeated at 48 and 72 hours.

Immediately after extraction the fourth-stage juvenile (J4) suspension was inoculated in onion seedlings (*Allium cepa* cv. Bedfordshire champion). The seedlings were placed individually in small glass containers (20 cm diameter and 5cm of height) containing 2ml of 1% agar and inoculated with fifty J4 when they were 4 and 7-day-old, with 2 to 4cm and 9 to 12cm of height respectively. Inoculated seedlings were kept at 20°C in a controlled temperature (CT) room, under a 12-hour light regime. Humidity ranged from 70 to 90%.

Four randomized containers per treatment (age of plant) were collected during four weeks. After collecting, the plants were weighted and stained in 0.5% acid fucsin in lacto-glycerol (Bridge et al., 1981). Plants were then destained in clear glycerol (50%) for one week and examined under a dissecting microscope. Each nematode stage was counted and the presence of eggs observed. Nematodes that remained in agar, were also counted after extracting by the modified tray technique.

The variance analysis was done using time of observation (weeks) and treatment (age of plants) as covariates (Hoel, 1961).

## RESULTS AND DISCUSSION

The total population of *D. dipsaci* teasel race on onion seedlings fluctuated until the 3rd week (Figure 1) with similar results for 4 or 7-day-old plants. During the fourth week nematode numbers increased on 4-day-old plants but decreased on 7-day-old plants. The latter started to show some senescence signals. These results suggest that this parasite prefers succulent host tissues instead of the senescent tissues as reported by Griffin (1974) and observed by us. By the end of the experiment (after 4-weeks), nematode numbers were larger (99/repl.) in the 4-day-old onion plants than in the 7-day-old plants (15/repl.).

Variance analysis of these data showed that there were significant differences ( $P < 0.01$ ) between observation time (weeks), and age of plants at infestation and their interaction.

Temperature has long been considered a primary factor in nematode reproduction but the host age is also important. It was shown in this experiment that age of the plant is important on the *D. dipsaci* life-cycle specially weeks later, as shown on the 4th week of observation.

Nematodes at J4 stages could invade onion seedlings at both ages (4 and 7-day-old) during the 1st week although they differed according to the age of plants, showing bigger number in 7-day-old plant. No significant differences in nematode numbers between 4 and 7-day-old-plants during the 2nd week were not found. Similar results were obtained at the 3rd week. The population of *D. dipsaci* built up clearly between the 3rd and 4th weeks, when 4-day-old plants were inoculated. This confirms the results obtained by Sayre & Mountain (1962) that the life-cycle of *D. dipsaci* can be completed between the 20th and the 28th days at 21°C. Therefore, J2 and J3 stages were found at 2nd week (Figure 1) while adults were found in the 1st week. Similar results were reported by Yuksel (1960).

The composition of different nematode stages (J2; J3; J4 and adults) showed differences in numbers between plants 4 and 7-day-old (Figure 1). J4 numbers that moulted to adults were similar in the first week (25%), for both plants (4 and 7-days-old). Proportionally more J3-J2 were found on 7-day plants at 2nd week. By the third week 7-day-old plants had the greater proportion of adults, but greater reproduction was evident on 4-day-old plants at week 4 (Table 1).

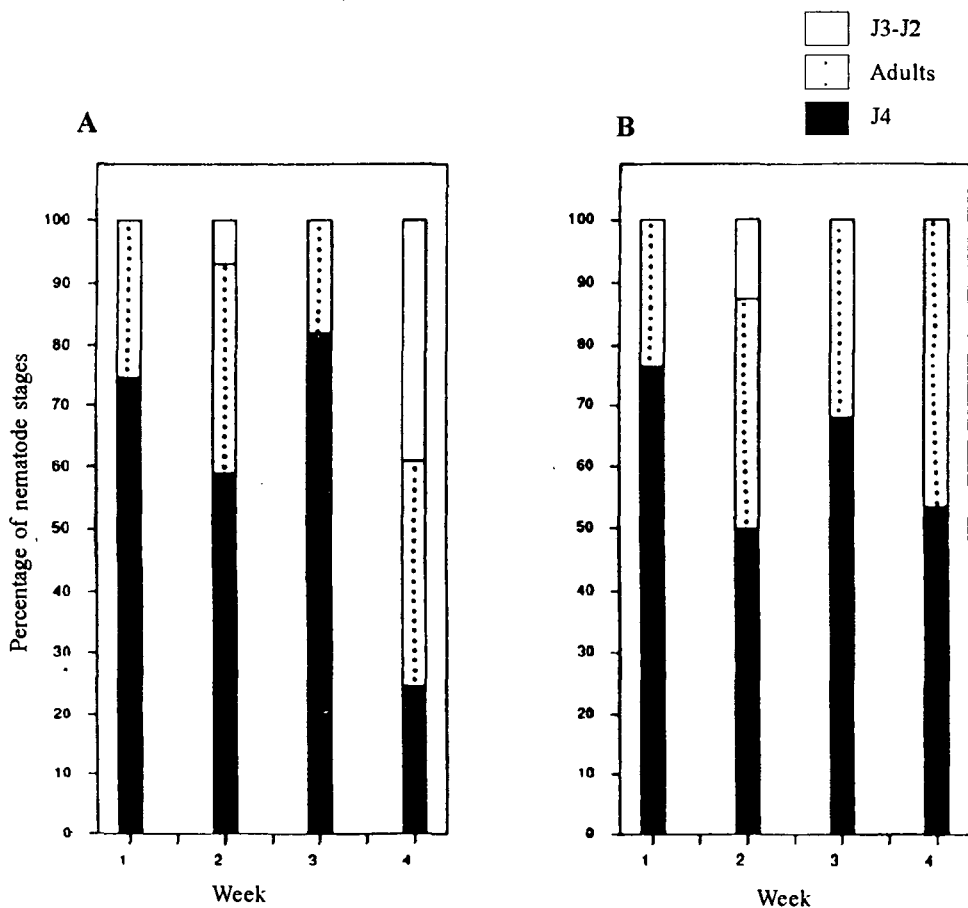


Figure 1. Percentage of nematode numbers produced on 4 and 7-day-old onion seedlings on study of life-cycle of *Ditylenchus dipsaci* teasel race.  
(A: 4-day-old seedlings; B: 7-day-old seedlings)

Table 1. Nematode numbers recovered from young and old onion plants during the study of life-cycle of *Ditylenchus dipsaci*.

Age of plants	Week of collection	Number of nematodes			
		J4 stage	Adult stages	J2-J3 stages	Total Population
4 days	1st	11.8a	4.0a	0.0a	15.8a
	2nd	30.4b	17.6b	3.6a	51.6b
	3rd	30.0b	6.6a	0.0a	36.6c
	4th	24.2b	35.8c	38.6b	98.6d
7 days	1st	22.8a	7.0a	0.0a	29.8a
	2nd	24.4a	18.4b	6.2b	49.0b
	3rd	17.8a	8.4a	0.0A	26.2a
	4th	8.0b	7.0a	0.0a	15.0a

Means followed by the same letter in vertical columns are not significantly different ( $p < 0.01$ ).

The weight of plants also decreased on 7-day-old plants during the experiment (Table 2) and only some 7-day-old plants survived at the end of observations (Table 3).

No significant differences were found in sex ratio (males/females) in the 4-day-old and 7-day-old onion seedlings during the life-cycle study, although numbers of males and females increased or decreased with time of sampling (Table 4).

Table 2. Weight of onion plants, in two different ages of germination, determined weekly during a month in the life-cycle study of *Ditylenchus dipsaci*.

Week of collection	Plant weight (g)	
	4-day-old (A)	7-day-old (B)
1st .....	0.020a	0.162a
2nd .....	0.028a	0.202b
3rd .....	0.026a	0.160a
4th .....	0.082b	0.044c

Means followed by the same letter in vertical columns are not significantly different ( $p < 0.01$ ).

Table 3. Mean number of plants collected during the life-cycle study on *Ditylenchus dipsaci* using two different periods of germination.

Week of collection	Number of plant	
	4-day-old (A)	7-day-old (B)
1st .....	3.40ab	4.00a
2nd .....	3.40ab	4.00a
3rd .....	3.00b	4.00a
4th .....	3.80a	3.60b

Means followed by the same letter in vertical columns are not significantly different ( $p < 0.01$ ).

Table 4. Proportion between males and females of *Ditylenchus dipsaci* determined during life-cycle study of this nematode on onion plants inoculated at two different ages.

Age of plants	Week of collection	Number of adults		
		Male	Female	Male/Female
4 days	1st	8a	12a	0.66a
	2nd	35b	53b	0.66a
	3rd	13a	20a	0.65a
	4th	5b	129c	0.39a
	1st	11a	24a	0.46a
	2nd	42b	50b	0.84ab
	3rd	15a	27a	0.55a
	4th	18a	17a	1.06b

Means followed by the same letter in vertical columns are not significantly different ( $p < 0.01$ ).

Therefore, the results showed above suggest that the study with *D. dipsaci* has to consider the age of plant specially if the evaluation is done three weeks after inoculation.

### LITERATURE CITED

- BRIDGE, J.; S.L.J. PAGE, & S.M. JORDAN, 1981. An improved method for staining nematodes in roots. In: Rothamsted Experimental Station Report for 1981, Harpenden: Lawes Agricultural Trust, 322p., pte. 1, p. 171.



- GRIFFIN, G.D., 1974. Effect of acclimation temperature on infection of alfalfa by *Ditylenchus dipsaci*. Journal of Nematology, 6: 57-59.
- HOEL, P.G., 1961. Estatística elementar. Rio de Janeiro. Fundo de Cultura, 312p.
- HOOPER, D.J. & J.A. COWLAND, 1987. Courgette marrows for the mass culture of some nematodes. Nematologica, 33:488-490.
- SAYRE, R.M. & W.B. MOUNTAIN, 1962. The bulb and stem nematode *Ditylenchus dipsaci*, on onion in Southwestern Ontario. Phytopathology, 52: 510-516.
- TENENTE, R.C.V. & A.A.F. EVANS, 1992. Ciclo vital de *Ditylenchus dipsaci* raça "teasel" sobre cebola em diferentes temperaturas. Fitopatologia Brasileira, 17(2): 22. (Resumo).
- WHITEHEAD, A.G. & J.R. HEMMING, 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann. Appl. Biol., 55: 25-38.
- YUKSEL, H., 1960. Observations on the life-cycle of *Ditylenchus dipsaci* on onion seedlings. Nematologica, 5: 289-296.