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REARING LARGE NUMBER OF *Ditylenchus dipsaci* INSIDE FRESH COURGETTE FRUITS (*Cucurbita pepo*) [1]

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SUMMARY

Tenente, R.C.V. & A.A.F. Evans, 1992. Rearing large number of *Ditylenchus dipsaci* inside fresh courgette fruits (*Cucurbita pepo*). Nematol. Brasileira 16: 27-34.

The multiplication of five populations of *D. dipsaci* inside the courgette fresh fruit, at 15°C, by eight weeks, was investigated. The best reproduction rate was of 375 times with the highest inoculum level of 500 J4 teasel race; with the 200 J4 level, this race increased 223.7 times. Bean race, with the 500 J4 of inoculum, multiplicated 5.8 times, while the red clover race, with its highest inoculum level of 200 J4, multiplicated 10.4 times. However, oat and lucerne races failed to increase their populations. The number of J3 plus J2 was higher than J4 for teasel and bean races, meaning that they were in active reproduction period by the 8th week. A similar experiment carried at 20°C was lost by microbial contamination.

Key words: bulb or stem nematode; *Ditylenchus dipsaci*; courgette fruits; rearing.

RESUMO

Tenente, R.C.V. & A.A.F. Evans, 1992. Criação massal de *Ditylenchus dipsaci* em frutos novos de abobrinha italiana (*Cucurbita pepo*). Nematol. Brasileira 16: 27-34.

Investigou-se a multiplicação de cinco populações *D. dipsaci* em frutos novos de abobrinha italiana, a 15°C, por 8 semanas. A melhor taxa de multiplicação foi de 375 vezes, com o inóculo de 500 J4s da raça 'teasel'. Com o inóculo de 200 J4s, esta raça aumentou 223,7 vezes. A raça 'gigante' do feijão, com o inóculo de 500 J4s, multiplicou 5,7 vezes e a do trevo vermelho, com o inóculo de 200 J4s, 10,4 vezes. As demais raças estudadas (alfafa e aveia) não multiplicaram. Nas raças 'gigante' do feijão

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e 'teasel', o número de J2 mais J3 era maior que o de L4, indicativo de estarem em fase de multiplicação ativa na 8ª semana de incubação. Um ensaio similar conduzido a 20°C foi perdido por contaminação microbiana.
Palavras-chaves: *Ditylenchus dipsaci*; multiplicação; nematóide dos bulbos ou caules.

INTRODUCTION

Some studies of plant nematode biology require a large number of the specific nematodes. Establishment of cultures of plant-parasitic nematodes in *Curcubita pepo* is reported for some species, such as *Ditylenchus dipsaci* (oat and giant races) and *Aphelenchoides ritzemabosi*, which can proliferate in courgette fruits under laboratory conditions (Hooper and Cowland, 1988). This method allows the rearing of large amounts of *D. dipsaci* even though this species is an obligatory plant parasite. Storage tissues like potato tubers (Baker, 1948), narcissus bulbs (Webster, 1964) and carrots (O'Bannon and Taylor, 1968) have been used to support the propagation of plant parasitic nematodes.

In this study, the objective was to find conditions for maximum reproduction of *D. dipsaci* when cultures are started with low numbers. This provides an alternative to the use of callus cultures to grow *D. dipsaci* for further experiments requiring a large number of this nematode.

MATERIAL AND METHODS

Fresh courgette fruits were freshly harvested from local farms or produced in the greenhouses at Silwood Park, Ascot and were used in a moderate size (4 cm-d and 20 cm long). They were superficially cleaned by washing under tap water with detergent, followed by soaking in ethanol (80%) for few minutes before placing on a tissue paper for drying

Nematodes for inoculation were obtained from infested tissue of several hosts of *D. dipsaci* (teasel, red clover, bean, oat and lucerne)

and were extracted during 12 to 48 hours by the modified tray technique (Whitehead and Hemming, 1965). The nematodes were treated with mercuric chloride (1%) and streptomycin sulphate (1%) for 20 minutes for superficial desinfection. Centrifugation concentrated the nematodes which were given 3 further rinses in distilled and sterilized water and then used to inoculate courgette fruits.

The different concentrations of fourth-stage juveniles (J4) of *D. dipsaci* were placed (with pipette) inside courgette fruits after making a small hole (3 mm-d and 2 cm-long) was made with a flamed cork-borer. Before plugging the role with the piece that was removed, a little part of the courgette was cut from the bottom, leaving enough space for the nematode suspension to remain and the plug was replaced (Fig. 1C & D). This part was modified from the original description of Hooper's technique in which he used a hypodermic syringe (1 ml). In preliminary tests, it was observed that some nematodes were lost as the needle was removed from the tissue, after injecting the suspension of nematodes into courgettes (Fig. 1A & B). This may not be a problem if the inoculation used is at a density of nematodes of 1000 individuals per fruit, or more, as reported by Hooper and Cowland (1988), but when used for lower initial number of nematodes, as in the present work, it failed to multiply the nematodes.

Parafilm was used to cover the inoculation hole because without it the courgette tissue dried quickly and left a hole, through which other pathogens could enter during the incubation period. Inoculated courgette fruits were incubated in 15 or 20°C controlled temperature rooms (CTRoom) for 6 or 8 weeks, when they were collected and placed for extraction, using the tray technique (previously described), to count the number of individual stages of *D. dipsaci*.

RESULTS AND DISCUSSION.

No nematodes were recovered from the courgettes kept at 20°C due to the high microbial contamination of the fruits; most of these

were lost, for each studied host race, whether 50, 100 and 200 nematodes were inoculated per fruit. Contamination by fungi began earlier than 8 weeks, appearing on the surface and penetrating into the fruits (Fig. 1B).

The infested courgette fruits kept at 15°C showed that, by 8 weeks, *D. dipsaci* had propagated, although large differences were found between host races, as well between the initial inocula (Table 1).

In this work, the teasel race reproduced best in infested courgettes after 8 weeks of incubation at 15°C, when the initial inoculum was higher than 200 J4 per fruit. Reproduction rate was 223.7-fold when the initial inoculum was 200 J4, and 374.7-fold when was 500 J4 per courgette (Table 1). The number of J3 plus J2 was higher than the number of J4, except when the initial inocula were 50 and 100 J4 per courgette.

However, the oat, bean and lucerne races, of which the highest level of inoculum was 200 J4 per fruit, failed to increase, compared at the same level of inoculum with red clover and teasel races (Table 1). These differences may be due to inherent differences between individual populations. Different behaviour is often seen within the same populations using the same test conditions (Seinhorst and Hooper - personal communications).

Different tissues of plant material have been reported to support the reproduction of some plant parasitic nematodes and two races of *D. dipsaci* were produced in freshly harvested courgette by Hooper and Cowland (1988). They recovered about 25,000 nematodes per gram of infested tissue, comparable with numbers obtained by Krusberg (1961) on callus of alfalfa.

The results obtained here with teasel race were similar to those obtained by Hooper and Cowland (1988). This enabled a large number of nematodes to be reared with fresh courgette fruits, and offers a simple, readily available substrate which is easy to manipulate due to the control of contamination, as well as being cheaper than producing nematodes on callus.

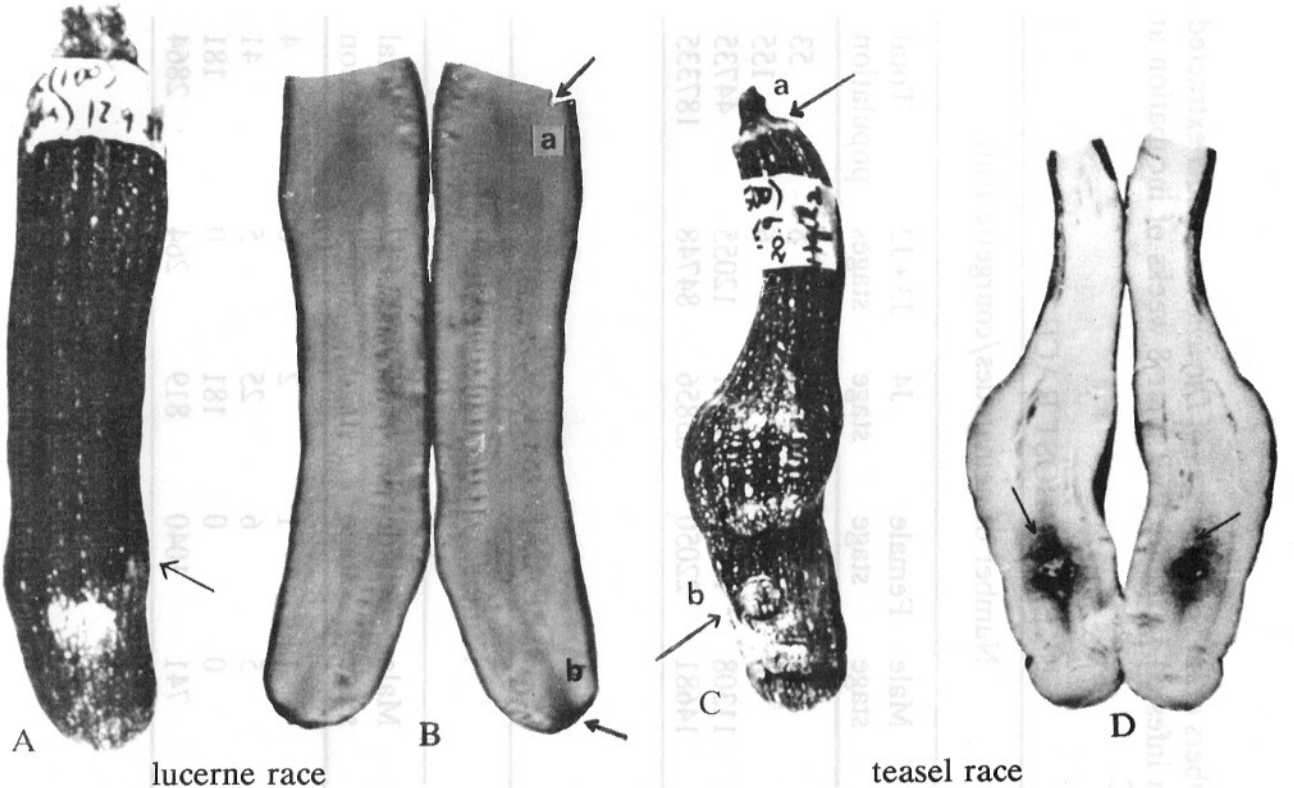

Fig. 1. Courgette fruits infested with *Ditylenchus dipsaci* by hypodermic syringe (A & B; lucerne race) and shrivelled fruits (C & D; teasel race): A) Whole fruit shows a slight symptom of lucerne race; B) Development point of the nematodes (a) and fungal infection (b); C) Severe symptom of teasel race: a) fungal infection and b) point of inoculation; D) Showing point of inoculation with heavily infected tissue.

Table 1. Numbers of individual stages of *Ditylenchus dipsaci* extracted from infested courgette fruit after 8 weeks of incubation at 15°C.

a. TEASEL HOST RACE

Initial inoculum population (J4)	Number of nematodes/courgette fruit				
	Male stage	Female stage	J4 stage	J3+J2 stages	Total population
50	20	15	10	8	53
100	26	102	21	6	155
200	11308	14758	6641	12055	44735
500	14681	22050	65856	84748	187335

b. BEAN HOST RACE

Initial inoculum population (J4)	Number of nematodes/courgette fruit				
	Male stage	Female stage	J4 stage	J3+J2 stages	Total population
50	1	1	2	0	4
100	5	6	25	5	41
200	0	0	181	0	181
500	741	1040	819	264	2864

Table 1 (contd.)

c. RED CLOVER HOST RACE

Initial inoculum population (J4)	Number of nematodes/courgette fruit				
	Male stage	Female stage	J4 stage	J3+J2 stages	Total population
50	22	41	23	16	102
100	5	3	2	0	10
200	381	802	456	437	2076

d. OAT HOST RACE

Initial inoculum population (J4)	Number of nematodes/courgette fruit				
	Male stage	Female stage	J4 stage	J3+J2 stages	Total population
50	26	28	39	40	133
100	10	17	9	3	39
200	8	14	11	2	35

e. LUCERNE HOST RACE

Initial inoculum population (J4)	Number of nematodes/courgette fruit				
	Male stage	Female stage	J4 stage	J3+J2 stages	Total population
50	3	7	0	0	10
100	8	8	4	0	20
200	1	0	0	0	1

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PRONÚNCIA DE *Meloidogyne*

1. A pronúncia dos nomes científicos de animais deve obedecer à gramática latina.

2. Nome com três ou mais sílabas é paroxítono quando a vogal da penúltima sílaba é longa, condição indicada nos dicionários latinos pela sobreposição do macro (pequeno traço reto). É proparoxítono quando essa vogal é breve, que os dicionários indicam sobrepondo-lhe o sinal bráquia (traço em meia lua).

3. O *y* de *gy̆ne* é breve, como indicam os dicionários latinos em palavras compostas como *androgy̆ne*. Em português existe a palavra **andrógino**, que os dicionários registram como proparoxítona, porque assim é em latim.

4. Conclusão: *Meloidogyne* é proparoxítona (pronúncia: meloidógine).

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