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No Reproduction of Two Populations of *Ditylenchus dipsaci* on Three Species of Fungi

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SUMMARY

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The possibility of two different populations of *Ditylenchus dipsaci* to multiply on three species of fungi, under laboratory conditions, at 20° C was investigated during one month. The initial inoculum per petri dish was 200 fourth-stage juveniles. No multiplication was observed, after nematode extraction by the Baermann funnel technique, since the majority of recovered nematodes was in fourth-stage juvenile. The results showed the same behaviour of both nematode populations. The largest, recovered numbers of nematodes were from *Macrophomina phaseolina* for populations named C and D, 106 and 96 fourth-stage juveniles (J4), respectively. From the two species of Fusarium, the numbers of recovered nematode was lower, about 13 J4 per petri dish. Our results indicate no reproduction of these two nematode populations when these species of fungi were used substract, although a few individuals moulted to adults, but no juveniles of second and third stages, were found.

Key words: *Ditylenchus dipsaci*, *Macrophomina phaseolina*, *Fusarium* spp., reproduction.

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RESUMO

Tenente R. C. V.; M. A. S. Mendes & E. Gomes Neto, 1995. Nenhuma reprodução de duas populações de *Ditylenchus dipsaci* em três espécies de fungos. **Nematol. Brasileira 19**:67-71.

Foi investigada a possibilidade de reprodução de duas diferentes populações de *D. dipsaci* sobre os fungos *Fusarium* spp. e *Macrophomina phaseolina*, usando um número inicial de nematóides de 200 juvenis do 4º estádio. Esse estudo foi realizado sob condições controladas de temperatura (20ºC) e a avaliação, feita um mês após o período de incubação, com a extração de nematóides mediante a técnica do funil de Baermann. Os resultados mostraram o mesmo comportamento de ambas as populações de nematóides, denominadas C e D, sendo os maiores números obtidos recuperados de *M. phaseolina*, respectivamente 106 e 96 juvenis do quarto estádio (L4) mas sempre inferiores ao número inoculado. Das duas espécies de *Fusarium* testadas, o total de nematóides recuperados foi bem inferior aos obtidos por *M. phaseolina*, ficando em torno de 13 L4 por placa de Petri. Os resultados não evidenciaram reprodução de *D. dipsaci* sobre essas espécies de fungos, embora bem poucos indivíduos se tornassem adultos. Não foram encontradas larvas do 2º e 3º estádios.

Palavras chaves: *Ditylenchus dipsaci*, *Macrophomina phaseolina*; *Fusarium* spp.; reprodução.

INTRODUCTION

The stem and bulb nematode, *D. dipsaci* (Kühn) Filipjev, is a bisexual species with vermiform juveniles and adults that parasitize stems and bulbs of large range of hosts (Hooper, 1972). This species is considered the worst parasite of garlic (*Allium sativum*) in temperate regions. In Brazil it is a serious problem in garlic fields (Charchar et al., 1980).

Some studies of plant nematode biology require large number of individuals. Rearing large number of nematodes in tissue culture and storage tissues is frequently described in the nematological literature. However, there is little information on reproduction of *D. dipsaci* on fungus cultures. Viglierchio (1971) found that a garlic isolate of *D. dipsaci* was able to reproduce on two fungi (*Verticillium theobromae* and *Cladosporium* spp.) out of nineteen fungus cultures tested. Hooper and Southey (1978) have suggested that feeding on soil fungi may contribute to persistence of *D. dipsaci* during periods of unfavourable climatic conditions or absence of host plant.

Therefore this work investigates the potential of *D. dipsaci* obtained from garlic plants to multiply on some fungus cultures.

MATERIAL AND METHODS

The fungus species tested for *D. dipsaci* multiplication were grown on potato dextrose agar (PDA), under controlled conditions of temperature 22° to 25° C with 12 hours light. They were *Fusarium* sp. and *Macrophomina phaseolina* from garlic roots and bulbs and *Fusarium* sp. from *Solanum quitoense*.

Two populations, named C and D, of *D. dipsaci* were collected in two different regions in Brazil, Inconfidentes, Minas Gerais State and Botucatu, São Paulo State, respectively. They were extracted for 12 to 48 hours from dried infested tissue of garlic bulbs by the modified tray technique (Whitehead & Hemming, 1965). After sieving, living nematodes were treated, for surface desinfection, with a 0.1% mercuric chloride and 1.0% streptomycin sulphate solution, followed by several washings with distilled water (Moody et al., 1973). Two hundred fourth stage juveniles (J4) were then, individually transferred to a drop of sterilized water, on the surface of the fungus colony. All operations were made in aseptic chamber to avoid contamination by other organisms. After inoculation, the cultures were kept in a dark incubator for one month at 20 C (Faulkner et al., 1974).

At the collecting time, water was added to each Petri dish and the nematodes, fungus and PDA medium were poured in Baermann funnel (Goodey, 1963). Nematode samples were then collected for three days, at 24-hour intervals. The nematodes were counted under microscope and each stage of them was recorded.

RESULTS AND DISCUSSION

Differences were observed, in recovering nematodes, between two populations of *D. dipsaci* (C and D) when compared among three different species of fungi (table 1). The majority of recovered nematodes was in the fourth-stage juvenile (J4) which indicates that no reproduction occurred. Therefore few adult specimens were found, but no young juveniles (2° and 3° stages) or eggs. For both populations of *D. dipsaci* nematode numbers decreased and 50% when *Fusarium* spp. and *M. phaseolina* were used as substrata, respectively.

The results of this study, suggest similarities with the results by Hooper & Southey, (1978) when mentioned that some fungi may contribute to keep *D. dipsaci* alive in the absence of host plants, because the nematode number decreased. Therefore, according to Viglierchio (1971) results, two out of nineteen species of fungi can give support to *D. dipsaci* reproduction, although in moderate and slight increase of

nematode number, when *Verticillium theobromae* and *Cladosporium* spp. were used as hosts. Similar results, between Viglierchio and ours regarding to *Fusarium*, showed that they did not multiply.

Many researchers mentioned that the ability of *D. dipsaci* to survive for long periods without any host, is due to the capacity of this parasite to feed on fungi. Our results can not suggest this capacity of *D. dipsaci* because 35% of the recovered juveniles already lost their reserves, indicating that the nematodes can not feed in these studied species of fungi, and probably used their reserves to remain alive.

The differences among our results and those from other researches, may be due to inherited characteristics of each nematode population of *D. dipsaci* as mentioned by Seinhorst and Hooper (personal communications). Therefore, the results from this experiment suggest that *D. dipsaci* populations, C and D, obtained from two different States of Brazil, can not multiply on studied fungi, *M. phaseolina* *Fusarium* spp.

Table 1. No reproduction of two populations of *Ditylenchus dipsaci* in selected fungi cultures, when 200 nematodes were inoculated as initial inoculum

Fungi/previous host	Populations of *D. dipsaci*	Stages of nematodes	Final nematode numbers			Mean
			R1	R2	R3	
Fusarium sp.	C	J4 adult	2	12	6	3.6
(S. quitoense isolate)			0	0	0	0.0
Fusarium sp.	C	J4 adult	3	8	13	8.0
(Garlic isolate)		2	1	0	0	0.3*
Macrophomina phaseolina	C	J4 adult	106	102	93	100.1
(Garlic isolate)			2	1	0	1.0**
Fusarium sp.	D	J4 adult	1	7	11	6.3
(S. quitoense isolate)			1	0	0	0.3*
Fusarium sp.	D	J4 adult	14	2	7	7.6
(Garlic isolate)			0	1	0	0.3*
Macrophomina phaseolina	D	J4 adult	96	86	91	91.0
(Garlic isolate)			1	1	0	0.6*

R1, R2 and R3 - Replicates.

(*) Males.

(**) Females.

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