Reproduction of the nematode *Zygotylenchus guevarai* (Nemata : Pratylenchidae) in monoxenic cultures

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SUMMARY

Zygotylenchus guevarai reproduced monoxenically on carrot disc and on *Agrobacterium rhizogenes* transformed potato root cultures. The life cycle was completed in 45 days at 25 °C. An initial inoculum level of 50 individuals provided three times more nematodes than ten or twenty individuals. In all tests most nematodes were found in the egg stage and only a low proportion of the population reached the adult stages.

RÉSUMÉ

Reproduction du nématode Zygotylenchus guevarai *(Nemata : Pratylenchidae) en culture monoxénique*

Zygotylenchus guevarai se reproduit en élevage monoxénique sur disques de carotte et sur cultures de racines de pomme de terre modifiées par *Agrobacterium rhizogenes*. Le cycle biologique est accompli en 45 jours, à 25 °C. Un inoculum de 50 individus produit trois fois plus de nématodes que des inoculums de 10 ou 20 individus. Dans tous les essais la plupart des nématodes sont rencontrés au stade d'œuf et seule une faible proportion de la population atteint l'âge adulte.

The migratory endoparasitic nematode *Zygotylenchus guevarai* (Tobar Jimenez) Braun & Loof, invades the root cortex causing root lesions and the formation of large cavities, destroying the cortical tissues (Varo Alcala, Tobar Jiménez & Muñoz Medina, 1970; Vovlas, Inserra & Lamberti, 1976). Although the nematode is widespread in the Mediterranean countries (Siddiqi, 1975; Lamberti, 1981), little information is available on its pathogenicity, perhaps due to difficulties found in rearing large numbers of individuals needed for such studies. Tobar Jiménez, Guevara Benítez and Martínez Sierra (1968), found that *Z. guevarai* increased only twelve fold on potted violet, *Viola tricolor* L. after 10 months in a lath house.

Carrot disc cultures have been used to propagate a number of root lesion nematodes providing large numbers of highly infective nematodes (O'Bannon & Taylor, 1968; Moody, Lownsbery & Ahmed, 1973; Huettel & Rebois, 1985; Lawn & Noel, 1986). Since contamination due to bacteria associated to the carrot tissue sometimes occurs in carrot disc cultures, *Agrobacterium rhizogenes* transformed-potato root cultures were considered as an alternative in this study to propagate *Z. guevarai*. Transformed root cultures have been successfully used to propagate sedentary endoparasitic nematodes (Paul *et al.*, 1987; Verdejo, Jaffee & Mankau, 1988). In this paper the monoxenic culture of *Z. guevarai* in both carrot and transformed potato root is reported.

Materials and methods

The nematode was isolated from soil around almond trees in Tarragona, Spain. Adult individuals recovered from soil were hand picked and surface disinfected in a 100 ppm mercuric chloride and 1 000 ppm streptomycin sulphate solution for 5 min. Nematodes were then pipetted to carrot disc cultures prepared as indicated by O'Bannon and Taylor (1968). Such cultures were used as the source of inoculum for the reproduction studies. Transformed potato roots were grown on solid Gamborg's B5 medium plus vitamins (Verdejo, Jaffee & Mankau, 1988) and incubated for 3 days at 25 °C before nematode inoculation.

To compare nematode reproduction on differentiated and non-differentiated tissues, 15 ± 1 surface disinfected females were added to each of 20 cultures containing either transformed-potato roots or carrot discs (one disc per culture). The number of *Z. guevarai* added per culture was determined after nematode inoculations. Cultures were maintainded at 25 °C in the dark for 68 days. Nematodes were recovered from carrot disc cultures by adding 5 ml distilled water per culture. Nematodes that migrated from the disc to the clear water were collected 24 h later. The carrot disc was then weighted and blended in a blender for a total of 30 s given as 10 second periods. Both suspensions were mixed and nematodes counted determining the number

of eggs, juvenile stages (J2, J3 and J4), males and females. To recover *Z. guevarai* from root cultures, the agar was melted in a microwave oven for 1-2 min, roots blotted dry and weighted. Nematodes remaining in the agar plate and in the roots were counted as indicated above.

The reproduction rate of the nematode was studied on carrot disc cultures inoculated with 10 ± 1 surface disinfected females. Nematode reproduction was assessed, as decribed previously, in ten cultures at 45, 85 and 125 days, respectively.

The effect of the initial inoculum level on nematode multiplication was determined after 125 days by adding 10, 20 and 50 surface disinfected nematodes (a mixture of all life cycle stages) to 250 ml flasks containing five carrot discs. Each inoculum level was replicated four times.

Results and discussion

Zygotylenchus guevarai was established in monoxenic cultures on both carrot discs and *A. rhizogenes* transformed roots (Table 1). The population increased 104 times on carrot discs in about 2 months. Clusters of nematodes were observed in the plate outside the carrot disc (Fig. 1 A). Although the nematode was able to complete its life cycle on transformed potato roots, as indicated by the presence of males, these cultures were apparently a poor host for the nematode. Nematodes were found in the agar but no nematode was observed into the root tissue. The nematode completed its life cycle in 45 days which seems to be the minimun time required to undergo development from adult to adult at 25 °C (Table 2). The reproduction rate of *Z. guevarai* was 106 and 1504 fold after 85 and 125 days, respect-

Table 1

Reproduction of *Zygotylenchus guevarai* on carrot disc and *Agrobacterium rhizogenes* transformed potato roots cultures inoculated with 15 ± 1 females after 68 days at 25 °C.

Substrate	*Tissue weight*	*No eggs/ culture*	*No juveniles/ culture*	*No females/ culture*	*No males/ culture*	*Total nem. culture*	*Reproduction rate*
Carrot discs	3.3*	808	440	92	78	1 417	104
Potato roots	1.1	1	21	9	2	33	2

* Mean of ten replications.

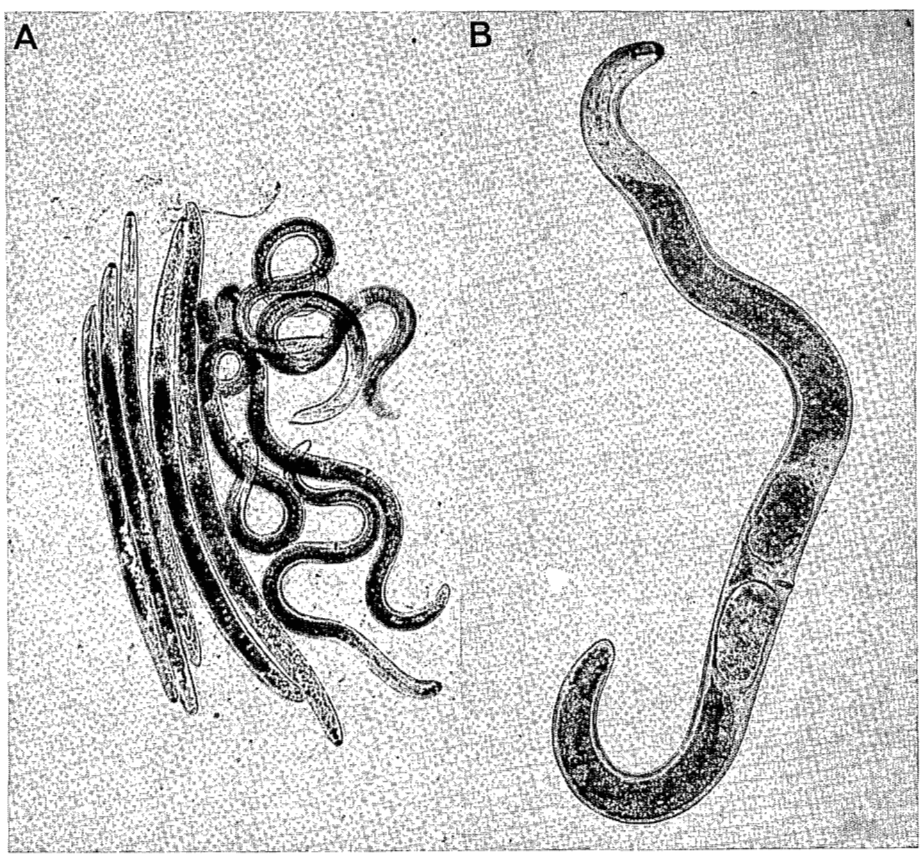

Fig. 1. Monoxenic culture of *Zygotylenchus guevarai*. A : Clusters of *Z. guevarai* observed in carrot disc cultures inoculated with 15 ± 1 females after 68 days at 25 °C; B : *Z. guevarai* female containing eggs from both ovaries found in carrot disc cultures.

ively. On occasion females with eggs from both ovaries were found but most of the observed females contained one or no eggs (Fig. 1 B). An initial inoculum level of ten or twenty individuals provided similar numbers of nematodes per culture after 107 days (Table 3). However, cultures inoculated with 50 individuals yielded three times more nematodes. The egg stage was predominant in carrot disc cultures (40-60 % of the population) in the three experiments except when the reproduction rate was assessed at 45 days. At this time, the population was composed mainly by females (52 %). The high proportion of females could be due to the lack of nematode reproduction in five of the ten cultures checked at 45 days, only females used as inoculum were recovered from those cultures. Carrot disc cultures of *Radopholus similis* and *Pratylenchus brachyurus* yielded large numbers of eggs (O'Bannon & Taylor, 1968). High numbers of eggs were also found in cultures of *P. brachyurus* on carrot callus tissue; however, those cultures of *Pratylenchus agilis, P. scribneri, Radopholus citrophilus* and *R. similis,* yielded low numbers of eggs (Reise, Huettel & Sayre, 1987). Only a low percentage of the population reached the adult stage, the proportion of males to females never being higher than 8 and 11 % of the population, respectively.

Table 2

Reproduction of *Zygotylenchus guevarai* on carrot discs inoculated with 10 ± 1 females after 45, 85 and 125 days at 25 °C.

Days after inoculation	*Carrot tissue weight (g)*	*No eggs/ culture*	*No juveniles/ culture*	*No females/ culture*	*No males/ culture*	*Total nem. culture*	*Reproduction rate*
45	3.4*	5	16	24	1	46	5
85	3.0	400	412	106	85	1 003	106
125	2.6	7 148	5 818	1 270	852	15 088	1 504

* Mean of ten replications.

Table 3

Reproduction of *Zygotylenchus guevarai* on carrot discs inoculated with 10, 20 and 50 nematodes after 107 days at 25 °C.

Inoculation level	*No eggs/ culture*	*No juveniles culture*	*No females/ culture*	*No males/ culture*	*Total nem. culture*	*Reproduction rate*
10	5 827*	4 715	441	186	11 169	1 117
20	7 786	4 582	379	234	12 981	649
50	22 230	13 264	1 499	949	37 942	759

* Mean of four replications.

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Accepté pour publication le 1er juin 1990.

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