RESEARCH NOTES—NOTAS DE INVESTIGACION

AN ENZYMATIC TECHNIQUE FOR OBTAINING *MELOIDOGYNE* FEMALES FOR BIOLOGICAL CONTROL STUDIES.

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RESUMEN

Godoy, G. and R. Rodríguez-Kábana. 1983. Una técnica enzimática para recolectar hembras de *Meloidogyne* para estudios sobre combate biológico. Nematrópica 13:75-78.

Este trabajo informa sobre una técnica para la extracción de hembras y masas de huevecillos de *Meloidogyne* spp. para estudios de control biológico. El uso de esta técnica, que consiste en macerar los tejidos de las raíces con enzimas, asegura una mayor recuperación de hembras o huevecillos, de nematodos endoparásitos para el estudio de hongos patógenos. La ténica consiste en incubar agallas en una solución de celulasas y pectinasas con sulfato de estreptomicina. La acción macerante de las enzimas facilita la recuperación de las hembras y masas de huevecillos.
Palabras claves adicionales: celulasas, pectinasas, masas de huevecillos.

Fungal parasitism associated with females of endoparasitic nematodes has been extensively studied in the past (4,5,6,7,8,9). Direct dissection from intact root systems is a procedure commonly used to recover females of these nematodes (4,5,8). During the process of dissection from hard root tissues, many females are partially or totally damaged and some never recovered. The procedure is also tedious and time-consuming and requires skillful personnel.

Tissue maceration of galled roots using commercial enzyme preparations is an established method for recovery of adult females of root-knot nematodes (*Meloidogyne* spp.) for biochemical and physiological studies. The procedure was introduced by Dropkin et al. (2) and modified by Dickson et al. (1) and Hussey (3). The last modification has the advantage of being rapid and providing large quantities of adult females. However, the use of sucrose solutions in the recovery process can favor the growth of microbial contaminants on the surface of the female body and affect the recovery or isolation of fungal parasites on agar plates.

In this paper we describe a modification of this method that eliminates the use of sucrose solutions.

We studied enzymatic maceration of roots from 'Rutgers' tomato (*Lycopersicun esculentum* Mill.), 'Ransom' soybeans [*Glycine max* (L). Merr.], and 'Summer Crookneck' squash (*Cucurbita pepo* L.). Gall sections (0.5 cm long) from roots of plants growing in soil infested with *Meloidogyne arenaria* (Neal) Chitwood were removed and rinsed in running tap water for 24 hr. This was followed by serial washings (3 times) in sterile demineralized water. The washed sections were placed in 50 ml-Erlenmeyer flasks containing 20 ml of a solution of pectinase and cellulase (Nutritional Biochemical Corporation, Cleveland, Ohio). The solution contained one gram of each enzyme diluted in 100 ml of sterile demineralized water. Streptomycin sulfate was added to have 1 mg/ml. The flasks were agitated for 5 minutes and incubated overnight at 30 C. Root tissues of squash and tomato were macerated within 24 hr, but soybeans required 40 hr of incubation to achieve an equal degree of maceration.

The softened gall sections were removed from the enzyme solution and placed in a petri dish with sterile water. The sections were easily split open with sterile needles. Females (at all stages of development) and large numbers of egg masses were released from the root tissues (Fig. 1).

Females and egg masses were collected with a sterile Pasteur pipette and rinsed in sterile water containing streptomycin sulfate (1 mg/ml). Females and egg masses were maintained at 5 C until analyzed for fungal content as described elsewhere (8).

In summary, the technique provides intact *Meloidogyne females* without exposure to sucrose solutions. This permits isolation from the females of slow-growing fungi free from contamination. Also, the technique is based on the use of two enyzmes that are commercially available in several levels of activity and from various sources. This permits the individual researcher to prepare his own mixture of the enzymes to suit his needs. Previous methods (1,2) were based on the use of enzyme preparations (e.g., Pectinol® 59L) which we have been unable to obtain. In contrast to other methods, our technique does not permit the rapid extraction of large quantities of *Meloidogyne* females but it allows *in situ* observation of adult females and other stages of development of the nematodes. The technique has been used successfully (unpublished data) in our laboratory to study parasitism of young females of *Heterodera glycines* Ichinohe by fungi and could be adapted easily to extract other endoparasitic nematodes.

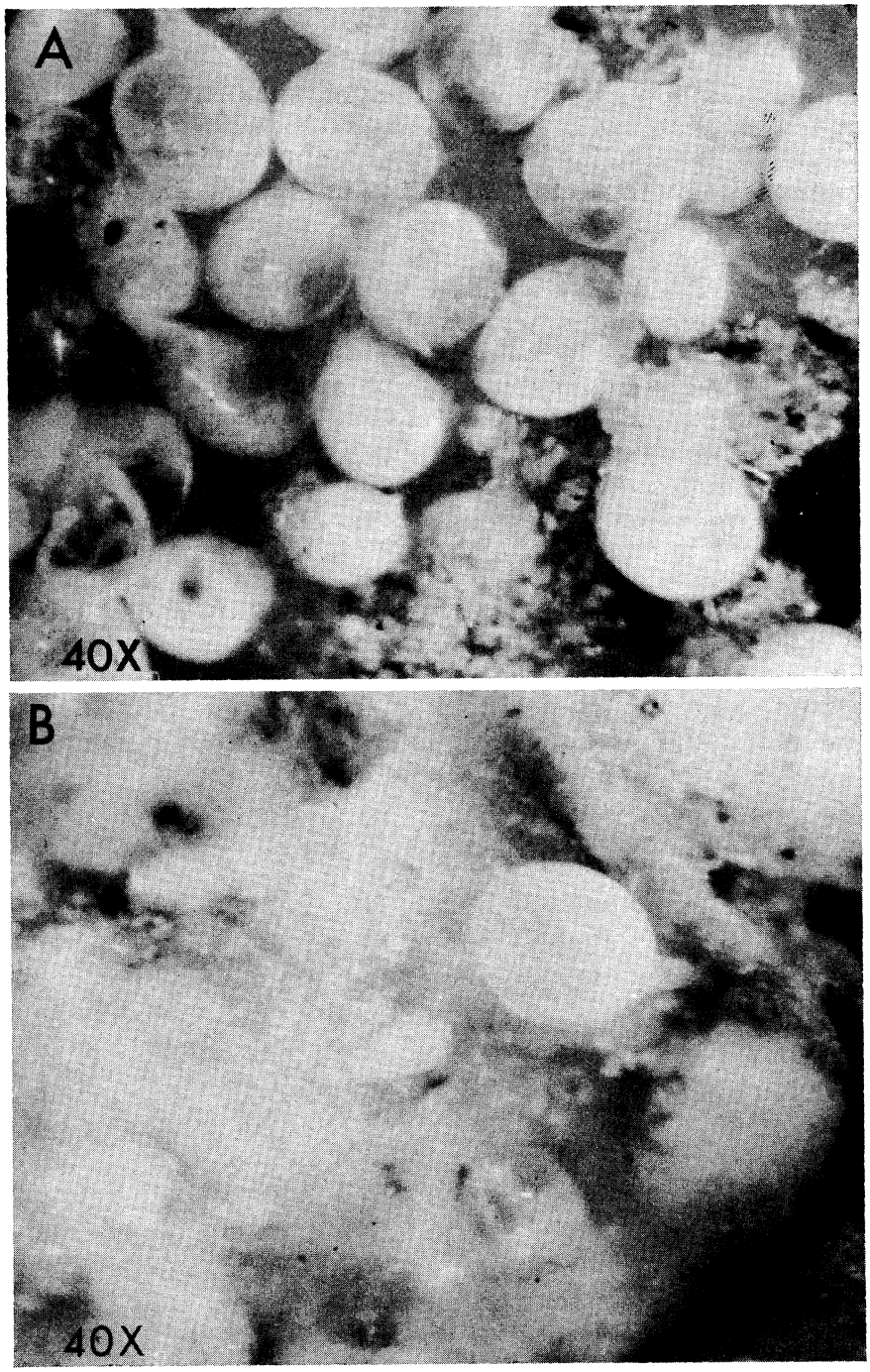

Fig. 1. Females of *Meloidogyne arenaria* obtained after enzymatic treatment of roots (A); (B) female and egg mass of the nematode in the root exposed by the treatment.

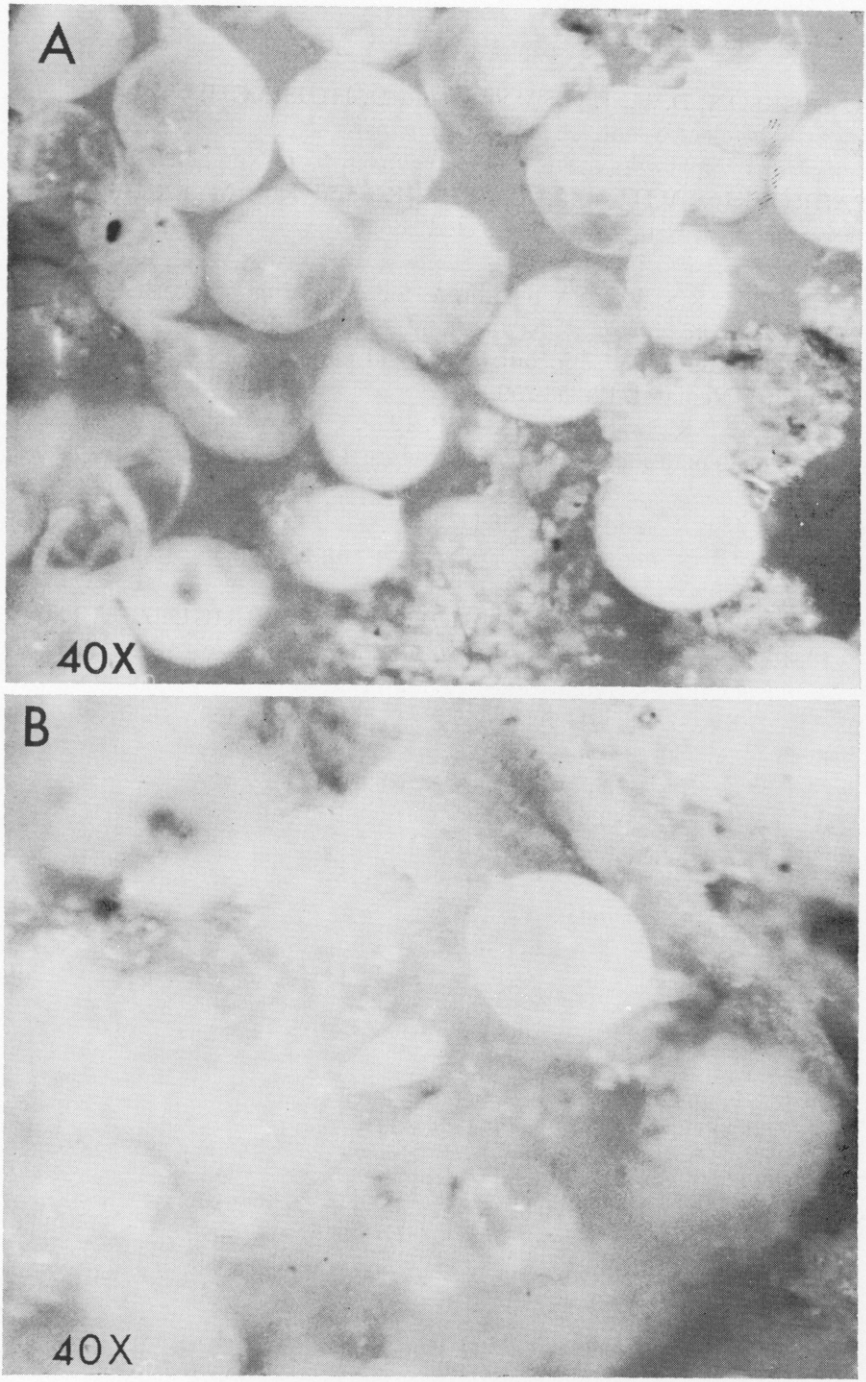

Fig. 1. Females of *Meloidogyne arenaria* obtained after enzymatic treatment of roots (A); (B) female and egg mass of the nematode in the root exposed by the treatment.

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