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INFLUENCE OF EXTRACTION PROCEDURES FROM ROOT SAMPLES ON THE RECOVERY AND INFECTIVITY OF *PRATYLENCHUS ZEAЕ* AND *HIRSCHMANNIELLA ORYZAE*

by

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Summary. The efficiency of three extraction techniques – Baermann funnel (Bf), maceration-filtration (M-F), and mistifier (Mi) – to extract *Pratylenchus zeaе* and *Hirschmanniella oryzae* from rice roots and their influence on the infectivity of *P. zeaе* were tested. M-F and Mi methods were the most efficient techniques for extracting *P. zeaе* and *H. oryzae*, respectively. When comparing infestation of rice plant roots using infested roots or nematodes obtained by Bf, M-F, or Mi methods as sources of inoculum, infectivity of *P. zeaе* was strongly decreased by these extraction techniques. However, more *P. zeaе* were able to infest rice roots when they were obtained using the Mi technique. The duration of extraction also affects the infectivity of *P. zeaе*. When estimating root infestation by migratory endoparasitic nematodes, it may be necessary to use different techniques to extract different nematodes. The effect of the method by which the nematodes have been obtained must also be considered in assessing their infectivity.

The determination of nematode populations from roots and soil provides better evaluations of endoparasitic nematode infestations than methods using populations obtained from soil alone. There are several techniques for the extraction of active endoparasitic nematodes from root tissues. Among the most commonly used are the Baermann funnel technique (Bf) (Baermann, 1917), the maceration-filtration technique (M-F) (Fallis, 1943; Stemmering, 1964), the mistifier technique (Mi) (Seinhorst, 1950), and the incubation technique (Young, 1954). Each method relies on nematode motility. Their efficiency can be affected by many factors such as duration and temperature of root sample storage, sample size, oxygen level, breakdown products from root tissues, and bacterial and fungal contaminants (Hooper, 1986). Other methods have been developed to extract immobile stages and eggs in addition to motile stages (Caveness and Jensen, 1955; Hussey, 1971; Coolen and D'Herde, 1972; Godoy and Rodriguez-Kabana, 1983). The efficiency of the different methods appears to depend on nematode-host combination (McSorley *et al.*, 1984; McSorley, 1987).

Most research on nematode extraction methods from soil or root tissues has aimed to improve their efficiency. The effect of extraction methods on the motility or infectivity of the nematodes has seldom been considered

(Viglierchio and Yamashita, 1983; Kaplan and Davis, 1990). However, nematodes extracted from roots are often used as inoculum for experiments conducted with sedentary and migratory endoparasitic nematodes. The present investigation compared the efficiency of three techniques (Bf, M-F, and Mi) used to extract *Pratylenchus zeaе* Graham and *Hirschmanniella oryzae* (Van Breda de Haan, 1902) Luc *et* Goodey from infested rice roots and to assess the influence of the extraction procedures on the infectivity of *P. zeaе*.

Materials and methods

Roots infested with *P. zeaе* were obtained from a permanent culture of the nematode maintained in a greenhouse on upland rice cv. UPL Ri-5. Roots infested with *H. oryzae* were collected in two irrigated rice fields of the IRRI experimental farm planted with rice cv. PSB RC4.

The efficiency of Bf, M-F and Mi methods for the extraction of *P. zeaе* and *H. oryzae* from rice roots were compared in two experiments. For each experiment, 1 kg of roots was washed in flowing water then cut into sections of approximately 1 cm and thoroughly mixed. All extractions were performed on aliquots of 3 g of roots. For the Bf extractions chopped roots were spread on a tissue

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paper supported on a 7.5 cm-diameter plastic sieve, 1.5 cm deep, made of mosquito net and immersed in water in a 9 cm-diameter Petri dish, 1.5 cm deep. For M-F extraction, chopped roots were comminuted for 15 seconds in a Waring blender and then transferred onto sieves similar to those used in the Bf method. Bf and M-F extractions were performed at room temperature (22-31 °C). In the Mi method, chopped roots were placed on coarse sieves attached to 7.5 cm-diameter PVC pipes, 12.5 cm deep, standing in 12.5 cm-diameter polystyrene beakers, 10 cm deep. The sieves were placed in a mistifier with an intermittent mist spraying for 15 min in every 30 min at a temperature between 24 and 28 °C. Nematodes were collected and counted after 6 h, and 1, 2, 3, 4, 5, 6, 7 and 8 days. Each experiment was conducted using a randomized complete block design with ten replications. Data were analyzed using standard ANOVA and means were separated using Duncan's new multiple range test (DMRT).

To assess the effect of the extraction method on *P. zaeae* infectivity, 6 kg of infected roots were washed in flowing water, chopped into sections of approximately 1 cm and thoroughly mixed. Extractions were done with 6 aliquots of 300 g of chopped roots for each method. For each method, an extraction was stopped and the nematodes collected after 6, 24, 48, 96, 144 and 192 hours. The nematodes were then inoculated on 10-day-old rice seedlings of cv. UPL Ri-5 (50 nematodes/plant) growing in clay pots containing 100 cm³ of autoclaved soil. The pots were kept in the greenhouse and treatments were arranged in a randomized block design with eight replications. The plants were harvested 1, 2, 4, 6, and 8 days after inoculation. The roots were processed using the M-F extraction method and nematode suspensions were collected after five days. Data on nematode counts were analyzed using standard ANOVA and means were separated using Duncan's new multiple range test.

In a second extraction experiment *P. zaeae* from 3 g root aliquots were inoculated on 10 day-old rice seedlings (UPL Ri-5) growing in clay pots containing 100 cm³ immediately after they were collected and counted. All nematodes obtained from the same 3 g root aliquot after 6 h, and 1, 2, 3, 4, 5, 6 and 7 days of extraction were inoculated on the same test plant. Simultaneously, at the beginning of the extraction experiment, 3 g root aliquots were incorporated into the 100 cm³ soil contained in ten clay pots where 10-days-old rice test plants were grown. Treatments were arranged in a randomized block design with eight replications under greenhouse conditions. Test plants were harvested 10 days after the first nematode inoculation. Their roots were processed by the M-F extraction method. Nematodes were collected and counted after 6 days of extraction. Data were analyzed using standard ANOVA, and using DMRT test after a square-root transformation for analysis of percentages of penetration.

Results

Greater numbers of *P. zaeae* were extracted from rice roots with the M-F method than with the Bf or Mi techniques (Fig. 1A and B). In the first experiment, the number of individuals recovered for all extraction periods from 6 hours to 7 days by the M-F technique were significantly greater ($P < 0.05$) than those obtained using the Bf or the Mi technique (Fig. 1A). In the second experiment, numbers of *P. zaeae* yielded using M-F and Bf did not differ significantly from each other but were significantly greater than those obtained using the Mi (Fig. 1B). With the three techniques, the number of nematodes recovered increased with the duration of the extraction period with more than 90% of the total number of nematodes recovered during the first three days.

In both experiments, the Mi technique yielded significantly ($P < 0.05$) more individuals of *H. oryzae* than the two other extraction methods (Figs. 1C and D). In the first experiment, the numbers of nematodes recovered by Bf or M-F were not significantly different (Fig. 1C). In the second experiment significantly greater numbers ($P < 0.05$) of *H. oryzae* were obtained by Bf than by M-F (Fig. 1D).

Figure 2 shows the average number of *P. zaeae* obtained after 6, 24, 48, 96, 144, and 192 hours of extraction using the three extraction methods and which were recovered from roots of a test plant after different periods of contact. When data from all extraction periods and different times of contact were combined, the number of *P. zaeae* obtained by Bf or Mi techniques which were able to infest the test plant root system were not significantly different. On the other hand, the number of *P. zaeae* obtained by the M-F technique which were able to infest the test plant root system were significantly ($P < 0.05$) fewer than those obtained by the other two extraction methods. In addition, the number of *P. zaeae* recovered from test plant roots significantly decreased when extraction time increased. The average number of *P. zaeae* recovered from test plant roots when inoculated with individuals obtained after 6, 24, 48, 96, 144, and 192 hours of extraction were 14.09, 12.57, 9.19, 8.38, 2.73, and 3.92, respectively. Moreover, for the three extraction techniques, the number of nematodes recovered from test plant roots after 8 days of contact were negatively correlated ($P < 0.05$) with extraction time.

The average number of *P. zaeae* recovered from test plant roots after 10 days of contact was significantly greater when infected roots were used as the source of inoculum than when the nematode inoculum was obtained by extraction from roots (Table I). The average number of *P. zaeae* recovered from the test plant roots were not significantly different when various extraction procedures were used to obtain the nematode inoculum. However, the pro-

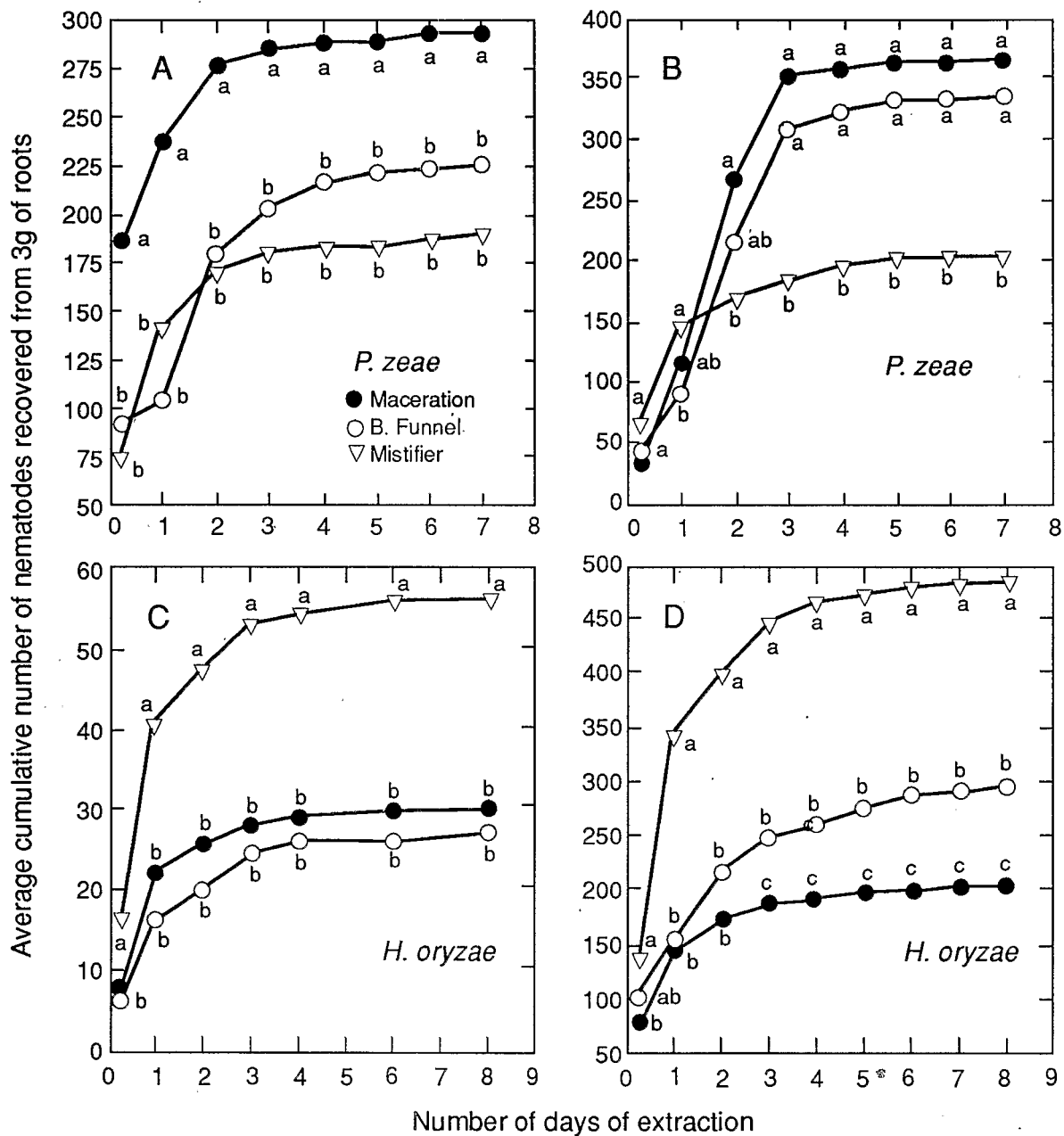


Fig. 1 - Average cumulative numbers of *Pratylenchus zeae* (A and B) and *Hirschmanniella oryzae* (C and D) recovered from infected rice roots with three extraction methods: Baermann-funnel, maceration-filtration, and mistifier. Each point represents the average of 10 replications. Nematode numbers recovered on a given day marked with the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

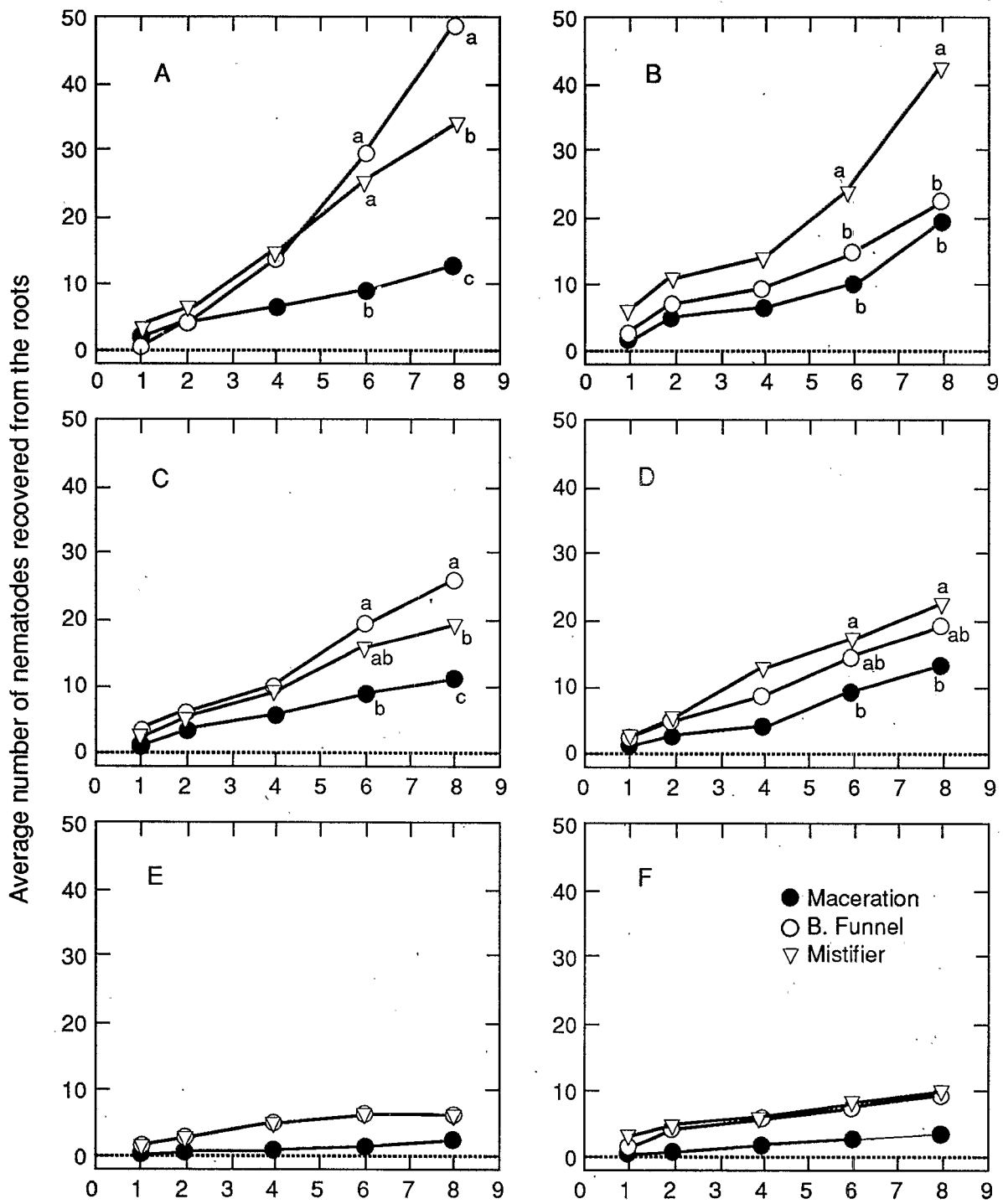


Fig. 2 - Average numbers of *Pratylenchus zeae* recovered from rice roots (cv UPL Ri-5) after 1, 2, 4, 6 and 8 days of contact. The initial inoculum having been obtained after 6 (A), 24 (B), 48 (C), 96 (D), 144 (E) and 192 (F) hours of extraction by Baermann-funnel, maceration-filtration, and mistifier extraction methods. Each point represents the average of 8 replications. Nematode numbers recovered on a given day marked with the same letter are not significantly different ($P=0.05$) according to Duncan's multiple range test. The absence of a letter on a given day indicates that the same letter applied to all treatments.

TABLE I - Effect of extraction procedures on the infectivity of *Pratylenchus zaei*.

	Source of inoculum			
	Nematodes obtained from 3 g of roots by different extraction techniques			3 g of Infected roots
	Maceration	B. Funnel	Mistifier	
Average inoculum	362 a	332 a	201 b	?
Average number of nematodes recovered from test plant roots	14 a	9 a	19 a	335.8 b
Average percentage of infective nematodes	5.1 a	2.7 a	9.9 b	-

In a row, treatment means having a common letter are not significantly different by DMRT at $P < 0.05$.

portion of nematodes able to infest the test plant roots was significantly greater when the inoculum was obtained by the Mi technique than by the M-F or Bf methods (Table D).

Discussion

The results indicate that different methods had different efficiencies when used to extract *P. zaei* and *H. oryzae* from rice roots. Moreover, the results suggest that when working with different migratory endoparasitic nematodes, it may be useful to use different extraction techniques to obtain the best estimation of nematode root infestation. The extraction method and the duration of the extraction procedure may also affect the infectivity of the recovered nematodes. Moreover, it is not always the most efficient extraction method that provides the nematodes with the highest infectivity. When performing root infestation experiments with migratory endoparasitic nematodes it is useful to correct for the effect of the extraction method on their infectivity.

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