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A Simple Method for Determining *Aphelenchoides besseyi* Infestation Level of *Oryza sativa* Seeds

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Abstract: A simple extraction method was developed for obtaining the white tip nematode, *Aphelenchoides besseyi* Christie, from single seeds of rice, *Oryza sativa*. The method was as follows: Individual rice seeds were split longitudinally and then transferred into single pipet tips. Tips containing a split seed were then singly placed upright in glass vials with water to extract the nematodes. This method was more efficient than the Baermann funnel technique and allowed nearly 100% recovery of living *A. besseyi* from single rice seeds within 4 hours.

Key words: Aphelenchoides besseyi, assay, extraction, methodology, nematode, nematode load, *Oryza sativa,* rice, white tip disease.

The white tip nematode (*Aphelenchoides besseyi* Christie), an ectoparasite of rice (*Oryza sativa* L.), enters rice flowers and hibernates beneath seed glumes (Hollis and Keoboonrueng, 1984) as adults and fourth-stage juveniles (Nandakumar et al., 1975). In Japan, rice seeds are harvested in autumn and stored. In the next spring, seeds are sowed directly in paddy fields or in seedling-raising boxes. Once the nematode-infected seeds are soaked in water, the nematodes revive and leave the seeds to attack rice seedlings (Tamura and Kegasawa, 1957, 1958). In 1998, an outbreak of this nematode occurred in Onomichi City, Hiroshima Prefecture, in western Japan. Our field survey revealed that infestation levels of the nematode varied among paddy fields even in a restricted area.

The number of *A. besseyi* per rice seed (nematode load) had been examined mostly by submerging seeds in shallow water after separating them from glumes (Uebayashi et al., 1971). This method is effective for nematode extraction but is laborious. The Baermann funnel technique is preferred instead for extracting nematodes from seeds after they have been split and macerated (Nandakumar et al., 1975). Nematode extraction from single seeds has not been studied in detail so far, even though it is very important for a precise evaluation of nematode population and plant damage. Here, we report a simple extraction method for determining the *A. besseyi* load on single rice seeds.

Materials and Methods

Rice seeds examined: Rice seeds from nematode-infected rice plants (cv. Hinohikari) previously showing ''white tip'' leaf symptoms were collected in three locations (Kinosho Nishi, Urasaki, and Minogo) in Onomichi City, Hiroshima Prefecture, on 24 and 29 September 1998. All of these seed samples were stored at 5 °C until their use for nematode extraction. Seed samples included blasted to ripe seeds.

Nematode extraction: Two extraction procedures were employed to compare the extraction efficiency of *A. besseyi* from rice seeds: the Baermann funnel technique and the simple extraction method described below. Extraction procedures were performed twice.

For the simple extraction method, rice seeds were cut longitudinally in two with small pruning scissors, then transferred into single plastic pipet tips (7 cm long, 1.0 mm and 7.4 mm top and bottom i.d., respectively) (Quality Scientific Plastics, Petaluma, CA) (Fig. 1). Tips containing a split seed were then singly placed upright in glass vials (6.5-ml capacity) with water. To examine the rate of nematode extraction, the tips were

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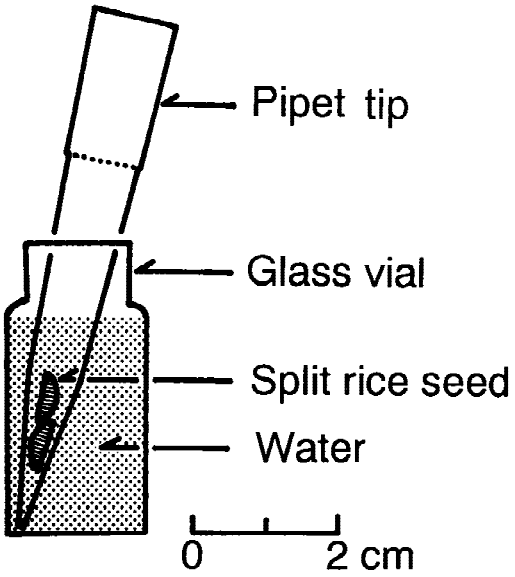

FIG. 1. Diagram of the simple extraction apparatus used for obtaining *Aphelenchoides besseyi* from an *Oryza sativa* seed.

successively transferred to new vials 2, 4, 8, and 24 hours after extraction was begun. Extracted nematodes were transferred to Syracuse watch glasses and counted as living or dead nematodes. Nematodes that did not move when prodded with a needle were considered dead. Seeds were dissected to count nematodes remaining in them 24 hours after the start of extraction. At the same time, nematodes on the inner tip wall were counted under a dissecting microscope.

Baermann funnel technique: Fifty seeds were cut in two and collectively placed on a Japanese paper (Improved paper 17, Heiwa Shigyou, Osaka, Japan) and soaked in water in a 9-cm-diam. Baermann funnel. Twenty-four hours after soaking, living and dead nematodes were collected from the funnel bottom, inner funnel wall, and rice seeds and then counted.

Nematode extraction with both procedures was conducted in darkness at 25 °C, as this temperature is within the range suitable for extraction of *A. besseyi* from soaked seeds (Tamura and Kegasawa, 1957).

Statistical analysis: The Mann-Whitney Rank Sum Test was used to compare the extraction efficiency of nematodes between the two procedures.

Results and Discussion

The simple nematode extraction method we employed in the present study was more efficient than the Baermann funnel technique for the extraction of *A. besseyi* from rice seeds (U value = 12.0, $P < 0.05$) (Table 1). Differing extraction efficiencies may be partly due to different travelling distances for nematodes sinking from the soaked

TABLE 1. Comparison of extraction methods for determining the level of *Aphelenchoides besseyi* infestation in *Oryza sativa* seeds.

Sample[a]	Number of seeds examined	Total number of live (dead) nematodes extracted or observed								Extraction efficiency
		0–2 hours	2–4 hours	4–8 hours	8–24 hours	On paper[b]	In seeds	On pipet or funnel wall	Total	
Simple extraction method										
Sample A	67	22 (0)	1 (0)	0 (0)	0 (0)	—[c]	0 (2)	0 (0)	23 (2)	0.92
Sample B1	50	31 (1)	0 (1)	0 (0)	0 (0)	—	0 (15)	0 (0)	31 (17)	0.69
Sample C1	50	28 (1)	1 (0)	1 (0)	0 (0)	—	0 (21)	0 (0)	30 (22)	0.60
Sample D1	50	43 (1)	1 (1)	0 (0)	0 (0)	—	0 (4)	0 (0)	44 (6)	0.92
Total	217	124 (3)	3 (2)	1 (0)	0 (0)	—	0 (42)	0 (0)	128 (47)	0.76
Baermann funnel technique										
Sample B2	50	—	—	—	1 (0)	0 (1)	11 (37)	0 (0)	12 (38)	0.02
Sample C2	50	—	—	—	1 (0)	0 (0)	0 (36)	0 (0)	1 (36)	0.03
Sample D2	50	—	—	—	10 (1)	0 (0)	1 (20)	1 (0)	12 (21)	0.33
Total	150	—	—	—	12 (1)	0 (1)	12 (93)	1 (0)	25 (95)	0.11

[a] Different letters indicate samples collected from different paddy fields.
[b] Examination of Baermann funnel paper or dissection of rice seeds was conducted 24 hours after the start of extraction.
[c] Not determined.

seeds to the bottom of the extraction container. In the case of the Baermann funnel, nematodes must pass through a paper sieve. In addition, some nematodes remained on the upper part of the funnel wall. The reason for higher nematode mortality in the Baermann funnel technique is unknown.

Kondo and Ishibashi (1986) used a pipet tip, in which tissue paper was placed as a sieve, to recover steinernematid nematodes from insect feces mingled with soil. However, the effect of tissue paper on nematode extraction was not determined. The method presented here does not have a sieve effect.

When the simple method was used, all live nematodes were extracted in 8 hours (Table 1). Nearly all nematodes (97%) were extracted in the first 2 hours from the seed samples (A, B1, C1, D1). Dead nematodes mostly remained on the underside of the glumes, but about 12% of the dead nematodes also were extracted. Because of high nematode extraction efficiency and easy preparation of the extraction apparatus, this method will be applicable for studying nematode populations and differing susceptibilities of rice plants to *A. besseyi.*

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