INTRODUCTION

The white tip nematode, *Aphelenchoides besseyi* Christie, is a seed-borne ectoparasite of rice, *Oryza sativa* L., and the causative agent of white tip disease of rice plants. The nematode enters rice florets where it rapidly proliferates, then desiccates and finally survives anhydrobiotically as fourth-stage juveniles and adults within seeds beneath the inner surface of paleae and lemmas (Huang et al., 1972; Huang and Huang, 1972, 1974; Nandakumar et al., 1975; Hollis and Keoboonrueng, 1984; Chiyonishio and Nakazawa, 1988). 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).

The white tip nematode, *A. besseyi*, is found between leaf sheaths at the seedling stage (Fukano, 1962), and then in the cavities above the growing tip of the rudimentary culm and finally on the young leaf surrounded by the innermost leaf sheath during tillering (Goto and Fukatsu, 1952).

Trade-off between dispersal and reproduction is observed in species of which individuals allocate a limited amount of resources into the two functions (Zera and Denno, 1997). In the case of parasites harbored in hosts, their dispersal depends on the locomotion of hosts. Consequently, a trade-off is likely to exist between dispersal and reproduction of the parasites due to reduced viability of host by parasites. In *A. besseyi*-rice plant system, an inoculation experiment of *A. besseyi* on potted rice plants suggested that the dispersal of rice seeds negatively correlates with the competition capability via seed specific gravity, because light seeds (specific gravity <1.0) floating on water germinated in a smaller ratio and required more time to protrude seminal roots than did heavy seeds (spe-
cific gravity \( \geq 1.0 \)), irrespective of their source, whether derived from nematode-inoculated or uninoculated plants (Togashi and Hoshino, 2003). The experiment also showed that nematode-inoculated plants produced a greater proportion of light seeds than un-inoculated plants and that nematode mortality was greater in light seeds than in heavy seeds. These results indicated a trade-off between dispersal and reproduction of nematodes harbored in the seed through seed specific gravity.

As the reproduction of *A. besseyi* after dispersal depends on the rice plant survival, the nematode might force the parent plant to supply a large amount of hydrocarbons and proteins to the light seeds harboring them. Intriguingly, the inoculation experiment indicated that as the number of nematodes in a light seed increased, the nematode mortality decreased and the degree of seed swelling increased, the latter suggesting a host manipulation by *A. besseyi* (Togashi and Hoshino, 2003). To our knowledge, similar type of host manipulation has never been reported for other nematode species that parasitize plant seeds.

The results of the inoculation experiment (Togashi and Hoshino, 2003) have not been confirmed for *A. besseyi*-infected rice seeds harvested in paddy fields yet. Thus, the objective of this study was to determine the relationships between initial seed specific gravity, endosperm development, the time required for seed to sink in water, the time required for seed germination, seedling growth, nematode load and nematode mortality in rice seeds obtained from a paddy field infested with *A. besseyi* and a non-infested field.

**MATERIALS AND METHODS**

**Rice seeds.** Ten hills of rice plants were randomly harvested from a paddy field heavily infested with white tip disease, where all rice plant hills showed “white tip” symptoms on the leaf blades. Plants were harvested at Nakano, Hiroshima City, Hiroshima Prefecture, on 24 September 1999. From the day of harvesting, the seeds were dried in a well-ventilated greenhouse by suspending the plants 1.5 m above the ground for 35 days. The mean air temperature was 17.4°C during the drying period. Six hills of rice plants at Hachi-Hon-Matsu, Higashi-Hiroshima City, Hiroshima Prefecture were harvested randomly from a paddy field on 24 September 2000. These were not infested with *A. besseyi*. Soon after harvesting, the seeds were dried as described previously. The mean temperature was 17.0°C in the greenhouse. After drying, all seeds were collected and then stored at 5°C in the dark until used. All rice plants were cultivar Hi-no-Hikari.

**Classification of seeds by specific gravity.** Seeds were placed in a beaker, covered with distilled water (specific gravity 1.00) and stirred vigorously for 10 seconds. After the seeds stopped moving, seeds that floated on the surface were collected and dried briefly on a paper towel. The seeds that floated (specific gravity less than 1.00) were referred to as “light seeds”. Seeds that sank to the bottom of the beaker were collected, blotted dry with paper towel, and transferred to salt solution (specific gravity 1.13 containing NaCl (129.35 g/L) and MgCl\(_2\) (0.65 g/L) in distilled water). Specific gravity was measured using a hydrometer. After stirring, seeds that floated and seeds that sank were collected separately and referred to as intermediate and heavy seeds, respectively. Salt solution was washed from the seed surface with distilled water and the seeds were blotted on a paper towel.

To obtain seeds with the three different specific gravity classes, 900 and 1,000 seeds were randomly sampled on 14 March 2000 and on 30 December 2000 from stored seeds collected from the infested and the non-infested field, respectively. These seeds were used for the following experiments soon after they were classified.

**Degree of seed swelling.** Degree of swelling was recorded for individual seeds as a measure of development of the endosperm and categorized as follows: least swollen, empty seeds (grade 1), less swollen seeds containing laminar endosperm (grade 2) or poorly developed endosperm (grade 3), swollen seeds containing well-developed endosperm (grade 4), and over-developed endosperm that had broken the interlocking structure at the edges of palea and lemma partially and extended through the wound (grade 5) (Togashi and Hoshino, 2001).

**Nematode infection.** The total number of live and dead nematodes harbored in each seed (referred to as ‘nematode load’) was determined by the method of Hoshino and Togashi (1999). Briefly, each rice seed was bisected using small pruning scissors and inserted into a pipette tip (7 cm long,
1.0 and 7.4 mm top and bottom inner diameter). The tip was placed into a 6.5-ml vial containing 6 ml water at 25°C. After four hours of incubation, the seed halves were removed and the nematodes in water and on the inner wall of the pipette tip were counted. In addition, the number of nematodes remaining on the inner surface of paleae and lemmas and on the surface of husked rice seeds was determined. Nematodes that did not move when prodded with a needle were considered dead.

**Relationship of seed specific gravity to seed swelling degree, nematode load, and nematode mortality.** One hundred seeds were randomly selected from each of the three seed groups of different specific gravity classes from the infested paddy field while fifty seeds were selected from each of three seed groups from the non-infested field. Degree of seed swelling and nematode load were determined individually. Data from the infested field were analyzed to determine the relationship of nematode number in a seed to nematode mortality and host viability for each of the three seed groups, based on the nematode number in a seed, and then nematode mortality and degree of seed swelling were calculated as related to each nematode load per seed.

**Relationship of seed specific gravity to time required for seed sinking, rhizogenesis, and seedling growth.** After rice seeds were recorded for degree of swelling, they were placed individually on the surface of 8 ml water in test tubes. The seeds were incubated at 25°C under about 9,530 lx with fluorescent lamps and they were examined daily at 7:30 a.m. for 21 days for sinking and rhizogenesis (seminal root emergence) characteristics. The length of coleoptile from base to leaf tip was determined 3 days after the start of rhizogenesis. Number of days required for the seed to sink was also recorded.

One hundred seeds randomly sampled from each of the three seed groups of different specific gravity classes collected from the infested field were examined for time required to sink in water, rhizogenesis, and early seedling growth for 21 days.

**Statistical analyses.** The Kruskal-Wallis test was used for comparison of the degree of seed swelling, nematode load on seed, time required for seed to sink, time required for rhizogenesis, and early seedling growth among the three seed groups of different specific gravity classes. A 2×3 contingency table was used for comparison of the proportions of the three seed groups between two infestation levels. When the analysis showed a significant difference, pairwise comparison was conducted by 2×2 contingency table for two seed groups (a seed group in question and the two other groups combined) and two infestation levels at an arranged significance level by the Bonferroni method (Yamamura, 1993). The proportion of seeds harboring the nematodes, the proportion of seeds with protruding seminal root (rhizogenesis), and nematode mortality were also compared among light, intermediate and heavy seeds sampled from each field by using a 2×3 contingency table for three seed groups and for two seed categories with or without nematodes or rhizogenesis or for two nematode physiological states. When the analysis showed a significant difference, pairwise comparison was conducted by 2×2 contingency table at an arranged significance level by the Bonferroni method (Yamamura, 1993). Linear regression analysis was used to show relationships between nematode load, nematode mortality, and degree of seed swelling. Spearman’s rank correlation coefficient (ρ) was used to indicate relationship between degree of seed swelling and nematode load and relationship between time required for rhizogenesis and time for seed sinking.

To determine the degree of aggregation of nematodes among seeds, the $I_δ$ value was calculated and its significance was tested with $F$-value (Morisita, 1959). The $I_δ$ value is defined as follows:

$$ I_\delta = \frac{q \sum_{i=1}^{q} n_i(n_i - 1)}{N(N - 1)} $$

and

$$ N = \sum_{i=1}^{q} n_i, $$

where $q$ is the number of seeds examined and $n_i$ is
the number of nematodes harbored in the $i$-th seed in $q$ seeds. The $I_q$ values less than, equal to, and more than unity indicate regular, random, and aggregated distributions of nematodes among seeds, respectively.

**RESULTS**

**Relationship of seed specific gravity to seed swelling degree, nematode load, and nematode mortality**

The proportions of light, intermediate and heavy seeds differed between *A. besseyi* infested and non-infested fields ($2 \times 3$ contingency table, $\chi^2 = 381.9$, $p < 0.001$). The proportion of light seeds was higher ($p < 0.001$) for the infested field (33.3%) than for the non-infested field (9.7%) (Table 1). This also was the case ($p < 0.001$) with the intermediate seed group, but the reverse was observed ($p < 0.001$) in the heavy seed group (Table 1).

Degree of seed swelling of rice differed between infested and non-infested fields on the light (Kruskal-Wallis test, $H = 20.5$, $p < 0.001$) and heavy seed ($H = 4.7$, $p < 0.05$) (Fig. 1). In the case of the light seed group, the percentage of grade 3 seed was highest (39.6%) for the infested field whereas that of least swollen seeds (swelling grade 1) was highest (55.0%) for the non-infested field (Fig. 1). In contrast, the majority of intermediate and heavy seeds had well-developed endosperm (swelling grades 4 and 5) in both fields (Fig. 1). The mean degree of seed swelling was smaller for the light seed group than for the two heavier seed groups in infested (Kruskal-Wallis test, $H = 177.5$, $p < 0.001$) and non-infested ($H = 123.1$, $p < 0.001$) paddy fields (Table 2).

*Aphelenchoides besseyi* was recovered from some seeds collected from the infested field whereas no nematodes were collected from 150 seeds from the non-infested field (Table 2). The proportion of seeds harboring *A. besseyi* (nematode prevalence) was higher for the light (69%) and intermediate (77%) seed groups than for the heavy seed group (29%) ($2 \times 3$ contingency table, $p < 0.001$, Table 2). Mean live, dead, or total nematode load on seed was higher ($p < 0.001$ for all cases) for light and intermediate seed groups than for the heavy seed group (Table 2). However, nematode load on individual seeds varied greatly, showing an aggregated distribution pattern for each of the three seed groups of different specific gravity classes (Table 2). The highest degree of aggregation occurred in heavy seed. Nematode mortality

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**Table 1. Frequency distribution of the specific gravity of *Oryza sativa* seeds sampled from paddy fields with or without *Aphelenchoides besseyi***

<table>
<thead>
<tr>
<th></th>
<th>No. of seeds examined</th>
<th>Percentage of seeds (specific gravity)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Light (&lt;1.00)</td>
</tr>
<tr>
<td>Infested paddy field</td>
<td>900</td>
<td>33.3 a</td>
</tr>
<tr>
<td>Non-infested paddy field</td>
<td>1,000</td>
<td>9.7 b</td>
</tr>
</tbody>
</table>

*aNumbers followed by different letters in each column indicate that the proportions of the total number of seeds sampled from infested or non-infested fields differ ($p < 0.001$) between the two fields by $2 \times 2$ contingency table for two seed groups (a seed group in question and the two other groups combined) and two infestation levels. Significance level was arranged by the Bonferroni method.*
was greater ($p<0.05$) for the light seed group than for the two heavier seed groups (Table 2). Inverse density-dependent mortality of *A. besseyi* in a seed was observed for the intermediate seed group but not for the light or heavy seed groups (Fig. 2).

Intriguingly, there was a positive correlation between the mean degree of seed swelling and the number of nematodes harbored in a seed for the light seed group (Fig. 3). The mean swelling degree (mean±SD=2.7±1.1) of light seeds harboring nematodes was greater than that (mean±SD=1.8±1.0) of light seeds obtained from non-infested paddy field (Kruskal-Wallis test, $H=20.1$, $p<0.001$) (Table 2). In contrast with the light seeds, intermediate and heavy seeds showed no correlation between the number of nematodes harbored in a seed and the mean degree of swelling of seeds harboring a specified number of nematodes for the infested field (Fig. 3).

**Relationship of seed specific gravity to time required for seed sinking, rhizogenesis, and seedling growth**

All intermediate and heavy seeds sank as soon as they were placed on the water surface. Mean time required for light seeds to sink was shorter for the infested field (2.3 days) than for the non-infested field (8.4 days) excluding seeds floating during a test period (Kruskal-Wallis test, $H=13.2$, $p<0.001$) (Table 3). Out of 50 light seeds obtained from the infested field, 19 sank in water within one day, 19 sank between days 2 and 7, and 12 floated throughout the 21-day test period. Out of 50 light seeds from the non-infested field, 14 sank on the first day, 29 sank between days 2 and 20, and 7 floated throughout the test period.

Rice seeds usually protruded seminal roots after they sank in water. More than 97% of intermediate and heavy seeds from the infested field protruded seminal roots while sitting in water and more than 66% from the non-infested field did so. For light seeds from the infested field, 7 of 25 that sank on days 1 and 2 protruded seminal roots, and 2 of 12 floating seeds did so on day 18 but did not sink during the test period. Other seeds did not show rhizogenesis. In the case of light seeds from the non-infested field, 10 of 17 that sank on days 1 and 2 protruded roots. Two of 3 floating seeds that protruded roots between days 10 and 16 sank 1 or 2 days after rhizogenesis while another did not sink.
Other seeds did not show rhizogenesis. The proportion of seeds protruding a seminal root was lower for the light seed group than for the intermediate and heavy seed groups obtained from the two paddy fields (2×3 contingency table, χ² = 216.91, p < 0.001 for infested field; χ² = 433.81, p < 0.001 for non-infested field) (Table 3). There was no difference in the proportion of seeds with rhizogenesis in the light seed group between infested and non-infested paddy fields (2×2 contingency table, χ² = 0.04, p > 0.05).

Time required for rhizogenesis after placing seed on the water surface was longer for the light seed group than for the two heavier seed groups from the infested field (Kruskal-Wallis test, H = 29.73, p < 0.001) and than for the heavy seed group from the non-infested field (H = 18.48, p < 0.001) (Table 3). Time required for rhizogenesis was shorter for each seed group with different specific gravity in the infested field than the non-infested field (Kruskal-Wallis test, H = 14.68, p < 0.001 for the light seeds; H = 53.81, p < 0.001 for the intermediate seeds; and H = 33.66, p < 0.001 for the heavy seeds). The time required for light seeds to protrude roots after being placed on the water surface was not related to the time required for sinking (ρ = −0.214, p = 0.662, n = 7 for the infested field; ρ = 0.232, p = 0.457, n = 12 for the non-infested field).

The coleoptile length 3 days after rhizogenesis did not differ among the three seed groups from the infested field (Kruskal-Wallis test, H = 3.74, p > 0.05) or from non-infested field (H = 2.89, p > 0.05) (Table 3). There was no difference in coleoptile length for each of the three seed groups between infested and non-infested fields (Kruskal-
Table 3. Relationships between the specific gravity of *Oryza sativa* seeds, degree of seed swelling, time required for seed to sink, percentage of seeds producing seminal root, time required for rhizogenesis, and coleoptile length 3 days after rhizogenesis. The seeds were obtained from paddy fields infested or non-infested with *Aphelenchoides besseyi*.  

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Seed group (specific gravity)</th>
<th>Light (&lt;1.00)</th>
<th>Intermediate (1.00 to 1.13)</th>
<th>Heavy (&gt;1.13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infested field</td>
<td>Non-infested field</td>
<td>Comparison&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Infested field</td>
</tr>
<tr>
<td>No. of seeds examined</td>
<td>150</td>
<td>50</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Degree of seed swelling&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4±1.1 b</td>
<td>2.0±1.2 c</td>
<td>*</td>
<td>3.9±0.4 a</td>
</tr>
<tr>
<td>Time for seed to sink (days)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3±1.6</td>
<td>8.4±7.1</td>
<td>*</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>% Rhizogenesis&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(38)a</td>
<td>(43)a</td>
<td></td>
<td>(100)b</td>
</tr>
<tr>
<td>Time for root to emerge (days)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.3 b</td>
<td>26.0 c</td>
<td>ns</td>
<td>97.0 a</td>
</tr>
<tr>
<td>Coleoptile length (cm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.5±3.0</td>
<td>14.5±3.6</td>
<td>*</td>
<td>7.0±1.4</td>
</tr>
<tr>
<td></td>
<td>(35)a</td>
<td>(13)a</td>
<td></td>
<td>(97)b</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean±SD. Means followed by the same letter in each row for the same paddy field are not different (p<0.05) by the Kruskal-Wallis test. Numerals in parentheses represent sample size.

<sup>b</sup> Percentages of rhizogenesis followed by the same letter for the same paddy field are not different (p<0.05) by 2×2 contingency table. Significance level was arranged by the Bonferroni method.

<sup>c</sup> * and ns indicate the presence and absence of difference (p<0.05), respectively between two fields in each parameter of each seed group by the Kruskal-Wallis test or 2×2 contingency table.
Wallis test, $H=0.274$, $p>0.05$; $H=3.243$, $p>0.05$; and $H=0.266$, $p>0.05$ for light, intermediate, and heavy seed groups, respectively).

**DISCUSSION**

The present study showed that when rice seeds were harvested from a field free of *A. besseyi*, a small proportion of them floated on water whereas the others sank soon after placing them carefully on the water surface. Because the rice plant originally was an inhabitant of the periphery of bodies of fresh water (Oka, 1988; Sato et al., 1991), forming light seeds with a reduced germination can be a means of long-distance dispersal. In contrast, intermediate and heavy seeds sank in water without floating and most of them germinated more rapidly than the light seeds. In most cases, light seeds have little or no endosperm compared with the intermediate and heavy seeds with fully developed endosperm. Light seeds required longer for the seminal roots to emerge although they extended coleoptiles at the same rate as intermediate and heavy seeds after rhizogenesis. Consequently, light seeds are regarded as inferior competitors for sunlight and soil nutrients compared with intermediate and heavy seeds. This indicates a trade-off between dispersal and competitive abilities in rice seeds through the difference in specific gravity.

*Aphelenchoides besseyi* harbored in light seeds have a greater chance to disperse a long distance than those in heavy seeds. When rice seeds disperse and germinate singly at a distance from the parent plant populations, the nematodes must live on a rice plant from which they emerged at the time of germination. Therefore, whether the rice seed germinates or not is crucial for survival and reproduction of the nematodes. From this consideration, the potential of reproduction for an individual nematode can be calculated from the product of the percentages of plant rhizogenesis and of nematode survival at the time of emergence from seed, which was estimated to be 0.159, 0.717, and 0.764 for individual nematodes in the light, intermediate, and heavy seeds, respectively. The nematodes harbored in the intermediate and heavy seeds may have a greater probability of reproduction than estimated because lots of rice seedlings are present near the seed that failed to protrude a seminal root. These observations suggested that a trade-off occurs between the dispersal and reproduction in the white tip nematode via the difference in the specific gravity of host rice seeds harboring the nematode.

An infestation of *A. besseyi* decreases rice grain yield by reducing the mean number of seeds per panicle and the mean endosperm size per seed in the field (Yoshii and Yamamoto, 1950; Todd and Atkins, 1958; Fukano, 1962). Our study confirmed that infection of *A. besseyi* on rice plants increased the proportion of light seeds. Intriguingly, light seeds from nematode-infected plants had a larger amount of endosperm, on average, than those from nematode-free plants. In addition, the mean degree of seed swelling increased as the nematode number in a seed increased for light seeds. Light seeds with fully developed endosperm are regarded as superior vehicles for long-distance dispersal of nematodes. Thus, light seeds with fully developed endosperm may be the result of host manipulation by the parasitic nematode.

What mechanism causes light seeds of nematode-infected rice plants to possess more endosperm than those of non-infected plants? What mechanism causes a positive correlation between nematode load and endosperm development in a light seed? We can postulate the actions of seeds on nematodes and those of nematodes on seeds. Temporal relationship between rice plant and nematode reproductions may help to select the most plausible hypothesis. *Aphelenchoides besseyi* enters rice florets before anthesis and begins reproduction (Huang and Huang, 1972). The nematode reproduction decreases drastically after anthesis and almost ceases two weeks after anthesis. The division of endosperm cells occurs between 3 and 10 days after anthesis (Hoshikawa, 1967a, b). The storage of starch in endosperm cells lasts between 4 and 35 days after anthesis (Hoshikawa, 1968). Thus, it is difficult to consider that a higher endosperm development resulted in more nematode reproduction prior to anhydrobiosis in the seed because the decrease in reproduction rate of the nematode starts before the endosperm development begins.

The proportion of grade-3 light seeds was greater in the infested field (13.2%) than in the non-infested field (1.6%), whereas the combined proportion of grade-4 intermediate and heavy seeds was smaller in the infested field (58.5%) than
in the non-infested field (70.6%). This may suggest that *A. besseyi* in seeds delays the development of the endosperm. However, the possibility that such a mechanism caused a positive correlation between nematode load and endosperm development in a seed was small, because the mechanism would produce a negative correlation between them.

The development of endosperm varies among rice seeds in a panicle of a rice plant without *A. besseyi* infection. For example, the floret that blossoms latest within a primary rachis branch produces a seed with a poor endosperm (Hoshikawa, 1989). After fertilization, florets on the primary rachis branches accumulate dry matter more rapidly and earlier than those on the secondary rachis branches (Nagato and Chaudhry, 1969). The former, referred to as “strong potential floret”, produces seeds with a well-developed endosperm while the latter, referred to as “weak potential floret”, produces seeds with a poor endosperm. A reduced photosynthesis by partial defoliation after heading causes weak potential florets to develop to empty seeds at a much greater rate than strong potential florets (Nagato, 1940). The nematode infection on rice plants reduces the foliage (Yoshi and Yamamoto, 1950) and may increase the proportion of grade 1 seeds on weak potential florets. If the nematodes in seeds stimulate the endosperm cells to accumulate hydrocarbons and proteins, many nematodes improve the endosperm development at a higher level than a few nematodes in seeds originating from weak potential florets, whereas the endosperm in seeds from strong potential florets attains a high developmental level irrespective of the number of nematodes harbored. The results of our study can be explained by such a process.

After rice seeds germinate, *A. besseyi* parasitizes the seedlings (Goto and Fukatsu, 1956; Tamura and Kegasawa, 1957; Fukano, 1962). The nematode also moves among rice plants at least until the developmental stage at which the sixth leaf emerges (Tamura and Kegasawa, 1958). Since the nematode is short-lived after emerging from seeds at 25°C (Tamura and Kegasawa, 1958), it is a short-distance disperser. Thus, the dispersal pattern of the nematode is considered to be a stratified diffusion in a combination of short-distance dispersal by the nematodes themselves with long-distance dispersal by means of light seeds floating on the water surface (Shigesada and Kawasaki, 1997).

This study, the results of which agreed with those of a nematode-inoculation experiment on potted rice plants (Togashi and Hoshino, 2003), was conducted in different paddy fields in different years. As weather conditions and management of paddy fields affect rice seed development and *A. besseyi* reproduction, the difference in characteristics of rice seeds and the nematode populations between the infested and non-infested fields must involve the effects of different fields and different years. Well-designed experiments in paddy fields are necessary to confirm the results of this study.

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