Journal of Nematology 32(3):303–308. 2000.

Effect of Water-Soaking and Air-Drying on Survival of *Aphelenchoides besseyi* in *Oryza sativa* Seeds

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Abstract: Several nematicides have been used to disinfest rice seeds contaminated with the white tip nematode, *Aphelenchoides besseyi*. We measured the efficacy of a standard disinfestation treatment that consists of soaking seeds in an aqueous emulsion of fenitrothion or fenthion and subsequently air-drying the seeds. We also measured the efficacy of the same treatments but without addition of nematicide to the soak water. Viability was monitored at key points during treatment. Our results showed that nematicides alone caused little nematode mortality within seeds. Most mortality occurred while seeds were being air-dried. Mortality caused by air-drying alone was 1.7 times greater than mortality caused by soaking seeds in water for 24 hours.

Key words: air-drying, *Aphelenchoides besseyi,* management, nematicides, *Oryza sativa,* rice seeds, water-soaking, white tip nematode.

The white tip nematode, *Aphelenchoides besseyi* Christie, is an ectoparasite of rice, *Oryza sativa* Linnaeus. The nematode enters rice florets, rapidly proliferates with a short generation time of about 10 days at 25 °C, then desiccates and survives anhydrobiotically as adults and fourth-stage juveniles within seeds beneath the glumes (Chiyonishio and Nakazawa, 1988; Hollis and Keoboonrueng, 1984; Huang and Chiang, 1975; Huang and Huang, 1972; Huang et al., 1972; Nandakumar et al., 1975). In Japan, rice is harvested in autumn and the seeds are stored until spring when they are sown in seedbeds in paddy fields, or else in seedling flats within shadehouses to be transplanted at regular intervals later into the paddy fields. Nematodes emerge from seeds soaked in water and attack seedlings (Tamura and Kegasawa, 1957, 1958).

Fukano (1962), Todd and Atkins (1958), and Yoshii and Yamamoto (1950a) demonstrated that infected plants are usually stunted. Leaves are dark green, considerably shortened, and usually twisted at the apex. Terminal leaf parts are often chlorotic. Panicles are reduced in length and produce fewer grains than in healthy plants. Unhulled grains are thinner, the percentage of abortive grains is increased, and husked rice grains have black spots. Consequently, infection with *A. besseyi* causes both yield loss and decreased quality of rice.

Management of *A. besseyi* in Japan usually involves soaking of rice seeds in aqueous emulsion or solution of nematicides such as fenitrothion (phosphorothioic acid O,O-dimethyl O-(3-methyl-4-nitrophenyl) ester), fenthion (phosphorothioic acid O,O-dimethyl O-[3-methyl-4-(methylthio)phenyl] ester), and cartap (carbamothioc acid *S, S′*-[2-(dimethylamino)-1,3-propanediyl] ester) for 24 hours. After soaking, the seeds are air-dried for a few days until they are soaked again in water for sprouting. Even when rice seeds are treated with nematicides, the infestation level of rice plants has been observed to vary greatly between paddy fields in restricted areas of Hiroshima Prefecture (pers. obs.). This led us to question the efficacy of nematicide treatment. Nematicide efficacy for seed disinfestation has been estimated based on several factors: mortality of nematodes within treated rice seeds (Chiyonishio and Nakazawa, 1988), percentage of diseased tillers in paddy fields (Fujimoto and Yamaguchi, 1971), and nematode density within rice seeds reproduced from treated seeds (Fujimoto and Yamaguchi, 1971). Chiyonishio and Nakazawa (1988) showed that nematode mortality was similar for aqueous seed treatments with and without nematicide. However, no

Received for publication 7 December 1999.

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The authors acknowledge Robert I. Bolla and Kazuyoshi Futai for reviewing the manuscript.

This paper was edited by A. F. Robinson.

study has determined when mortality occurs during seed treatment, i.e., during soaking or during subsequent air-drying of the seeds. The objective of this study was to determine the independent effects of soaking and subsequent air-drying of seeds on nematode mortality.

Materials and Methods

Rice seeds: Rice seeds were collected on 23 October 1998 from two potted rice plants in Higashi-Hiroshima City, Hiroshima Prefecture (Sample A). Other seeds were sampled from 30 representative hills of rice plants in two paddy fields at Kinosho-Nishi (Sample B) and at Urasaki (Sample C) in Onomichi City, Hiroshima Prefecture, on 29 September 1998. Soon after sampling, seeds were stored in the dark at 4 ± 1 °C and were 3–4 months old when used. Sample A was used for Experiment 1 and B for a repeat of Experiment 1. Sample C was used for Experiment 2. All seeds used were from cultivar Hi-no-Hikari. Both ripe and abortive seeds were included in the samples.

Viability of nematodes within seeds: In all experiments, nematode mortality within dry and wet rice seeds was determined using the method of Hoshino and Togashi (1999). Individual seeds were bisected longitudinally with small pruning scissors and then placed into single, translucent pipet tips (7 cm long with 1.0-mm and 7.4-mm top and bottom i.d., respectively, Quality Scientific Plastics, #111). Tips containing a split seed were then singly placed upright in glass vials (6.5-ml capacity) containing 6 ml water at 25 ± 0.1 °C. Tips containing split seeds were incubated in the dark for 4 hours. Water was transferred from vials to Syracuse watch glasses where living and dead nematodes were counted. Additionally, split seeds were removed from the tips and dissected in water to determine the number of nematodes present. Nematodes that did not move when prodded with a needle were considered dead.

Experiment 1: Four hundred seeds of Sample A were divided into four equal groups. Group 1 was soaked in 300 ml of an aqueous emulsion (1:1,000) of fenitrothion (50% e.c.) and group 2 in the same concentration of fenthion e.c. After 24 hours at 25 ± 0.1 °C, the supernatant was poured through a 20-µm-pore sieve and nematodes retained on the sieve were collected and counted. Seeds were then washed with three additional 300-ml volumes of water from which nematodes were collected and counted. Seeds were then air-dried for 24 hours at 25 ± 0.5 °C, and viability of nematodes remaining in the dry rice seeds was determined as described previously. Seed group 3 was soaked in water but without nematicide at 25 ± 0.1 °C for 24 hours and then air-dried at 25 ± 0.5 °C for 24 hours in the same way as groups 1 and 2. Numbers of nematodes emerging from seeds and viability of nematodes remaining in seeds were determined as before. Group 4 (control) was examined for nematode viability just after removing seeds from storage.

In the repeat of Experiment 1, the following treatment was added. Seeds were soaked in an aqueous emulsion (1: 1,000) of fenitrothion (50% e.c.) for 72 hours at 25 ± 0.1 °C, then washed three times with water and air-dried for 24 hours at 25 ± 0.5 °C. Nematode mortality in air-dried seed was assessed as in the other treatments.

Experiment 2: To measure independently the nematode mortality caused by soaking seeds in water and the mortality caused by subsequent air-drying, 400 randomly selected seeds were divided into four equal groups. The percentages of nematodes alive and dead within rice seeds in group 1 as untreated control were estimated immediately after removing seeds from storage. Group 2 was soaked for 24 hours in 300 ml water at 25 ± 0.1 °C and then washed three times with 300 ml water. After that, nematodes that had emerged from seeds were counted and the viability of nematodes remaining in seeds was determined without drying seeds. Group 3 was soaked in water in the same way as group 2, and then air-dried for 24 hours at 25 ± 0.5 °C. Viability of nematodes remaining in seeds was determined. Group 4 was held in air at 25 ± 0.5 °C for 48 hours, at which time the percentage of

nematodes alive was determined, to measure mortality due solely to warming from 4 °C to 25 °C.

Statistical methods: Contingency tables made by numbers of surviving and dead nematodes were used for comparison of mortality among treatments. When a significant difference was identified among treatments, Fisher's exact probability was calculated to compare mortality between any two treatments. Significant level was arranged by the Bonferroni method (Yamamura, 1993). Additionally, corrected mortality was calculated as described by Abbott (1925) to exclude the initial mortality in samples, i.e.,

$$\frac{(\%\ \text{survival for untreated control}) - (\%\ \text{survival after treatment})}{(\%\ \text{survival for untreated control})} \times 100\ (\%),$$

where % survival is obtained by subtracting % mortality from 100.

Results

Most nematodes remained within rice seeds when soaked 24 hours in water or nematicide emulsion (Tables 1, 2, 3). In Experiment 1, the mortality of *A. besseyi* was significantly greater in nematicide-treated and air-dried groups and in groups that had been water-soaked and air-dried than in the untreated control (Tables 1, 2). Mortality for nematicide-treated and air-dried seeds, however, was not significantly greater than mortality for seeds that were soaked in water and air-dried (Tables 1, 2).

In Experiment 2, mortality of *A. besseyi* was greater when seeds were both water-soaked and air-dried than when only water-soaked (Table 3). Mortality was lowest in the warm air control treatment (Table 3). Correcting mortality values to eliminate the effect of initial mortality at the start of the experiment made treatment differences more evident (Table 3). Since a portion of the survivors of water-soaking of seeds were killed by subsequent air-drying, net mortality from the combined treatments (m_3) must satisfy the following equation,

$$m_3 = 1 - (1 - m_1)(1 - m_2),$$

where m_1 and m_2 express mortalities caused by water-soaking and air-drying, respectively. Table 3 gives m_1 and m_3 values as 0.340 and 0.724, respectively. Consequently, mortality due to air-drying (m_2) is estimated to be 0.582. This indicates that mortality caused by air-drying is 1.7 times greater than that by water-soaking.

Discussion

The reason for nematode mortality during seed-soaking is unknown. In rice seeds, *A. besseyi* is distributed in the space between the inner surface of glumes (lemma and palea) and epicarp, especially in the basal part of a seed (Huang and Huang, 1972). When a rice seed is soaked in water, it ab-

Table 1. Net mortality of *Aphelenchoides besseyi* when infested rice (*Oryza sativa*) seeds previously stored at 4 °C were soaked for 24 hours at 25 °C in water or in a 0.1% aqueous emulsion of fenitrothion or fenthion, and then air-dried for 24 hours at 25 °C.

Seed treatment[a]	No. of surviving nematodes[b]	No. of dead nematodes[b]	Mortality (%)[c]	Corrected mortality (%)[d]
Soaked in fenitrothion emulsion followed by air-drying	12 (0)	52 (2)	81.3a	79.5
Soaked in fenthion emulsion followed by air-drying	9 (0)	42 (8)	82.4a	80.7
Soaked in water followed by air-drying	19 (2)	89 (0)	82.4a	80.7
Untreated control	167	16	8.7b	0.0

[a] One hundred rice seeds were used for each treatment.
[b] Numbers in parentheses are nematodes emerging from rice seeds into nematicide emulsion or water during soaking. Such nematodes are included in the numbers of nematodes surviving or dead nematodes.
[c] Mortality of nematodes was different among the four treatments at the 5% level (2 × 4 contingency table). Different letters indicate significant differences at the 5% probability level according to Bonferroni's method (Fisher's exact probability).
[d] Corrected mortality is obtained from mortality modified by Abbott's method, i.e., (% survival for untreated control) − (% survival after treatment) / (% survival for untreated control) × 100 (%), where % survival is obtained by subtracting % mortality from 100.

TABLE 2. Net mortality of *Aphelenchoides besseyi* when infested rice (*Oryza sativa*) seeds previously stored at 4 °C were soaked for 24 or 72 hours at 25 °C in water or in a 0.1% aqueous emulsion of fenitrothion or fenthion, and then air-dried for 24 hours at 25 °C.

Seed treatment[a]	No. of surviving nematodes[b]	No. of dead nematodes[b]	Mortality (%)[c]	Corrected mortality (%)[d]
Soaked in fenitrothion emulsion for 24 hours followed by air-drying	19 (0)	51 (6)	72.9a	68.7
Soaked in fenitrothion emulsion for 72 hours followed by air-drying	14 (0)	80 (3)	85.1a	82.8
Soaked in fenthion emulsion for 24 hours followed by air-drying	10 (0)	57 (3)	85.1a	82.8
Soaked in water for 24 hours followed by air-drying	28 (6)	38 (0)	57.6a	51.1
Untreated control	66	10	13.2b	0.0

[a] One hundred rice seeds were used for each treatment.
[b] Numbers in parentheses are nematodes emerging from rice seeds into nematicide emulsion or water during soaking. Such nematodes are included in the numbers of nematodes surviving or dead nematodes.
[c] Mortality of nematodes was different among the five treatments at the 5% level (2 × 5 contingency table). Different letters indicate significant differences at the 5% probability level according to Bonferroni's method (Fisher's exact probability).
[d] Corrected mortality is obtained from mortality modified by Abbott's method, i.e., (% survival for untreated control) – (% survival after treatment) / (% survival for untreated control) × 100 (%), where % survival is obtained by subtracting % mortality from 100.

sorbs water and swells quickly (Morinaga and Tajiri, 1941; Hoshikawa, 1975). Swelling may increase the pressure within rice seeds. Respiration also rapidly increases (Takahashi, 1955). Thus it can be proposed that some nematodes are killed and others are stressed by high pressure, CO_2, and low oxygen content within imbibed seeds. If so, then respiratory stress may contribute to nematode mortality during air-drying after soaking. Another plausible explanation for mortality during air-drying is that freshly hydrated nematodes are physiologically unprepared to survive desiccation. Adults and juveniles of *A. besseyi* in rice seeds are, in most cases, in a dormant (anhydrobiotic) state (Huang and Huang, 1972; Nandakumar et al., 1975) and remain viable in dry-stored seeds for up to 3 years (Yoshii and Yamamoto, 1950b). Soaking of seeds in water induces the nematodes to terminate dormancy (Huang and Chiang, 1975; Huang and Huang, 1974; Nandakumar et al., 1975). This process is sufficiently rapid for 50% of the nematodes within rice seeds to emerge when incubated in water at 25 °C for 78–88 hours (Tamura and Kegasawa, 1957). More than 90% of anhydrobiotic nematodes revive 3 hours after absorbing water (Chiyonishio and Nakazawa, 1988). In our experiments, this quick response may have been responsible for the fatal effect of air-drying

TABLE 3. Net mortality of *Aphelenchoides besseyi* when infested rice (*Oryza sativa*) seeds previously stored at 4 °C were held in air at 25 °C for 48 hours before assay, soaked in water for 24 hours before assay, or both soaked in water 24 hours and air-dried 24 hours before assay.

Seed treatment[a]	No. of surviving nematodes[b]	No. of dead nematodes[b]	Proportion of survivors (%)[c]	Corrected mortality (%)[d]
Soaked in water at 25 °C but not air-dried	179 (1)	182 (0)	49.6a	34.0
Soaked in water and air-dried at 25 °C	51 (0)	195 (0)	20.7b	72.4
Held in air at 25 °C	301	126	70.5c	6.1
Untreated control	410	136	75.1c	0.0

[a] One hundred rice seeds were used for each treatment.
[b] Numbers in parentheses are nematodes emerging from rice seeds into water during soaking. Such nematodes are included in the numbers of nematodes surviving or dead nematodes.
[c] Proportion of living nematodes was different among the four treatments at the 5% level (2 × 4 contingency table). Different letters indicate significant differences at the 5% probability level according to Bonferroni's method (Fisher's exact probability).
[d] Corrected mortality is obtained from mortality modified by Abbott's method, i.e., (% survival for untreated control) – (% survival after treatment) / (% survival for untreated control) × 100 (%).

after water-soaking. This reasoning also explains Sivakumar's (1987) observation that nematode mortality from heating rice seeds in the sun is enhanced by pre-soaking in water.

Nematode mortality from soaking and drying seeds is sometimes reported to match that from fenitrothion or fenthion treatment (Chiyonishio and Nakazawa, 1988). When Chiyonishio and Nakazawa seeds were soaked in water again to assess the effect of nematicides, all living nematodes appeared stressed because the nematodes were curled or kinked and moved slowly or intermittently. We considered that such symptoms were due to nematicide residue on seed surfaces, because seeds were not washed before air-drying (Chiyonishio and Nakazawa, 1988). In our case, because rice seeds were washed with water after soaking of seeds in nematicide emulsion, we assume residual nematicide was removed from the surface of the rice seeds. Absence of nematicide residue would be consistent with our inability to find nematodes exhibiting symptoms of physiological stress after the seeds were resoaked in water. If rice seeds are not washed after nematicide treatment, residual nematicides may kill nematodes as they emerge from seeds after sowing. If so, the amount of water used for sprouting the rice seeds is important because it determines the concentration of nematicide to which emerging nematodes are exposed. Erratic nematicide dilution would explain the variation in nematicide efficacy that we typically observe.

We finally note that, to manage *A. besseyi,* it sometimes is recommended to apply nematicides to seedling starter flats. Our results suggest that a combination of water-soaking plus air-drying of seeds followed by nematicide application to seedlings before transplanting may be the most effective practice for control of *A. besseyi* in rice because a low percentage of *A. besseyi* survive water-soaking and air-drying.

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