Biological Control of Soil Pests by Mixed Application of Entomopathogenic and Fungivorous Nematodes¹

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Abstract: In greenhouse experiments, massive application of the fungivorous nematode, Aphelenchus avenae, in summer at 26-33 C (1×10^5 nematodes/500 cm³ autoclaved soil) or in autumn at 18-23 C (5×10^4 nematodes/500 cm³ autoclaved soil) suppressed pre-emergence damping-off of cucumber seedlings due to Rhizoctonia solani AG-4 by 67% or 87%, respectively. Application of 2×10^5 A. avenae to sterilized soil infested with R. solani caused leafminer-like symptom on the cotyledons, which did not occur in mixed inoculations with the entomopathogenic nematode, Steinernema carpocapsae. When 1×10^6 A. avenae were applied 3 days before inoculation with 100 Meloidogyne incognita juveniles, gall numbers on tomato roots were reduced to 50% of controls. Gall numbers also were suppressed by S. carpocapsae (str. All). Reduction in gall numbers was no greater with mixed application of A. avenae and S. carpocapsae than with application of single species, even though twice the number of nematodes were added in the former case. These nematodes were positively attracted to tomato root tips. Aphelenchus avenae suppressed infection of the turnip moth, Agrotis segetum, but not the common cutworm, Spodoptera litura, by S. carpocapsae.

Key words: Aphelenchus avenae, biological control, Meloidogyne incognita, Rhizoctonia solani, Steinernema carpocapsae.

The entomopathogenic nematode Steinernema carpocapsae is a promising biological agent for a broad range of soil-inhabiting insect pests. Practical control of insect pests by these nematodes was demonstrated on common cutworm, Spodoptera litura, and turnip moth, Agrotis segetum, under greenhouse and field conditions (16). Inundative soil application of these nematodes also affected the population densities of native nematodes; free-living nematodes increased, whereas plant-parasitic nematodes decreased (15-17). Bird and Bird (4) clearly demonstrated the same phenomena with Meloidogyne javanica and Steinernema glaseri. They suggested that penetration of M. javanica into roots was suppressed due to competition for space with S. glaseri, which also was attracted to the tomato roots.

The fungivorous nematode Aphelenchus avenae is ubiquitous in soil. The nematode can be propagated on axenically cultured tissues of carrot and on tobacco and tomato callus; therefore it was regarded as plantparasitic (2). On the other hand, *A. avenae* failed to parasitize many plants under greenhouse conditions (22). Because *A. avenae* lives on many kinds of soil fungi (24), trials have been conducted to see if this nematode controlled soil-borne pathogenic fungi. The nematode appears to have promise as a biological control agent of certain pathogenic fungi (1,3,6,7,9,13).

This paper reports the efficacy of A. avenae as a biological control agent against pre-emergence damping-off of cucumber caused by *Rhizoctonia solani*, the suppressive effect of A. avenae and (or) S. carpocapsae as nonparasitic, nonpredacious antagonists against M. incognita juveniles, and the possibility of simultaneous control of fungal and insect pests and plant-parasitic nematodes by mixed application of these nematodes.

MATERIALS AND METHODS

Aphelenchus avenae from a spinach field at Kagoshima, Japan, was cultivated on Botrytis cinerea growing on wheat bran at 25 C (10). About 30 days after incubation, fourth-stage and adult nematodes were collected by sieving and used in the exper-

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iments. Steinernema carpocapsae (str. All) was propagated on chicken offal medium for 2 months at 25 C (19). The infective juveniles were used.

Egg masses of *Meloidogyne incognita* from severely galled roots of tomato were surface sterilized with 1% NaOCl and rinsed in sterilized distilled water three times. Second-stage juveniles (J2) hatched from egg masses were used as inocula.

A virulent strain of *Rhizoctonia solani* AG-4 was grown on autoclaved wheat bran medium (10 g bran plus 15 ml water) at 25 C for 5 days. The freshly colonized medium was used as inoculum for the fungus.

Spodoptera litura and Agrotis segetum were reared at 25 C on artificial diet (20) under a photoperiod of 16 hours light and 8 hours dark. Sixth-instar larvae of the insects were used for experiments.

Control of pre-emergence damping-off by A. avenae: Autoclaved sandy loam soil (500 cm³) containing 1 g inoculum of R. solani plus 0, 1×10^4 , 3×10^4 , 6×10^4 , 1×10^5 , or 2×10^5 A. avenae was added to each of five replicate clay pots. Control pots received neither fungus nor nematodes. The pots were placed in a greenhouse. After 2 weeks, three cucumber (*Cucumis sativus* L. cv. Soeyoh) seeds were sown in each pot. Four weeks later, the surviving plants in each pot were counted. The experiment was performed once in late August (26–33 C) and again in October (18–23 C).

Effect of mixed inoculation with A. avenae and (or) S. carpocapsae and (or) M. incognita on pre-emergence damping-off: The following soil treatments were established: R. solani: R. solani and M. incognita; R. solani and A. avenae; R. solani, A. avenae, and S. carpocapsae; R. solani, A. avenae, S. carpocapsae, and M. incognita; and a sterilized soil control. Inoculum levels were 1 g R. solani, 2 \times 10⁵ A. avenae, 5 \times 10⁵ S. carpocapsae, and 1×10^2 M. incognita per pot. Procedures for preparation of pots, sowing of seeds, and growing of plants were the same as for the previous experiment. Pots were maintained at 18-23 C, and seedling survival and condition were assessed 30 days after sowing. The experiment was performed once.

Aphelenchus avenae and S. carpocapsae as antagonists of M. incognita: One tomato seedling (7-8 cm high) was planted in each of 200 plastic pots containing 250 cm³ autoclaved sandy loam soil. At the same time, two glass tubes (0.5 cm d, 5 cm long) were inserted in the soil 2 cm deep, on opposite sides and 2 cm from the plant stem. Thirteen days later, 100 J2 of M. incognita in 10 ml water (5 ml per tube) were added. A range of A. avenae and (or) S. carpocapsae $(10^2 \text{ to } 5 \times 10^6 \text{ in } 10 \text{ ml water})$ was added to the tubes either 3 days before, at the same time as, or 3 days after the addition of M. incognita. Ten replications of the following four treatments were maintained: M. incognita alone as controls; M. incognita and A. avenae; M. incognita and S. carpocapsae; and M. incognita, A. avenae, and S. carpocapsae. For the last treatment, the total number of nematodes was twice that for other treatments at each dose level. The roots of five replicate plants per treatment were stained (5) and galls were counted 15 and 30 days after inoculation with M. incognita.

Orientation of S. carpocapsae and A. avenae to tomato root tips: Tomato seeds were sonicated in 2.5% NaOCl for 5 minutes, then washed several times with sterile water before placement on 0.75% agar with Gamborg's B-5 medium (14) in petri dishes (9 cm d). Seeds were placed in light after germination. When seedlings had a radicle ca. 3 cm long and cotyledons were green, ca. 5×10^3 Å. avenue and (or) 5×10^3 S. car*pocapsae* concentrated on a 0.45-µm-pore membrane filter were brushed onto an inoculation zone (1 cm d) 1 cm from the root tip (12). Five replications were maintained at room temperature for each treatment. The behavior of the nematodes was qualitatively assessed for 1 hour. This experiment was conducted four times.

Effect of A. avenae on infectivity of S. carpocapsae: A nematode suspension (0.4 ml) containing 50 S. carpocapsae and 0, 50, 500, or 5,000 A. avenae was added to a circular filter paper (5 cm d) in a plastic petri dish (5.3 cm d). In an additional treatment, the suspension contained no *S. carpocapsae* and 5,000 *A. avenae*. One last-instar larva of *S. litura* or *A. segetum* was placed in each dish and incubated at 25 C. There were 20 dishes per level of *S. carpocapsae--A. avenae*. Mortality of insects was determined by touch response at 8-hour intervals from 24 to 120 hours. The experiment was performed once.

Effect of S. carpocapsae and A. avenae on pre-emergence damping-off: A nematode suspension (50 ml) containing $1 \times 10^4 A$. ave*nae* and 0, 1×10^4 , 1×10^5 , or 1×10^6 S. carpocapsae was sprayed on the colonized medium (1 g wheat bran) of R. solani, which was evenly spread on the top of 450 cm³ autoclaved sandy loam soil in a clay pot. Immediately after the addition of the nematodes, 50 cm³ soil was layered on top so that the final volume was 500 cm³. Pots were wrapped in polythene bags to prevent desiccation and placed in a greenhouse at 18-25 C. There were five replications per level of A. avenae-S. carpocapsae. Emergence of seedlings was followed for 10 days. This experiment was performed once.

RESULTS

Control of pre-emergence damping-off by A. avenae: Pre-emergence damping-off of cucumber due to R. solani was reduced significantly by A. avenae (Table 1). No postemergence damping-off was observed during the experiment. In summer, when the greenhouse temperature ranged from 26 to 33 C, no seedlings emerged in soil inoculated with R. solani alone, whereas 33-80% emerged with application of A. avenae. In fall, when the temperature ranged from 18 to 23 C, a low percentage of seedlings emerged in soil inoculated with R. solani alone. Application of nematodes again increased emergence; however, inoculation with 2×10^5 or more nematodes per pot caused a leafminer-like symptom on the cotyledons of young cucumber seedlings and a slight decrease in emergence. No retardation in the further growth of these plants was observed. The symptom on the TABLE 1.Suppression of pre-emergence damp-ing-off of cucumber seedlings due to Rhizoctonia solaniAG-4 by Aphelenchus avenae.

Inoculum level†	Seedling emergence (%)‡	Seedlings (N) alive/dead		
Ex	periment 1 (26–33	C)		
0	0 ± 0	0/15 a		
1×10^{4}	33 ± 26	5/10 b		
3×10^{4}	33 ± 26	5/10 b		
6×10^{4}	40 ± 45	6/9 b		
1×10^{5}	66 ± 40	10/5 b		
2×10^{5}	80 ± 8	12/3 c		
Control	100 ± 0	15/0 с		
Ex	periment 2 (18–23	C)		
0	13 ± 8	2/13 a		
5×10^4	86 ± 8	13/2 bc		
1×10^{5}	86 ± 8	13/2 bc		
2×10^{5}	80 ± 8	12/3 bc		
5×10^5	66 ± 26	10/5 b		
1×10^{6}	73 ± 5	11/4 bc		
Control	100 ± 0	15/0 с		

Number of alive/dead followed by the same letter in each experiment are not different (P = 0.05).

 \dagger Numbers of A. avenae added per pot containing 1 g wheat bran medium colonized by R. solani. Control pots received neither nematodes nor fungus.

 \ddagger Values are means \pm SD of five replicate pots.

cotyledons did not occur in the summer experiment.

Effect of A. avenae and (or) S. carpocapsae and (or) M. incognita on pre-emergence damping-off: Cucumber seedlings failed to emerge in soil inoculated with R. solani alone or R. solani plus M. incognita. All seedlings emerged in soil inoculated with R. solani plus A. avenae, R. solani plus A. avenae and S. carpocapsae, and in uninoculated soil. In soil inoculated with R. solani plus A. avenae, S. carpocapsae, and M. incognita, 80% of the seedlings emerged. A leafminer-like symptom of the cotyledons was caused by inoculation with R. solani and A. avenae but was not observed in the other treatments. Seedling height was less (P = 0.05) in the presence of R. solani and A. avenae (23.1 \pm 1.3 cm) than in the uninoculated control $(26.3 \pm 1.3 \text{ cm})$. Seedling height in the other treatments was not different from the control.

Aphelenchus avenae and S. carpocapsae as antagonists of M. incognita: Numbers of M. incognita-induced galls in tomato roots decreased with application of A. avenae or S.

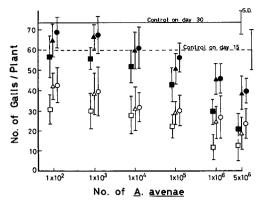


FIG. 1. Numbers of *Meloidogyne incognita*-induced galls on tomato as affected by *Aphelenchus avenae*. Aphelenchus avenae was applied 3 days before (square), simultaneously with (triangle), or 3 days after (circle) inoculation with 100 *M. incognita* second-stage juveniles. White and black symbols indicate the number (mean \pm SD of five replicates) of galls per plant 15 and 30 days, respectively, after inoculation with *M. incognita*. Horizontal lines indicate numbers of galls after 15 and 30 days on plants inoculated with *M. incognita* only.

carpocapsae (Figs. 1, 2). Application of these nematodes 3 days before M. incognita tended to reduce gall formation more than did simultaneous application or application 3 days after; however, the timing of inoculation was not significant (P > 0.05), except at day 30 and with application of 1×10^5 or more nematodes 3 days before addition of M. incognita. Although there tended to be more reduction of gall formation with A. avenae than with S. carpocapsae, the difference was not significant (P > 0.05). Despite the twofold increase in total nematodes at each dosage level, mixed application of these nematodes had less effect than did the single application of either nematode (Fig. 3).

Orientation of A. avenae and S. carpocapsae to root tips: A. avenae moved rapidly to the root tips on agar plates and swarmed around the meristematic region (Fig. 4). In contrast, 40-50% of S. carpocapsae juveniles stayed in the inoculation zone and became motionless within 1 hour after inoculation; the remainder moved to the root tips and to the differentiation zone. When S. carpocapsae was placed on the root tip, very few nematodes dispersed beyond the

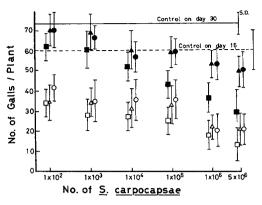


FIG. 2. Numbers of *Meloidogyne incognita*-induced galls on tomato as affected by *Steinernema carpocapsae*. *Steinernema carpocapsae* was applied 3 days before (square), simultaneously with (triangle), or 3 days after (circle) inoculation with 100 *M. incognita* second-stage juveniles. White and black symbols indicate the number (mean \pm SD of five replicates) of galls per plant 15 and 30 days, respectively, after inoculation with *M. incognita*. Horizontal lines indicate numbers of galls after 15 and 30 days on plants inoculated with *M. incognita* only.

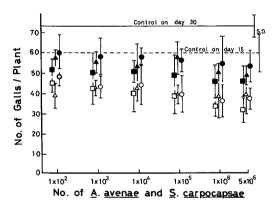


FIG. 3. Numbers of Meloidogyne incognita-induced galls on tomato as affected by mixed application of Aphelenchus avenae and Steinernema carpocapsae. Each nematode species was applied with the number of individuals on abscissa. Nematodes were applied 3 days before (square), simultaneously with (triangle), or 3 days after (circle) inoculation with 100 secondstage juveniles of M. incognita. White and black symbols indicate the number (mean \pm SD of five replicates) of galls per plant 15 and 30 days, respectively, after inoculation with M. incognita. Horizontal lines indicate numbers of galls after 15 and 30 days on plants inoculated with M. incognita only. Regressions for day 30 and pre-application data were Y = -7.6X+ 62.6 (r = -0.9482) for A. avenae, Y = -6.2X +61.2 (r = -0.9731) for S. carpocapsae, and Y = -1.1X+ 51.8 (r = -0.7527) for the mixture, where Y is the number of galls per plant and X is the number of applied nematodes on logarithmic scale.

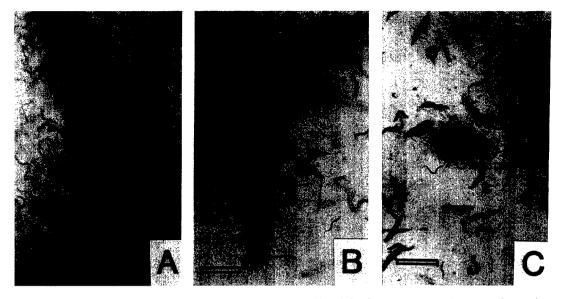


FIG. 4. Attraction of nematodes to tomato root on agar. A) Aphelenchus avenae swarming around root tip. B) Steinernema carpocapsae clinging onto root surface. C) Clusters of S. carpocapsae induced by mixed inoculation with A. avenae. Scale bars = 1 mm.

inoculation zone. When added together, A. avenae did not aggregate at the root tip but dispersed in the agar plate; S. carpocapsae formed clusters in the inoculation zone.

Infectivity of S. carpocapsae as affected by A. avenae: Infection of S. litura by S. carpocapsae was unaffected by A. avenae, whereas infection of A. segetum was decreased (P =0.05) by high number of A. avenae (Table 2).

Effect of S. carpocapsae and A. avenae on pre-emergence damping-off: No seedlings of cucumber emerged in the soil inoculated with R. solani alone. Application of 1×10^4 A. avenae without S. carpocapsae resulted in 100% emergence 11 days after sowing. Addition of *S. carpocapsae* resulted in 100% emergence on days 8, 8, and 7 for 1×10^4 , 1×10^5 , and 1×10^6 *S. carpocapsae*, respectively. In the latter two treatments, emergence occurred sooner (P = 0.05) than with *A. avenae* alone.

DISCUSSION

In the present study, A. avenae substantially suppressed R. solani-induced damping-off of cucumber. Suppression was greater at 18-23 C than at 26-33 C. We suggest that this is due to the higher temperature optimum for R. solani (21) than

TABLE 2. Infection (% mortality) of Spodoptera litura and Agrotis segetum by Steinernema carpocapsae (str. All) as affected by Aphelenchus avenae.

Inoculum/insect		S. litura		A. segetum					
S. carpo- capsae	+	A. avenae	48 hr	72 hr	96 hr	48 hr	72 hr	96 hr	120 hr
0	+	0	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Ō	+	5.000	0 a	0 a	0 a	0 a	0 a	0 a	0 a
50	+	0	85 b	95 b	100 b	50 b	80 d	90 с	90 с
50	+	50	95 b	100 c		40 b	60 cd	80 c	90 c
50	+	500	95 b	100 c		10 a	40 bc	60 bc	60 bc
50	+	5,000	95 b	100 c		0 a	20 b	40 Ь	60 bc

Each treatment had 20 petri dishes each containing one last-instar larva with artificial food. Values followed by the same letter within a column are not significantly different (P = 0.05) based on χ^2 -test.

for A. avenae. Because the experiments were not repeated or conducted at the same time, additional research is needed on the effect of temperature. A leafminer-like symptom on cotyledons of cucumber seedlings was induced by massive application of A. avenae in sterilized soil (7) and by combined inoculation of A. avenae and R. solani in the present study. This symptom did not occur when both A. avenae and S. carpocapsae were added to R. solani-infested soil. Although A. avenae appears to have some detrimental effects on the host plant, we have not observed substantial damage to plants under field conditions (unpubl.).

That plant-parasitic nematodes were suppressed by inundative application of entomopathogenic nematodes to soil (4,15,17) was confirmed by the present experiments. Bird and Bird (4) reported the attraction of S. glaseri to tomato root tips on agar plates and a decrease in the numbers and reproductive capacity of M. javanica when S. glaseri was applied in pot experiments. They suggested that S. glaseri was attracted to CO₂ from the root tips and that M. javanica juveniles failed to compete for root surface required for penetration because S. glaseri is much larger and more active than M. javanica. We also observed that A. avenae and S. carpocapsae were positively attracted to, and aggregated around, root tips. Both A. avenae and S. carpocapsae suppressed gall number on tomato roots. Mixed application of these nematodes was expected to cause greater reduction in gall formation than single application, but the opposite was true. This suggests an antagonistic interaction between A. avenae and S. carpocapsae. The mechanism is unknown, but contact stimulation may cause nematodes to disperse as appeared to occur when both nematodes were added to roots on agar plates in the present study. Infection of A. segetum but not S. litura by S. carpocapsae was decreased by A. avenae, perhaps because A. segetum is more resistant than is S. litura to S. carpocapsae (23). In contrast, S. carpocapsae did not lessen the suppression of pre-emergence damping-off by A. avenae.

Commercial application of A. avenae at the rates used in the present experiments will be possible. Aphelenchus avenae can be mass produced on industrial by-products such as wheat bran, beet pulp, bagasse, brewer's grain (malt barley), or combinations of these materials (8,10). One kilogram (dry weight) of these materials will produce 200-300 million nematodes (8). In a field trial, complete control of R. solani-induced damping-off of spinach was achieved by application of A. avenae together with its culture substrates (beet pulp plus brewer's grain) as a basal dressing at the rate of 1 kg/2 m of plant row (18). Massive application of entomopathogenic nematodes $(0.5-1 \text{ million}/\text{m}^2)$ is also commercially possible.

Although further basic studies are needed on the interaction between entomopathogenic and fungivorous nematodes, mixed application of these beneficial nematodes may provide simultaneous control of soil insect pests, plant-parasitic fungi, and plant-parasitic nematodes. These nematodes may also be used with certain chemical pesticides (11,16).

LITERATURE CITED

1. Barker, K. R. 1964. On the disease reduction and reproduction of the nematode *Aphelenchus avenae* on isolates of *Rhizoctonia solani*. Plant Disease Reporter 48:428-432.

2. Barker, K. R., and H. M. Darling. 1965. Reproduction of *Aphelenchus avenae* on plant tissue in culture. Nematologica 11:162–166.

3. Barnes, C. L., C. Russel, C. D. Foster, and R. W. McNew. 1981. *Aphelenchus avenae*, a potential biological control agent for root rot fungi. Plant Disease 65:423-424.

4. Bird, A. F., and J. Bird. 1986. Observations on the use of insect parasitic nematodes as a means of biological control of root-knot nematodes. International Journal for Parasitology 16:511-516.

5. Byrd, D. W., Jr., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15:142-143.

6. Caubel, G., B. Jouan, P. Queneherve, and J. Radwan. 1981. Control of *Rhizoctonia solani* Khün, parasitic on cotton, with the nematode *Aphelenchus avenae* Bastian. Revue de Nématologie 4:93–98.

7. Choi, D.-R., N. Ishibashi, and K. Tanaka. 1988. Possible integrated control of soil insect pests, soilborne diseases, and plant nematodes by mixed application of fungivorous and entomogenous nematodes. Bulletin of the Faculty of Agriculture, Saga University 65:27-35. (In Japanese; English summary.)

8. Choi, D.-R., and N. Ishibashi. 1989. Propagation of *Aphelenchus avenae* isolates on six species of fungi and five substrates. Japanese Journal of Nematology 19:13-17.

9. Choo, P. H., and R. H. Estey. 1985. Control of the damping-off disease of pea by *Aphelenchus avenae*. Indian Journal of Nematology 15:1-4.

10. Evans, A. A. F. 1970. Mass culture of mycophagous nematodes. Journal of Nematology 2:99-100.

11. Fedorko, A., M. Kamionek, J. Kozlowska, and M. Mianowska. 1977. The effects of some insecticides on nematodes of different ecological groups. Polish Ecological Studies 3:89–93.

12. Gaugler, R., J. F. Campbell, and T. R. Mc-Guire. 1989. Selection for host-finding in *Steinernema feltiae*. Journal of Invertebrate Pathology 54:363–372.

13. Gupta, M. C. 1986. Biological control of Fusarium moniliforme Sheldon and Pythium butleri Subramanian by Aphelenchus avenae in chitin and cellulose-amended soils. Soil Biology and Biochemistry 18: 327-329.

14. Huettel, R. N., and R. V. Rebois. 1985. Culturing plant parasitic nematodes using root explants. Pp. 155–158 *in* B. M. Zuckerman, W. F. Mai, and M. B. Harrison, eds. Plant nematology laboratory manual. The University of Massachusetts Agricultural Experiment Station, Amherst, MA.

15. Ishibashi, N., and E. Kondo. 1986. Steinernema feltiae (DD-136) and S. glaseri: Persistence in soil and bark compost and their influence on native nema-todes. Journal of Nematology 18:310-316.

16. Ishibashi, N. 1987. Integrated control of insects/nematodes by mixing application of steinernematid nematodes with chemicals. Pp.165–176 in N. Ishibashi, ed. Recent advances in biological control of insect pests by entomogenous nematodes in Japan. Research Project on Biological Control, Ministry of Education, Japan. (In Japanese; English summary.) Saga University, Saga 840, Japan. 17. Ishibashi, N., and E. Kondo. 1987. Dynamics of entomogenous nematodes, *Steinernema feltiae*, applied to soil with and without nematicide treatment. Journal of Nematology 19:404-412.

18. Ishibashi, N., and D.-R. Choi. 1990. Possible simultaneous/integrated biological control of soil pests by mixed application of entomopathogenic and fungivorous nematodes. 3rd International Conference of Plant Protection in the Tropics; March 1990; KL, Malaysia (in press).

19. Kondo, E., S. Honda, and N. Ishibashi. 1985. Growth and propagation of entomogenous nematodes, *Steinernema* spp., on several kinds of media prepared by chicken liver and intestine. Japanese Journal of Nematology 15:1–10. (In Japanese; English summary.)

20. Koyama, M., and S. Kamano. 1976. Mass rearing of common cutworm, *Spodoptera litura*, on the artificial diet. Shokubutu Boeki 31:470–474. (In Japanese.)

21. Shehata, M., D. W. Davis, and N. A. Anderson. 1984. Resistance to *Rhizoctonia* stem rot in peas as influenced by temperature, watering method, and period of disease development. Plant Disease 68:22-24.

22. Sutherland, J. R. 1967. Failure of the nematode Aphelenchus avenae to parasitize conifer seedling roots. Plant Disease Reporter 51:367-369.

23. Tojo, S., Y. Nishimoto, M. Noda, and N. Ishibashi. 1987. Physiological analysis of different infectivities of *Steinernema feltiae* (str. DD-136) to the larvae of two lepidopteran insects. Pp.133–138 in N. Ishibashi, ed. Recent advances in biological control of insect pests by entomogenous nematodes in Japan. Research Project on Biological Control, Ministry of Education, Japan. (In Japanese; English summary.) Saga University, Saga 840, Japan.

24. Townshend, J. L. 1964. Fungus hosts of Aphelenchus avenae Bastian 1865 and Bursaphelenchus fungivorus Franklin and Hooper, 1962 and their attractiveness to these nematode species. Canadian Journal of Microbiology 10:727-737.