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Steinernema feltiae (DD-136) and *S. glaseri*: Persistence in Soil and Bark Compost and Their Influence on Native Nematodes[1]

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Abstract: Infective juveniles (J3) of the entomogenous nematodes *Steinernema feltiae* DD-136 (ca. 10,000 J3/100 ml) and *S. glaseri* (ca. 2,500 J3/100 ml) were incubated in steam-sterilized and nonsterilized sandy soil and bark compost for 8 weeks at 25 C. The nematodes were recovered by a two-step extraction procedure at 1-week intervals, and their infectivity to lepidopterous larvae (*Spodoptera litura* and *Galleria mellonella*) and their effect on the population and community of native nematodes in soil were determined. Survival of inoculated nematodes and mortality of insects were enhanced in sterilized media. Nonsterilized bark compost proved to be equally as suitable a medium as sterilized compost. In nonsterilized soil, the survival curve of *S. feltiae* declined more rapidly than that of *S. glaseri* which was less infective to insects despite its greater persistence even in nonsterilized soil. Soon after the addition of steinernematids to soil, the population of native nematodes showed a fluctuation with an increase in rhabditids and a decrease in other kinds of nematodes.

Key words: entomogenous nematodes, inundative soil application, survival, infectivity, *Steinernema glaseri, S. feltiae, Spodoptera litura, Galleria mellonella.*

Steinernematid nematodes, parasitic to many insect species, have been used with limited success to control foliage-feeding insects because the nematodes desiccate and die on the exposed leaf surface. In contrast, these nematodes show considerable promise as biological control agents of certain soil insects because soil is their natural habitat (1,2,4,5,7–9,13,16,18). When applied to soil, however, these nematodes do not seem to become established. Experimentally, the best control of soil insects by nematodes was obtained in sterilized media (14,16,17). We observed that infective stages (J3) of steinernematids persisted longer in sterilized soil than in nonsterile soil. Accordingly, we compared survival and infectivity of *Steinernema feltiae* and *S.*

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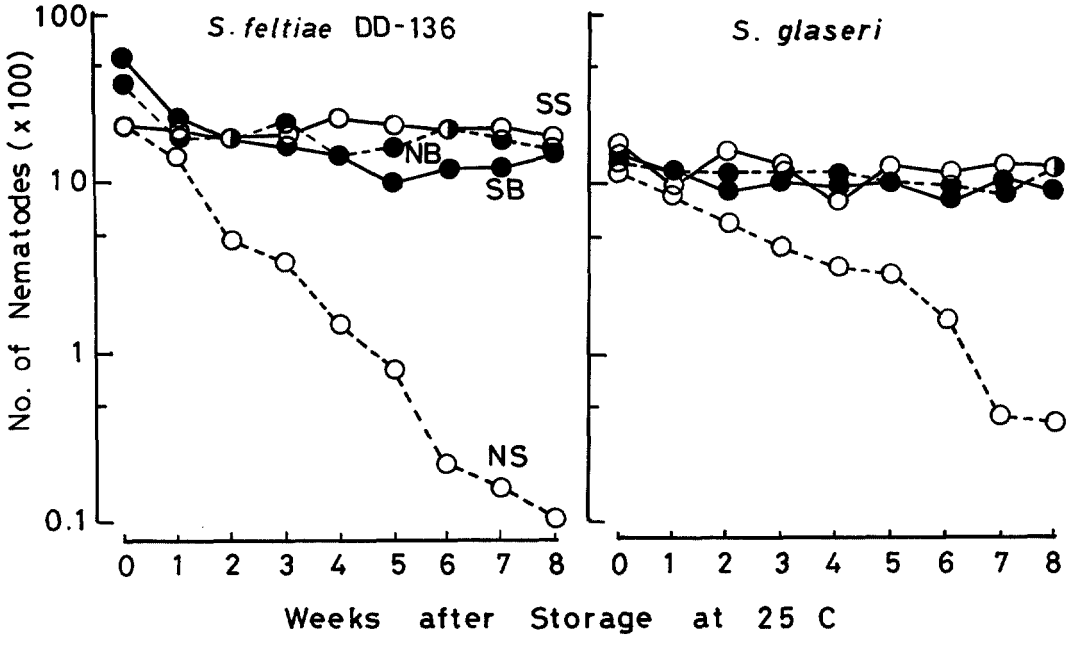

FIG. 1. Persistence of infective juveniles (J3) of *Steinernema feltiae* DD-136 and *S. glaseri* incubated in bark compost or sandy soil with and without steam-sterilization. SS (open circle, solid line) = sterilized soil. SB (solid circle, solid line) = sterilized bark. NS (open circle, broken line) = nonsterilized soil. NB (solid circle, broken line) = nonsterilized bark. Nematodes were inoculated with ca. 10,000 J3 for DD-136 and ca. 2,500 for *N. glaseri*.

glaseri in steam-sterilized and nonsterilized soil and bark compost. The biological impact of these entomogenous nematodes on native nematodes in the nonsterilized media was also investigated. Based on our results, a possible method of soil application of steinernematid nematodes is suggested.

MATERIALS AND METHODS

Steinernema feltiae (DD-136) was obtained from the University of California, Berkeley, and *S. glaseri* from Biotechnology Australia Pty., Ltd., Sydney. The nematodes were reared on dog food–agar (10) or greater wax moth larvae (*Galleria mellonella*). Sandy soil from under the canopy of pine trees and commercial bark compost (Kojin) were passed through a 2-mm-pore sieve. These media were divided into two parts: steam-sterilized at 120 C for 20 minutes and nonsterilized. One milliliter of nematode suspension containing about 10,000 J3 of *S. feltiae* DD-136 or 2,500 J3 of *S. glaseri* was placed on the bottoms of polyethylene containers (8.5 cm d × 4.5 cm) containing 100 ml steam-sterilized or nonsterilized sandy soil or bark compost at 25 C. After the addition of the nematode suspension, the water contents were 60% (w/w) for bark compost and 26% for sandy soil. This water content was maintained by keeping the containers tightly sealed throughout the experimental period. Five containers were taken from each group at 1-week intervals for 8 consecutive weeks. Nematodes were recovered from the total sample (100 ml) of the container by Baermann funnel overnight at 25 C. After the Baermann procedure (BF), the soil in the funnel was subjected to the sucrose centrifugal-flotation technique (CF) (sp. gr. = 1.18). Nematodes recovered from each recovery procedure were totaled and represented the nematodes persisting in the container. Dead individuals were not collected since they were discarded in the CF precipitate.

Infectivity of nematodes to insects was bioassayed every 2 weeks by placing three fifth-instar larvae of *Spodoptera litura* or three sixth-instar larvae of *Galleria mellonella* on the upper surface of the medium, incubating in the dark at 25 C, and observing daily for 7 days. Each treatment was replicated five times. The data were analysed by χ-square test for the days re-

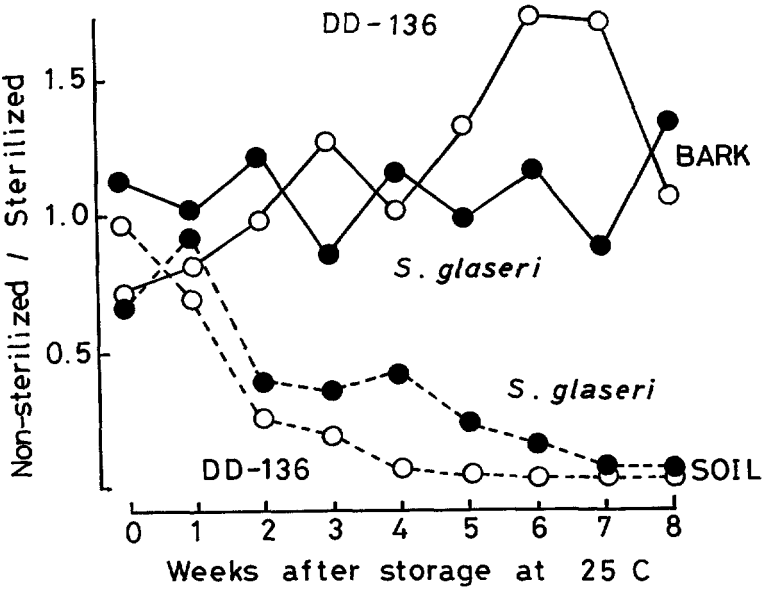

FIG. 2. Recovery ratios (nonsterilized/sterilized) of *Steinernema feltiae* DD-136 and *S. glaseri* from bark compost and sandy soil. Solid line = bark compost. Broken line = sandy soil. Open circle = DD-136. Solid circle = *S. glaseri.*

quired to give insect mortality. Dead insects were dissected and examined for nematode infection.

RESULTS

Survival: The number of entomogenous nematodes recovered by the two extracting procedures indicated that inoculated nematodes were least persistent in nonsterilized sandy soil, from which the number of entomogenous nematodes recovered precipitously decreased with time (Fig. 1). Nematodes survived longest in bark compost. The consistent 1:1 ratios of nematodes from nonsterilized : sterilized compost indicated that sterilization was not needed to maintain inoculated nematodes in bark compost (Fig. 2). Generally, recovery rates were lower for DD-136 than for *S. glaseri.* Even immediately after inoculation, the respective recovery rates for DD-136 were 56.3, 40.4, 22.8, and 22.1% from sterilized bark compost (SB), nonsterilized bark compost (NB), sterilized soil (SS), and nonsterilized soil (NS), while those for *S.*

TABLE 1. Mortality (%) of *Galleria mellonella* larvae placed on the surface of media preincubated with infective juveniles of *Steinernema feltiae* DD-136 (ca. 10,000/100 ml) or *S. glaseri* (ca. 2,500/100 ml) for up to 6 weeks at 25 C. Numerals in parentheses indicate the days required to give the mortality percentage indicated.

	Preincubation (weeks)							
	S. feltiae				*S. glaseri*			
Medium	0	2	4	6	0	2	4	6
Bark compost								
Sterilized	100 (2) a	100 (2) a	100 (2) a	100 (2) a	100 (4) a	100 (3) a	100 (4) a	100 (5) a
Nonsterilized	100 (2) a	100 (2) a	100 (2) a	100 (5) b	100 (5) a	100 (3) a	100 (4) a	100 (4) a
Sandy soil								
Sterilized	100 (2) a	100 (2) a	100 (2) a	100 (7) c	100 (4) a	100 (3) a	100 (4) a	100 (5) a
Nonsterilized	100 (2) a	100 (3) a	100 (7) b	13 (7)	100 (4) a	100 (7) b	100 (7) b	13 (7)

Data followed by the same letter in columns are not different ($P = 0.05$) for days required to give the mortality according to χ-square test. Figures without letters were omitted from the test.

TABLE 2. Mortality (%) of *Spodoptera litura* larvae placed on the surface of media preincubated with infective juveniles of *Steinernema feltiae* DD-136 (ca. 10,000/100 ml) or *S. glaseri* (ca. 2,500/100 ml) for up to 6 weeks at 25 C. Numerals in parentheses indicate the days required to give the mortality percentage indicated.

	Preincubation (weeks)							
	S. feltiae				*S. glaseri*			
Medium	0	2	4	6	0	2	4	6
Bark compost								
Sterilized	100 (3) a	100 (2) a	100 (3) a	100 (3) a	100 (4) a	100 (7) a	73 (7) a	73 (7) a
Nonsterilized	100 (3) a	92 (7) b	100 (3) a	100 (5) b	100 (4) a	100 (6) a	67 (7) a	93 (7) a
Sandy soil								
Sterilized	100 (4) a	100 (6) b	100 (4) a	92 (7) c	100 (4) a	83 (7) a	53 (7) a	33 (7)
Nonsterilized	100 (3) a	92 (7) b	27 (7)	13 (7)	100 (7) b	8 (7)	7 (7)	0 (7)

Data followed by the same letter in columns are not different ($P = 0.05$) for days required to give the mortality according to χ-square test. Figures without letters were omitted from the test.

glaseri were 60.8, 69.0, 76.8, and 51.1%, respectively.

Infectivity: As seen in Tables 1 and 2, *G. mellonella* larvae were killed more rapidly than *S. litura* larvae. Nematodes were observed through the cuticle of all insect cadavers at the beginning of infection. All cadavers, however, did not produce J3; particularly in nonsterilized soil cadavers were often observed from which nematodes disappeared a few days after infection. Disappearance of nematodes may have been caused by contamination with other pathogens. DD-136 killed the lepidopterous larvae more quickly than did *S. glaseri*, though the number of invading J3 was not

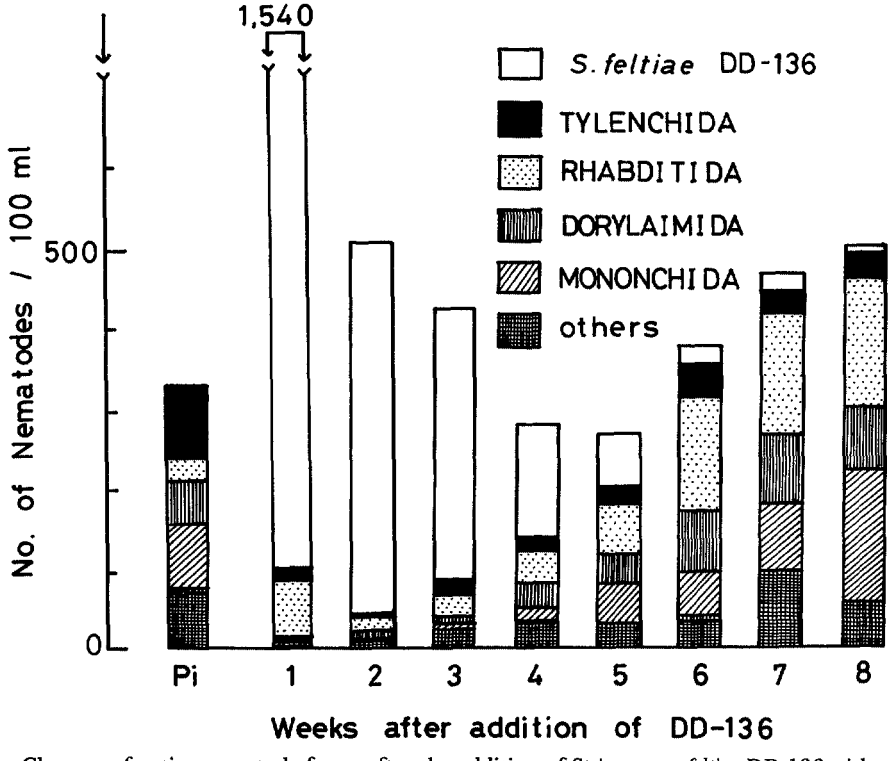

FIG. 3. Changes of native nematode fauna after the addition of *Steinernema feltiae* DD-136 with ca. 10,000 J3 to 100 ml sandy soil under canopy of pine trees. Column at left indicates original population structure of native nematodes.

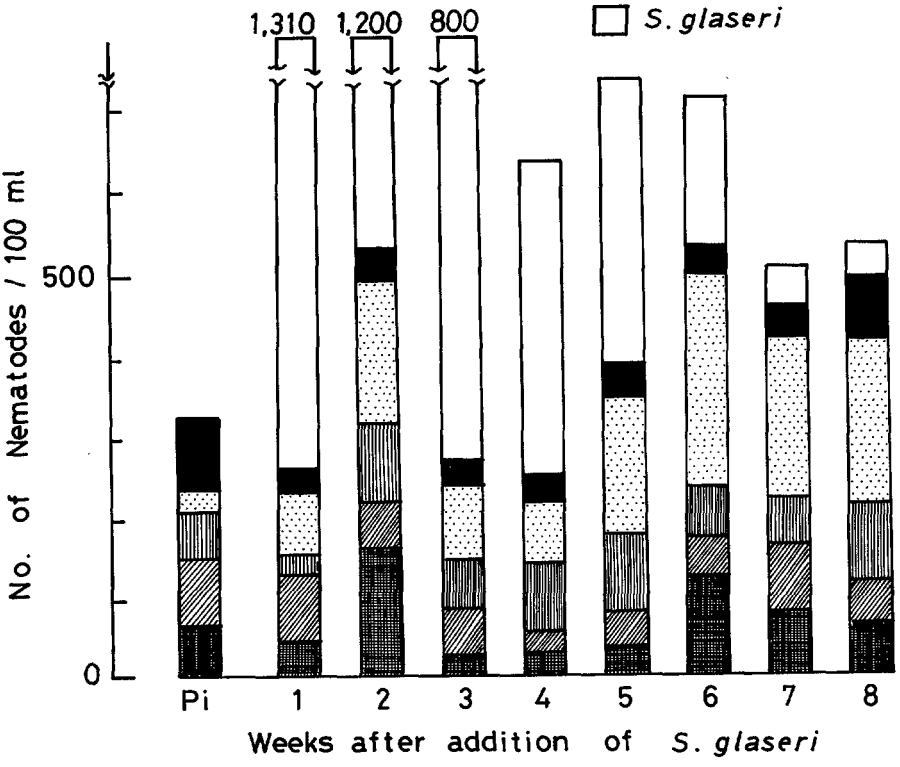

FIG. 4. Changes of native nematode fauna after the addition of *Steinernema glaseri* (ca. 2,500 J3/100 ml soil). Column at left indicates original population structure of native nematodes.

investigated. Entomogenous nematodes in bark compost gave higher insect mortality than did those in soil. For instance, all wax moth and common cutworm larvae (*S. litura*) used were killed by 5 days when placed on bark compost with DD-136 incubated for up to 6 weeks. *S. glaseri* also caused higher mortality of lepidopterous larvae in bark compost than in soil. *S. glaseri*, however, was less infective than DD-136 to insects used in this experiment. No larvae of *S. litura* were killed in nonsterilized soil that had contained *S. glaseri* for 6 weeks, even though more than 200 J3 were recovered from the container. On the other hand, DD-136 killed 13% of the *S. litura* larvae on the same media, despite lower recovery of ca. 50 J3 after 6 weeks. No insect larvae died in the control treatments during the 7-day experimental period.

Impact on native nematodes: The population structure of native nematodes in nonsterilized soil just before inoculation with steinernematids consisted of 17% Tylenchida, 8% Aphelenchida, 15% Rhabditida, 10% Dorylaimida, 20% Mononchida, and 5% Areolaimida. The remaining 25% contained a mixture of Monhysterida, Chromadorida, Enoplida, etc. These percentages represent ca. 350 individuals extracted from 50 g of soil. The addition of steinernematids resulted in a rapid decrease in the population density of native nematodes (Figs. 3, 4). Although there was a concomitant reduction in the number of inoculated nematodes, the population of native ones soon recovered or surpassed their original levels by the end of the experiments (8 weeks). The population densities of the plant-parasitic nematodes, such as stubby-root, ring, and spiral nematodes, were suppressed throughout the experiments, while native rhabditids increased several times above the initial level. In the control soil, population densities of native nematodes fluctuated slightly without the drastic changes observed in the experimental groups. Bark compost originally contained very few rhabditids, represented by only one or two species, but the population den-

sities increased, as they did in soil, after the addition of entomogenous nematodes.

Discussion

Despite many reports that entomogenous nematodes are prospective control agents of soil insect pests, soil applications of steinernematids have not always given satisfactory results. Soil-inhabiting insects may possess some resistance to steinernematids, having presumably been subjected to selection pressures by parasitic rhabditid nematodes (1). It has also been suggested that soil application may be inefficient because the nematodes may be clumped (12). On the other hand, inundative soil application was reported as promising for control of soil insect pests (15). Thus, we still have no definite view on the efficacy of soil application of entomogenous nematodes. We have to take into consideration that soil application of nematodes could be affected by biotic factors (e.g., predation, parasitism, occupation of space) as well as abiotic factors (e.g., temperature, desiccation, sunlight) that are fatal to nematodes applied on exposed plant surfaces.

The present investigation corroborated our earlier observations that entomogenous nematodes in sterile soil survived and retained infectivity longer than in nonsterile soil. In bark compost, however, nematode persistence was almost equally maintained in both sterilized and nonsterilized media. It is known that compost can reach 60 C during fermentation, which would cause a reduction of soil organisms to such a degree that only a few rhabditids could survive. Although we did not examine the microflora, the poor soil biota of bark compost may have provided the applied entomogenous nematodes with favorable conditions for their persistence. Moreover, these nematodes caused higher rates of insect mortality than did those in sterilized soil. Physical conditions in bark compost may have been favorable to nematodes as well. Thus, bark compost provides a promising medium for the control of soil insects; entomogenous nematodes can be mixed into the compost as base fertilizers or added later.

S. glaseri was reported to be less infective to lepidopterans than were other steinernematid nematodes (3). In our experiment infectivity of *S. glaseri* to lepidopterous larvae over time was lower than that of DD-136, despite the greater persistence of *S. glaseri*.

An interesting result from our experiment was disturbance of the native nematode population by the addition of large numbers of steinernematid nematodes; native rhabditids increased and plant nematodes decreased. These data were not statistically analyzed, but there was an obvious change from the initial population levels. There is no prey–predator relationship between entomogenous nematodes and plant-parasitic ones, but we wonder why plant nematodes were suppressed after the application of steinernematids. No detrimental metabolite from the entomogenous nematodes was detected; the filtrate from a DD-136 suspension at a concentration of 5×10^6 nematodes/50 ml incubated overnight did not affect *Meloidogyne incognita* juveniles. In addition, an inundative soil application of DD-136 ($5 \times 10^6/m^2$) lowered the population densities of native nematodes in nonsterilized soil and suppressed recovery of the population density in chemically sterilized soil (Ishibashi, unpubl.). It is generally accepted that there is an inverse relationship in abundance of free-living vs. plant-parasitic nematodes in cultivated soils. We are inclined to suspect that competition for space or habitat exists between these nematodes, though it may be presumptuous to speculate based on the data from the present investigation.

It is well known that soil chemically treated to have low species diversity values (e.g., β-index) favors the infestation of invading or surviving soil pests, since there may be few antagonists. This concept can be also applied to the survival or infectivity of applied entomogenous nematodes. Consequently, application of entomogenous nematodes is recommended to chemically treated soils, where they will attack more effectively the invading soil insect pests. This hypothesis was also supported by our previous field trials (11). Our proposal suggests that a pest control strategy similar to the application of *Trichoderma harzianum* in fumigated soil against soil-borne disease organisms (6) is occurring with these nematodes. Inundative soil application of entomogenous nematodes after chemical treat-

ments could lead to integrated control of soil insects and nematodes.

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