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# Dynamics of the Entomogenous Nematode *Steinernema feltiae* Applied to Soil with and without Nematicide Treatment[1]

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*Abstract:* The dynamics of *Steinernema feltiae* strain DD-136 in soils with different fauna was investigated to determine the best method for the biological control of soil insects. Infective juveniles (J3) were applied to field plots with and without 1,3-D (Telone II) fumigation. Recovery of J3 and changes in native nematode fauna were monitored until the applied J3 were no longer recovered by Baermann funnel (BF). Recovery of J3 by BF or by a two-step extraction procedure from steam-sterilized or nonsterilized sandy or silty soil with different fauna was investigated. More DD-136 J3 were recovered from the 1,3-D treated soil than from nontreated soil, while native nematodes in the treated soil fluctuated more with the addition of DD-136 than those in nontreated soil. The J3 persisted longer in silty than in sandy soils. The inundative soil application of DD-136 increased native rhabditids and decreased plant-parasitic nematodes. DD-136 in chemically treated soil not only effectively attacked the invading soil insect pests but also suppressed the recovery of plant nematodes.

*Key words:* biological control, *Neoaplectana carpocapsae,* nematode displacement fauna, nematode persistence, Telone II.

Steinernematid nematodes are promising biological control agents of insects and are commercially available (e.g., from BIOSIS in California and Biotechnology Australia in Sydney). Soil-inhabiting insects are logical targets because these nematodes occur naturally in soil where they are protected from environmental extremes. Unlike chemical pesticides and other microbial control agents, steinernematids are attracted to their hosts and actively find them. However, many problems remain as obstacles to successful field application, because nematodes applied to soil are affected by biotic factors, and the applied steinernematids also alter the naturally occurring nematode fauna in the soil. Previous research (4) demonstrated that infective juveniles (J3) of *Steinernema feltiae* and *S. glaseri* persisted longer in steam-sterilized than in nonsterilized soil. Native nematode populations fluctuate considerably after inundative application of entomogenous nematodes. Our objective was to determine the persistence and influence of the entomogenous nematode, *S. feltiae* strain DD-136, in soils with and without fumigation with Telone II or sterilization by autoclave. The study was also extended to give a biological rationale to empirical observation that biocontrol of soil insects by nematodes is more effectively achieved in soil with low species diversity (= low biological buffering action).

## Materials and Methods

*Steinernema feltiae* strain DD-136, originally from the University of California, Berkeley, was cultured on dog food agar or chicken offal medium (5). The infective juveniles (J3) were collected from the medium for about 1 month.

*Persistence of J3 in the field:* Experiments were conducted for 2 years in a field with loam (60% sand, 30% silt, 10% clay) soil. Four plots (2 × 3 m) were treated with 200 liters/ha Telone II (1,3-dichloropropene 98%) in February 1984 and in March 1985 to reduce the soil fauna, and four plots were not treated. Cuttings of sweet potato, *Ipomoea batatas* Poiret, were transplanted into all plots and conventional fertilizing practice was followed each year. Application of J3 was performed in September, October, and November in different plots for each time, when the sweet potato was

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TABLE 1. Population structure and species diversity ($\beta$-index†) of nematode fauna at the time of DD-136 application to soil with and without Telone II (200 liters/ha) treatment.

| | No. and $\beta$-index of nematodes isolated from 50 g soil | | | |
|---|---|---|---|---|
| | 1984 | | 1985 | |
| Nematodes‡ | Nontreated | Telone II§ | Nontreated | Telone II‖ |
| Plant-parasitic | 88.0 | 10.2 | 53.0 | 5.2 |
| Bacteriophagous | 95.0 | 29.0 | 86.0 | 13.3 |
| Fungivorous | 14.6 | 10.8 | 40.0 | 20.4 |
| Omnivorous | 18.2 | 8.0 | 33.0 | 5.5 |
| Predacious | 8.0 | 0.4 | 18.0 | 0.0 |
| Total | 223.8 | 58.4 | 230.2 | 44.4 |
| $\beta$-index | 5.9705 | 3.7187 | 5.3824 | 3.2328 |

Each value is the mean of four replicates.
† The reciprocal of the $\lambda$-value of Simpson (11).
‡ Collected by Baermann funnel method.
§ Telone II applied 20 February and soil sampled 3 September.
‖ Telone II applied 10 March and soil sampled 25 September.

harvested. Immediately after harvest, 30 polyvinyl chloride tubes (5.5 × 30 cm) were inserted 15 cm deep in the soil in each plot; 15 tubes at 15-cm intervals each as two rows 100 cm apart. On one side, 50 ml of water containing 12,000 J3 (240 J3/ml or 520 J3/cm$^2$) was pipetted onto the soil surface in 15 tubes, and 15 tubes on the other side received 50 ml of water without nematodes. Soil in four tubes randomly sampled from each plot was thoroughly homogenized, and nematodes were extracted from four 50-g aliquants over 16 hours by the Baermann funnel (BF) procedure at 25 C. After the BF extraction, the soil in each funnel was extracted by the sucrose centrifugal-flotation (CF) technique (sp. gr. = 1.18) (2). The nematodes collected were totaled and represented the persisting DD-136 nematodes. Soil samplings were continued until no DD-136 J3 were recovered by the extraction procedure.

To assess downward movement of applied J3 over time, nematodes were extracted from soil depths of 0–2, 2–7, and 7–12 cm within a tube by the BF + CF procedure.

*Influence on native nematodes:* Native nematodes recovered during the extraction of DD-136 were grouped according to their feeding habit as plant-parasitic (Tylenchida, mainly *Meloidogyne* sp. and spiral nematodes), bacteriophagous (Rhabditida, Areolaimida, Diplogasteirida, Teratocephalida, etc.), fungivorous (Aphelenchida and Tylenchida such as *Tylenchus* spp.), omnivorous (Monhysterida, Dorylaimida, Chromadorida, Enoplida, etc.), and predacious (mainly Mononchida). At the time of DD-136 application, the species diversity of native nematodes was assessed and is reported as the reciprocal of the $\lambda$-value (11):

$$\lambda = \sum_{i=1} ni(ni - 1)/N(N - 1)$$

where ni is the total number of individuals of species i found in the sample, and N is the total number of all species sampled (Table 1). The reciprocal was called $\beta$-index (7).

The influence of application of DD-136 on native nematodes was expressed as the ratio of population density in the tubes where DD-136 was applied to the density in the tubes where DD-136 was not applied in each plot at each sampling time. The fluctuation of each feeding-habit group was also assessed in the same manner.

*Persistence of DD-136 in soils with different fauna:* Persistence of applied DD-136 was investigated in three soil textures with different fauna. The soils were sandy (70% sand, 20% silt, 10% clay) from a sweet potato nursery bed, loamy (60% sand, 30% silt, 10% clay) under the canopy of pine

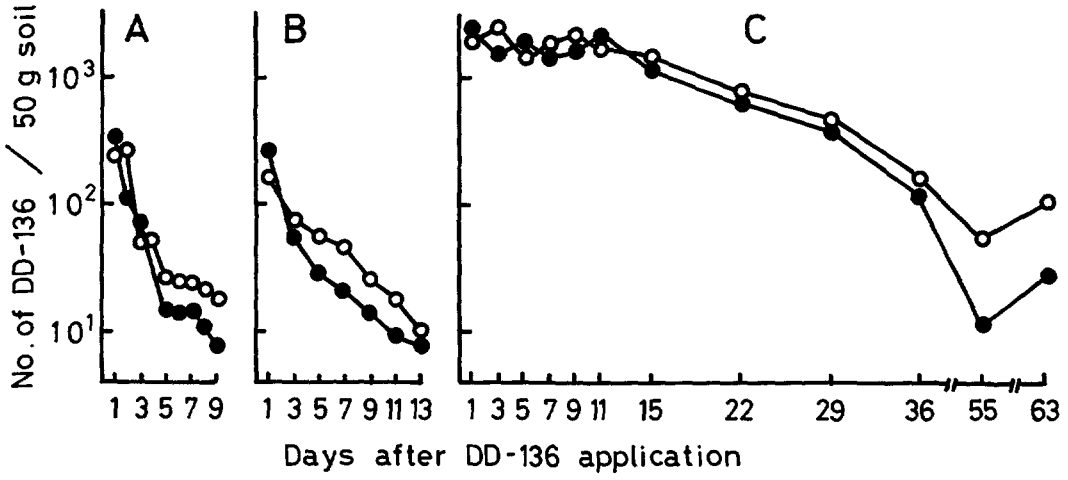


FIG. 1. Population changes of *Steinernema feltiae* DD-136 infective juveniles (J3) in soil with and without Telone II treatment in February 1984. Nematodes were applied at the rate of 520/cm² and recovered by a two-step Baermann funnel + centrifugal flotation. A) Applied 3 September. B) Applied 3 October. C) Applied 15 November. Open circle = Telone II-treated soil. Solid circle = nontreated soil.

trees (*Pinus thunbergii* Parlatore), and silty (undetermined) under the canopy of loquat trees (*Eriobotrya japonica* Lindley). Four liters of soil were taken from each site, half of which was steam sterilized (1.5 kgf/cm², 120 C, 20 minutes 2 times). The soil mesofauna was collected by BF or modified Tullgren apparatus (8) at the beginning of the experiment to determine their initial population densities. Fifty milliliters of soil was placed in a polystyrene vial (3.5 × 6.0 cm), and 1 ml of water with 3,000 J3 was pipetted onto the soil surface. Soil moisture (w/w) was kept near field capacity; sandy 24%, loamy 38%, and silty 40%. Forty vials of each soil texture were placed in the dark at 25 C. At 2–3-day intervals for the first 2 weeks and 1-week intervals thereafter, four vials from each group were extracted by the two-step (BF + CF) procedure to recover nematodes from the sterilized soils. Nonsterilized soils were extracted by BF only to study the effect on native fauna, because previous research demonstrated that this was sufficient (4). Sterilized sandy soil was employed in both BF only and two-step procedure to compare the recovery of DD-136 J3 between the two procedures. Duncan's multiple-range test was used to determine significant differences in the recovery of DD-136 among the soil types 30 days after application.

TABLE 2. Number of infective juveniles of *Steinernema feltiae* DD-136 recovered at intervals after application to soil nontreated or treated with Telone II.

| Days after DD-136 application | Number of nematodes recovered per 50 g soil | | | |
|---|---|---|---|---|
| | Exp. 1 | | Exp. 2 | |
| | Nontreated | Telone II† | Nontreated | Telone II† |
| 1 | 402 a | 480 a | 402 a | 382 a |
| 2 | 263 a | 374 a | 198 a | 222 a |
| 3 | 60 a | 95 a | 65 a | 90 a |
| 5 | 3 b | 28 a | 5 b | 33 a |
| 7 | 2 | 3 | 0 | 4 |
| 9 | 2 | 2 | 2 | 3 |
| 11 | 0 | 1 | 2 | 2 |
| 14 | 0 | 0 | 0 | 1 |

Infective juveniles (J3) of DD-136 were applied at the rate of 520/cm² on 25 September (Exp. 1) and on 28 October (Exp. 2) to the separate plots and J3 were recovered by Baermann funnel method.

Maximum and minimum daily temperatures at 10 cm deep were 28 and 20 C for Exp. 1 and 24 and 14 C for Exp. 2.

Values within a row followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

† Telone II applied 10 March 1985.

## RESULTS

*Persistence of DD-136 in the field:* Declining population density of DD-136 in the soils with and without Telone II was ob-

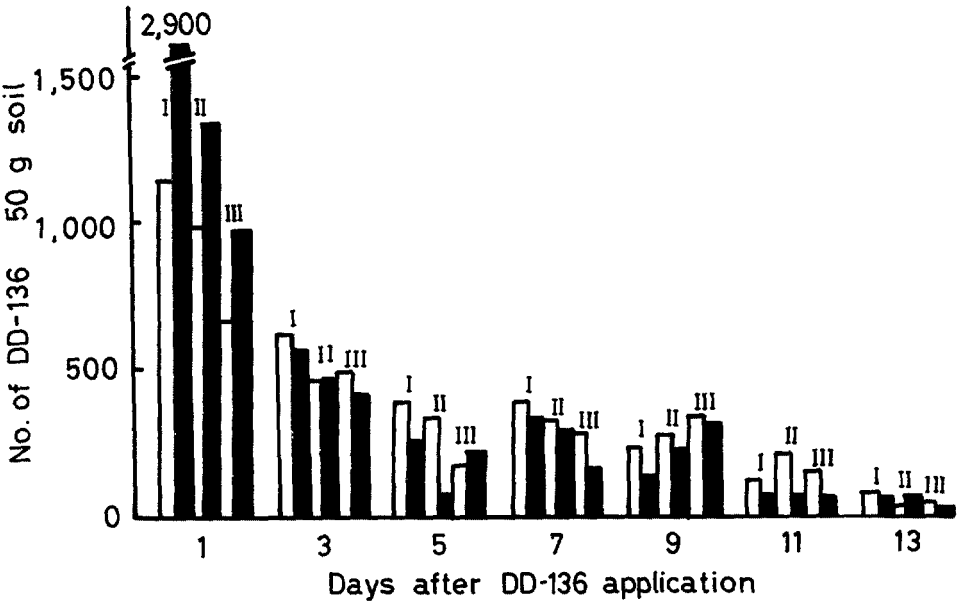


FIG. 2. Downward movement of *Steinernema feltiae* DD-136 J3 applied on 3 October 1984 at the rate of 520/cm² on the soil surface with and without Telone II treatment on 20 February 1984. Open column = Telone II-treated soil. Solid column = nontreated. I, II, and III indicate 0–2, 2–7, and 7–12 cm, respectively, below the soil surface.

served over time (Fig. 1). Experiments initiated in September and October had no significant difference between chemically treated and nontreated soils for the first 3 days after application. Five days after application, however, significantly more J3 were recovered from the treated than nontreated soil (Fig. 1A, B). In these experiments, the soil temperatures ranged from 20 to 30 C 5–10 cm deep. On the other hand, J3 did not decrease significantly in the experiment started in November (Fig. 1C). Soil temperatures during the experimental period were below 7 C 5–10 cm deep. Nevertheless, 2 months after application, more J3 were recovered from the chemically treated than from nontreated soil.

The decline of recovery of DD-136 applied in 1985 was similar to that obtained in 1984, although numbers of nematodes recovered were significantly less in 1985 because nematodes were extracted by BF alone (Table 2). No significant difference of J3 recovered from the chemically treated and nontreated soils during the first 3 days after nematode application was observed, but discernible differences were observed on day 5. Subsequently no difference was seen due to negligible recovery from either soil.

One day after application, DD-136 applied onto the soil surface was recovered from depths to 12 cm (Fig. 2). The recovery of J3 from the nontreated soil was greater than from treated soil 1 day after application, but after 3 or more days greater nematode numbers were recovered from the treated soil. There was no conspicuous difference between depths.

*Influence on native nematodes:* The population density of native nematodes in both treated and nontreated soil declined immediately after the application of DD-136 but rose above the control level after 5–7 days and fluctuated around the control level (Fig. 3). Thus, inundative soil application of *S. feltiae* disturbed the native nematode fauna, and fluctuations were greater in Telone II treated soil than in nontreated soil. Similar trends were observed in duplicate experiments the next year. The main difference in the fluctuating population structure was the increase in bacteriophagous nematodes (Figs. 4A, 5A). This increase in bacteriophagous nematodes (mainly

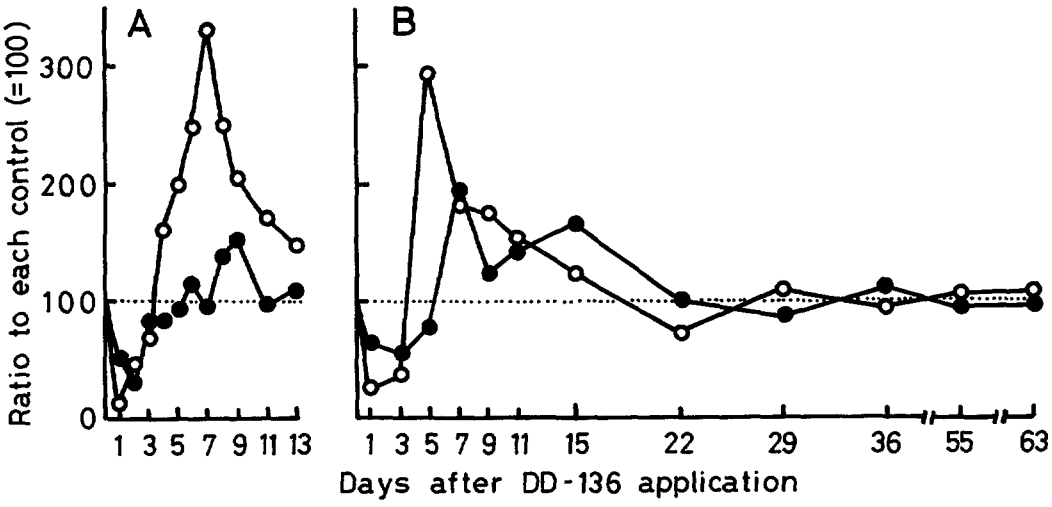


FIG. 3. Changes in relative population ratio of native nematodes after the application of *Steinernema feltiae* DD-136 J3 (520/cm²) to soil with and without Telone II treatment in February 1984. The dotted lines indicate the level (100%) of control, which did not have DD-136 applied, at the respective sampling time. A) Applied 3 October. B) Applied 15 November. Symbols are the same as those in Figure 1.

Rhabditida) after DD-136 application was consistent throughout the experimental period for 1984 and 1985. In 1984 the plant-parasitic nematodes appeared to be suppressed by the addition of DD-136, but in 1985 they were not. Similarly, fungivorous nematodes such as *Tylenchus* spp. and *Aphelenchus* spp. did not follow any consistent trends. Interestingly, the ratio of predacious nematodes to those in plots without application of DD-136 conspicuously increased after the application of DD-136 (Figs. 4B, 5B). Although the ratios may be exaggerated because these nematodes

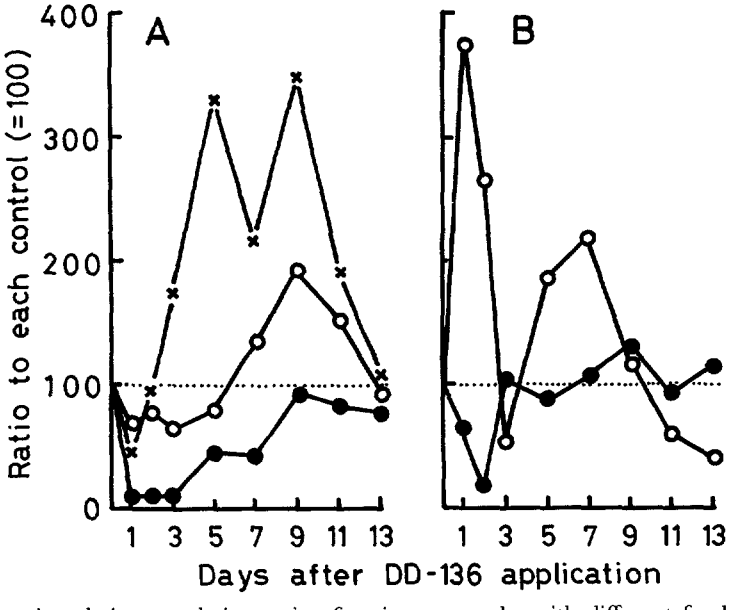


FIG. 4. Changes in relative population ratio of native nematodes with different food habits after the application (3 October) of DD-136 J3 (520/cm²) in the soil, showing the control level (dotted line = 100%) without DD-136 application at the respective sampling time. A) Cross = bacteriophagous. Solid circle = plant parasitic. Open circle = fungivorous. B) Open circle = predacious. Solid circle = omnivorous.

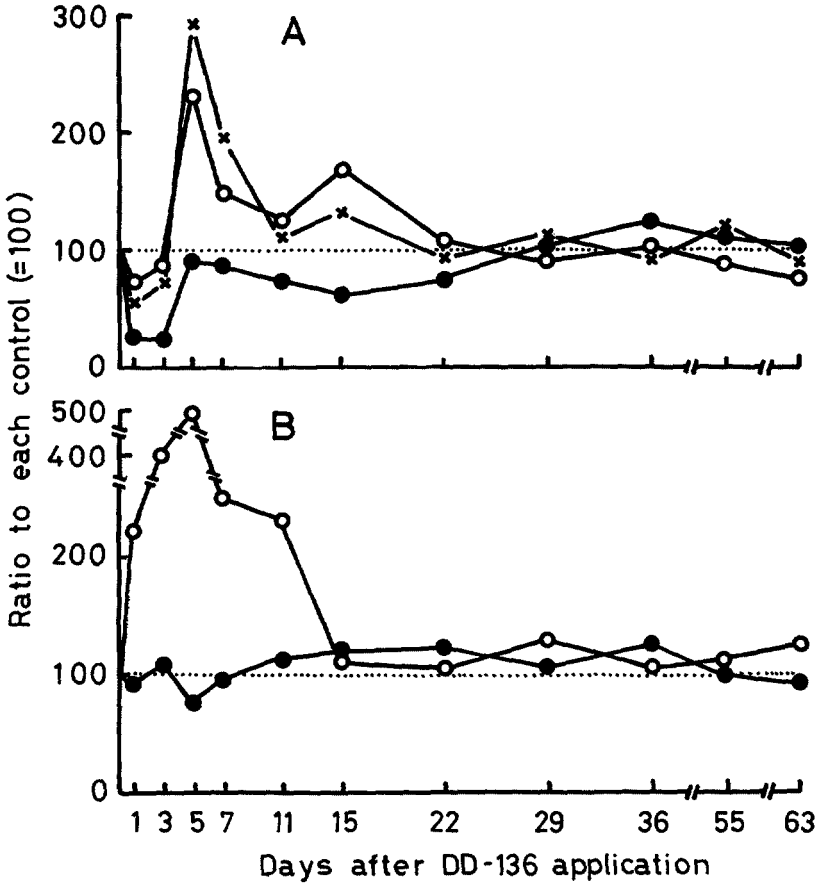


FIG. 5. Changes in relative population ratio of native nematodes with different food habits after the application (3 October) of DD-136 J3 (520/cm²) in the soil, showing control level (dotted line = 100%) without DD-136 application at the respective sampling time. Symbols are identical to those in Figure 4.

usually occur in small numbers (5–10 individuals/50 g soil), greater numbers of mononchid nematode juveniles were apparent.

*Persistence in various soil types:* Entomogenous nematodes declined sharply in sandy, loamy, and silty textured soils with different soil fauna. Sandy soil contained more spiral and root-knot nematodes and acaridid mites, whereas loamy soil contained relatively numerous predacious mononchid nematodes and silty soil had many tardigrades and fewer free-living nematodes (Table 3). The species diversity ($\beta$-index) of nematode fauna was the highest in the loamy, followed by silty and sandy soils. The reduction of DD-136 was the greatest in the nonsterilized sandy, followed by nonsterilized loamy and nonsterilized silty soils in that order (Fig. 6). However, J3 recovered from sandy and loamy soils were not significantly different 30 days after application. Even by BF procedure alone, sterilized sandy soil had more DD-136 than nonsterilized silty soil. Consequently, recovery of DD-136 was greatest in sterilized silty soils using BF + CF followed by sterilized loamy and sandy soils. There was no significant difference in the last two.

## DISCUSSION

The present field investigations confirmed the results of our previous laboratory experiment in which the infective juveniles (J3) of DD-136 persisted more in sterilized soil and the application induced an increase in rhabditid nematodes in nonsterilized soil (4). The J3 persistence in the

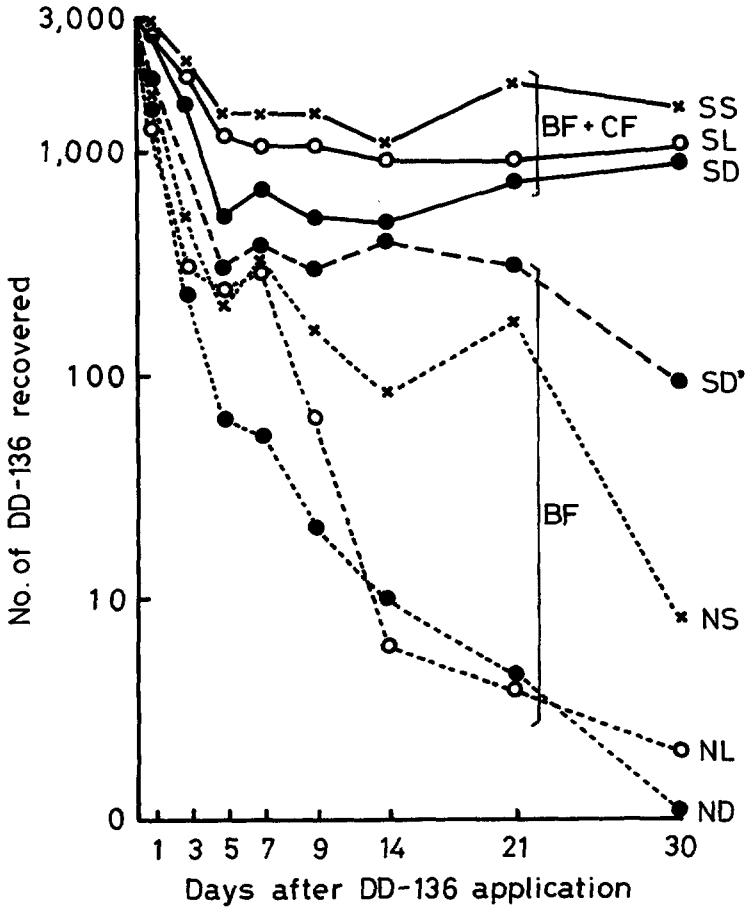


FIG. 6. Recovery of *Steinernema feltiae* DD-136 J3 from silt, loam, and sand inoculated with 3,000 J3/50 $cm^3$ soil and incubated at 25 C. BF = Baermann funnel method. BF + CF = centrifugal flotation technique after BF. SS = steam-sterilized silt; SL = sterilized loam; SD = sterilized sand; SD' = sterilized sand by BF alone for comparison between the two extraction procedures; NS = nonsterilized silt; NL = nonsterilized loam; ND = nonsterilized sand.

soil seems to be affected more by biotic than abiotic factors. Soil mites (especially mesostigmatids), mononchid and dorylaimid nematodes, and tardigrades may constitute major predators on the applied entomogenous nematodes (unpubl.). We also have observed J3 infected by an endoparasitic fungus similar to that reported previously (10).

Persistence of DD-136 also seems to be affected by soil texture because the nematodes persisted longer in the silty than in the sandy soil. This survival may be related to the higher level of water content in the silty soil. Although survival is enhanced in silty soils, the percentage of *S. feltiae* J3 able to migrate and infect insect hosts in soil decreased as the percentage of clay and silt increased (3), suggesting the lowered mobility of nematodes in these heavy soils. Other studies (12,13) have also shown an inverse relationship between survival and infectivity of plant-parasitic nematodes in soil.

Sharp decline of DD-136 recovery from soil also may be explained by nematode dispersal. About 20% of the total individuals remained at the point of application for more than 6 weeks (9). Since we extracted all the soil in the tubes, lateral dispersal is not a factor, but consideration must be given to downward movement below

TABLE 3. Mesofauna in three soil types at application (15 January 1985) of DD-136.

| | No. of mesofauna organisms and β-index of nematode fauna isolated from 50 g soil | | |
|---|---|---|---|
| | Sandy soil | Loamy soil | Silty soil |
| Rotifers | 177 | 2 | 1 |
| Tardigrades | 0 | 25 | 43 |
| Mesostigmatid mites | 2 | 12 | 2 |
| Acaridid mites | 42 | 0 | 0 |
| Nematodes | | | |
| Plant-parasitic | 298 | 18 | 2 |
| Free-living | 65 | 218 | 61 |
| Predacious | 3 | 27 | 5 |
| Total | 366 | 263 | 68 |
| Species diversity (β-index)† of nematodes | 3.8571 | 7.8685 | 5.2355 |

Soils were sampled from sweet-potato nursery bed in glasshouse, rhizosphere of pine trees, and rhizosphere of loquat trees for sandy, loamy, and silty, respectively.

Nematodes, rotifers, and tardigrades were collected by Baermann funnel method. Soil mites were collected by Tullgren apparatus.

† The reciprocal of the λ-value of Simpson (11).

the tube. *S. feltiae* tends to remain in the upper layer of soil and our data support this. Accordingly, we think that few DD-136 dispersed beyond the bottom of the tube. Nematode vigor in soil needs to be assessed by the infectivity to host insects rather than motility through the Baermann sieve (6).

The greater fluctuation or disturbance of native nematode populations in soils treated with 1,3-D than in nontreated soils, after inundative application of entomogenous nematodes, may have resulted from a lower biological buffering action of the treated soil with low species diversity. Some native rhabditid nematodes increased after addition of entomogenous nematodes, but plant-parasitic nematodes were suppressed for a few weeks in 1984. This phenomenon was not repeated in 1985, however. Field trials in October showed a slight decline of spiral and root-knot nematodes, and field trials in winter showed no difference in plant-parasitic nematodes, but trials in September had lower root-knot nematode populations. Inundative application of DD-136 to chemically treated fields suppressed the recovery of plant-parasitic nematodes. The degree of impact upon these nematodes appeared to depend upon their original population density; i.e., lower original densities were suppressed more. Bird and Bird (1) demonstrated that the root-knot nematode *Meloidogyne javanica* had reduced gall activity when mixed with high numbers of *S. glaseri*, suggesting competition between root-knot nematodes and *S. glaseri* in the vicinity of roots. We also have similar results (unpubl.) showing that gall formation of tomato seedlings by 600 *M. incognita* J2 per plant in 800 ml soil was significantly reduced when the soil was simultaneously inoculated with $10^5$–$10^6$ DD-136 J3. There may be a competition for habitat space as well as predation or parasitism by other soil organisms upon the plant-parasitic nematodes. This may also account for the suppression of plant nematodes by the inundative application of free-living nematodes in the chemically treated soil. The simultaneous application of entomogenous nematodes with chemicals (e.g., oxamyl) into fumigated soil will lead to integrated control of soil pests; the applied J3 will effectively attack the invading insect pests and also suppress the recovery of plant nematodes, because these nematodes are not only extremely tolerant to chemicals but also are activated by low doses of chemicals (unpubl.) to invade insect pests.

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