Infectivity of Nictating Juveniles of Steinernema carpocapsae (Rhabditida: Steinernematidae)*

Nobuyoshi Ishibashi**, Shinji Takii*** and Eizo Kondo**

The rates of nictating Steinernema carpocapsae infective juveniles (IJs) were at most 20% and 10% for nematodes harvested from insect cadaver, Galleria mellonella, and for those from chicken offal medium, respectively. Repeated employment of nictating IJs for the nictation performance did not raise the rate, but kept almost the initial levels. Accordingly, after four performances, the number of nictating individuals became 0.16% of the initial population. The nictating IJs were more attracted to the host cue (larval plasma of Spodoptera litura) and more penetrated to the host insect than did non-nictating IJs obtained from nematode–water suspension. There were no significant differences in the migration/penetration between the nictating IJs with and without insecticides; acephate, permethrin, and oxamyl at 50 μg/ml each. The experiments confirmed that nictating behavior was a positive or precursory behavior for infection of S. carpocapsae. Jpn. J. Nematol. 24(1): 20–29 (1994).

Key words: entomopathogenic nematode, infective juvenile, nictating behavior, migration, penetration, search behavior

Entomopathogenic steinernematid nematodes have gained a stable position as biological control agents against insect pests (1, 5, 20) and are commercially available in many countries. The infective juvenile (IJ) is applied against the target insect and initiates the infectious process. However, all IJs are not uniformly infectious, particularly for Steinernema carpocapsae and S. feltiae (4, 17, 18). Migration of S. carpocapsae IJs to host cues is usually very low, though through repeated selection pressure infectivity has been enhanced (6, 7, 8).

The entomopathogenic nematodes have been grouped into two searching behavioral types (11, 25, 26); ambushers and cruisers. These two searching behaviors represent the extremes, and there is a continuum between these two extremes. The ambusher group, e.g., S. carpocapsae, adopts a sit–and–wait strategy and tends to migrate upwards or remain near the point of inoculation (9, 12, 25, 27), while the cruiser group, e.g., S. glaseri, actively forages through the soil in search of hosts and tends to disperse deeper into the soil, presumably searching for subterranean insect hosts (10, 19).

Another behavioral difference between the two groups is nictation, which is seen in S. carpocapsae but is very rare in S. glaseri. This behavior features the IJ standing on its tail on a substrate and waving its whole body above the substrate (16). The purpose of this nictation has

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been purported to be one of several behavioral steps leading to infection of the host. However, we have not yet obtained the direct evidence that this nictating behavior is an important component leading to host infection. We have observed that the nictating IJs killed the host insects faster than did non-nictating ones which were obtained from a nematode–water suspension (17). In addition, effective control of insects by S. carpocapsae corresponded with soil moisture levels that provided the highest nictation rate (23, 24).

In this paper, we confirmed that more nictating S. carpocapsae IJs migrated to the host cue (larval plasma of Spodoptera litura) and penetrated to the host body than did the non-nictating ones. However, only 10–20% of the individuals in the nematode population nictate and we will discuss why such low population levels are supposed to be actually infective.

MATERIALS AND METHODS

Nematodes: Infective juveniles of Steinernema carpocapsae strain All from Biosys (Palo Alto, CA, USA) were cultivated on chicken offal medium with symbiotic bacterium, Xenorhabdus nematophilus, which was inoculated 2 days before. The nematodes were incubated for 1 month at 25°C. Another batch of nematodes was maintained on greater wax moth larvae, Galleria mellonella. After host death, the cadavers were placed on the upper portion of filter paper (Wako No. 1) strip in an 18-cm long test tube with a small quantity of 0.1% formalin, and incubated at 25°C until the emergence of new IJs (ca. 14 days after nematode inoculation). Nematodes isolated from the medium or from insect cadavers were immersed in 1% sodium dodecyl sulfate for 20 min to recover only the IJs. The IJs were centrifuged five times with distilled water and then passed through a 30-μm-pore nylon cloth to obtain only ensheathed infective juveniles (EnIJs). The collection of desheathed infective juveniles (DeIJs) was conducted as described by Ishibashi and Takii (17); the EnIJs were immersed in 0.1% NaOCl for 20 min, centrifuged five times with distilled water, then passed through the nylon cloth, and left without agitation for 24 hr at 25°C until used.

Nictation on the bark compost arena: About 20,000 individuals of EnIJs or DeIJs collected from chicken offal medium and about 10,000 individuals of EnIJs collected from the insect cadavers, were placed in a 6-cm-d petri dish containing 4 ml of distilled water, and 2 g of bark compost (Linnai, Ohji Paper Co., Tokyo). The bark compost was previously dried for 2 hr at 170°C and sieved through a 0.840-mm-pore screen, and was layered at 4-5 mm deep in the petri dish. After 1, 2, and 3 days at 25°C in the dark, nictating nematodes on the top of the bark compost were counted in 10 randomly chosen stereomicroscopic field (0.785 cm²/field). The mean number of nictating nematodes was multiplied by 36 to obtain the total number of nictating nematodes in a dish, since the area of arena was 28.26 cm².

Collection of nictating nematodes: Nematodes were placed on the bark compost arena for nictation performance as described above and a nylon cloth (125-μm aperture, 6 cm d) placed on the top surface of the bark compost. After incubation for 24 hr in the dark at 25°C, the cloth with the nictating nematodes on it was removed. The nematodes were rinsed from the cloth with distilled water or phosphate buffered saline (PBS) solution (20 mM potassium phosphate, 150 mM NaCl, pH 7.2) in a 15 ml plastic centrifuge tube. Collected nematodes were used within 30 min after removal from the nylon cloth.

Repeated performance of nictation: About 10,000 individuals of EnIJs collected from the
Cadavers of wax moth larvae were allowed to nictate on the nylon cloth as above. One day after incubation, the nictating nematodes on the cloth were rinsed with distilled water and counted. These nictating IJs were collected and again allowed to nictate in the same situation, then counted. Again the nictating nematodes were subjected to the same arena. Thus, four successive collections were made for the experiment. Five replicates were taken for each collection.

**Insecticides:** The insecticides used were the same ones as previously reported (17). Briefly, they were oxamyl, technical grade 42.5% w/v (Sankyo Co., Tokyo); acephate, 50% (w/v) active ingredient (a.i.) wettable powder (Ortran, Sankyo Co., Tokyo); dichlorvos, 50% (w/v) (a.i.) emulsifiable concentrate (Des, Sankyo Co.); methomyl 45% (w/w) (a.i.) wettable powder (Lannate, Nihon Noyaku Co., Osaka); and permethrin 20% (w/v) (a.i.) emulsifiable concentrate (Adion, Sankei Kagaku Co., Kagoshima). Stock solutions of these chemicals were freshly prepared in distilled water.

**Migration to insect plasma:** Migration test on agar plates was conducted as previously reported (22). The attractive source, hemolymph of full-grown last-instar larvae of the common cutworm, *Spodoptera litura*, was used. Immediately after the collection, the hemolymph was centrifuged at 5,000g for 10 min at 0°C to remove the hemocytes. A few crystals of phenylthiourea were added to the plasma to prevent melanization and the plasma was lyophilized. The lyophilized plasma was kept at -20°C until use. For use, PBS was added to the dried plasma to the initial volume of hemolymph.

A 4-mm layer of 0.6% agar in PBS was prepared in a 4.5-cm-d petri dish. Two wells (0.5 cm d, 0.2 cm deep) were made in the agar on opposite sides and 1 cm from the central inoculation well (0.2 cm d, 0.2 cm deep). Fifty μl of insect plasma were applied in one side well and the same volume of PBS in the opposite well (control). One μl of PBS containing 30 nictating EnIJs or DeIJs, with or without an insecticide, was applied in the central inoculation well 2 hr after addition of the solutions to the side wells. Non-nictating infective juveniles, which had been kept in distilled water for 24 hr at 25°C, were also used in this bioassay. After the nematodes were allowed to move for 2 hr at 25°C in the dark, the plates were exposed to -20°C for 10 min to stop the nematode movement. The nematodes in the plasma well in the petri dish was counted and expressed as percentages of all inoculated individuals. The experiment was performed three times with five replications for each treatment.

**Penetration to insect host:** One hundred nictating EnIJs or DeIJs with and without an insecticide treatment were inoculated with 0.4 ml of distilled water onto a filter paper (Wako, No. 1) in a petri dish (5.5 cm d) containing a starved last-instar larva of *S. litura*. The dishes covered with lids but unsealed were placed in the dark at 25°C. The insects were divided into two groups for the exposure to nematodes; 6 and 12 hr. After each incubation, the insect larva was surface-washed with a 0.01% formaldehyde solution followed by distilled water rinse, and then transferred onto a new filter paper in a dish with no nematodes. After 48 hr from the commencement of incubation with nematodes for each exposed group, all insects living and dead were dissected in a phosphate buffered saline solution (2.2 mM ammonium phosphate, monobasic; 1.4 mM potassium phosphate, dibasic; 0.4 mM magnesium phosphate; 42.8 mM sodium chloride), and left for 24 hr. The emerged nematodes were counted and expressed as percentages of the initial inoculum population. Non-nictitating EnIJs or DeIJs, which had been kept in a distilled water for 24 hr at 25°C, were also used in this bioassay. The experiment was performed three times with ten
replications for each treatment.

Statistical analysis: Percentages obtained from each experiment were arcsine-transformed to ensure normality and analyzed using a contingency table. Multiple comparisons were made with Duncan's multiple-range test. All comparisons used a 0.05 level of significance.

RESULTS

Nictation: Nictating rate of the EnIJs from chicken offal medium was 4-5% on day 1 and gradually increased to ca. 10% on day 4 (Table 1). There were no significant differences ($p > 0.05$) in the rates between EnIJs and DeIJs from the chicken offal medium. However, the rates of the EnIJs from the insect cadavers were significantly higher ($p < 0.05$) than those from the above chicken offal medium (Table 1).

Repeated performance of nictation: The experiment was carried out by using IJs from Galleria cadavers. The nictating rate for the first performance was $21.8 \pm 2.4\%$ followed by $21.5 \pm 2.8$, $25.7 \pm 3.4$, and $23.1 \pm 3.8\%$ for the 2nd, 3rd, and 4th repetition, respectively. No significant difference ($p = 0.05$) was observed among these values by $t$-test. Although the nictation rate remained stable, the actual numbers of nictating IJs decreased. That is, the number of nictating nematodes at the 1st performance was more than 2,000, decreasing to ca. 16 after the

<table>
<thead>
<tr>
<th>Nematodes collected from</th>
<th>% Nictation on day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Ensheathed juveniles</td>
<td>$5.5 \pm 1.2a$</td>
</tr>
<tr>
<td>Desheathed juveniles</td>
<td>$4.5 \pm 0.8a$</td>
</tr>
<tr>
<td>Galleria mellonella cadaver</td>
<td></td>
</tr>
<tr>
<td>Ensheathed juveniles</td>
<td>$18.5 \pm 3.4b$</td>
</tr>
</tbody>
</table>

Each value is the mean ± S. D. Values followed by the same letter in a column are not significantly different ($p = 0.05$) according to the $t$-test.

1) A 6-cm-d petri dish in which 20,000 IJs harvested from chicken offal medium or 10,000 IJs from insect cadavers were placed. Nematodes were incubated in the dark at 25°C.

2) Desheathed juveniles were obtained by treating ensheathed juveniles with a 0.1% NaOCl solution for 20 min to remove the 2nd-stage cuticle.

Table 2. Migration of nictating and non-nictating infective juveniles of Steinernema carpocapsae towards Spodoptera littura plasma.

<table>
<thead>
<tr>
<th>Nematodes harvested from</th>
<th>% Migration to Spodoptera littura plasma$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-nictating juveniles</td>
</tr>
<tr>
<td>Insect cadaver</td>
<td>$8.2 \pm 2.8a$</td>
</tr>
<tr>
<td>Chicken offal medium</td>
<td>$0.2 \pm 0.2b$</td>
</tr>
</tbody>
</table>

Each value is the mean ± S. D. Values followed by the same letter are not significantly different ($p = 0.05$) according to the $t$-test.

$^1$ Migration was recorded 2 hr after setting nematodes on the petri dish.
4th repetition.

*Migration to insect plasma:* Nematode migration to host insect plasma was significantly greater \((p<0.05)\) by the nictating IJs than non-nictating ones, whether EnIJs or DeIJs. The differences were more noticeable in the nematodes collected from *G. mellonella* cadavers than those from artificial cultivation on chicken offal medium (Table 2). There were no significant differences \((p>0.05)\) in the migration rates between nictating IJs from the chicken offal medium whether they were exposed to insecticides or not (Table 3). However, so few nictating DeIJs from the oxamyl and methomyl treatments were obtained and therefore these were not employed for this experiment. In addition, no nictating nematodes were obtained from the dichlorvos treatment for either EnIJs or DeIJs.

Table 3. Migration of insecticide-treated nictating infective juveniles of *Steinernema carpocapsae* towards *Spodoptera litura* plasma\(^{1)}\).

<table>
<thead>
<tr>
<th>Treatments(^{2)})</th>
<th>% Migration to <em>S. litura</em> plasma</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ensheathed juveniles</td>
<td>Desheathed juveniles</td>
</tr>
<tr>
<td>Water (control)</td>
<td>29.1±12.3 a</td>
<td>32.0±7.8 a</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>24.1±1.5 a</td>
<td>— (^{10})</td>
</tr>
<tr>
<td>Acephate</td>
<td>16.3±6.8 a</td>
<td>33.7±9.0 a</td>
</tr>
<tr>
<td>Permethrin</td>
<td>23.3±13.5 a</td>
<td>38.5±18.1 a</td>
</tr>
</tbody>
</table>

Each value in the mean ± S.D.
Values followed by the same letter are not significantly different \((p=0.05)\) according to DUNCAN's multiple range test.

1) Nematodes used were harvested from chicken offal medium.
2) Migration of non-nictating juveniles with water were 0.2-0.4% as shown in Table 2.
3) Concentration of tested chemicals was 50 µg/ml each.
4) Nictating nematodes were few in number to conduct the bioassay.

Table 4. Penetration rate (%) of nicating and non-nicating infective juveniles of *Steinernema carpocapsae* harvested from chicken offal medium and from insect cadavers, to *Spodoptera litura* last-instar larvae for 6 or 12 hours exposure\(^{1)}\).

<table>
<thead>
<tr>
<th>Nematodes</th>
<th>Ensheathed juveniles</th>
<th>Desheathed juveniles(^{2)})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nematodes from</td>
<td>Nematodes from</td>
</tr>
<tr>
<td></td>
<td>Insect cadaver</td>
<td>Chicken offal medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chicken offal medium</td>
</tr>
<tr>
<td>Non-nictating</td>
<td>4.5±3.2 b (100.0)</td>
<td>0.7±0.3 a (30.0)</td>
</tr>
<tr>
<td></td>
<td>4.5±3.2 b (100.0)</td>
<td>0.7±0.3 a (30.0)</td>
</tr>
<tr>
<td>Nictating</td>
<td>15.8±4.6 b (100.0)</td>
<td>8.3±3.2 b (100.0)</td>
</tr>
<tr>
<td></td>
<td>15.8±4.6 b (100.0)</td>
<td>8.3±3.2 b (100.0)</td>
</tr>
<tr>
<td>Non-nictating</td>
<td>15.1±5.2 b (100.0)</td>
<td>4.3±2.9 a (50.0)</td>
</tr>
<tr>
<td></td>
<td>15.1±5.2 b (100.0)</td>
<td>4.3±2.9 a (50.0)</td>
</tr>
<tr>
<td>Nictating</td>
<td>26.3±8.8 b (100.0)</td>
<td>18.8±6.9 b (100.0)</td>
</tr>
<tr>
<td></td>
<td>26.3±8.8 b (100.0)</td>
<td>18.8±6.9 b (100.0)</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.D.
Values followed by the same letter within a same exposure time are not significantly different \((p=0.05)\) according to the t-test.

1) The values in parentheses are the mortality (%) of insects recorded 48 hr after the commencement of exposure to nematodes.
2) Desheathed juveniles were not employed from insect cadavers.
Table 5. Penetration of insecticide-treated nictating infective juveniles of *Steinernema carpocapsae* to *Spodoptera litura* last-instar larvae.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% Penetration</th>
<th>6-hr exposure to insect</th>
<th>12-hr exposure to insect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ensheathed juveniles</td>
<td>Desheathed juveniles</td>
<td>Ensheathed juveniles</td>
</tr>
<tr>
<td>Water (control)</td>
<td>8.2±3.2 a</td>
<td>7.5±2.3 a</td>
<td>18.7±6.9 a</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>6.4±2.6 a</td>
<td>7.4±2.6 a</td>
<td>15.8±8.3 a</td>
</tr>
<tr>
<td>Acephate</td>
<td>7.1±1.5 a</td>
<td>8.1±2.4 a</td>
<td>20.1±1.6 a</td>
</tr>
<tr>
<td>Permethrin</td>
<td>7.4±1.7 a</td>
<td>7.1±1.5 a</td>
<td>20.4±1.6 a</td>
</tr>
</tbody>
</table>

Each value is the mean ± S. D. Values followed by the same letter in a column within the same exposure group are not significantly different (p = 0.05) according to DUNCAN’S multiple range test.

Penetration: Higher penetration rates were obtained from nictating nematodes than from non-nictating ones for both 6 and 12 hr exposures (Table 4). In this experiment, the IJs collected from insect cadavers penetrated more into the host insect, *Spodoptera litura*, than did those from chicken offal medium (p < 0.05). However, nematodes from chicken offal medium gave more reproducible and consistent results. There were no significant differences (p > 0.05) in the penetration rates between DeIJs and EnIJs at the 6 or 12-hr exposure to the host insects. All host insects died 48 hr after exposure to nematodes in the 6-hr and 12-hr exposures to nictating EnIJs harvested from the insect cadavers and 12-hr exposure to nictating EnIJs and DeIJs from chicken offal medium. There were no significant differences (p = 0.05) in the penetration rates between nictating IJs with and without insecticides (Table 5).

DISCUSSION

Previously, we suggested that nictating behavior of *S. carpocapsae* could be adopted as an indicator for screening pesticides for compatibility with nematodes (17). The nictating IJs, regardless of chemical treatments, killed the host insects faster than did non-nictating IJs obtained from aqueous suspension. Accordingly, a given concentrations of insecticides such as oxamyl, acephate, or permethrin that enhance nictating behavior may be used for mixed application with nematodes. Insects exposed to nictating IJs were killed faster than non-nictating ones (17) and this observation can be explained by higher rates of migration to the host cues and greater penetration of the insect’s body in the present experiments.

For nictating IJs, there were no significant differences (p = 0.05) in these migration and penetration activities between treatment with or without exposure to the chemical insecticide or among the tested chemical insecticides at the concentration of 50 μg/ml. Therefore, compatibility of a chemical at a certain concentration with nematodes can be established using the nictation behavior.
Nictation has been taken as a behavior in quest for a host insect (16, 20); waving the extended body from side to side may indicate their sampling for the source of chemical stimulants. However, from our observations, immobile standing IJs also can respond quickly to a chemical stimulus. Since nictation is a typical behavior of ambushers such as S. carpocapsae, it has been conceived as a mechanism by which ambush strategists attach to insect hosts (20). It is likely that the nictating nematodes may gain a greater opportunity to attach to the host insect by occupying the space more widely than do non-nictating ones on the surface. Under proper moisture conditions, the IJs can also leap (16); leaping behavior may tremendously expand their effective range.

Most S. carpocapsae IJs seem to become quiescent in soil after application (14, 17, 18). Nictation is usually observed by a low percentage (20%) of all nematodes applied onto the bark compost. The majority of them were stayed on the bottom of the petri dish or crawled on the surface of the bark compost. In our tests, we should have employed these IJs as the control, instead of non-nictating juveniles which had been kept quietly in a distilled water, though the latter nematodes were the source material for the experiments. If the IJs initiating the nictating behavior are the ones positive to cause an infection and the immobiles do not, the percentage of infectious nematodes represent a small population of the total population. We expected the gradual increase in the nictating rates by the successive use of nictating juveniles; however, the rates were almost on the plateau (ca. 20%, IJ collected from Galleria cadavers) in the four repeated performances. Consequently, numbers of nictating juveniles on the last performance became only 0.16% of the initial population. If the nematodes kept the active behavior for infection at a certain yield rate, the individual nematode should bear some indigenous property; 20% infectious and 80% noninfectious or at recess. When we observed a nictating juvenile, it would nictate for a given period. On the other hand, S. feltiae and S. carpocapsae IJs are supposed to become gradually more infectious with time or to have a survival strategy staggering their period of infectivity (3, 4, 13, 20). Steinernema carpocapsae IJs, after 2 months storage at 5-10°C, tended to be exsheathed. These nematodes migrated to insect plasma by 80% or more, but ensheathed IJs soon after emerged from insect cadaver responded only at a 20% or less to the same cue. Therefore, we employed the stored and exsheathed nematodes for the previous attraction bioassay (21, 22) to obtain a clear difference between attraction and non-attraction.

Two steinernematids isolated in Britain (S. feltiae and an undescribed Steinernema sp.) had a tendency for bimodal infection with time when stored at 5°C. Initially, they were infectious and then a few nematodes infected as the period of storage increased, but after this time, the numbers that invaded the Galleria larvae began to increase and eventually attained the original level (4). The authors suggested that the nematodes entered a diapause when kept at a low temperature. If this tendency is genetically programmed, it may be conducive for the maintenance of the species. If nematodes are all infectious at the same time and no hosts are available, the nematodes may become locally extinct (20). Steinernema carpocapsae may have developed at least two surviving strategies to avoid extinction. These survival strategies are 1) an increase in infectivity of the IJs with time and 2) immobility of the majority of the population after emergence from the host. Thus, predacious mites have a tendency not to eat immobile nematodes that adopt a straight posture (2, 15).

Male IJs of S. carpocapsae have been reported to be more active to disperse, locate, and to
establish in distant live host insects than female ones (12). If so, nictating IJs emerging soon after being placed on the arena for nictation should have been mostly male IJs. However, we did not investigate sexual development of the IJs to confirm this hypothesis. We are presently investigating the sex ratio of nictating juveniles. The results have come out to accept the above hypothesis; the nictating juveniles at earlier emergence became male by 70% or more after penetration to host insect (unpublished).

Infective juveniles from insect cadavers had a higher migrating rate to S. litura plasma than those produced from chicken offal medium. Nictating rates were also higher in the former IJs; with ca. 20% for the IJs from insect cadavers and 10% for those from chicken offal medium. No significant differences were seen in the mortality of insects between the two sources of nematodes when G. mellonella or S. litura larvae were used in the bioassay. However, the nematodes produced on the artificial medium are more likely to have a tendency to stagger their period of infectivity than those harvested from insect cadavers. These behavioral differences between the two sources of nematodes should be further investigated. The IJs produced on artificial medium would affect the efficacy of nematodes, particularly in large scale application-programs.

ACKNOWLEDGEMENT

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LITERATURE CITED

和文摘要

昆虫病原性線虫 Steinernema carpocapsae におけるニクテイティング感染態幼虫の感染性

石橋 信義・瀧井 新自・近藤 栄造

Steinernema carpocapsae 感染態幼虫のニクテイティング率は、ハチミツガ幼虫死体から得られた幼虫で20％、ニワトリ内臓培地で増殖した線虫で10％程度であった。ニクテイティング個体を集め、4回繰り返してニクテイティングさせても、その行動率は変わらなかった。かくして、4 回目にニクテイティングした線虫は最初に用いた総個体数の0.16％になった。ニクテイティング幼虫のハスモンヨトウ幼虫血清への誘引率、宿主昆虫への侵入率は、殺虫剤（オキサミル、アセフェート、ペルメトリル）処理・無処理に関わらず、非ニクテイティング幼虫（静水中で静止した幼虫）よりも明らかに高かった。従って、ニクテイティング行動を水よりも高めるか、または水と同等の殺虫剤は、その濃度において、線虫との混合施用が可能である。ニクテイティング行動を線虫の感染行動とみれば、線虫の感染率は概して低くなることになる。しかし、種維持戦略からみれば、このほうが、反って好都合であろうと考察する。