ヤガ類幼虫に対する防除能力をスクリーニングされた日本産昆虫病原性線虫(Steinernema属)の病原性に温度がおよぼす影響

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<td>p. 27-40</td>
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Influence of temperature on pathogenicity of some entomopathogenic nematode isolates (Steinernema spp.) from Japan screened for ability to control some noctuid moth larvae \(^1,2\)

Mutsuhiro Yoshida \(^3,*\)

Pathogenicity of indigenous steinernematids against some noctuid larvae was examined to screen candidates in a biological control program. First, 17 isolates from ten species (Steinernema feltiae, S. kraussei, S. kushidai, S. litorale, S. monticolum and five RFLP types) were screened for pathogenicity against the middle instar larvae of Peridoroma saucia, and S. feltiae and S. litorale were selected. Secondly, three isolates of S. feltiae and eight isolates of S. litorale were screened for pathogenicity against the middle instar larvae of Agrotis segetum, and S. feltiae HkBr36 and S. litorale IbKt142 were selected. Both isolates showed high pathogenicity at 25°C and below, but reduced pathogenicity at 30°C. Therefore, with the addition of a subtropical species S. abassi collected after the second screening, the screened isolates were compared with an introduced nematode, S. carpocapsae All, for their pathogenicity against the late instar larvae of A. segetum and Spodoptera litura. The isolate S. litorale IbKt142 showed high pathogenicity between 7 and 25°C, and caused significantly higher mortality of A. segetum at 7, 10 and 15°C and S. litura at 7 and 10°C than S. carpocapsae All did. Moreover, S. abassi Onlu181 caused significantly higher mortality of A. segetum at 30 and 35°C and S. litura at 35°C than S. carpocapsae All did. Nematol. Res. 40 (2), 27-40 (2010).

Key words: Agrotis segetum, Peridoroma saucia, Spodoptera litura, Steinernema litorale, S. abassi

INTRODUCTION

Nematodes belonging to the genera Steinernema Travassos, 1927 and Heterorhabditis Poinar, 1976 are called "entomopathogenic nematodes (EPNs)". As EPNs have outstanding beneficial traits such as high pathogenicity against insects, durable survivability and host-searching ability of the infective third-stage juvenile, reproduction on artificial medium, symbiotic association with entomopathogenic bacteria, and so on (Gaugler, 2002), scientific and commercial interests in EPNs discovered numerous geographic isolates (Hominick, 2002), are still searching for new species/isolates, are developing mass-production and formulation technologies and creating commercial insecticide products composed of EPNs for pest management (Georgis et al., 2006; Kaya et al., 2006). In Japan, a series of intensive research projects for the practical utilization of the EPNs, especially an introduced species, S. carpocapsae (Weiser, 1955), were conducted from the 80s to the 90s (Ishibashi, 1992) and two introduced nematode insecticide products composed of S. carpocapsae and S. glaseri (Steiner, 1929) are being utilized in Japan nowadays. Especially an introduced nematode insecticide product composed of S. carpocapsae is widely being applied to turfgrass, vegetables, fruit trees, flowers, ornamental plants, and so on (JPPNET, 2009). On the contrary, although more than ten steinernematid species have been recorded from Japan (Mamiya and Ogura, 1990; Mamiya et al., 1995; 2001; Phan et al., 2006; Stock et al., 1998; Yoshida, 2003; 2004; 2007; Yoshida et al., 1998), so far only S. kushidai Mamiya, 1988 has been applied to control white grubs mainly in golf course turfgrass and sweet potato fields (Ando, 1995; Ogura and Oya, 1992; Oya and Kamiwada, 1990; Suzuki et al., 2000).

Temperature conditions have a significant impact on infectivity of EPNs and low and high temperatures usually restrict the use of the EPNs (Griffin, 1993). For example, S. carpocapsae is generally recommended to be used at ambient temperatures between 20 and 30°C with the efficacy declining at temperatures below 15°C and above 35°C (Fujie, 1998). The lethal effect of S. kushidai also declined on the third instar larvae of Anomala cuprea (Hope, 1839) at 10, 15 and 35°C (Fujie et al., 1995). When examining the efficacy of EPNs against insects, it is important to monitor the temperature range at which the tested EPNs demon-
strate pathogenicity.

Consequently, a series of screenings of indigenous EPNs have been conducted in order to evaluate the potential of the indigenous isolates as biological control agents against soil inhabiting pests, especially to select indigenous isolates/species highly pathogenic at low and/or high temperatures in addition to being highly pathogenic at normal temperatures. Among soil inhabiting pests, the noctuid moth larva is one of the important agricultural pest groups. The cutworm is known as a serious agricultural pest causing damage to crops, vegetables and flowers by cutting the plant stem (Ohiro and Ozaki, 1975). The common cutworm is one of the most important agricultural pests, due to a wide host range, species of crops and vegetables (Okamoto and Okada, 1968) and resistance to chemical pesticides (Takeda, 2008). The present study reports the characteristics of some indigenous steinernematids concerning the relationship between pathogenicity and temperature, derived by screening the three noctuid moth larvae. In addition to the results of the screening, the potential of selected isolates as biological control agents mainly against the cutworm is discussed.

**MATERIALS AND METHODS**

Sources of nematodes and insects:

The steinernematid isolates used in the present study (Table 1) were recovered from soils in Japan and Sakhalin by using a *Galleria* baiting technique (Bedding and Akhurst, 1975). An introduced nematode insecticide product *Steinernema carpocapsae* All was obtained from Mr. Tanabe of SDS Biotech K.K. The Sakhalin isolate of *S. feltiae* (Filipjev, 1934) was collected by the Genebank Project (Exploration and Introduction of Microbial Genetic Resources) of National Institute of Agrobiological Sciences. The oriental grub *Exomala orientalis* (Waterhouse, 1875) was used to culture *S. kushidai* in the laboratory and others were cultured in late instar larvae of the greater wax moth, *Galleria mellonella* (Linnaeus, 1758). Infective third-stage juveniles (J3s) of *S. abbreviata* Elawad, Ahmad, and Reid, 1997, *S. kushidai* and *Steinernema* sp. RFLP type MY8 were

<table>
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1 Isolates used for the first screening test.
2 Isolates used for the second screening test.
3 Isolates used for the first and second screening tests.
stored at 10-12°C and other isolates were stored at 4-7°C until used.

The noctuid moth larvae used in the present study were cultured in the laboratory at room temperature (20-25°C) on an artificial insect diet “Insecta LFS” (NIHON NOSAN Co. Ltd). The variegated cutworm, *P. saucia*, was obtained from Dr. Saito, formerly of National Institute for Agro-Environmental Sciences, the cutworm, *A. segetum*, was obtained from Dr. Yoshimatsu, National Institute for Agro-Environmental Sciences and the common cutworm, *S. litura*, was obtained from Mr. Oida, Chiba Prefectural Agriculture and Forestry Research Center and Mr. Tanabe, SDS Biotech K.K. The culture of *S. litura* was kept as a mix of two populations.

First screening test:

The pathogenicity of ten Japanese species including 17 isolates (Table 1) was examined by using the middle instar larvae of *P. saucia* in the presence of soil at 20°C. As all isolates used in the first screening test successfully reproduced at 20°C in the insects, *S. kushidai* in white grubs and the other 16 isolates in greater wax moth larvae, the pathogenicity of each isolate against the middle instar larvae of *P. saucia* was examined at 20°C at first. Some species causing 50% mortality and above at 20°C, were used for the next screening in order to examine the influence of temperature on their pathogenicity by using the middle instar larvae of *P. saucia* in the presence of soil under variable temperature conditions at 7, 10, 15, 25, and 30°C.

About 150 ml of sterilised loamy soil containing about 25% water content (by weight) was put into a polythene cup (90 mm diameter at top, 75 mm diameter at bottom, 50 mm depth, 250 ml volume), then 2,500 IJs (10,000 IJs per 1 ml distilled water) were applied to the soil surface and each cup was incubated at 7, 10, 15, 20, 25, and 30°C. The application rate in the first screening corresponds to about two times the standard application rate of nematode insecticide products (25 IJs/cm² (Shapiro-Ilan et al., 2006)). After one day, five middle instar larvae of *P. saucia* were added with artificial diet to each cup, which was then incubated at the same temperature as the pre-incubation. The noctuid larvae were used after incubating for about one day at the test temperature. Three cups were prepared as three replicates for each test temperature and kept for 15 days at 7°C and 10 days at 10-30°C. Three cups containing soil, insects, artificial diet for insects, and 0.25 ml of distilled water in the same manner as above, were prepared as three replicates for the control at each test temperature. Each cup was checked for larval mortality every day from the 2nd day after treatment.

Second screening test:

The second screening test was conducted by using the middle instar larvae of *A. segetum* at 4, 7, 10, 15, 20, 25, and 30°C, in order to select isolates showing higher pathogenicity at a broader temperature range among isolates belonging to the screened species. Three geographic isolates of *S. feltiae* and eight of *S. litorale*, which had been kept in good condition when conducting the second screening tests, were used for the second screening test (Table 1). As another Hokkaido isolate of *S. feltiae* (see Yoshida, 2003; 2004) was lost in the rearing process before the screening test, only two Hokkaido isolates and one Sakhalin isolate of *S. feltiae* were used for the second screening test. The screening examined their pathogenicity for 15 days at 4-10°C and 10 days at 15-30°C in the same manner as the first screening test.

After the first test, all *P. saucia* suddenly died and the author could not get more of the lepidopteran species. Therefore, *A. segetum*, which was then being kept in another laboratory, was used for the tests, as it is easy to keep large numbers of the cutworms and large numbers of middle instar larvae could be obtained for all the screening tests.

Third screening test:

The pathogenicity of the selected isolates in the second screening test was examined by using the late instar larvae of the cutworm, *A. segetum* and the common cutworm, *S. litura*, as compared with *S. carpocapsae* All and *S. abbasi* Onr1181 which was isolated after the second screening test was conducted.

The inoculation numbers of nematode individuals/cup in the third screening test was determined judging from the mortality of the late instar larvae of *A. segetum* caused by *S. litorale* IbKt142. The pre-test was conducted at 15°C and prepared in the same manner mentioned below. On the nematode inoculation, approximately 500, 1,000, 2,500, 5,000, or 10,000 IJs were applied in 1 ml distilled water to each cup containing soil.

About 300 ml of sterilized loamy soil containing about 25% water content was put into a polythene cup (130 mm diameter, 50 mm depth, 430 ml volume), then IJs were applied to the soil surface and each cup was incubated at 7, 10, 15, 20, 25, and 35°C. After one day, five late instar larvae of *A. segetum* or *S. litura* were added with artificial diet to each cup, which was then incubated at the same temperature as the pre-incubation. The noctuid larvae were used after incubating for about one day at the test temperature. Three cups were prepared as three replicates for each test temperature and kept for 30 days at 7°C, 15 days...
at 10°C, 10 days at 15 and 20°C, and 5 days at 25, 30 and 35°C for A. segetum, and for 15 days at 7°C, 10 days at 10°C, 5 days at 15°C, 2 days at 20 and 25°C, and 3 days at 30 and 35°C for S. litura. Three cups, containing soil, insects, artificial diet and 1 ml of distilled water in the same manner as above, were prepared as three replications for the control at each test temperature and with each test insect, A. segetum or S. litura. Each cup was checked for larval mortality every day from the 2nd day after treatment at 15°C and above and every five days at 7 and 10°C.

Statistical analysis:

Statistical analysis of differences among mean values was performed according to Tukey’s HSD test, using Excel Statistics Ver. 4.0 software, Esumi Co. Ltd., Tokyo, Japan.

RESULTS

First screening test:

Steinernema feltiae HkHm26, HkEr36 and RsSh131, S. litorale ItSi144 and Steinernema sp. RFLP type MY3 SaMt28 showed high pathogenicity as compared with other species. They caused more than 80% mortality of P. saucia larvae at 20°C. The isolates S. litorale HkEr37 and Steinernema sp. RFLP MY3 NnSk47 and HkSb84 caused about 50% mortality and Steinernema sp. RFLP MY3 YnEn82 and FsKy29 caused only about 20% mortality. Steinernema sp. RFLP type MY6 caused about 50% mortality and S. kushidai and S. kraussei (Steiner, 1923) caused less than 30% mortality. In contrast, S. monticolum Stock, Choo & Kaya, 1997 and Steinernema spp. RFLP types MY5 & MY8 showed no pathogenicity against P. saucia and Steinernema sp. RFLP type MY7 caused nearly 0% mortality (Fig. 1). Consequently, S. feltiae, S. litorale and Steinernema spp. RFLP types MY3 & 6 were examined for their pathogenicity at different temperatures (Fig. 2).

Among three isolates belonging to S. feltiae, Japanese isolate HkEr36 caused more than 80% mortality between 7 and 25°C and HkHm26 caused more than about 50% mortality, between 7 and 25°C. The Sakhalin isolate RsSh131 from a subarctic region caused more than 80% mortality at 15 and 20°C but did not cause high mortality at 10°C. Steinernema litorale ItSi144 caused more than 70% mortality between 7 and 20°C. All five examined isolates of Steinernema sp. RFLP type MY3 showed the highest pathogenicity at 15°C among 10, 15, 20, and 25°C. The pathogenicity of isolLeS SaMt28, NnSk47 and HkSb84 was

![Fig. 1. Effect of 17 steinernematid isolates (ten species) on mortality (mean ± SE, n = 3) of the middle instar larvae of Peridoroma saucia at 20°C at 10 days after treatment.](image-url)

1 See Table 1.
almost comparable to that of *S. feltiae* and *S. litorale* at 15°C. However at 10, 20 and 25°C, the pathogenicity of *Steinernema* sp. RFLP type MY3 was relatively lower than that of *S. feltiae* and *S. litorale*. *Steinernema* sp. RFLP type MY6 NnSk50 at 10, 15 and 20°C showed almost the same level of pathogenicity, which was relatively lower than that of *S. feltiae* and *S. litorale* (Fig. 2). Accordingly judging from the first screening test, *S. feltiae* and *S. litorale* were selected for the next screening test.

Second screening test:

*Steinernema feltiae* HkEr36 caused more than 80%...
Fig. 3. Effect of different temperature conditions on mortality (mean ± SE, n = 3) of the middle instar larvae of Peridoroma saucia, caused by treatment with eight isolates of Steinernema litorale and two isolates of S. feltiae. Mortality of larvae was recorded at 15 days (4-10°C) and 10 days (15-30°C) after treatment. Means with the same lower case letter are not significantly different at 5% level between temperature conditions in each isolate according to Tukey's HSD test.

1 See Fig. 2.
mortality at 7 to 20°C, which was significantly higher than mortality at 4, 25 and 30°C. The mortality of the larvae was highly reduced at 4 and 25°C. Isolate HkHm26 caused more than 80% mortality at 10, 15 and 20°C, which was significantly higher than mortality at 7 and 30°C. At 25°C there was no significant difference between the pathogenicity of HkEr36 and that of HkHm26, and at 7°C the pathogenicity of HkEr36 was significantly higher than that of HkHm26 (Fig. 3). Accordingly, isolate HkEr36 was selected as a candidate for the next screening test among S. feltiae isolates. The Sakhalin isolate RsSh131 caused no mortality in the second screening. Therefore, the mortality data of RsSh131 is not given in Fig. 3.

Steinernema litorale AiAt199 caused more than 80% mortality at 15 and 20°C, and the mortality of the larvae was significantly reduced at 10 and 30°C. Isolates CbSr95 and IbKt142 caused more than 70% mortality between 10 and 25°C, and the mortality was significantly reduced at 7 and 30°C. Isolate CbWd140 caused more than 80% mortality between 10 and 20°C, and the mortality was significantly reduced at 7 and 30°C. Isolates IbDg157, HkSf16 and HkEr37 caused more than 80% mortality between 15 and 25°C, and the mortality was significantly reduced at 7 and 30°C. Isolate ItSi144 caused more than 80% mortality between 10 and 20°C, and the mortality was significantly reduced at 7 and 30°C. Isolates IbDg157, HkSf16 and HkEr37 caused more than 80% mortality between 15 and 25°C, and the mortality was significantly reduced at 7 and 30°C. Isolate ItSi144 caused more than 80% mortality between 10 and 25°C. The pathogenicity of isolate IbKt142 at 10 and 25°C was relatively higher than that of CbSr95. Therefore, isolate IbKt142 was selected as a candidate isolate for the

![Graph 1](image1.png)

**Fig. 4.** Effect of *Steinernema litorale* IbKt142, applied at 500, 1,000, 2,500, 5,000, and 10,000 infective juveniles (IJs)/cup, on mortality (mean ± SE, n = 3) of the late instar larvae of the cutworm, *Agrotis segetum*, at 15°C at 10 days after treatment.

![Graph 2](image2.png)

**Fig. 5.** Effect of different temperature conditions on mortality (mean ± SE, n = 3) of the late instar larvae of *Agrotis segetum*, caused by treatment with four steinernematid isolates (four species) respectively. Mortality of larvae was recorded at 30 days (7°C), 15 days (10°C), 10 days (15 and 20°C), and 5 days (25, 30 and 35°C) after treatment. Means with the same lower case letter are not significantly different at 5% level between temperature conditions and means with the same upper case letter are not significantly different at 5% level between steinernematid species at the same temperature condition, according to Tukey's HSD test.  

1 See Fig. 2.
next screening test among *S. litorale* isolates.

Judging from the second screening test, *S. feltiae* HkEr36 and *S. litorale* IbKt142 were selected for the next screening test.

Third screening test:

The first and second screening tests did not identify any steinernematid species/isolates which showed high pathogenicity at temperatures greater than 25°C. In contrast Yoshida (2007) reported that *S. abassi* Onlr181 from "Iriomote-jima Is." in a subtropical region showed high pathogenicity against late instar larvae of *A. segetum* at 25 and 30°C. Therefore *S. abassi* was added to the present test. The mortality of *A. segetum* caused by *S. abassi* Onlr181 was used in "Results" and "Discussion" after correcting and modifying the mortality data from Yoshida (2007).

As *S. litorale* IbKt142 showed 100% mortality against middle instar larvae of *A. segetum* at 15°C within 10 days after treatment (Fig. 3), the nematode numbers which caused 100% mortality against the late instar larvae of *A. segetum* at 15°C within 10 days was also adopted in the third screening test. At 15°C, 10,000 IJs caused 100% mortality in all three replications, 5,000 and 2,500 IJs caused more than 67% mortality, 1,000 IJs caused 40% mortality, and 500 IJs caused about 27% mortality (Fig. 4). Accordingly, 10,000 IJs/cup was adopted as the application rate. This application rate corresponds to about three times the standard application rate of nematode insecticide products (25 IJs/cm²) (Shapiro-Ilan et al., 2003).

The third screening test using the late instar larvae of two noctuid species selected *S. litorale* IbKt142 and *S. abassi* Onlr181 and the low temperature pathogenicity of the former and the high temperature pathogenicity of the latter were recognized as being superior to *S. carpocapsae* All as described below.

Pathogenicity comparison by using the late instar larvae of *A. segetum*:

*Steinernema litorale* IbKt142 caused more than 80% mortality at 7 to 25°C, and the mortality was significantly reduced at 30°C. The isolate *S. feltiae* HkEr36 caused more than 60% mortality at 10, 15, 20, and 25°C, and the mortality was significantly reduced at 7 and 30°C. *Steinernema abassi* Onlr181 caused more than 80% mortality at 25 and 30°C, and the mortality was significantly reduced at 20 and 35°C (the modified data derived from Yoshida (2007)). *Steinernema carpocapsae* All caused more than 70% mortality at 20 and 25°C, and the mortality was highly reduced at 10 and 35°C (Fig. 5).

Among these four isolates, *S. litorale* IbKt142 consistently caused high mortality between 7 and 25°C and *S. abassi* Onlr181 caused high mortality between 25 and 30°C. Only *S. abassi* Onlr181 caused mortality at 35°C (Fig. 5). The pathogenicity of *S. litorale* IbKt142 was significantly higher than that of *S. feltiae* HkEr36 at 7 and 10°C and that of *S. carpocapsae* All at 7, 10 and 15°C, and relatively higher than that of *S. feltiae* HkEr36 at 15, 20 and 25°C, and that of *S. carpocapsae* All at 20 and 25°C. The pathogenicity of *S. abassi* Onlr181 was significantly higher than that of *S. carpocapsae* All at 30 and 35°C and relatively higher than that of the latter at 25°C. At 25°C, *S. litorale* IbKt142 and *S. abassi* Onlr181 caused high mortality between 25 and 30°C. Only *S. abassi* Onlr181 caused mortality at 35°C (Fig. 5).

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The pathogenicity of *S. litorale* IbKt142 was significantly higher than that of *S. feltiae* HkEr36 at 7 and 10°C and that of *S. carpocapsae* All at 7, 10 and 15°C, and relatively higher than that of *S. feltiae* HkEr36 at 15, 20 and 25°C, and that of *S. carpocapsae* All at 20 and 25°C. The pathogenicity of *S. abassi* Onlr181 was significantly higher than that of *S. carpocapsae* All at 30 and 35°C and relatively higher than that of the latter at 25°C. At 25°C, *S. litorale* IbKt142 and *S. abassi* Onlr181 caused high mortality between 25 and 30°C. Only *S. abassi* Onlr181 caused mortality at 35°C (Fig. 5).

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Abbasi Onlr181 showed the same level of pathogenicity. However, there was no significant difference among these four isolates in mortality at 25°C (Fig. 5). Consequently, *S. litorale* IbKt142 and *S. abbasi* Onlr181 were examined for pathogenicity against *S. litura* in order to compare it with that of *S. carpocapsae* Al.

Pathogenicity against the late instar larvae of *S. litura*:

*Steinernema litorale* IbKt142 caused 100% mortality between 7 and 25°C, and the mortality was significantly reduced at 30°C. *Steinernema abbasi* Onlr181 caused more than 75% mortality at 25, 30, and 35°C, and did not cause any mortality at 20°C. *Steinernema carpocapsae* Al caused almost 100% mortality at 15 to 30°C and the mortality was slightly reduced from 15 to 7°C but significantly reduced at 35°C (Fig. 6).

Among these three isolates, *S. litorale* IbKt142 consistently caused high mortality between 7 and 25°C, *S. abbasi* Onlr181 caused high mortality between 25 and 35°C, and *S. carpocapsae* Al consistently caused high mortality between 15 and 30°C. There was no significant difference among these three isolates in mortality at 25°C. The pathogenicity of *S. litorale* IbKt142 was significantly higher than that of *S. carpocapsae* Al at 7 and 10°C. The pathogenicity of *S. abbasi* Onlr181 and *S. carpocapsae* Al was significantly higher than that of *S. litorale* IbKt142 at 30°C. The pathogenicity of *S. abbasi* Onlr181 was significantly higher than that of *S. carpocapsae* Al at 35°C (Fig. 6).

Low temperature pathogenicity of *S. litorale* IbKt142:

As it took more than 25 days for *S. litorale* IbKt142 to cause 80% mortality of the late instar larvae of *A. segetum* at 7°C, changes in mortalities of *A. segetum* at 7°C and 10°C and *S. litura* at 7°C and 10°C were graphed as the cumulative mortality respectively (Fig. 7). It took more than 10 days at 7°C and more than five days at 10°C until the isolate caused the death of *A. segetum*. On the other hand, it took more than five days at 7°C and five days or less at 10°C until the isolate caused the death of *S. litura* (Fig. 7).

The isolate caused 100% mortality of *A. segetum* at 10°C (15 days) and *S. litura* at 7°C (15 days) and 10°C (10 days), but did not cause 100% mortality of *A. segetum* at 7°C (Fig. 7). At 7°C, *S. litorale* IbKt142 had extremely reduced pathogenicity against the late instar larvae of *A. segetum*.
DISCUSSION

Through the first and second tests at variable temperature conditions, *S. litorale* IbKt142 and *S. feltiae* HkEr36 showed high pathogenicity at the broadest temperature range among the tested indigenous steinernematid species/isolates. In the third test *S. litorale* IbKt142 caused higher mortality of the late instar larvae of *A. segetum* at 7 and 10°C than *S. feltiae* HkEr36 did and showed high pathogenicity consistently between 7 and 25°C (Fig. 5). Accordingly the present screening test selected *S. litorale* IbKt142 as a promising biological control agent, especially since it was characterized as having low temperature pathogenicity. Moreover, *S. litorale* IbKt142 showed higher pathogenicity against the late instar larvae of *A. segetum* at 7, 10 and 15°C (Fig. 5), *S. litura* at 7 and 10°C (Fig. 6) and corn earworm, *Helicoverpa armigera* (Hübner, 1808) (Noctuidae), at 25°C (Yoshida, unpublished data) than *S. carpocapsae* All did. However, *S. litorale* IbKt142 showed lower pathogenicity against *A. segetum* and *S. litura* at 30°C than *S. carpocapsae* All did and all indigenous isolates used for the first and second tests had reduced pathogenicity at 25 or 30°C and above (Figs. 2 and 3). Consequently, with the entry of a subtropical species *S. abbasi* OnIr181, the screened isolates were compared for their pathogenicity against *A. segetum* with *S. carpocapsae* All. Although the mortality at 25°C was almost at the same level among them, *S. abbasi* OnIr181 caused significantly higher mortality at 30 and 35°C (Fig. 5) (Yoshida, 2007). *Steinernema abbasi* OnIr181 was also highly pathogenic against the late instar larvae of *S. litura* at 25, 30 and 35°C (Fig. 6). However, *S. abbasi* OnIr181 had significantly reduced pathogenicity at 20°C. Consequently, it is considered that *S. abbasi* OnIr181 could complement the efficacy of *S. litorale* IbKt142 at 30 and 35°C. As both species showed the same level of pathogenicity at 25°C, their interaction between 20 and 30°C should be examined in order to evaluate the possibilities of their combined use. The combined utilization of *S. litorale* IbKt142 and *S. abbasi* OnIr181 might provide a powerful pest management strategy which could control various lepidopteran pests over a wide temperature range throughout the four seasons.

In the present study, the target of control by the EPNs was the soil-dwelling larval stage, i.e. middle and late instar larvae of *A. segetum* and late instar larvae of *S. litura*. The late instar larval stage of *A. segetum* and *S. litura* are important agricultural pests and it is difficult to control them by chemicals as they stay in soil. The middle and late instar larvae of the former cut plant stems of young plants around the soil surface or injure the vegetative growing point and the late instars stay dispersed in soil around young plants during daytime. Therefore, cutworms over the middle instar often cause significant yield losses, though the population density usually decreases remarkably at early instar (Kubota et al., 1974; Ohiro and Ozaki, 1975; Ozaki, 1976). The early instar larvae of the latter usually cause almost no damage to plants, over the fourth instar the amount of food ingested increases and the sixth instar larvae ingest 85% of the total amount of food consumed by larvae. As the species usually has a nocturnal habit over the fourth instar, it often hides under fallen leaves, covertures or the soil surface during daytime. Accordingly, overlooking the feeding marks caused by the early instar larvae can result in losses on crops and vegetables by the late instar larvae (Oyama, 1979). The normal habitat of EPNs is in soil and the main target of the pathogenic study was the late instar larvae of noctuid moths. The control of the late instar larvae suppresses the next generation and should cause a reduction in the field population of the target pest.

*Agrotis segetum* overwinters in the late instar larval state, the larvae after overwintering injure young plants of various crops and vegetables in spring and the next generations injure plants from spring to autumn being emergent a few times/year (Kubota et al., 1974; Ohiro and Ozaki, 1975; Ozaki, 1976). Therefore in the control of *A. segetum*, it is important to suppress the overwintering population. The low temperature pathogenicity of *S. litorale* IbKt142 could provide a biological control technique against the cutworms after and before hibernation. In the present screening, it took for more than ten days at 7°C until the nematode killed the late instar larvae of *A. segetum* (Fig. 7) and the screening was conducted under conditions that nematodes could reach the test insects without any difficulty. In order to estimate whether *S. litorale* IbKt142 could demonstrate efficacy in the field at low temperatures, the host-searching and infection abilities at low temperatures should be studied. In an agricultural field at Tsukuba, it was confirmed that *S. litorale* IbKt142 showed the ability to survive for about one year (Yoshida, unpublished data). Accordingly, if *S. litorale* IbKt142 possesses low temperature infection ability and can demonstrate the ability in agricultural fields, we can expect *S. litorale* IbKt142 to provide a technique for preventive application against pest populations before and after hibernation between late autumn and early spring. However, Hasegawa and Chiba (1969) reported that the lower threshold temperature for development of *A. segetum* larvae was 4.2°C. So for management of *A. segetum* larvae in winter season, it might be necessary to examine the pathogenicity of *S. litorale* IbKt142 at 4°C, or to search for new isolates...
with activity at temperatures lower than 4°C. As *A. segetum* occurs and invades from surrounding areas also in summer season and *S. litorale* IbKt142 was less pathogenic at temperatures greater than 25°C, in order to control *A. segetum* larvae throughout the four seasons, a technique combining a preventive application of *S. litorale* IbKt142 and an additional application of *S. abbreviatus* OnIr181 against the populations occurring/invasing in summer season needs to be developed.

The first and second tests also indicated the intraspecific variation in the influence of temperature on the pathogenicity. *Steinernema feltiae* and *S. litorale* showed interesting intraspecific variation. Some isolates detected from warmer regions caused higher mortality at lower temperature than some isolates detected from cooler regions did. In the case of *S. feltiae* HkEr36 and HkHm26 from Hokkaido, a cool temperate region, it was consistently highly pathogenic against the middle instar larvae of *P. saucia* between 7 or 10 and 20°C. However RShH131 from Sakhalin, a subarctic region, had significantly reduced mortality at 10°C (Table 2, Fig. 2). At the second screening test using the middle instar larvae of *A. segetum*, HkEr36 also consistently showed high pathogenicity between 7 and 20°C and HkHm26 did between 10 and 20°C (Fig. 3). The isolation site of HkEr36 is considered to be warmer than that of HkHm26 (Table 2). A similar situation to that in *S. feltiae* was observed also in the case of *S. litorale*, i.e. Honshu isolates, CbSr95, CbWd140, IbKt142, and IsSi144, caused higher mortality at low temperatures than Hokkaido isolates did. Especially CbSr95 and CbWd140, which were detected from a warm region, southern Boso Peninsula, showed high pathogenicity at 10°C, the same as IbKt142 and IsSi144 from a northern region of Honshu and *S. feltiae* (Table 2, Fig. 3). The lower side of the temperature range of Honshu isolates of *S. litorale* was the same as that of *S. feltiae*, whereas the lower side of the temperature range of Hokkaido isolates of *S. litorale* was higher than that of *S. feltiae*. *Steinernema litorale* morphologically and molecularly closely resembles *S. feltiae*, the reproductive isolation between them is incomplete and both species were isolated from similar habitats in Hokkaido and have been isolated sympatrically (Kuwata et al., 2006; Yoshida, 2004). Accordingly the difference in the temperature range causing significantly high mortality might act as an ecological advantage.
isolation on their infectivity and host selection between these closely related species occurring in the same habitat. In contrast, unlike with EPN fauna in Hokkaido, *S. feltiae* has not been detected in Honshu and Honshu isolates of *S. litorale* occur in a similar habitat to that of Hokkaido isolates (Yoshida, 2004). Therefore it is possible that some Honshu isolates of *S. litorale* acquired or re-established infectivity at lower temperatures.

In contrast, *Steinernema* sp. RFLP type MY3 almost did not indicate the intraspecific variation in the temperature range showing high pathogenicity, although the species occurs widely in Japan, from Hokkaido in a subarctic region to Amami-Oshima Is. in a subtropical region (Yoshida et al., 1998; Yoshida, unpublished data). Namely, all of the four isolates of *Steinernema* sp. RFLP type MY3 from Kyushu, Honshu and Hokkaido caused the highest mortality at 15°C against the middle instar larvae of *P. saucia* and had drastically reduced pathogenicity at 10°C and abruptly reduced at 25°C (Fig. 2, data of FsKy29 was not shown), independent of the mean temperature of the isolation site, *i.e.* SaMt28 was detected from a warm temperate region and NnSk47, FsKy29 and HkSb84 were detected from a cool temperate region (Table 2). In the preliminary test at 15°C, FsKy29 showed high pathogenicity against the late instar larvae of *Mythimna separata* (Walker, 1865) (Noctuidae) and SaMt28 also showed high pathogenicity against the middle instar larvae of *S. litura*. *Steinernema* sp. RFLP type MY3 might be adapting to the natural host occurring at around 15°C.

In the case of the three steinernematid species mentioned above, it is considered that the ambient temperature condition of their isolation sites did not affect the temperature range causing high mortality. That is to say, isolates collected from cool environments have not always showed high pathogenicity at lower temperatures. Meanwhile, *S. monticolum* and *S. kraussei* are EPNs active at cold temperatures (Koppenhöfer et al., 2000; Long et al., 2000; Willmott et al., 2002). Koppenhöfer et al. (2000) indicated that the thermal activity range of *S. monticolum* corresponds well to the climate of the area where *S. monticolum* was isolated in a mountainous region of southern Korea. Gwynn and Richardson (1996) collected EPNs from areas of the UK with lower average temperatures and Long et al. (2000) selected an isolate of *S. kraussei* among the isolates as a potential biocontrol agent against the black vine weevil, *Otiorhynchus sulcatus* (Fabricius, 1775) (Curculionidae, Coleoptera) at low temperatures. The Japanese isolate of *S. kraussei* HkHm22 also showed high pathogenicity against the last instar larvae of the pine sawyer beetle, *Monochamus alternatus* Hope, 1842 (Cerambycidae, Coleoptera), at 7 and 10°C (Aikawa and Yoshida, 2006). These reports and the results from the present screening indicate that in order to identify isolates which show high pathogenicity at low temperature, we should examine the pathogenicity of isolates from warm environments as well as isolates from cool environments in Japan. Whereas in order to identify isolates which are highly pathogenic at high temperatures, another type of survey and research are needed, as all of the isolates used in the first and second screening tests had reduced pathogenicity at more than 25°C and *S. abbasi* had reduced pathogenicity at 35°C.

**ACKNOWLEDGEMENTS**

The author is grateful to Dr. O. Saito, National Institute for Agro-Environmental Sciences (NIAES) (Present: Bio-oriented Technology Research Advancement Institution), and Dr. S. Yoshimatsu, NIAES, for providing some noctuid species and instructions on how to rear the noctuid species. I am indebted to Mr. H. Tanabe, SDS Biotech K.K., for providing *S. carpocapsae* All and *S. litura*, Mr. H. Oida, Chiba Prefectural Agriculture and Forestry Research Center, for providing *S. litura*, and Ms. A. Nakamura, formerly of National Agricultural Research Center, Ms. I. Mikawa, NIAES, and Ms. Suenaga, formerly of NIAES, for rearing noctuid moths.

This work was supported in part by project research programs of Ministry of Agriculture, Forestry and Fisheries, “Integrated research on development of innovative techniques for implementation of sustainable agriculture” (1999-2003) and “Development of new biorational techniques for sustainable agriculture” (2004-2008), and Genebank Project (Exploration and Introduction of Microbial Genetic Resources) of National Institute of Agrobiological Sciences.

**LITERATURE CITED**


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Received: January 19, 2010

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ヤガ類幼虫に対する防除能力をスクリーニングされた日本産昆虫病原性線虫（Steinernema属）の病原性に温度がおよそ影響

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生物防除に供しうる土着のSteinernema属昆虫病原性線虫を選定するために、ヤガ類幼虫に対する病原性を調査した。最初に、土着種10種17アイソレートの病原性をニセタマナヤガ中齢幼虫を使って比較したが、S. feltiaeとS. lituraeが選定された。次いで、前者2、後者8アイソレートの病原性をカブトヤガ中齢幼虫を使って比較した結果、更にアイソレートと北茨城アイソレートがそれぞれ選定された。両種とも25℃以下で高い病原性を示したが、30℃では低下した。そこで、S. abbagi西茨城アイソレートを加えて、3アイソレートのカブトヤガ、ハスモンヨトウ老齢幼虫に対する病原性を導入種S. carpocapsae Allと比較した。S. liturae北茨城は7〜25℃でヤガ類に対し高い病原性を示し、S. carpocapsae Allと比較して7・10℃でカブトヤガ、7・10℃でハスモンヨトウに対し有意に高い病原性を示した。S. abbagi西茨城はS. carpocapsae Allと比較して30・35℃でカブトヤガ、35℃でハスモンヨトウに対し有意に高い病原性を示した。

土塚締め法あるいはボールミル法により調整された土塚中のジャガイモ逆面種セントチュウの直接定量のための検量線の比較
後藤友一、佐藤恵利、李方剛、藤田晃一、杉戸哲子 .......................... 41

土塚締めとリラタイムPCRからなる定量方法を、土塚中のジャガイモ逆面種セントチュウ（PCN）の定量に適用した。また、線虫締めの破壊と同一の土塚サンプルの入手の効率、2つの前処理法（ボールミルと土塚締め）を比較した。3種類の黒ポタクを用いてこれらの実験を行った。いずれの土塚、いずれの前処理法でも、土塚20g当り5pcのPCNを2個幼虫（J2）を検出でき、Cr値とJ2の添加数との間には、r>0.9897の有意な相関が認められた。Cr値と土塚中のJ2密度との関係を示す検量線は、両者の土塚では2つの前処理方法で同じであったが、両者の土塚ではCr値がボールミル法で低く、1回以内の値を示したところ、DNA抽出効率はボールミル法の方が良好であると推察された。ボールミル法で得られた検量線は3土塚ではほぼ同一であったが、締め法では異なっていたことから、ボールミル法の方が線虫の直接定量には相応しいと考えられました。

ビジネスカメシミに自然状態で接続するCaenorhabditis japonica耐久型幼虫の生存性
田中隆篤、園井幸恵、吉賀豊司 ................................. 47

Caenorhabditis japonica Kiontkle, Hironaka and Sudhausはビジネスカメシミに接続する細菌食性線虫である。休止状態のC. japonica耐久型幼虫は常にカメシミ細胞体表皮から一年を通じて検出される結果、カメシミ上で長期生存することが考えられるが、実際の生存期間は明らかでない。形態的特徴をみるためクリオ凍結電子顕微鏡観察をおこなったところ、カメシミ上的線虫は緩やかに卵殻状態であったが、無性生存性を有するイネシンガレセントチュウのような耐性の発達はみられなかった。野外から採取したカメシミを実験室内でいくつかの湿度条件下におき、3か月間保持後に卵殻を酸洗し、カメシミ上の耐久型幼虫の生存を調べた。その結果、湿度100％においてはカメシミ上にほとんど線虫は残存しておらず、湿度97％では1頭のカメシミあたり19頭の線虫が検出され、その生存率は33%であった。一方、木製の箱で維持したカメシミではカメシミの生存率は100%となかった。2頭のカメシミあたり67頭の線虫が検出され、その生存率は55%であった。以上の結果より、C. japonica耐久型幼虫はビジネスカメシミ上で緩やかに卵殻状態にあり、数か月間生存可能であることが明らかになった。

Apelheuschens avenae（アフェレンクス科）とFilenchus misellus（ディレンクス科）にRhizoctonia solani菌系7系統を闘として与えた場合の増殖性
岡田浩明、原田啓基、野崎真奈 ................................. 60

アフェレンクス科のApelheuschens avenae（Aa）とディレンクス科のFilenchus misellus（Fm）について、土壌伝染性植物病原性状菌Rhizoctonia solani（Rs）の7つの系統を供試させた場合の増殖性を調べた。いずれか1つのRs系統の菌糸が生育したジャガイモ素地にAaとFmのいずれか1種を30匹播種し、25℃で40日間増殖させた後、ベールマン漏斗で線虫を分離し、個体数を調べた。負の二項分布を仮定した統計モデルにより検討したところ、線虫種、Rs系統及び両者の交互作用のいずれもが線虫個体数に影響したと考えられた。全菌株をAaでは個体数がFmより著しく多く、供試したRs系統のうち、特にダイズとキャベツから採取された系統で良く増殖した。一方Fmは、ほとんど増殖しなかったRs系統があったが、イネ、ダイズ、キャベツに由来する系統である程度の増殖が認められた。