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CONTROL OF INSECT PESTS WITH ENTOMOPATHOGENIC NEMATODES

I. Control of Weevils with Entomopathogenic Nematodes

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II. Control of Turfgrass Insect Pests with Entomopathogenic Nematodes in Japan

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FOREWORD

This important Bulletin is based on two papers first presented by their authors at an international seminar on the "*Use of Biological Control Agents under Integrated Pest Management*", held in October 1993 in Fukuoka, Japan, and co-sponsored by the University of Kyushu and the University of Saga. The two papers discuss the use of entomopathogenic nematodes to control insect pests.

While some nematode species are serious pests in their own right, other species attack insects. They carry in their gut bacterial cells which are pathogenic to insects (i.e. entomopathogenic). Infective juveniles of entomopathogenic nematodes usually live in the soil. When they find an insect host they enter it through its natural body openings and release their bacterial cells. The host dies within a few days, and the nematode then enters its reproductive stage, the dead body of the insect providing a food source for the young nematodes.

Since nematodes are living organisms, it has not been easy to find a formulation which delivers the nematode to the target site and target insect. Now they are being used in commercial preparations, and their potential is enormous. The first paper discusses the use of two genera of nematode, *Steinernema* and *Heterohabditis*, to control billbugs and various species of weevil. The second paper discusses the use of entomopathogenic nematodes to control turfgrass insect pests on golf courses in Japan.

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(Chinese Abstract)

摘 要

已成功的利用斯氏線蟲屬和異小桿線蟲屬等蟲生線蟲之商品化生物防治劑來防治各種象鼻蟲屬之害蟲。已採用線蟲生物防治劑防治之害蟲有(1)耳喙象鼻蟲；(2)*Sphenophorus*屬象鼻蟲；(3)*Diaprepes abbreviatus*象鼻蟲；(4)桔根象鼻蟲。而許多象鼻蟲科的其他種類和近緣科屬之害蟲未來都有可能利用此種商品化生物防治劑來防治。

(Japanese Abstract)

摘 要

Steinernema と *Heterorhabditis* 属の昆虫寄生性線虫はゾウムシ科の各種害虫に対する生物的防除剤として商品化されている。この線虫剤は次の4種のゾウムシにも有効である。すなわち *Otiorhynchus* spp., *Sphenophorus* spp., *Diaprepes abbreviatus*, *Pachnaeus litus* である。そのほかの数種のゾウムシ及びゾウムシ科に近縁の科の種類が将来の対象害虫として有望である。

(Korean Abstract)

초 록

바구미류(Curculionid species)의 여러 빈종에 대한 생물적방제 인자로써 *Steinernema*와 *Heterorhabditis* 속(屬)에 속하는 곤충병원성 선충의 상품화가 성공되었다. 선충을 기본으로 한 상품은 1) root weevils, *Otiorhynchus* spp. 2) billbugs, *Sphenophorus* spp. 3) apopka weevil, *Diaprepes abbreviatus*, 그리고 4) citrus root weevil, *Pachnaeus litus* 등에 도입되었다. 근연관계가 있는 과(科)에 대한 상품화 가능성 역시 높다.

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ABSTRACT

Entomopathogenic nematodes in the genus Steinernema and Heterorhabditis have been successfully commercialized as biological control agents for a variety of curculionid species. Nematode-based products have been introduced for: 1) root weevils, Otiorhynchus spp. 2) billbugs, Sphenophorus spp. 3) the apopka weevil, Diaprepes abbreviatus, and 4) the citrus root weevil, Pachnaeus litus. Several other Curculionidae species and other closely related families are potential candidates for future commercial introductions.

INTRODUCTION

Gaugler and Kaya (1990) recently published a complete review of current knowledge of entomopathogenic nematodes. Klein (1990) gave a condensed introduction regarding nematode efficacy to the order Insecta. The intent of this paper is to provide a brief overview of entomopathogenic nematode efficacy against weevils, and the recent technological developments which have made possible the commercial success of nematode-based products.

Entomopathogenic nematodes in the genus *Steinernema* and *Heterorhabditis* and their associated bacteria (*Xenorhabdus* spp.) have been successfully commercialized as biological control agents for a variety of curculionid species. They can kill hosts rapidly, are easy to apply, and are exempted from federal and local registration requirements in most countries because of their safety to mammals and plants (Georgis *et al.* 1991). Difficulties in production, storage, formulation, quality control, and application technology limited their commercial success in the past. Recent public pressure to limit environmental contamination associated with chemical insecticide use has resulted in a dramatic

increase in research conducted by scientists in government, universities and industry to overcome some of these technical difficulties (Smith *et al.* 1992). There are now three major biotechnology companies that have been successful in introducing nematode-based products into some commercial and consumer markets.

Production

Since their discovery as biological control agents, nematodes have been produced *in vivo*, with an insect host serving as the media for nematode-bacterial growth and production. This method has limitations, because it requires a constant source of healthy insects. It is also sensitive to biological variation, and costs of production are high in terms of equipment and man-hours. More efficient methods of production using *in vitro* methods have recently been developed. *Steinernema* spp. are now commercially produced in monoxenic liquid culture systems that use fermentation tank technology. This approach is the most economical of all known methods. Nematode production is taking place in tanks of up to 80,000 liters in volume, which has lowered

Keywords: billbugs, biological control, entomopathogenic nematodes, weevils.

costs considerably, allowing successful introductions into markets requiring large numbers of nematodes or markets of low cash crop value.

Formulation

The successful market introduction of an entomopathogenic nematode based-product requires a reliable and stable formulation. This has been a difficult task, because most larger markets are demanding a product with a minimum shelf-life of six months when stored at room temperatures (20 -25 C). Nematode products contain living animals that have certain temperature, oxygen and moisture requirements necessary for their survival and effectiveness as control agents. While no nematode formulation has been completely successful in reaching these goals, some have come very close (Georgis 1992).

Application Technology

Strategies must also be developed which insure the successful delivery of the nematode to the target site and target insect, thereby increasing the probability of nematode-insect interaction. This has not been a simple task. Many parameters must be investigated to improve performance. Determining target insect life-stage susceptibility is critical, since different life stages of different species are not equally susceptible. Research by the biosys company has shown that pest population levels and behavior have a great influence on nematode performance and must be considered carefully (unpublished data). Often, larval stages of insects such as borers are not accessible to nematodes. Selecting the most appropriate nematode species and/or strain is important for efficacy and commercial development. Abiotic factors such as soil type, soil temperature and moisture, and biotic factors, including pathogens and predators, can greatly influence the nematodes' ability to effectively kill the target pest.

Application strategies, including field dosage, volume, irrigation and appropriate application methods, are very important, especially if nematodes are to be integrated with other control strategies. Compatibility with a wide range of agrichemicals has been demonstrated (biosys, unpublished data). This

has benefited the successful introduction with existing Integrated Pest Management programs. Crop morphology and phenology must be considered in predicting whether nematodes are viable control candidates. Additional research has shown the potential for entomopathogenic nematodes to be used in other habitats (e.g. aquatic, foliar, and cryptic), and in manure.

Efficacy

Steinernematids and heterorhabditids differ in host seeking behavior, tolerance to environmental parameters, behavior in the soil and pathogenicity to various insect species (Gaugler 1988). The success of entomopathogenic nematodes is largely due to the extensive amount of scientific research conducted, both in the laboratory and in the field.

Probably the most studied group of weevil pest species are the so-called root weevils such as black vine weevil, *Otiorynchus sulcatus*, and strawberry root weevil, *O. ovatus*. These insects are serious pests on several crops throughout western Europe, and North America. Table 1 summarizes several field studies of black vine weevil in containerized ornamental plants supervised by the biosys company. Our experience with this insect has shown that rates of 7.5×10^6 nematodes per hectare are necessary for consistent control. Both *S. carpocapsae* and *H. bacteriophora* under appropriate environmental conditions give control equal to, or better than, registered insecticides when targeted against the immature life stages. In North America, Helix and BioSafe N (*S. carpocapsae*) are sold commercially for use on cranberry. BioVector is sold for other berry crops and also mint, while Exhibit is sold for use on ornamental plants. Several thousand hectares have been treated to date.

Table 2 shows data on the pathogenicity of various *Steinernema* spp. against neonate larvae of black vine weevil (Shanks, unpublished data). Other studies have shown that all larval and pupal stages are susceptible.

Billbugs, *Sphenophorus* spp., have become significant pests of turf in North America and Japan. All immature stages are susceptible to entomopathogenic nematodes. Table 3 summarizes field trial results of

Table 1. Field efficacy of entomopathogenic nematodes against black vine weevil, *Otiorhynchus sulcatus*

Treatment	Rate a.i./ha	No. of trials	% reduction	Median % reduction
Exhibit*	7.5x10 ⁹	26	86.4 ± 2.8**	91.1
	5.0x10 ⁹	9	84.5 ± 5.1	86.3
	2.5x10 ⁹	3	95.0 ± 2.6	94.0
<i>Steinernema carpocapsae</i> (UK strain)	7.5x10 ⁹	13	86.3 ± 3.8	85.0
	5.0x10 ⁹	1	61.0	61.0
	2.5x10 ⁹	1	92.0	92.0
<i>Steinernema feltiae</i>	7.5x10 ⁹	6	58.6 ± 9.5	51.2
	5.0x10 ⁹	5	85.8 ± 6.1	83.0
	2.5x10 ⁹	3	70.3 ± 18.7	86.0
<i>Heterorhabditis</i> spp.	7.5x10 ⁹	9	84.2 ± 5.7	90.0
	5.0x10 ⁹	10	92.4 ± 3.7	96.9
	2.5x10 ⁹	3	97.0 ± 3.0	96.5
Insecticide	Standard	10	77.8 ± 7.8	86.2

* Exhibit = *Steinernema carpocapsae* (All strain).

** Mean ± standard error 3-4 week post application evaluation. Soil temperature above 16°C. Container trials with various ornamental plant species.

Table 2. Susceptibility of neonate black vine weevil larvae, *Otiorhynchus sulcatus* to various steinernematid species

Treatment	Rate no./cm ²	Av. no. of living larvae per container
<i>Steinernema carpocapsae</i>	50	0.0 a*
	75	0.0 a
<i>Steinernema feltiae</i>	50	0.3 a
	75	0.0 a
<i>Steinernema glaseri</i>	50	0.4 a
	75	0.6 a
Untreated	—	16.8 b

* Potted strawberry plants. Means in the column followed by different letters are significantly different (P>0.05). Duncan's Multiple Range Test.

Table 3. Field efficacy of entomopathogenic nematodes against bluegrass billbug, *Sphenophorus purvulus*, in the U.S.A.

Treatment	Rate a.i./ha	No. of trials	% reduction	% range
Exhibit*	2.5x10 ⁹	10	78.4 ± 7.4**	70.4-91.2
<i>Heterorhabditis bacteriophora</i>	2.5x10 ⁹	4	74.1 ± 8.0	67.0-84.1
Isazophos 4EC	2.3 kg	9	89.6 ± 6.1	82.5-95.4

* Exhibit = *Steinernema carpocapsae* All strain.

** Mean ± standard error; 2-4 weeks evaluation. Significant differences between treated and control plots (P<0.05), using the Kruskal-Wallis test.

Table 4. Field efficacy against hunting billbug, *Sphenophorus venatus venatus*, in Japan

Treatment	Rate a.i./ha	No. of surviving insects	% reduction	% range
BioSafe*	2.5x10 ⁹	4.7 ± 2.2a**	83.7	77.3-96.2
Chlorpyrifos EC 40%	x 1000 2.01/m ²	9.1 ± 3.8a	68.6	59.4-75.0
Untreated	—	29.0 ± 10.3b	—	—

* BioSafe = *Steinernema carpocapsae* (All strain).

** Means ± standard error followed by the same letter are not significantly different at the P<0.05 level, using the Kruskal-Wallis test; 10-16 day evaluation.

Exhibit (*S. carpocapsae*) against bluegrass billbug, *S. purvulus*, in the United States. Although it was less effective on billbug populations than the insecticide isazophos, the control effect is within acceptable limits. In Japan (Table 4), field trials conducted by SDS Biotech in cooperation with biosys has displayed good levels of control against hunting billbug, *S. venatus*, with BioSafe (*S. carpocapsae*). Adults of this species are also susceptible. These results have made it possible to register BioSafe for commercial sale in Japan.

Another commercial success story in the United States has been the introduction of BioVector (*S. carpocapsae*) for control of the apopka weevil, *Diaprepes abbreviatus*, and the citrus root weevil, *Pachnaxius litus*. These

two insects cause extensive root damage to citrus in the state of Florida. Thousands of hectares of citrus orchards and nurseries have been treated with BioVector. Tables 5a and 5b show the results of a seven month study examining adult insect emergence after a single, early-season application (Bullock, unpublished data).

The sweetpotato weevil, *Cylas formicarius*, is a serious pest of sweet potato all over the world. Research efforts have shown that there is a good potential for controlling this pest with entomopathogenic nematodes. Table 6 shows results from one recent study (Jansson *et al.* 1990).

Table 7 lists various "weevil" pests that are reported in the literature to be susceptible to entomopathogenic nematodes. As

previously mentioned, some of these pests are now being controlled effectively with

commercial entomopathogenic nematode-based products.

Table 5a. Field efficacy of entomopathogenic nematodes against apopka weevil, *Diaprepes abbreviatus*

1990: Monthly capture of adults				
Treatment	Av. no. of weevils caught in ten traps**			
	April	May	June	July
BioVector*	1.9 ± 1.0	1.0 ± 0.3a	1.4 ± 0.5a	0.3 ± 0.2
Untreated	2.6 ± 1.3	3.5 ± 0.8b	3.9 ± 1.0b	0.5 ± 0.2
Treatment	August	September	October	
	BioVector	1.9 ± 1.0	1.0 ± 0.3a	1.4 ± 0.5a
Untreated	2.6 ± 1.3	3.5 ± 0.8b	3.9 ± 1.0b	
1990: Cumulative capture of adults				
Treatment	Av. no. of weevils caught in ten traps			
	April-May	April-June	April-July	
BioVector	2.9 ± 1.3a	4.3 ± 1.7a	4.6 ± 1.7a	
Untreated	6.1 ± 1.3b	10.0 ± 1.9b	10.5 ± 1.9b	
Treatment	April-August	April-September	April-October	
	BioVector	4.6 ± 1.7a	4.8 ± 1.7a	4.8 ± 1.7A
Untreated	10.7 ± 2.0b	10.8 ± 2.0b	11.0 ± 2.0	

* BioVector = *Steinernema carpocapsae* (All strain).

** Means (+ standard error) in column followed by different letters are significantly different, according to two sample F-tests (P<0.05)

Table 5b. Field efficacy of entomopathogenic nematodes against citrus root weevil, *Pachnaeus litus*

1990: Monthly capture of adults

Treatment	Av. no. of weevils caught in ten traps**			
	April	May	June	July
BioVector*	0.4 ± 0.2a	0.5 ± 0.2a	0.2 ± 0.2	0.0
Untreated	4.5 ± 1.0b	2.1 ± 0.8b	1.1 ± 0.7	0.0
	August	September	October	
BioVector	0.0	0.4 ± 0.2	0.1 ± 0.1	
Untreated	0.2 ± 0.1	0.8 ± 0.5	0.3 ± 0.2	

1990: Cumulative capture of adults

Treatment	Av. no. of weevils caught in ten traps		
	April-May	April-June	April-July
BioVector	0.9 ± 0.3a	1.1 ± 0.3a	1.1 ± 0.3a
Untreated	6.6 ± 1.2b	7.7 ± 1.3b	7.7 ± 1.3b
	April-August	April-September	April-October
BioVector	1.1 ± 0.3a	1.5 ± 0.3a	1.6 ± 0.4a
Untreated	7.9 ± 1.4b	8.7 ± 1.5b	9.0 ± 1.6b

* BioVector = *Steinernema carpocapsae* (All strain)

** Means (± standard error) within a column followed by different letters are significantly different according to two sample T-tests (P>0.05).

Table 6. Field efficacy of entomopathogenic nematodes against sweetpotato weevil, *Cylas formicarius elegantulus*

Treatment	Sample period 9-17 September*			
	Rate a.i./ha	Vine	Root	Whole plant
<i>Heterorhabditis bacteriophora</i> (HP88)	1.0x10 ⁹	3.2a**	2.3d	5.6c
	2.2x10 ⁹	3.2a	7.2cd	10.4bc
	3.1x10 ⁹	3.2a	4.1cd	7.3bc
<i>Steinernema carpocapsae</i> (All)	1.1x10 ⁹	4.0a	7.5abc	11.5abc
	3.0x10 ⁹	3.3a	14.1abc	17.4b
	4.9x10 ⁹	2.6a	10.5abc	13.1abc
Insecticide	Standard	5.6a	26.5ab	32.1a
Untreated	—	6.0a	20.0a	26.0a
Sample period 4-14 October				
<i>Heterorhabditis bacteriophora</i> (HP88)	1.0x10 ⁹	1.3b	5.4a	6.7b
	2.2x10 ⁹	1.6b	8.1a	9.7ab
	3.1x10 ⁹	1.7ab	3.8a	5.6b
<i>Steinernema carpocapsae</i> (All)	1.1x10 ⁹	1.1b	4.6a	5.7b
	3.0x10 ⁹	1.8b	8.7a	10.6b
	4.9x10 ⁹	1.7b	7.3a	9.0ab
Insecticide	Standard	5.2a	25.3a	30.5a
Untreated	—	1.9ab	20.8a	22.7a

* Plant parts examined for presence of larvae.

** Means within a column followed by different letters are significantly different (P>0.05), according to Duncan's Multiple Range Test.

Table 7. Weevils that are susceptible to entomopathogenic nematodes

Scientific name	Common name	Author(s)
<i>Conotrachelus nenuphar</i>	Plum curculio	Tedders 1982
<i>Cosmopolites sordidus</i>	Banana root weevil	Figueroa 1990
		Peña & Duncan 1991
		Schmitt <i>et al.</i> 1992
		Treverrow <i>et al.</i> 1991
<i>Curculio caryae</i>	Pecan weevil	Nyczepir <i>et al.</i> 1992
		Smith <i>et al.</i> 1993
<i>Curculio dentipes</i>	Chestnut curculio	Nagata 1987
<i>Cylas formicarius</i>	Sweet potato weevil	Jansson <i>et al.</i> 1990
		Mannion & Jansson 1992
<i>Cyrotrechalus longimanus</i>	Bamboo weevil	Liu <i>et al.</i> 1989

Table 7. continued

<i>Diaprepes abbreviatus</i>	Apopka weevil	Figuroa & Roman 1990 Roman & Figuroa 1985 Schroeder 1987 Schroeder 1990 Simanton & Bullock 1973
<i>Hylobius abietis</i>	Large pine weevil	Burman <i>et al.</i> 1979 Burman 1981 Pye & Burman 1977
<i>Hylobius pales</i>	Pales weevil	Thomas 1970
<i>Lissorhoptrus oryzophilus</i>	Rice water weevil	Kisimoto <i>et al.</i> 1987 Mesneses-Carbonell 1983 Nagata 1987
<i>Listronotus oregonensis</i>	Carrot weevil	Bélair & Boivin 1985 Boivin & Bélair 1989
<i>Naupactus durius</i>	White-fringed weevil	Ahmad 1974 Harlan <i>et al.</i> 1971 Swain 1943
<i>Nemocestes incomptus</i>	Strawberry weevil	Georgis & Poinar 1984
<i>Otiorrhynchus</i> spp.	Root weevil	Bedding & Miller 1981 Bluel & Kaserer 1989 Burlando & Kaya 1993 Deseö & Costanzi 1987 Dorschner <i>et al.</i> 1989 Hanula 1993 Jackson <i>et al.</i> 1985 Klingler 1990 Rutherford <i>et al.</i> 1987 Shanks <i>et al.</i> 1990 Simons 1981
<i>Pachnaeus litus</i>	Blue green weevil	Diaz & Hernandez 1978 Hernandez & Mracek 1984 Montes & Montejo 1991
<i>Pantomorus cervinus</i>	Fuller rose beetle	Ahmad 1974
<i>Sitona hispidulus</i>	Clover root weevil	Jaworska & Wiech 1988
<i>Sitona</i> spp.	<i>Sitona</i> weevils	Wiech & Jaworska 1990
<i>Sphenophorus</i> spp.	Billbugs	Shetlar 1989

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DISCUSSION

In the Discussion, after the first session on entomopathogenic nematodes, one participant was interested in the fact that some nematodes are effective against Lepidoptera, while other nematode species are not. He wondered whether this might be related to some internal defence mechanism and/or external morphology which might determine whether the nematode can penetrate the membrane of the insect. Dr. Kaya pointed out that the dense hairs on the body of some species of larvae protect them against nematodes. Furthermore, *Steinernema carpocapsae* is one of the few nematode species which goes through nictating behavior standing on its tail, and can actually jump onto an insect host. Dr. Smith was asked about the cost of nematode applications to control e.g. turf billbugs. He replied that the cost varies according to the species of nematode, but at application rates of half a billion to three billion nematode nematodes per acre (1 to 7 billion per hectare), the cost would range from US\$20 to US\$200 per acre (approximately US\$50 to 500 per hectare).

Participants were also interested in formulation techniques. Dr. Smith explained that an infective juvenile has no mouth and cannot absorb food directly from a nutrient source. One formulation had extended the shelf-life to a commercially viable period by reducing the nematodes' metabolic rate. He pointed out that farmers do not like radically new products, and prefer those which resemble the conventional formulations they are used to. For this reason, commercial firms are now exploring the possibility of granule applications, and also of aerial spraying.

The important question was raised whether entomopathogenic nematodes have an effect on beneficial insects such as natural enemies of pests. Dr. Kaya replied that in general, if the natural enemies of an insect pest live above the ground, they are not affected by nematodes. If they are living in the soil there may be a possibility of infection, although in practice this did not seem to happen. He suggested that more work needs to be carried out on natural enemies which live in the soil, particularly predators. He also pointed out that insects such as scarab beetles are exposed to infection for only a short period of time during the larval stage, since the adults have a hard carapace which is resistant to nematode attack. One participant was interested in what would happen if a scarab beetle were to eat a nematode. Dr. Kaya answered that eating the nematode would probably not lead to infection of the beetle unless infective juveniles were very abundant. However, if a natural enemy were a parasitoid developing inside a larva, it would be exposed to entomopathogenic nematodes and would be killed by them. Dr. Smith pointed out that if a cockroach eats a nematode, the structure of the cockroach's stomach is such that the nematode is torn apart and there is no infection. There is considerable concern in Europe on the effect of nematodes on non-target species, and many studies are under way, but as yet no effect has been found. He pointed out that many different crops in the United States receive nematode

applications every year. There is no information on whether there has been any change in the microbial populations of the soil as a result, but suggested that this would be an interesting topic of study. Dr. Smith felt that soil microorganisms seem to have little influence on nematode effectiveness, compared to abiotic factors such as soil moisture. He was asked whether any nematodes are known to eat insect eggs, and replied that in general, insect eggs do not seem to be susceptible. However the nematode *Deladenus siricidicola* in Australia invades the ovary of *Sirex noctilio*, which then lays eggs which contain nematodes. The eggs of bark beetles can also be infected with nematodes.

It was pointed out that not all infective juveniles are infectious, and that at most only 10-20% are infectious at any one time. An increase in this rate to e.g. 90% would greatly increase the effectiveness of nematode applications. Dr. Smith was asked whether the efficiency of infective juveniles in terms of their behavior had been considered. Dr. Smith replied that nictating behavior is dominant in young juvenile nematodes. As they age, their lipid content falls and they change from ambushing to hunting behavior. When *S. carpocapsae* was applied to insects such as weevils which are difficult to kill, there was a population reduction of 20%. Four weeks later, there was a population reduction of 60%. He was interested in the reason for this change in mortality rate, which might reflect a change in nematode behavior, and pointed out that it may not be advantageous for all nematodes to behave in the same way. Dr. Kaya supported this view, and pointed out that nematodes are part of an evolutionary process adapted to survival of the species. From the point of view of efficient use of available resources, it would be disadvantageous for all nematodes to become infective at the same time, however desirable this might be from the commercial point of view.

CONTROL OF INSECT PESTS WITH ENTOMOPATHOGENIC NEMATODES

II. Control of Turfgrass Insect Pests with Entomopathogenic Nematodes in Japan

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(Chinese Abstract)

摘 要

自1987年以來，在高爾夫球場施用農業化學藥劑已成為大眾傳播媒體上所熱烈爭論的話題，因此在果嶺上要求發展出安全有效的蟲害防治方法及減低農業化學藥劑的用量。在日本已進行利用蟲生線蟲防治果嶺害蟲之田間及室內測試研究，同時評估蟲生線蟲對果嶺重要害蟲的感染防治力。小卷蛾線蟲對日本草坪切根蟲*P. teterrcla*及球葉蛾的幼蟲有很高的感染率。但這種線蟲對Sacarabacid屬之金龜子幼蟲則幾乎完全無法感染，而在小卷蛾線蟲中混合低濃度的化學藥劑，則對*A. schönfeldti*金龜子的第三齡幼蟲有很高的感染力。

(Japanese Abstract)

摘 要

1987年以來ゴルフコースの芝草への農薬散布の是非がマスコミで問題にされている。結論としてはゴルフコースに使用する農薬量を減らしかつ安全な使用法を開発することにある。そのため日本では芝草害虫防除に昆虫寄生性線虫の利用が野外試験を含め真剣に検討されている。*Steinernema carpocapsae*はスジキリコトウ、シバツトガ、タマナヤガ幼虫に高い罹病性を示す。本線虫はコガネムシ科の幼虫にはほとんど効果がないので、*S. carpocapsae*の散布には低濃度の殺虫剤を混用することによってチビサクラコガネの3令幼虫にも高率の感染をもたらすことができる。

(Korean Abstract)

초 록

1987년 이래, 골프장 잔디에 대한 농약사용은 대중매체를 통하여 주로 토의되어온 논쟁거리이다. 골프장 잔디해충을 방제하기 위하여 농약의 양을 줄이면서 안전하고 효과적인 방제방법을 개발하기 위한 요구가 필연적으로 야기되었다. 잔디의 해충방제를 위한 근충병원성 선충의 실험적 이용이 일본에서 집중적으로 연구되어 왔고 실제 포장시험이 수행되었다. 주요 잔디해충 방제를 위한 근충병원성 선충의 감입평가가 실험실과 포장에서 수행되었다. *Steinernema carpocapsae*는 잔디밤나방(*Spodoptera depravata*), *Parapediasia teterrcla*, 김저세미나방(*Agrotis ipsilon*)의 유충에 높은 감입률을 보였다. 이 선충은 sacarabacid beetles에는 거의 감입하지 않기 때문에 낮은 농도의 농약과 함께 *S. carpocapsae*를 혼합 살포하면 쉐펠트풍뎅이(*Anomala schönfeldti*)의 3령 유충에 높은 감입률을 보인다.

CONTROL OF INSECT PESTS WITH ENTOMOPATHOGENIC NEMATODES

II. Control of Turfgrass Insect Pests with Entomopathogenic Nematodes in Japan

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ABSTRACT

Since 1987, the application of agricultural chemicals to golf course turf has been a controversial issue often discussed in the mass media. Demand has consequently arisen for the development of safe effective methods, using reduced amounts of agricultural chemicals, for the control of turfgrass insect pests on golf courses. The experimental utilization of entomopathogenic nematodes for the control of turfgrass insects is being studied intensively in Japan and practical field trials are being conducted. Infective evaluation of entomopathogenic nematodes to control important turfgrass insects has been conducted under laboratory and field tests. *Steinernema carpocapsae* shows a higher infectivity to larvae of *Spodoptera depravata*, *Parapediasia teterrela*, and *Agrotis ipsilon*. As this nematode is almost uninfected to larvae of scarabaeid beetles, a mixed application of *S. carpocapsae* together with a low concentration of chemicals has shown a higher infectivity to the 3rd instar larvae of *Anomala schönfeldti*.

MATERIALS AND METHODS

- Nematodes.** Four species of entomogenous nematodes were used in this experiment: *Steinernema carpocapsae*, *S. feltiae*, *S. glaseri*, and *S. kushizai*. These nematodes were donated by SDS Biotech KK and Kubota Co. Both types of nematode had been cultured on chicken guts and dog food media.
- Insects.** Larvae of *Spodoptera depravata*, *Parapediasia teterrela*, *Agrotis ipsilon*, and adults and larvae of *Scarabaeidae* were used. These lepidopterous larvae and scarabaeid adults were collected from the turfgrass of golf courses. Scarabaeid larvae were reared at 25°C on an artificial diet consisting mainly of leaf mold.
- Chemicals.** The agricultural chemicals used in this study were fenitrothion

(50%EC) and diazinon (40%EC).

4. Laboratory tests.

- The impregnated filter paper method.** A 1ml. solution containing (ca. 125 to 500) of the infective 3rd stage juvenile (J₃) was inoculated into a plastic petri dish (9cm in inner diameter and 1.3 cm in height), the bottom of which was covered with a sheet of filter paper (Toyo No. 2). Twenty minutes after the solution had been put in the petri dish, the insect larvae were individually introduced into the dish. Ten to 20 insects were used for each of the various tests, and the mortality was observed each day after application.
- The nematode medium method.** Scarabaeid larvae or adults were reared in a plastic container in a medium

Keywords: biological control, entomopathogenic nematodes, important turfgrass insects.

containing nematodes (7.0 cm inner diameter, and 5 cm in height, about 250ml). The rearing medium consisted of 20g of leaf mold (or sawdust) containing 40% to 50% water and 2ml of aqueous suspension of J_1 (ca. 1.5×10^4 to 16.5×10^4).

- c) *Application of S. carpocapsae with chemicals.* Scarabaeid larvae were reared in soil, in a plastic container measuring 29.4 x 22.0 x 6.3 cm. The 3rd instar larvae of *Anomala schönfeldti* were introduced by placing 10 larvae in each container. At 24 hr after introduction of the larvae, the mixing application of *S. carpocapsae* ($1.2 \times 10^6/m^2$) with fenitrothion or diazinon (x1000, 5,000 dil.) was carried out.
 - d) *Pot tests.* For practical use, the infectivity of *S. kushidai* to the larvae of *A. cuprea* was evaluated in a plastic pot (16cm inner diameter and 19cm in height) of cultivated turfgrass. Ten larvae were introduced into each pot. At 24 hr after the treatment, J_1 nematodes were applied at a rate of $2 \times 10^6/m^2$ to 1×10^7 , either by a sprayer onto the turfgrass surface or by being injected 5 cm below the soil surface. Mortality was observed 2 weeks after the treatment.
5. **Field trials.** These nematodes were tested in the field from 1987 to 1988. As a means of controlling the important insects that attack golf course turfgrass, small areas of turfgrass (9 m²) infested with the larvae of these insects were plotted, and the J_1 (ca. 1×10^6 , $1 \times 10^7/m^2$) water suspension was sprayed uniformly.

RESULTS

1. Laboratory tests.

- a) According to the impregnated filter paper method, the infectivity of *S. carpocapsae* (All) to the last instar larvae of *S. depravata* was higher than that of *S. feltiae* or *S. glaseri* (Table 1). However, the level of infectivity tended to decrease with a reduction in the number of nematodes per dish. This became clear with the increasing

percentage of adult emergence. In the same test, Mexican and All strains of *S. carpocapsae* showed an equal infective effect (Table 2). These larvae were individually inoculated onto a dish containing ca. 700 J_1 for 2 periods of 30, 60, and 120 min. After nematodes on the body surface were washed off with water, these larvae were put in a plastic container (250ml.) containing turfgrass (Table 3). Not all adults had emerged after the exposure times of more than 60 min.

- b) Infective effects of 4 species of nematodes to scarabaeid larvae were lower in these experiments (Tables 4, 5). Table 4 shows that the mortality of 1st or 2nd instar larvae of *A. cuprea* was 55%, compared to 40% for *S. glaseri* (ca. 16.5×10^3).
- c) Effects of mixing application of *S. carpocapsae* with chemicals. As infectivity of *S. carpocapsae* to scarabaeid larvae was lower, the effects of mixing the application of *S. carpocapsae* ($1.2 \times 10^6/m^2$) with fenitrothion (x 1,000, 5,000, dil.) or diazinon (x 1,000, 5,000, dil.) were evaluated using the larvae of *A. schönfeldti* (Tables 6, 7). These mixtures were significantly more effective than insecticides alone.
- d) Pot tests. *S. kushidai* from soil in Hamakita, Shizuoka Prefecture, proved to have strong infectivity to scarabaeid larvae (Koizumi *et al.*, 1987). Insecticidal effects of *S. kushidai* J_1 against the 3rd instar larvae of *A. cuprea* are shown in Table 8. Both spraying and injecting *S. kushidai*, at an application rate of $1 \times 10^6 J_1/m^2$, achieved effective control of scarabaeid larvae.

2. **Field trials.** Results obtained from golf courses from August 1987 to August 1989 are shown in Table 9. In these field trials, a higher mortality of insects was obtained in autumn than in summer. In all probability this was due to a difference in soil moisture. The infective activity of nematodes to insects tends to decrease under dry conditions (Kondo and Ishibashi 1968).

Table 1. Infectivity of three species of entomogenous nematodes for 5th and 6th instar larvae of *Spodoptera depravata*, using impregnated filter paper inoculated with an aqueous suspension of infective 3rd stage juveniles (J₃)

Nematode species	No. of nematodes per petri dish	No. of larvae ^{a)}	% mortality on days after application ^{b)}				% adult emergence ^{b)}
			4	8	12	20 days	
<i>S. carpocapsae</i> (All)	125	20	10	55	67	76	24
	250	20	15	60	83	82	18
	500	20	25	65	83	100	0
<i>S. feltiae</i>	125	20	15	45	61	65	35
	250	20	20	30	67	94	6
	500	20	15	50	61	94	6
<i>S. glaseri</i>	125	20	10	15	50	76	24
	250	20	20	35	39	94	6
	500	20	10	35	50	94	6

a) Replications were performed 10 times

b) All data were corrected for control mortalities (Abbott 1925).

Table 2. Infectivity of *Steinernema carpocapsae* (Mexican) for 3rd and 4th instar larvae of *S. depravata*, using impregnated filter paper inoculated with an aqueous suspension of infective 3rd stage juveniles (J₃)

No. of nematodes per petri dish	Replications	No. of larvae	% mortality on days after application			% adult emergence
			4	8	12 days	
125	5	10	30	80	100	0
250	5	10	30	80	100	0
500	5	10	60	80	100	0

Table 3. Mortality of 5th and 6th instar larvae of *S. depravata* exposed for different periods to moist filter paper containing ca. 700 infective juveniles of *Steinernema* spp.

Nematode species	Exposure time (min)	No. of larvae	% mortality on days after application				% adult emergence
			2	4	6	8 days	
<i>S. carpocapsae</i> (Mexican)	30	20		5	20	20	64
	60	20	25	70	70	70	0
	120	20	15	90	100		0
<i>S. carpocapsae</i> (All)	30	20		10	10	10	91
	60	20	10	45	55	55	21
	120	20	35	45	60	75	7
<i>S. feltiae</i>	30	20				0	100
	60	20		20	20	20	71
	120	20		5	55	60	21
<i>S. glaseri</i>	30	20				0	100
	60	20	10	10	10	10	78
	120	20	0	30	30	45	35

Replications were performed 20 times.

Larvae exposed for different times to nematodes were washed and put in 250ml. plastic containers provided with turfgrass.

Table 4. Infectivity of *S. glaseri* and *S. bibionis* to scarabaeid larvae reared in medium containing nematodes (J₃)

Scarabaeidae	Nematode species	No. of nematodes/ container	No. of larvae	% mortality 20 days after application	
				1st instar	2nd instar
<i>Anomala osakana</i>	<i>S. glaseri</i>	1.5x10 ⁴	20	10	15
		4x10 ⁴	20	25	15
		8x10 ⁴	20	45	40
		16.5x10 ⁴	20	55	40
<i>A. cuprea</i>	<i>S. feltiae</i>	1.5x10 ⁴	20	10	10
		4x10 ⁴	20	20	15
		8x10 ⁴	20	25	15
		16.5x10 ⁴	20	30	15

The rearing medium was mixed with 20g of leaf mold containing 40% - 50% water and 2ml. aqueous suspension of J₃ in a 250 ml. plastic container

Table 5. Infectivity of 2 species of entomogenous nematodes to 2nd and 3rd instar larvae of *Anomala cuprea*, immersed for different periods in an aqueous suspension of J₃

Nematode species	No. of nematodes/5mL petri dish	Immersion time (min)	No. of larvae	% Mortality 16 days after application	
				2nd instar	3rd instar
<i>S. carpocapsae</i> (Mexican)	5x10 ⁴	5	12	8.3	8.3
		60	12	8.3	8.3
	1.3x10 ⁴	5	12	8.3	8.3
		60	12	8.3	16.7
<i>S. feltiae</i>	5x10 ⁴	5	12	8.3	16.7
		60	12	8.3	25.0
	1.3x10 ⁴	5	12	16.7	16.7
		60	12	25.0	33.3

larvae immersed to nematodes were moved into the rearing medium containers, and were reared at 25 °C

Table 6. Efficacy of the mixture of *S. carpocapsae* (Mexican strain) and diazinon (40EC) to 3rd instar larvae of *Anomala schönfeldti* in containers*

Treatment	No. of larvae	% Mortality at various days after application		
		5	8	11
J ₃	20	0	0	0
Diazinon x 1.000,dil.	20	45	65	75
J ₃ + diazinon x 1.000,dil.**	20	55	100	100
Diazinon x 5.000,dil.	20	30	30	30
J ₃ + diazinon x 5.000,dil.**	20	50	65	70
Diazinon x 10.000,dil.	20	0	5	10
J ₃ + diazinon x 10.000,dil.**	20	5	10	25

* A plastic container 29,5 x 20,0 x 6,3 cm.

** Spray of J₃ (1.2x10⁹/1L/m²) after each application of diazinon (1L/m²)

Table 7. Efficacy of the mixture of *S. carpocapsae* (Mexican strain) and fenitrothion (50EC) to 3rd instar larvae of *Anomala schonfeldti* in containers*

Treatment	No. of larvae	% Mortality at various days after application		
		5	8	11
J ₃	20	0	0	0
Fenitrothion. x 1.000,dil.	20	30	30	65
J ₃ + fenitrothion. x 1.000,dil.**	20	60	75	95
Fenitrothion. x 5.000,dil.	20	20	25	30
J ₃ + fenitrothion. x 5.000,dil.**	20	50	55	60
Fenitrothion. x 10000,dil.	20	0	5	5
J ₃ + fenitrothion. x 10000,dil.**	20	10	20	20

* A plastic container is 29.5 x 20.0 x 6.3 cm.

** Spray of J₃ (1.2 x 10⁷/lit/m²) after each application of fenitrothion (lit/m²).

Table 8. Insecticidal effect of *S. kusidai* J₃ on 3rd instar larvae of *A. cuprea* in a plastic container

Methods of application	No. of nematodes applied (no./m ²)	No. of larvae tested	No. of dead larvae	Mortality ²⁾ (%)
Injection ¹⁾	2 x 10 ⁵	50	22	26
	5 x 10 ⁵	50	35	60
	1 x 10 ⁶	50	44	84
Spraying	2 x 10 ⁵	50	20	21
	5 x 10 ⁵	50	36	63
	1 x 10 ⁶	50	42	79
Control		50	12	0

Five replications were made.

1) J₃ nematodes were injected 5 cm below the soil surface.

2) Mortality was calculated by Abbott's formula.

Table 9. Mortality of the 3rd-4th instar larvae of *S. depravata* as a result of applying *S. carpocapsae* (All strain) to turfgrass in five field trials.^{a)}

Field trial	Application date	No. of nematodes per m ²	No. of replications	% Mortality at various days after application				Place
				4	5	8	10	
Exp. 1	Aug. 13, 1987	5.0 x 10 ⁵	3		47.4	45.5	Kikugawa	
		1.0 x 10 ⁶	3		52.5	54.5	Country	
		Control	3		0	0	Club	
Exp. 2	Sept. 21, 1987	5.0 x 10 ⁵	3		54.2	66.7	Hamaoka	
		1.0 x 10 ⁶	3		70.8	81.5g	Golf	
		Control	3		0	0	Course	
Exp. 3 ^{b)}	Sept. 30, 1987	5.0 x 10 ⁵	3		57.1	83.3	Hamaoka	
	Oct. 3, 1987	1.0 x 10 ⁶	3		78.6	83.3	Golf	
		Control	3		0	0	Course	
Exp. 4	Oct. 3, 1988	1.0 x 10 ⁶	3	57.1		84.5	Hamaoka	
		Control	3	0		0	Golf Course	
Exp. 5	Aug. 31, 1989	2.5 x 10 ⁵	3	92.0		95.0	Laboratory	
		5.0x 10 ⁶	3	97.0		99.0	Farm of Shizuoka	
		Control	3	0		0	University	

a) Experiments were carried on turfgrass damaged by larvae of *S. depravata* in the golf courses and a laboratory farm.

b) Nematodes applied twice.

DISCUSSION

According to Poinar (1979), *S. carpocapsae* has the broadest host range, covering over 59 families of insects. *S. glaseri* discovered in the larvae of the Japanese beetle prefers those beetles. *S. feltiae* isolated in bionid fly larvae infects the same insects. Inoculation experiments on the effect of these nematodes on turfgrass insects under laboratory conditions and in field tests were carried out. In laboratory tests, *S. carpocapsae* (All and Mexican) were more infective to lepidopterous larvae than *S. glaseri* or *S. feltiae*. However, the infectivity of these nematodes to scarabaeid larvae was significantly lower, due to differences in the defenses of the midgut wall of the hosts. A mixed application of *S. carpocapsae* with a low concentration of chemicals achieved a higher synergistic effect on larvae of *A. schönfeldti*. The application of nematodes

after chemicals had the same effect as nematodes or chemicals alone. The mechanism of the action of mixed applications may be related to the effect of the chemicals on the larval defenses against nematodes. In pot tests, *S. kushidai* showed a higher insecticidal effect on the larvae of *A. cuprea*. This nematode promises to be an effective biological control agent against scarabaeid larvae. In practical field trials, the optimum time for control of lepidopterous insects by nematodes is in the autumn, when soil moisture is high.

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