Entomopathogenic Nematodes(Steinernematidae and Heterorhabditidae) from Korea with a Key to *Steinernema*

Choo, Ho-Yul · Joon Bum Kim* and Dong Woon Lee

(Department of Agricultural Biology, Gyeongsang National University, Chinju, Gyeongnam 660-701, Korea)

(*Division of Forestry Entomology, Research Institute of Forestry, Seoul 130-012, Korea)

한국산 곤충병원성 선충과 *Steinernema* 속의 검색표

추호렬 · 김준범* · 이동운

(경상대학교 농생물학과, 진주, 경남, 660-701)

(*임업연구원 산림곤충과)

ABSTRACT

A survey for entomopathogenic nematodes was conducted throughout the nine provinces and within three city limits during the summer of 1990 and 1991. Six of the nine provinces and one of the three cities were positive for entomopathogenic nematodes. Out of the total 499 soil samples, 23(4.6%) were positive for entomopathogenic nematodes with 19(3.8%) containing *Steinernema* and 4(0.8%) containing *Heterorhabditis*. *Heterorhabditis bacteriopora* and three distinct groups of *Steinernema* species were identified. One group was identified as *S. carpocapsae*, another *S. glaseri* and the other *S. monticola* based on cross breeding studies. Positive sample sites in each habitat included 15 of the 415(3.6%) from forests including regrowth areas with shrubs, 1 of the 27(3.7%) from turfgrass including golf courses and parks, 3 of the 24(12.5%) from agricultural fields, 2 of the 16(12.5%) along riparian areas, and 2 of the 17(11.8%) near the seashore. We advocate that more surveys be conducted for entomopathogenic nematodes before commercial sources of nematodes are widely applied which may obscure the naturally-occurring nematodes. A key to *Steinernema* is provided for the identification.

Key words *Steinernema, Heterorhabditis,* entomopathogenic nematodes, biological control.

INTRODUCTION

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae are being commercially produced as biological control agents of insect pests, especially for those inhibiting the soil and cryptic habitats(Gaugler and Kaya 1990, Gaugler et al. 1992). These nematodes are associated with symbiotic bacteria in the genera *Xenorhabdus* for Steinernematidae and *Photorhabdus* for heterorhabditids(Boemare et al. 1993). Upon finding a suitable host, the infective juvenile stage of the nematode enters in through natural openings and penentrates into the hemocoel. The bacterial cells, located in the gut

of the infective juvenile, are released and kill the insect host within 48 hours after the nematode has breached the host's hemocoel. The nematodes feed on the bacterial cells and host tissues, and after two or three generations in the cadaver, emerge as infective juveniles into the soil environment. The infective juveniles initiated the life cycle again when they infect a new insect host. These nematodes have a worldwide distribution and have been isolated from many islands and all continents(Kaya 1990) except Antarctica(Griffin et al 1990). Kaya(1990) summerized the results of extensive surveys conducted in many countries showing the distribution of these nematodes in various habitats. Other surveys not cited by Kaya(1990) have documented their distribution in Puerto Rico(Roman and Beavers 1982), Hawaiian Islands, USA(Hara et al 1991), various parts of the continental USA(Gaugler et al 1992, Liu and Berry 1995, Ruedo et al 1991), Scotland(Boag et al 1994), Repubric of Ireland(Griffin et al 1991), western Canada(Mracek and Webster 1993), Catalogne. Spain(Dedoucet and Gobarra 1994), Azores, Portugal(Rosa et al 1994), Argentina(Stock 1994) and Sri Lanka (Amarasinghe et al 1994). Such surveys have led to a number of newly described species, new isolates of already described species, and many more that still need to be described. An addition source of their distribution can be determinded by reading the literature of newly species discription whitch provides information on the locality of their isolation(Cabanillas et al 1994, Gardner et al 1994, Stock 1993).

Although the isolation of entomopathogenic nematodes has been documented in many countries, no systemic survey has been conducted for Korea. However, *Heterorhabditis bacteriopora* has been isolated from the People's republic of Korea(North Korea)(Mracek et al 1988) suggesting that entomopathogenic nematodes would also be present in the southern part of the Korean Peninsula. Accordingly, we initiated a survey to determine their occurence in the Korea. Such isolations may eventually result in their use as biological control agents against Korean insect pests. In addition, a key to *Steinernema* is provided for the identification.

METERIALS AND METHODS

Soil samples were collected from diverse habtats(forests, agricultural fields, turfgrass, seashores, and riparian areas) throughout the nine Provinces and within the city limits of Pusan, Daegue, and Gwangju during July and August of 1990 and 1991(Fig. 1). Samples were taken 3 to 16 km apart within 200 m of a road. Each sample site was 2-4㎡, and 5 subsamples($10 \times 10 \times 15$ cm each) were collected with a small hand shovel. The subsamples were combined resulting in ca. 800 ml of soil which was placed in a plastic bag and kept cool(10 to 15℃). If any insect were observed, they were examined for nematode infection. However, no concentrated effort was made to find insects because of time constraints. In addition, the dominant flora of each sample site was recorded. Between sampling sites, the hand shovel was washed with water and air dried.

The soil was transported to the laboratory for the extraction of entomopathogenic nematodes. The soil was thoroughly mixed in the bag, and a 250 ml subsamples of soil was removed and placed into a 300 ml plastic container. The remaining soil was stored at 4℃ and a second isolation for nematodes was done 2 to 4 weeks later. Six last instar *Gallerria mellonella* larvae were added to each container(Bedding and Akhurst 1975), and all containers were stored at room temperature (25 ± 3℃) for 1 week. Dead larvae from each container were set up in a
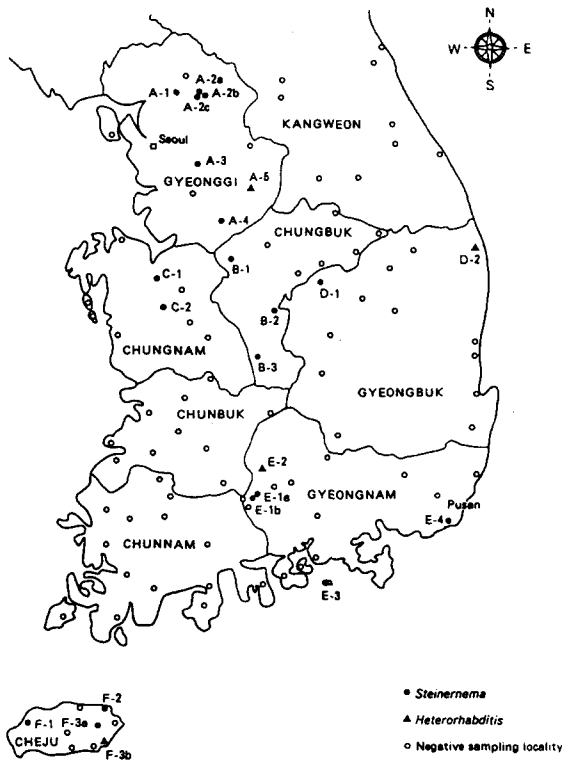
Fig. 1. Map of showing the actual positive sampling sites as indicated by the black dot for *Steinernema* and the black triangle for *Heterorhabditis*. The open circles indicated the negative localities which contained a number of sampling sites. That is, there were 499 sampling sites but not all negatives ones are shown.

WHITE trap(Woodring and Kaya 1988) to collect emerging infective juveniles.

Nematodes isolated from the WHITE traps were used to infect another group of *Galleria* larvae to verify that they were pathogenic. The colors of the cadavers were noted 4 to 8 days after death. Ocher or brown cadavers were dissected 4 to 5 days postinfection and orange or red ones were dessected at 8 to 10 postinfection. The adult males were used to identify the nematodes to genus and whenever possible to species. In a few instances, cross breeding studies using the hanging drop method(Poinar 1975)

were conducted to determine whether the isolates were the same species or not. In one instance, cross breeding was conducted with *S. carpocapsae* from a laboratory culture.

RESULTS

A total of 499 soil samples were collected throughout the nine Provinces and within the three cities(Fig. 1). Positive samples in each habitat included 15 out of 415(3.6%) from forests including regrowth areas with shrubs, 1 of the 27(3.7%) from turfgrass including golf courses and parks, 3 out of 24(12.5%) from agricultural fields, 2 out of 16(12.5%) along riparian areas, and 2 out of 17(11.8%) near the seashore. Six of the nine provinces and one of the three cities were positive for entomopathogenic nematodes (Table 1). In all cases, the second isolation confirmed the results of the first; i.e., positive samples for entomopathogenic nematodes remained positive and those that were negative remained negative. Soil types of all positive sites were determined by the Gyeongnam Provincial Rural Development Administration and were classified as sandy, sandy silt, or sandy clay(Table 1). The nematodes were identified as *Steinernema* and *Heterorhabditis* based on the color of the cadavers and on the morphology of the male.

Out of the 499 samples, 23(4.6%) were positive for entomophathogenic nematodes with 19 (3.8%) containing *Steinernema* and 4(0.8%) containing *Heterorhabditis*. Fourteen of the *Steinernema* positive sites were isolated from coniferous, deciduous or regrowth forest habitats; two were isolated from near the seashore with one isolate from a grassy area and one among black pine trees; two were isolated from agricultural fields, with one fallow and the other weedy; and one was from turfgrass at a golf course(Table 1). No natural infections of insects were found at any

site except in turfgrass at Pusan(Dongrae isolate) where larval cadavers of unknown scarab grubs, probably *Anomala sp.*, were infected with a steinernematid.

We were only able to maintain nine populations of *Steinernema*. Ten populations were lost during the second to third subcultures before samples for morphometric studies were made. Of the remaining nine populations, three distinct groups(A, B, and C) were evident. Isolates

Table 1. Location, soil texture, habitat, and vegitation of entomopathogenic nematodes isolated from Korean soils.

Location[a]	Soil texture	Habitat	Dominant vegitation	Nematode genus
			Gyeonggi Province	
Dongducheon(A-1)	Sandy clay	Regrowth forest	Korean bush clover (*Lespedeza maximowiczii*)	*Steinernema*
Pocheon 1(A-2a)	Sandy clay	Regrowth forest	Korean azalea (*Rhododendron mucronulatum*) Arrowroot (*Pueraria thunbergiana*)	*Steinernema*[b]
Pocheon 2(A-2b)	Sandy clay	Forest	Korean pine (*Pinus koraiensis*)	*Steinernema*[b]
Pocheon 3(A-2c)	Sandy clay	Forest	Red pine (*P. densiflora*)	*Steinernema*[b]
Seongnam(A-3)	Sandy clay	Forest	Red pine	*Steinernema*
Anseong(A-4)	Sandy clay	Forest	Red pine	*Steinernema*
Yeoju(A-5)	Sandy	Riparian	Weed (*Persicaria biumei*)	*Heterorhabditis*
			Chungbuk Province	
Jincheon(B-1)	Sandy clay	Forest	Red pine	*Steinernema*
Boeun(B-2)	andy clay	Forest	Larch (*Larix leptolepis*)	*Steinernema*
Okcheon(B-3)	Sandy clay	Forest	Red pine	*Steinernema*
			Chungnam Province	
Yesan(C-1)	Sandy clay	Forest	Red pine False acasia (*Robina psedoacasia*)	*Steinernema*
Cheongyang(C-2)	Sandy clay	Forest	Red pine	*Steinernema*
			Gyeongbuk Province	
Mungyeong(D-1)	Sandy clay	Agricultural	Common lambsquarters	*Steinernema*
Uljin(D-2)	Sandy clay	Riparian	Cogongrass (*Imperata cylindrica*)	*Heterorhabditis*
			Gyeongnam Province	
Sancheong 1(E-1a)	Sandy clay	Forest	Larch	*Steinernema*
Sancheong 2(E-1b)	Sandy clay	Forest	Larch	*Steinernema*
Hamyang (E-2)	Sandy	Agricultural	Peanut (*Arachis hypogaea*)	*Heterorhabditis*
Samcheonpo(E-3)	Sandy clay	Forest	Black pine (*P. thunbergii*)	*Steinernema*
			City of Pusan	
Dongrae(E-4)	Sandy	Golf course	Turfgrass[c]	*Steinernema*
			Cheju Province	
Hanrim(F-1)	Sandy	Seashore	Unknown grass(weed)	*Steinernema*
Keumyung(F-2)	Sandy	Seashore	Black pine[d]	*Steinernema*
Namcheju(F-3a)	Sandy clay	Agricultural	Fallow[e]	*Steinernema*
Namcheju(F-3b)	Sandy clay	Forest	Black pine	*Heterorhabditis*

[a]The letter and number in the parenthesis refer to the location as shown on Figure 1.
[b]Identified as *Steinernema carpocapsae* based on cross breeding studies with laboratory cultures of *S. carpocapsae*.
[c]Only site where larval cadavers of unknownscarab grubs, probably *Anomala* sp., were found
[d]Pine trees growing along the seashore near a resort area.
[e]Cultivated field that was fallow at time of sampling.

Pocheon 1, 2, and 3(Gyeonggi Province) were placed in Group A. Isolates in this group interbred with each other and with laboratory cultures of *S. carpocapsae*. We concluded that these isolates were *S. carpocapsae*. Isolates Dongrae(Pusan city), Mungyeong (Gyeongbuk Province), and Namcheju and Hanrim(Cheju Province) were placed Group B and they were *S. glaseri*. Isolates Sancheong 1 and 2(Gyeongnam Province) were placed in Group C and identified as new species. No interbreeding studies within Groups B and C were conducted. Group C was named *S. monticola* and is being published.

Two *Heterorhabditis* isolates were collected from riparian areas(Gyeonggi and Gyeongbuk Provinces), one was collected in a peanut field adjacent to a riparian area (Gyeongnam Province), and one was collected from a forest(Cheju Province)(Table 1). Based on morphometrics, the *Heterorhabditis* isolates were identified as *H. bacteriophora*.

DISCUSSION

We focused our survey in the mountainous forests because they have the greatest diversity of insect fauna consisting of oriental and Palearctic species(Paik 1993). Sixty-six percent of the land mass in Korea is mountainous consisting primarily of coniferous or mixed coniferous and deciduous forests. Twenty-one percent of the land mass is agricultural of which 14% is in rice production and 7% is in other crops. The remaining 13% of the land mass is in residential/industrial or other uses. The rice cultivation areas are not considered to be a natural habitat for steinernematids and heterorhabditids because the fields are flooded. Moreover, the agricultural practices rely heavily on pesticides for insect suppression which limits the available insect fauna.

Our results suggest that areas with greater human activity have equal or better chances of successfully yielding entomophathogenic nematodes than areas with less human activity. A higher percentage of positive samples was obtained from nonforested areas suggesting human activity may greatly influence the distribution of these nematodes. This suggestion is reinforced by Mracek and Webster(1993) who observed that entomopathogenic nematodes were more prevalent in areas where human impact was more substantial than in natural habitats. Similarly, Akhurst and Brooks(1984) and Griffin et al.(1991) had more positive sites in agricultural areas than in forests in both North Carolina, USA and the Republic of Ireland. In England, more entomopathogenic nematodes were isolated from agricultural fields(48%) than in woodlands(42%), but roadside verge had the highest percentage of nematodes(66%)(Hominick and Briscoe 1990). In Scotland, permanent pastures(4.1%) had more isolations of nematodes than coniferous (3.2%) or deciduous(1.6%) forests(Boag et al 1992). However, Hara et al.(1991) and Liu and Berry(1995) had more positive nematode sites along the seashore than any other habitat. After the seashore sites, Liu and Berry(1995) found that forests and orchards had the next most abundant positive sites and agricultural situations had the lowest frequency of nematode recovery sites. In contrast, Mracek(1980) found more steinernematids in forests than in agricultural lands in Czechoslovakia.

Initially, we postulated that already described species may be more prevalent from areas with high human activity compared with areas with low human activity. This does not appear to be the case because *S. carpocapsae* was isolated from forests and *H. bacteriophora* was isolated from agricultural fields and forests.

Our survey was similar to other surveys in two ways that are worthy of comment. First, there was a general trend of sandy and sandy loam

soils yielding more positive sites for entomopathogenic nematodes than clay soils(Blackshaw 1988, Griffin et al 1991, Hara et al 1991, Hominick and Briscoe 1990, Liu and Berry 1995). And second, nematode-infected insects were not found during most surveys. In our Korean survey, only one site revealed field infected insects. In part, when surveys are conducted, a vast area is being covered and there is very little time to conduct a systematic search for insects. Yet, epizootics of entomopathogenic nematodes have been documented(Akhurstv at al 1992, Kaya 1990), and many nematode-infected insects have been recovered from nature during the course of other investigations(Poinar 1975). For most field studies including surveys, Hominick and Reid(1990) commented that they are "snapshots" in time and provide no information on recycling or persistence.

Surveys serve an important purpose for a number of reasons. They document the occurrence of these nematoeds in various habitats and localities, are a source of new species and isolates that form the basis for additional opportunities for commercial products, offer these new species and isloates for potential use as classical biological control agents, and serve as a source of genetic diversity. It is critical to conduct these surveys to document the occurrence of native entomopathogenic nematode species or isolates before areas are "polluted" by inundative and inocultive applications of commercial speices. Applied nematodes may outcompete and replace the native entomopathogenic nematodes resulting in their local extinction.

Key to the males of *Steinernema* species

1. Tip of tail containing a cuticular mucron ······ 2
1.a. Tip of tail lacking a cuticular mucron ······ 8
2. Average length of mucron 1-4μm ··············· 3
2.a. Average length of mucron 4-13μm ············· 4

3. Number of genital papillae 21
···································*S. rara* Doucet, 1986
3.a. Number of genital papillae 22-23············· 5
4. Average distance from anterior end to excretory pore 74(range: 65-85μm): average length of testis reflexion 578(range: 475-675)
······························ *S. feltiae*(Filipjev, 1934)
4.a. Average distance from anterior end to excretory pore 94(range: 75-113μm): average length of testis reflexion 370(range: 334-398)
··················· *S. monticola* Stock, Choo & Kaya
5. Spicules lacking a velum, lamina of spicules without a rostrum ····················· *S. neocurtillis* Nguyen & Smart, 1992
5.a. Spicules lacking a velum, lamina of spicules with a rostrum ······································ 6
6. Six pairs of preanal papillae
···························*S. carpocapsae* (Weiser, 1955)
6.a. Five pairs of preanal papillae ···················· 7
7. Length of testis reflexion more than 400μm ··· 8
7.a. Length of testis reflexion less than 400μm
··············· *S. scapterisci* Nguyen & Smart, 1990
8. Length of spicules 67μm or more: length of tail 45μm or more: width at the cloaca more than 50μm ························· *S. affinis*(Bovien, 1937)
8.a. Length of spicules 67μm or less: length of tail less than 45μm: width at the cloaca less than 50μm ·················· *S. kraussei* (Steiner, 1923)
9. Spicules tip notched nor swollen ··············· 10
9.a. Spicules tip neither notched or swollen······ 12
10. Lamina of spicules with a velum
··············· *S. longicaudum* Shen & Wang, 1991
10.a. Lamina of spicules without a velum ······ 11
11. Tip of spicules notched or scarred: 7-8 pairs of preanal papillae ···*S. glageri* (Steiner, 1929)
11.a. Tip of spicules swollen or rounded: 9-10 pairs of preanal papillae ··· *S. anomali*(Kozodoi, 1984)
12. One single ventral preanal papillae ············ 13
12.a. Two single ventral preanal papillae
······························· *S. kushidai* Mamiya, 1985
13. Lamina of spicules without a rostrum ······ 14
13.a. Lamina of spicules with a rostrum ········· 15

14. Length of spicules more than 70μm

······ *S. puertorricensis* Roman & Figueroa, 1994

14.a. Length of spicules less than 70μm ··· *S. cubana*
    Mracek, Arteage-Hernandez & Boemare, 1994

15. Length of testis reflexion more than 283μm

··*S. riobravis*
                Cabanillas, Poinar & Raulston, 1994

15.a. Length of testis reflexion less than 283μm ··· 16

16. Spicules length less than 80μm; length of tail
    less than 35μm ······························· *S. ritteri*
                            Doucet & Doucet, 1990

16.a. Spicules length more than 80μm; length of
tail more than 35μm ··· *S. intermedia*(Poinar, 1985)

Key to the infective juveniles of *Steinernema* species

1. Average length more than 1100μm ··············· 2

1.a. Average length less than 1100μm ·············· 5

2. Average distance from anterior end to
   excretory pore from 76-90μm; ratio D from
   0.50-0.79 ··· *S. anomali* (Kozodoi, 1984) and
   longicaudum Shen & Wang, 1991(Characters
   separating the infective juveniles of these
   species have not yet been found)

2.a. Average distance from anterior end to
   excretory pore from 90-110μm; ratio D from
   0.58-0.71 ··· 3

3. Average distance from anterior end to pharynx
   base from 112-130μm; tail length less than 75μm
   ······························ *S. glageri* (Steiner, 1929)

3.a. Average distance from anterior end to
   pharynx base from 135-147μm; tail length more
   than 75μm ··· 4

4. Greatest width from 47-54μm

·········*S. puertorricensis* Roman & Figueroa, 1994

4.a. Greatest width from 33-46μm

········· *S. cubana* Mracek, Arteaga-Hernandez &
                                    Boemare, 1994

5. Average length from 800-905μm ··················· 6

5.a. Average length less than 800μm ·············· 8

6. Greatest width 22-29μm; ratio A is 31(22-33)

···································· *S. feltiae* (Filipjev, 1934)

6.a. Greatest width more than 29μm; ratio A less
    than 31 ··· 7

7. Average distance from anterior end to
   excretory pore is 18μm(range: 14-22)

··············*S. neocurtillis* Nguyen & Smart, 1992

7.a. Average distance from anterior end to
   excretory pore is 63μm(range: 56-66)

····························· *S. kraussei* (Steiner, 1923)

8. Average length less than 600μm(range 440-600)

··· 9

8.a. Average length less than 600μm ·············· 12

9. Average distance from anterior end to pharynx
   base less than 102μm ······························· 10

9.a. Average distance from anterior end to
   pharynx base is more than 102μm ············ 11

10. Average distance from anterior end to
    excretory pore is 37μm(range:34-40); ratio D
    is less than 0.42(0.34-0.41)

····································*S. rara* Doucet, 1986

10.a. Average distance from anterior end to
    excretory pore is 43μm(range:40-46); ratio D
    is greater than 43(0.44-0.50)

····················· *S. ritteri* Doucet & Doucet, 1990

11. Ratio E is 0.73(0.60-0.80)

·············· *S. scapterisci* Nguyen & smart, 1990

11.a. Ratio E is 0.60(0.54-0.66) ··· *S. carpocapsae*
                                        (Weiser, 1955)

12. Average distance from anterior end to nerve
    ring from 76-87μm(range:70-89) ··············· 13

12.a. Average distance from anterior end to nerve
    ring from 93-95μm(range:85-104) ············ 14

13. Average distance from anterior end to
    excretory pore is 50μm or less(42-50):ratio D is
    0.41(0.38-0.44) ······ *S. kushidai* Mamiya, 1985

13.a Average distance from anterior end to
    excretory pore more than 50μm(51.2-64):ratio
    D is 0.49(0.45-0.55) ··· *S. riobravis* Cabanillas,
                            Poinar & Raulston, 1994

14. Minute refractile spine in tail tip present
··································· *S. affinis* (Bovien, 1937)

14.a. Minute refractile spine in tail tip absent ··· 15

15. Ratio A is 23(20-26); ratio E is 0.96(0.89-
　1.08) ·············· *S. intermedia* (Poinar, 1985)
15.a. Ratio A is 19(14-22); ratio E is 0.76(0.63-
　0.8) ·········*S. monticola* Stock, Choo & kaya

적 요

1990년과 1991년 여름동안 한국내의 9개도 3개 직
할시에서 곤충기생성선충의 분포를 조사하였는바 6개
도와 1개 직할시에서 선충이 검출되었다. 499곳의 샘
플중 23곳 샘플에서 선충이 검출되었는데(검출율
4.6%), 그중 19곳의 샘플(3.8%)이 *Steinernema*였다.
*Steinernema*가 검출된 샘플중 1개군은 *S. carpocapsae*
였고 1개군은 *S. glaseri*, 1개군은 *S. monticola*로 동정
되었다. 그리고 4곳(0.8%)은 *Heterorhabditis*속으로서
*H. bacteriophora*로 동정 되었다. 환경별로는 삼림토양
의 415곳중 15곳(3.6%), 공원과 골프장및 잔디밭 27곳
중 1곳(3.7%), 해안지역 12곳중 2곳(11.8%)에서 선충
이 검출되었다. 곤충병원성 선충은 우리나라 토양에
널리 분포하고 있는것으로 확인 되었는데, 상품화된
선충을 살포하게되면 자연분포 선충의 검출이 곤란하
기 때문에 시판 선충이 살포되기전 보다 많은 분포조
사가 이루어 져야 겠다. 그리고 *Steinernema* 속의 동
정을 위하여 검색표를 마련하였다.

검색어 *Steinernema, Heterorhabditis*, 곤충병원성선
충, 생물적 방제

REFERENCES

Akhurst, R. J., Bedding, R. A., Bull, R. M. &
　Smith, D. R. J. 1992. An epizootic of
　Heterorhabditis spp. (Heterorhabditidae:
　Nematoda) in sugar cane scarabaeids
　(Coleoptera). Fundam. appl. Nematol. 15, 71-73.
Akhurst, R. J. & Brooks, W. M. 1984. The
　distribution of entomopathogenic nematodes
　(Heterorhabditidae and Steinernematidae) in
　North Carolina. J. Invertebr. Pathol. 44, 140-145.
Amarasinghe, L. D., Hominick, W. M., Briscoe, B.
　R. & Reid, A. P. 1994. Occurrence and
　distribution of entomopathogenic nematodes in

Sir Lanca. J. Helmintol. 68, 277-286
Bedding, R. A. & Akhurst, R. J. 1975. A simple
　technique for the detection of insect
　pathogenic nematodes in soil. Nematologica 21,
　109-110.
Blackshaw, R. P. 1988. A survay of insect
　parasitic nematodes in Northern Ireland.
　Ann. appl. Biol. 113, 561-565.
Boag, B., Neilson, R. & Gordon, S. C. 1992.
　Distribution and prevalence of the
　entomopathogenic nematode *Steinernema feltiae*
　in Scotland. Ann. appl. Biol. 121, 355-360.
Boemare, N. E., Akhurst, R. J. & Mourant, R. G.
　1993. DNA relatedness between *Xenorhabdus*
　spp.(Enterobacteriaceae), symbiotic bacteria of
　entomopathogenic nematodes, and a proposal to
　transfer *Xenorhabdus luminescens* to a new
　genus, *Photorhabdus* gen. nov. Intern. J. Syst.
　Bacteriol. 43, 249-255.
Cabanillas, H. R., Poinar, G. O. Jr. & Raulston, J.
　R. 1994. *Steinernema riobravis* n. sp.
　(Rhabditida: Steinernematidae) from Texis.
　Fundam. appl. Nematol. 17, 123-131.
Dedoucet, M. M. A. & Gabarra, R. 1994. On the
　occurrence of *Steinernema glaseri* (STEINER,
　1929)(Steinernematidae) and *Heterorhabditis
　bacteriopora* Poinar, 1976(Heterorhabditidae) in
　Catalogne, Spain. Fundam. appl. Nematol. 17,
　441-443.
Gardner, S. L., Stock, S. P. & Kaya, H. K. 1994.
　A new species of *Heterorhabditis* from the
　Hawaiian Islands. J. Parasiol. 80, 100-106.
Gaugler, R. & Kaya, H. K. (eds.) 1990.
　Entomopathogenic nematodes in *Biological
　control*. CRC Press, Boca Raton, 365pp
Gardner, S. L., Stock, S. P. & Kaya, H. K. Wis,
　E. E. 1992. Large-scale inoculative releases of
　the entomopathogenic nematode *Steinernema
　glaseri*: assessment 50 years later. Biol. Contr.
　2, 181-187.
Griffin, C. T., Downes, M. J. & Block, W. 1990.
　Tests of Antarctic soils for insect parasitic
　nematodes. Antarctic Sci. 2, 221-222.

Griffin, C. T., Moore, J. F. & Downes, M. J. 1991. Occurence of insect-parasitic nematodes(Steinernematidae, Heterorhabditidae) in the Republic of Ireland. Nematologica 37. 92-100.

Hara, A. H., Gaugler, R., Kaya, H. K. & Lebeck, L. M. 1991. Natural papulations of entomopathogenic nematodes(Rhabditida: Heterorhabditidae, Steinernematidae) from the Hawaiian Islands. Environ. Entomol. 20, 211-216.

Hominick, W. M. & Briscoe, B. R. 1990. Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in British soils. Parasitology 100, 295-302.

Hominick, W. M. & Reid, A. P. 1990. Perspectives on entomopathogenic nematology. In: *Entomopathogenic Nematodes* in *Biological control.*(Gaugler, R. & Kaya, H. K., eds.), CRC Press, Boca Raton, 327-345.

Husberg, G. B., Vanninen, I. & Hokkanen, H. 1988. Insect pathogenic fungi and nematodes in fields in Finland. Vaxts-kyddsnoister 52, 38-42.

Kaya, H. K. 1990. Soil ecology. In: *Entomopathogenic Nematodes in Biological Control.* (Gaugler, R. & Kaya, H. K., eds.), CRC Press, Boca Raton, 93-115.

Kaya, H. K. & Gaugler, R. 1993. Entomopathogenic nematodes. Annu. Rev. Entomol. 38, 181-206.

Liu, J. & Berry, R. E. 1995. Natural distribution of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) in Oregon soils. Environ. Entomol.24, 159-163.

Mracek, Z. 1980. The use of "*Galleria traps*" for obtaining nematode parasites of insects in Czechoslavakia(Lepidoptera: Nematoda, Steinernematidae). Acta Entomol. Bohemoslo. 77, 378-382.

Mracek, Z., Hanzal, R. & Kodrik, D. 1988. Sites of penetration of juvenile Steinernematidis and heterorhabditis(Nematoda) into larvae of *Galleria mellonella*(Lepidoptera). J. Invertebr. Pathol. 52, 477-478.

Mracek, Z. & Webster, J. M. 1993. Survey of Heterorhabditidae and Steinernematidae (Rhabditida, Nematoda) in Western Canada. J. Nematol. 25, 710-717.

Paik, W. H. 1993. *Agricultural Entomology.* Hyangmunsa, Seoul. 475pp.(In Korean).

Poinar, G. O. Jr. 1975. *Entomogenous Nematodes.* E. J. Brill, Leiden, 317pp.

Poinar, G. O. Jr. 1990. Taxanomy and biology of Steinernematidae and Heterorhabditidae. In: *Entomopathogenic nematodes in Biological Control.*(Gaugler, R. & Kaya, H. K., eds.), CRC Press, Boca Raton, 23-61.

Roman, J. & Beavers, J. B. 1982. A survey of Puerto Rican soils for entomopathogenic nematodes which attack *Diaprepes abbreviatus*(L.)(Coleoptera: Curculionidae). J. Agric. Univ. Puerto Rico. 67, 311-316.

Rosa, J. S., Martins, A., Mendes, C., Amaral, J. -J., Lacey, L. A. & Simones, N. 1994. Natural occurrence of soil entomopathoges in the Azores Islands. Ⅵth Intern. Colloq. Invertebr. Pathol. Micro. Contr. Abstracts 2, 275-276.

Ruedo, L. M., Osawaru, S. O., & Harrison, R. E. 1993. Natural occurrence of entomopathogenic nematodes in Tennessee nursery soils. J. Nematol. 25, 181-188.

Stock, P. 1994. Isolation of entomopathogenic nematodes from the Pampean region of Argentina. Ⅵth Intern. Colloq. Invertebr. Pathol. Micro. Contr. Abstracts 2, 13.

Stock, S. P. 1993. A new species of the genus *Heterorhabditis* Poinar, 1976 (Nematoda: Heterorhabditidae) parasitizing *Graphognathus* sp. larvae(Coleoptera: Curculionidae) from Argentina. Res. Rev. Parasitol. 53, 103-107.

Woodring, J. L. & Kaya, H. K. 1988. Steinernematid and heterorhabditid nematodes: a handbook of techniques. Southern Coop. Series Bull. 331, 30 pp.