Isolation of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) from Korea*

Ho Yul CHOO**, Harry K. KAYA*** and S. Patricia STOCK****

A survey for entomopathogenic nematodes was conducted throughout the nine provinces and within three city limits in the Republic of Korea during the summers 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 bacteriophora* and three distinct groups of *Steinernema* species were identified. One of the three steinernematid groups was identified as *S. carpocapsae* 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. *Jpn. J. Nematol.* 25(1): 44-51(1995).

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 inhabiting the soil and cryptic habitats (11, 20). These nematodes are associated with symbiotic bacteria in the genera *Xenorhabdus* for steinernematids and *Photorhabdus* for heterorhabditids (7). Upon finding a suitable host, the infective juvenile stage of the nematode enters it through natural openings and penetrates 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 initiate 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 (19) except Antarctica (13). KAYA (19) summarized the results of extensive

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surveys conducted in many countries showing the distribution of these nematodes in various habitats. Other surveys not cited by KAYA (19) have documented their distribution in Puerto Rico (28), Hawaiian Islands, USA (15), various parts of the continental USA (12, 21, 30), Scotland (6), Republic of Ireland (14), western Canada (24), Catalogne, Spain (9), Azores, Portugal (29), Argentina (31) and Sri Lanka (3). 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 determined by reading the literature of newly species description which provides information on the locality of their isolation (8, 10, 32).

Although the isolation of entomopathogenic nematodes has been documented in many countries, no systematic survey has been conducted for the Republic of Korea. However, *Heterorhabditis bacteriophora* has been isolated from the People's Republic of Korea (North Korea) (23) suggesting that entomopathogenic nematodes would also be present in the southern part of the Korean Peninsula. Accordingly, we initiated a survey to determine their occurrence in the Republic of Korea. Such isolations may eventually result in their use as biological control agents against Korean insect pests.

MATERIALS AND METHODS

Soil samples were collected from diverse habitats (forests, agricultural fields, turfgrass, seashores, and riparian areas) throughout the nine provinces and within the city limits of Pusan, Daegu, and Gwangju in the Republic of Korea 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 m^2, and 5 subsamples (10 × 10 × 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°C). If any insects 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 subsample of soil was removed and placed into a 300 ml plastic container. The remaining soil was stored at 4°C, and a second isolation for nematodes was done 2 to 4 weeks later. Six last instar *Galleria mellonella* larvae were added to each container (4), and all containers were stored at room temperature (25 ± 3°C) for 1 week. Dead larvae from each container were set up in a WHITE trap (33) 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 dissected at 8 to 10 days 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 (26) 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.

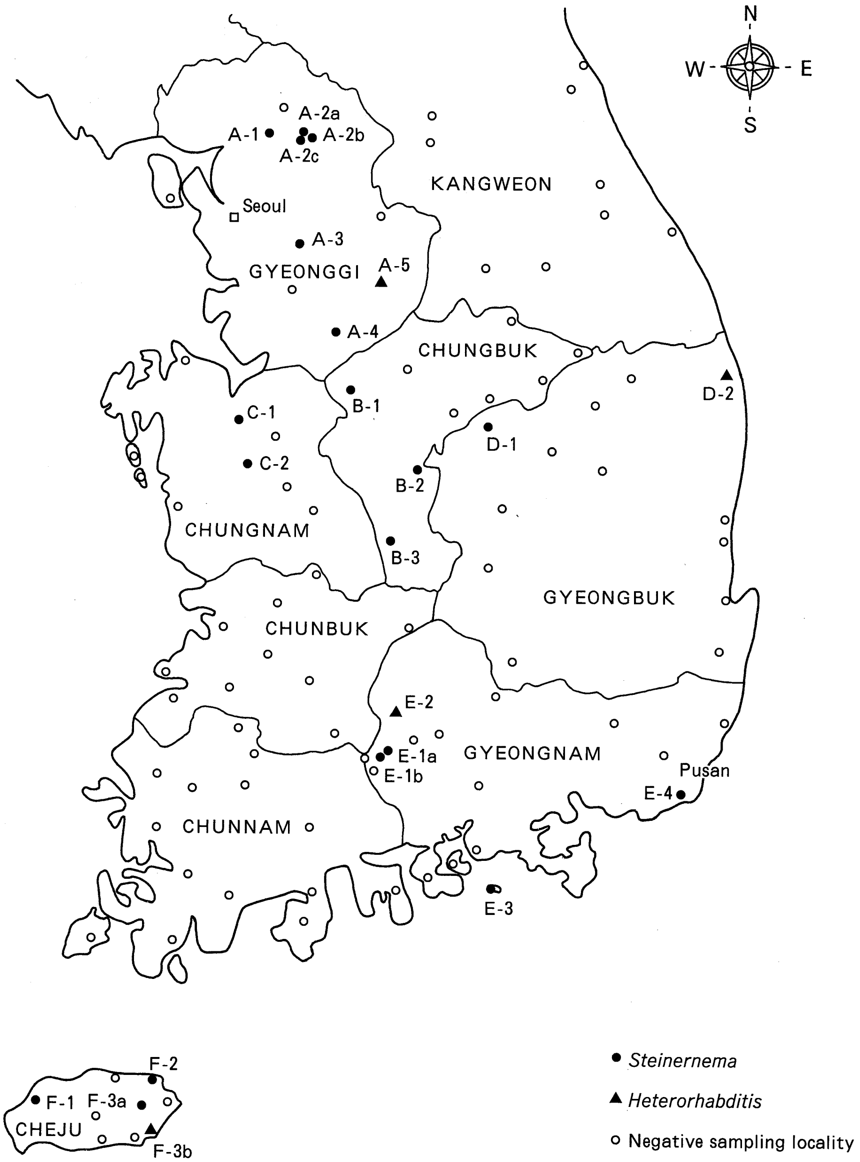

Fig. 1. Map of the Republic of Korea showing the actual positive sampling sites as indicated by the black dot for *Steinernema* and the black triangle for *Heterorhabditis*. The open circles indicate the negative localities which contained a number of sampling sites. That is, there were 499 sampling sites but not all negatives ones are shown.

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,

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

Location (see Fig. 1)[a]	Soil texture	Habitat	Dominant vegetation	Nematode genus
			Gyeonggi Province	
Dongducheon (A-1)	Sandy clay	Regrowth forest	Korean bush colver (*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 blumei*)	*Heterorhabditis*
			Chungbuk Province	
Jincheon (B-1)	Sandy clay	Forest	Red pine	*Steinernema*
Boeun (B-2)	Sandy 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 acacia (*Robina pseudoacacia*)	*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	Riparian	Cogongrass (*Imperata cylindrica*)	*Heterorhabditis*
			Gyeongnam Province	
Sancheong 1 (E-1a)	Sandy silt	Forest	Larch	*Steinernema*
Sancheong 2 (E-1b)	Sandy silt	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 unknown scarab grubs, probably *Adoretus* sp., were found.
[d]Pine trees growing along the seashore near a resort area.
[e]Cultivated field that was fallow at time of sampling.

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 entomopathogenic 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 isolated 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 *Adoretus* 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 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 (Gyeonbuk Province), and Namcheju and Hanrim (Cheju Province) were placed in Group B and they showed characteristics of *S. intermedia* and *S. glaseri*. Isolates Sancheong 1 and 2 (Gyeongnam Province) were placed in Group C and appeared to be related to *S. feltiae*. No interbreeding studies within Groups B and C were conducted.

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 (25). Sixty-six percent of the land mass in the Republic of 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 entomopathogenic nematodes than areas with less human activity. A higher percentage of positive samples was obtained from nonforested areas suggesting that human activity may greatly influence the distribution of these nematodes. This suggestion is reinforced by MRACEK & WEBSTER (24) who observed that entomopathogenic nematodes were more prevalent in areas where human impact was more substantial than in natural habitats. Similarly, AKHURST & BROOKS (2) and GRIFFIN *et al.* (14) 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%) (16). In Scotland, permanent pastures (4.1%) had more isolations of nematodes than coniferous (3.2%) or deciduous (1.6%) forests (6). However, HARA *et al.* (15) and LIU & BERRY (21) had more positive nematode sites along the seashore than any other habitat. After the seashore sites, LIU & BERRY (21) 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 (22) 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 (5, 14, 15, 16, 21). 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 (1, 19), and many nematode-infected insects have been recovered from nature during the course of other investigations (26). For most field studies including surveys, HOMINICK & REID (17) 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 nematodes 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 isolates 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 the areas are "polluted" by inundative and inoculative applications of commercial species. Applied nematodes may outcompete and replace the native entomopathogenic nematodes resulting in their local extinction.

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和文摘要

韓国からの昆虫病原性線虫（Steinernematidae 科および Heterorhabditidae 科）の検出

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1990年夏から1991年にかけて韓国内（９道、３直轄市）の昆虫病原性線虫の分布調査をした。９道のうち６道と１市から線虫は検出された。全土壌サンプル499のうち23から線虫は検出され（検出率4.6%)、その内の19（3.8%）は *Steinernema* 属で明瞭に識別された。そのうち１群は交配実験で *S. carpocapsae* と同定された。残りの４（0.8%）は *Heterorhabditis* 属で、*H. bacteriphora* と同定された。生息環境別にみると、低木の改植林を含む森林土壌は415のうち15（3.6%)、公園・ゴルフ場など芝地は27中１（3.7%)、海岸域は12中２（11.8%）であった。これらの線虫は、朝鮮半島南部に普遍的に分布するものと結論する。広範囲な市販線虫の施用は、自然に存在する昆虫病原性線虫の分布調査を困難にするであろう。従って、線虫が広く施用される前に、より詳細な分布調査が必要であると我々は提唱する。