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ENTOMOPATHOGENIC NEMATODES (RHABDITIDA: HETERORHABDITIDAE AND STEINERNEMATIDAE) IN SRI LANKA AS BIO-CONTROL AGENTS

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A survey for endemic and climatically adapted entomopathogenic nematodes was carried out in Sri Lanka. Their habitat specificity and distribution in part of the coastal areas was documented. The dry season and monsoon rain had no influence on the prevalence of these nematodes. Nematodes found in these soils belong to both *Steinernema* (2 types) and *Heterorhabditis* (2 types). Except one Heterorhabditid isolate the rest were identified as new compared to the known species to date.

INTRODUCTION

Entomopathogenic rhabditid nematodes (*Steinernema* and *Heterorhabditis* spp.), which are one of the bio-control agents of insect pests have a third stage juvenile termed a "duer larva" which is the survival and infective stage. These are non-feeding and harbour cells of a symbiotic bacterium. This bacterium causes the death of the host. Once the nematodes contact a potential host, they enter through natural openings and release the bacteria, which rapidly multiply and colonise in the host haemocoel. The nematodes go through several generations in the cadaver until nutrients become depleted. They then differentiate into duer larvae which leaves the host, usually in their thousands, to enter the soil until they contact another host or perish.

While these nematodes are ubiquitous in soil and have great potential for the biological control of many important insect pests, different species and strains exhibit differences in pathogenicity or infectivity to a given insect pest. Also, indigenous nematodes may provide isolates best suited for inundative release against local insect pests because of adaptation to local climate and population regulators (Bedding, 1990). With this in mind, entomopathogenic nematodes have been isolated from many parts of the world, frequently using a simple baiting technique involving Wax moth *(Galleria mellonella)* larvae (Bedding and Akhurst, 1975).

The survey conducted for this study commenced in July 1991 and continued until August 1992 to document the prevalence and distribution of entomopathogenic nematodes in Sri Lanka to provide isolates for future pest management programmes.

MATERIALS AND METHODS

Soil samples were collected to a depth of 10 cm using a soil corer with a volume of 250 ml. At each site, four random samples were taken over an area of approximately 10 m^2, pooled in a polythene bag and placed in a thermal box for transport to the laboratory. Samples were bioassayed not more than 12 hours after bringing them in the laboratory. Three aliquots, each of approximately 200 ml were taken from the pooled soil from each site and were placed in separate plastic containers (8.5 cm x 6 cm). Three late instar *Galleria* larvae were placed in each and the covered containers were kept at room temperature (22 - 25°C). Tap water (10 ml/200 ml soil) was used to moisten soil collected during the drought period. After 3 days, the *Galleria* cadavers were collected without disturbing the soil and after 6 days the soils were disturbed and searched for cadavers of the larvae which had tunnelled into the soil. Since a single bioassay does not recover all nematodes present (Hominick and Briscoe, 1990), the soil in the three containers was pooled to make two of 200 ml each. Three fresh *Galleria* larvae were introduced to each and the containers were kept until the larvae died from nematode infection or starvation.

Galleria cadavers suspected of having a nematode infection were cleaned and nematodes isolated were used to re–infect *Galleria* larvae to confirm that they were entomopathogenic nematodes. Identification to species was done using Restriction Fragment Length Polymorphism (RFLP) analysis (Reid and Hominick, 1993).

Effect of season on nematode prevalence

In general, from January to early March, the rainfall that can be expected with 75% probability is less than 100 mm, which is considered as the dry period throughout the island. Normally, in the wet zone, at least 100 mm of rainfall can be expected 3 years in 4, in all other months. The South-West monsoon, which provides rain to the wet zone starting in May, continues up to July, while the North–East monsoon which provides rain to the dry zone, starts in October, and continues until December (Anon, 1988). The year 1992, when this work was done, experienced an unusual prolonged dry period, which continued for more than three months from January to late April.

Soil collection along the coast of the wet and intermediate zone continued for three sampling rounds, one during the drought in March 1992 (n=18), one in June 1992, six weeks after the monsoon rain began (n=51) and the other in July 1992, twelve weeks after the monsoon rain began (n=42). Although the number of sites sampled in each sampling round was different, 18 sites were sampled on all three occasions to document any seasonal effect on nematode prevalence.

Influence of dose and soil type on infectivity of nematodes

Typical soil from tea plantation (sandy clay loam, pH=5.1) and sand from the southern coast of Sri Lanka (pH=7.9), where no native entomopathogenic nematodes

were found, was tested. As described earlier, soil and sand containers were prepared. Tap water (10 ml) was added to moisten the soil. Treatments of 1000, 500, 100, 50, 25, 10 and 0 nematodes (*Steinernema carpocapsae*; obtained from Biosys, California) were applied with 1 ml of water to the center of the containers in a centrally made well which was then obliterated. All were replicated 5 times and maintained at a room temperature of 23±3° C. A last instar *Galleria mellonella* larva was added to each and the cadavers were replaced every 3 days until mortality stopped. The number of nematodes which infected were counted by dissecting the cadavers in Ringers solution. The relationship between the mean number of nematodes per host and dose was estimated by regression analysis (Fan and Hominick, 1991).

RESULTS AND DISCUSSION

In all, 189 inland sites were sampled from July 1991 until February 1992. They were classed as tea plantations (n=129), grass fields (n=21), natural forests (n=28) and, other habitats (n=11). Since none provided a positive assay, further examination of soil from these areas was terminated. The frequency of the soil types in descending order was sandy clay loam (n=73), sandy loam (n=46), clay loam (n=42) and loam (n=28).

Sampling along the coast was then begun because of a recent discovery of *Heterorhabditis* spp. from the coastal area of Hawaii (Hara *et al.*, 1991). By contrast to our inland survey, both *Steinernema* and *Heterorhabditis* spp. were recovered from the sandy soils of the South–West coastal area, within 5–100 m of the ocean. In this belt, the nematodes were confined to the wet and intermediate ecological zones (30/100 and 3/11 positive sites, respectively). There were no nematode positive sites (0/8) in the dry zone. There was no obvious relationship between presence of nematodes and distance from the sea (5–100 m). This is the first extensive survey of Sri Lanka and the first record of both heterorhabditids and steinernematids from tropical coast. Among the 33 positive sites along the coast there were two genetic types of *Heterorhabditis* and two of *Steinernema* were found. A similar survey to the present one by Hara *et al.* (1991) in Hawaii and Griffin *et al.* (1993) in Britain produced only *Heterorhabditis* isolates within 100 m to a few hundred metres of the sea respectively. They recovered no *Steinernema* isolates in close proximity to the sea. The question of why the entomopathogenic nematodes are restricted to a narrow coastal area is an intriguing one. It is unknown how they survive and what hosts they utilise.

Of all positive sites, 4 sites yielded heterorhabditids and steinernematids simultaneously, but never in the same host. One site produced two genetically different *Heterorhabditis* isolates from the same soil sample. Of the 38 isolates, 17 were steinernematids and 21 were heterorhabditids. The 21 *Heterorhabditis* isolates established cultures in *Galleria* larvae in the laboratory for indentification, but seven *Steinernema* isolates failed to produce infectives or died in transit to the UK.

The established nematode types were categorized according to differences in their recombinant DNA patterns. There were two distinct RFLP types for *Heterorhabditis*

and two for *Steinernema*. The most common heterorhabditid (all except one isolate) was identical to the D1 strain from Darwin (obtained from Dr. Curran, CSIRO, Canberra) and is believed to be *H. indicus* (Poinar, Karunakar and David, 1992) recently described from India (Curran, per. comm.). The other was unlike any other heterorhabditid known to date. The two steinernematids are new to science, and maps of restriction enzyme cutting sites indicate that they are closely related.

Biotic and abiotic factors at the sampling sites

The associated vegetation of all the sites positive for nematodes along the coast was *Cocos nucifera* (Coconut), *Ipomoea* (Bimthamburu) creepers, and common grass. Insects were only occasionally found in the soil samples, but none was parasitized. The soil of the nematode positive had pH in the range of 7.8–8.9. Soil texture analysis showed that prevalence of the nematodes in sand, loamy sand, sandy loam, and loam was 70.4%, 18.5%, 11.1% and 0% respectively (n=33). Temperature taken at the time of sampling indicates that the nematodes are capable of surviving temperatures of 30°C or even higher.

Seasonal effect on recovery of nematodes

There was no relationship between the number of sites with nematodes and the rainy season. Thus, 33.3% (6/18) of the sites were positive during the height of the drought, while 38.9% (7/18) and 27.8% (5/18) of the same sites were positive on the two sampling rounds after the monsoon rain. Also, individual sites converted from positive to negative or negative to positive unpredictably during the study. Only one site produced nematodes on all 3 occasions, while 5 sites were negative all the time. There were no specific characters of the sites to correlate with nematode availability or scarcity.

Influence of dose and soil type on infectivity of nematodes

This test was carried out to investigate whether coastal compared to tea plantation soil had any effect on nematode recovery. The results showed that 37% of the nematodes in sand containers (slope of the regression line, b=0.3711; R^2 =0.986) and 46.5% of the nematodes in tea soil containers (slope of the regression line, b=0.465; R^2=0.914) were recovered from the insects, regardless of the dose applied. This shows that infectivity of the nematodes in the sandy clay loam soil and the sand are similar to each other and to infectivity documented by Fan and Hominick (1991). Thus, if the nematodes were present in the sandy clay loam typical of tea plantations, they should have been recovered by the bioassay. Hence, the negative bioassay probably reflect rarity of nematodes rather than failure of the bioassay.

Seasonal variation in the prevalence of entomopathogenic nematodes has been examined before. Some studies revealed that there was a seasonal effect whilst others did not. If there is a seasonal effect, results suggest that prevalence is higher in cooler seasons than warm. On the coast of Sri Lanka, season is marked by rain rather than temperature and there was no detectable effect of the monsoon rain on entomopathogenic

nematode prevalence. Indeed, our first positive sites were sampled in the height of the drought. This supports Beavers *et al.* (1983) who mentioned that in Florida, there was no correlation between rainfall and seasonal abundance of the entomopathogenic nematodes recovered during their survey.

Some literature suggests that prevalence of steinernematids is higher in regions with temperate and cooler climates while heterorhabditids predominate in the soils of warmer and tropical regions. For example, Hara *et al.* (1991) found that the prevalence of heterorhabditids was higher than that of steinernematids in their survey of the coasts and some inland areas of the Hawaiian islands. By contrast, Hominick and Briscoe (1990) found that steinernematids were common while heterorhabditids were rare in their UK survey, which concentrated on inland areas. However, Griffin *et al.* (1993) recently found that heterorhabditids in Ireland, the north of Scotland, and the south of Wales usually exist within a few hundred metres of the sea. None were recovered from the inland sites sampled. Griffin *et al.* (1993) documented that *Heterorhabditis* is also widespread in the temperate islands of Britain and Ireland. Now we have found both genera in Sri Lanka. The broad generalisation that steinernematids are temperate while heterorhabditids are tropical must now be questioned.

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