Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from the southwestern parts of South Africa

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Soil samples were collected in the southwestern parts of South Africa to obtain entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae. In total, 498 samples were randomly taken from cultivated and uncultivated habitats, including deciduous fruit orchards, vineyards and natural vegetation. Entomopathogenic nematodes were isolated from 36 samples (7 %) by baiting with larvae of *Galleria mellonella* (greater wax moth). *Heterorhabditis* was the dominant genus isolated, while *Steinernema* was rare. The most common species was *Heterorhabditis bacteriophora* Other species identified were *Heterorhabditis zealandica* and *Steinernema khoisanae.* The isolation of *H. zealandica* represents a new record for South Africa, whereas *S. khoisanae* has thus far been recorded only from South Africa.

Key words: entomopathogenic nematodes, *Heterorhabditis,* South Africa, *Steinernema,* survey.

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* occur naturally in soil, where they parasitise different life stages of various soil-inhabiting insects. The nematodes are synergistically associated with bacteria and together they kill and utilise their insect host. Infective juveniles (IJs) are the only free-living stage in soil and carry the bacteria in their intestines, releasing them once the haemocoel of the host is penetrated. The IJ is a special third stage of development, highly resistant to adverse conditions in the soil, with some species capable of surviving for several months or even years without feeding. They kill their hosts within 1–2 days, can be produced commercially and can be applied with standard spraying equipment or through certain types of irrigation systems. The main interest in these nematodes is their potential as biological control agents in integrated pest management systems.

The first record of EPNs in South Africa was from the maize beetle *Heteronychus arator* (Fabricius) (*Heteronychus sanctae-helenae* Blanch.) in Grahamstown, Eastern Cape Province (Harington 1953). Three isolates of *Steinernema* and a *Heterorhabditis* were evaluated in KwaZulu-Natal against the African sugarcane stalk-borer, *Eldana saccharina* Walker, in laboratory and field tests (Spaull 1988, 1990). A further survey was conducted in 1991 to obtain isolates more virulent against *E. saccharina,* during which seven *Heterorhabditis* and 15 *Steinernema* isolates were found (Spaull 1991), but they were not identified to species level. A new species of *Steinernema* for South Africa, *S. khoisanae,* was described by Nguyen et al. (2006).

In South Africa, fruit storage bins are a major source of re-infestation of apple orchards by codling moth (*Cydia pomonella* (Linnaeus)) (Proverbs & Newton 1975). Phytosanitary authorities in South Africa have adopted a precautionary policy with regard to exotic biological control agents, including EPNs. It is therefore not possible to import exotic commercially-available EPNs for local evaluation without a full impact study. The purpose of this survey was to obtain EPNs from South African soils, particularly species such as *Steinernema carpocapsae* (Weiser, 1955) Wouts, Mracek, Gerdin & Bedding, 1982, and *Steinernema feltiae* (Filipjev, 1934) Wouts, Mracek, Gerdin & Bedding, 1982, for use as biological control agents against codling moth in the cryptic habitat of stacked storage bins.

Materials and methods

Collection of soil

Soil samples were collected in 2004/2005 from different habitats throughout the southwestern parts of South Africa, including disturbed agricultural and undisturbed natural soil. Samples of approximately 1 kg were taken to a depth of up to

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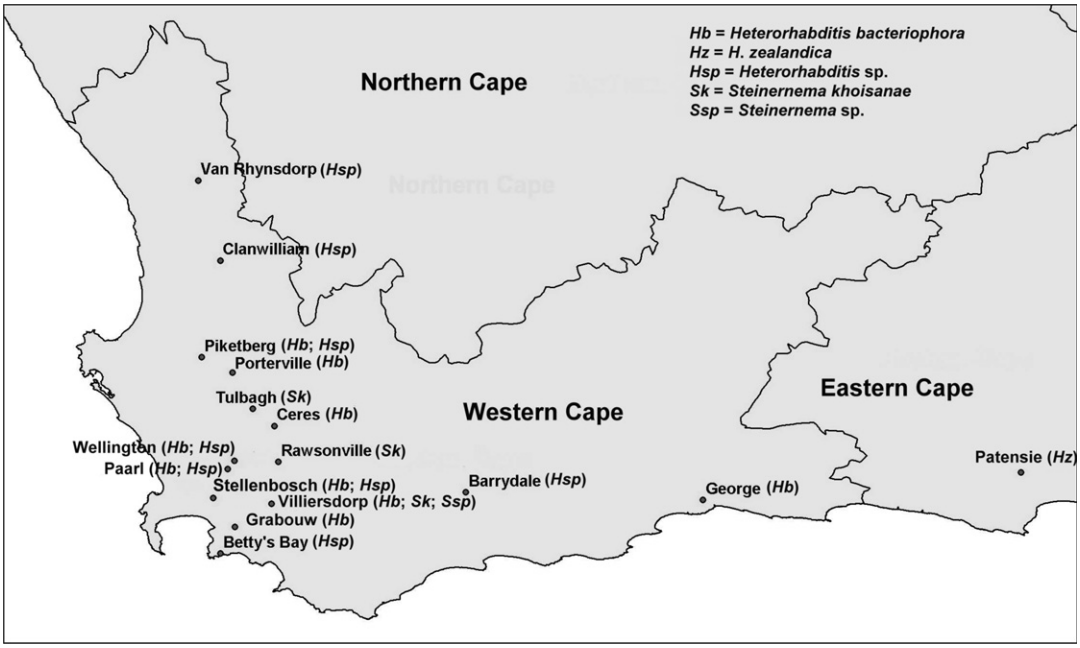

Fig. 1. Distribution and occurrence of entomopathogenic nematodes in the southwestern parts of South Africa.

20 cm from moist, shady areas and transported to the laboratory in plastic bags.

Trapping and storage of nematodes

The soil in each sample was mixed and five greater wax moth *(Galleria mellonella* L.) larvae were added to two 250 ml sub-samples in separate plastic containers. The containers were closed with lids and incubated in the dark at 25 °C (Bedding & Akhurst 1975). After a period of 6–7 days, dead larvae were removed and individually placed on a modified White's trap (White 1927). IJs were harvested during the first week of emergence and stored together at 14 °C in 150 ml filtered water in 500 ml flat culture flasks with vented lids. The IJs of *Heterorhabditis* species were maintained by recycling through *G. mallonella* every three months and those of *S.khoisanae* every six months (Dutky et al. 1964; Nguyen 1988).

Nematode identification

Specimens used for identification were obtained by infecting *G. mellonella* larvae with 200 IJs each, in 8.5-cm-diameter Petri dishes lined with moistened filter paper, and kept in a growth chamber at 25 °C. First-generation males and females of *Steinernema* and hermaphrodites of *Heterorhabditis* were obtained 3–4 days after the *G. mellonella* larvae had died by dissecting the cadavers in Ringer's solution, and those of second-generation males and females after 5–7 days. Specimens were killed and fixed in 5 % formalin at 80 °C.

The nematodes were identified to genus or species level by microscopical examination of males and females or hermaphrodites of the first generation and morphology and morphometrics of the IJs and males (first and second generation) (Nguyen & Smart 1996). Hermaphrodites or first-generation females were preserved in 95 % alcohol for molecular characterisation. Species verification was done by sequencing the ITS regions and alignment with EPN sequences deposited in Genbank (Nguyen et al. 2004).

Results and discussion

In total, 498 soil samples were collected, mostly from the Western Cape Province. EPNs were found in 36 samples (7 %) representative of all the habitats (Fig. 1, Tables 1, 2). The species identified were *Heterorhabditis bacteriophora* Poinar 1975, *Heterorhabditis zealandica* Poinar, 1990 and *S. khoisanae,* with seven unidentified *Heterorhabditis* and one *Steinernema* species (Table 2). Three *S. khoisanae* isolates were recovered, two from agricultural soil under apple and grape-

Table 1. Species and isolates of entomopathogenic nematodes identified from a survey of sites in the southwestern region of South Africa.

Species	Isolate no.	Nearest town	Habitat
Heterorhabditis bacteriophora	SF523	Ceres	Apple (*Malus domestica* Baumg.)
H. bacteriophora	SF525, SF529	Ceres	Apple
H. bacteriophora	SF584, SF585, SF586	Ceres	Peach (*Prunus persica* Sieb. & Zucc.)
H. bacteriophora	SF19	George	Grass
H. bacteriophora	SF1	Grabouw	Apple
H. bacteriophora	SF347	Paarl	Plum (*Prunus salicina* Lindl.)
H. bacteriophora	SF378	Paarl	Plum
H. bacteriophora	SF10	Piketberg	Pear (*Pyrus communis* L.)
H. bacteriophora	SF134	Piketberg	Apple
H. bacteriophora	SF145	Porterville	Grass
H. bacteriophora	SF286	Porterville	Vegetables
H. bacteriophora	SF8, SF160, SF179	Stellenbosch	Grass
H. bacteriophora	SF381	Stellenbosch	Apple
H. bacteriophora	SF285	Villiersdorp	Apple
H. bacteriophora	SF311, SF351, SF407, SF413	Wellington	Grapevine (*Vitis vinifera* L.)
Heterorhabditis zealandica	SF41	Patensie	Natural vegetation
Heterorhabditis sp.	SF379	Barrydale	Peach
Heterorhabditis sp.	SF52	Betty's Bay	Natural vegetation
Heterorhabditis sp.	SF597	Clanwilliam	Grass
Heterorhabditis sp.	SF291	Paarl	Grapevine
Heterorhabditis sp.	SF281	Piketberg	Peach
Heterorhabditis sp.	SF593	Stellenbosch	Garden
Heterorhabditis sp.	SF401	Van Rhynsdorp	Current (*Ribes* sp.)
Heterorhabditis sp.	SF288	Wellington	Grapevine
Steinernema khoisanae	SF362	Rawsonville	Grapevine
S. khoisanae	SF80	Tulbach	Grass
S. khoisanae	SF87	Villiersdorp	Apple
Steinernema sp.	SF207	Villiersdorp	Grapevine

vine and one from soil under grass on a road reserve.

According to a review of the biogeography and habitats of EPNs (Hominick 2002), *Steinernema* is generally recovered more often than *Heterorhabditis,* although exceptions do occur. *Heterorhabditis* species were rare in most European surveys, with *H. bacteriophora* being geographically the most widespread (Hominick 2002). In the present survey *Heterorhabditis* was the dominant genus, with *H. bacteriophora* the most common species for the Western Cape Province, while *Steinernema* species were rarely detected. *H. bacteriophora* and *S. khoisanae* are the only EPN species previously recorded in South Africa, also from the Western Cape Province (Grenier et al. 1996). In KwaZulu-Natal, however, *Steinernema* species were more common than *Heterorhabditis* species (Spaull 1990, 1991). Future surveys should provide a better indication of the distribution patterns of EPNs in South Africa.

Other reports on the presence of entomopathogenic nematodes on the African continent were from Kenya (Waturu 1998), Egypt (Atwa 2004) and Ethiopia (Mekete et al. 2005). In Kenya, *H. bacteriophora, Heterorhabditis indica* Poinar, Karunakar & David and *Steinernema karii* Waturu, Hunt & Reid (Waturu et al. 1997) were isolated. *H. bacteriophora* was the predominant species in Egypt, while *Steinernema* was the least frequently found genus (Atwa 2004). In Ethiopia the dominant species detected was *Steinernema yirgalemense* Nguyen, Mekete, Gozel, Gaugler & Adams (6.3 %), with only two isolates of *H. bacteriophora* (0.7 %) (Mekete et al. 2005). These results and those of the present survey showed *H. bacteriophora* to be common in Africa.

G. mellonella infected with *H. bacteriophora*

Table 2. South African isolates of entomopathogenic nematodes from different habitats.

Habitat	No. of samples	Positive samples	*Hb*	*Hz*	*Hsp*	*Sk*	*Ssp*
Apple	43	8	7	–	–	1	–
Peach	84	5	3	–	2	–	–
Pear	8	1	1	–	–	–	–
Plum	6	2	2	–	–	–	–
Natural vegetation	46	2	–	1	1	–	–
Current	1	1	–	–	1	–	–
Grass	36	7	6	–		1	–
Grapevine	183	8	3	–	3	1	1
Vegetable	10	1	1	–	–	–	–
Garden	3	1	–	–	1	–	–
Total	498	36	23	1	8	3	1

Hb = *Heterorhabditis bacteriophora.*
Hz = *H. zealandica.*
Hsp = *Heterorhabditis* sp.
Sk = *Steinernema khoisanae*
Ssp = *Steinernema* sp.

isolated from different sites showed two phenotype variants in the colour of the cadaver. Most were the usual brick-red, but those infected with SF160 from undisturbed vegetation in the Stellenbosch area were bright golden-yellow in colour. Green and red phenotypes of *H. bacteriophora* and pink to yellow and grey to purple-brown ones of *H. downesi* have previously been reported from different sites (Rolston et al. 2005). The colour variation can possibly be ascribed to the bacteria associated with the two *H. bacteriophora* isolates producing different colour pigments (Richardson et al. 1988). The difference in colour between isolates is an interesting subject for future studies. *H. zealandica* is a new record for South Africa. The type locality of *H. zealandica* is near Auckland, New Zealand, but it has also been recorded from the USA (Duncan et al. 2003), Lithuania, Russia and Australia (Poinar 1990). As this species was found in the Baviaanskloof near Patensie (Fig. 1), in a mountainous area without previous cultivation, it can be regarded as indigenous to South Africa.

S. khoisanae belongs to the glaseri-group (Nguyen 2006) of described *Steinernema* species, with mean size of IJs in excess of 1000 µm. With a mean length of 1075 µm (Nguyen et al. 2006) the IJs of *S. khoisanae* are the fourth-largest of the 47 described species in the genus *Steinernema* (Nguyen 2006). By comparison, the IJs of *S. carpocapsae,* for instance, have a mean length of 558 µm (Adams & Nguyen 2002). Commercial and application problems are expected when using an EPN with such large IJs. The cost of production of large IJs is considerable. Application problems such as clogging of nozzles and filters and sedimentation in spray tanks and irrigation systems may also occur (Wright et al. 2005). They could, however, have special characteristics that justify their commercialisation.

S. carpocapsae and *S. feltiae* have been used successfully for the control of codling moth in orchards (Kaya et al. 1984; Unrush & Lacey 2001) and for treatment of fruit bins (Lacey & Chauvin 1999; Lacey et al. 2005). Hominick (2002) speculated that *S. carpocapsae* and *S. feltiae* could be ubiquitous in temperate regions. Expectations to isolate these species from local soils were therefore high, but unfortunately they have thus far not been found. *H. bacteriophora* was the most common species present in apple orchards (Table 2) in the Western Cape Province. Due to production problems expected with the large IJs of the indigenous *S. khoisanae,* evaluation of the 32 *Heterorhabditis* isolates will be the main focus in further research on the treatment of fruit bins to control codling moth in apple orchards.

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