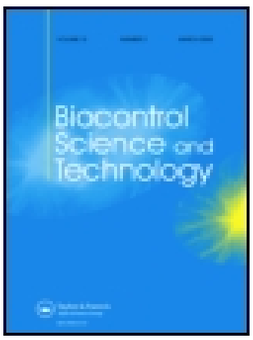

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REVIEW ARTICLE

Commercialisation of entomopathogenic nematodes: should import regulations be revised?

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ABSTRACT

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are obligate insect pathogens. Their favourable characteristics as biocontrol agents have resulted in some species of EPNs being released globally and widely used for the control of diverse insect pests. In this review, we consider the occurrence of currently described EPN species, including those that have been released globally for commercial purposes. We also discuss the contribution of regulation policies to the global distribution of these species and issues that influence import regulations. Possible non-target effects, the use of commercial versus native EPNs and the possible interaction between these species are considered. Finally, we provide a view as to whether existing policies adequately deal with the risks associated with the global movement of EPNs and we suggest future directions that should be considered for the use of EPNs as biological control agents.

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Biological control; *Heterorhabditis*; macrobial; microbial; non-target effects; *Steinernema*

1. Introduction

Nematodes are considered to be the most numerous Metazoa on earth (Decraemer, Hunt, Perry, & Moens, 2006). Estimates of nematode diversity range between 0.5 and 1 million species, with only about 26,000 species currently described. Of these described species, approximately 14,000 are free-living, invertebrate and plant-associated nematodes, and these reside in more than 30 orders (Hodda, Peters, & Traunspurgen, 2009). In the case of the invertebrate parasitic nematodes, there are 30 families (Kaya & Stock, 1997; Stock & Hunt, 2005), of which seven (Mermithidae, Allantonematidae, Neotylenchidae, Sphaerularidae, Rhabditidae, Steinernematidae and Heterorhabditidae) have the potential to be considered as biological control agents (BCAs) (Stock & Goodrich-Blair, 2012). Of these, the latter two families are the most widely studied and currently receive considerable attention as BCAs of soil insect pests.

Steinernematidae and Heterorhabditidae are obligate insect pathogens in nature. The successful application of these families as BCAs is attributed in part to their symbiosis

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with bacteria of the genera *Xenorhabdus* and *Photorhabdus*, which are carried in the intestines of free-living infective juveniles (IJs) of *Steinernema* and *Heterorhabditis*, respectively (Forst & Clarke, 2002; Renn, 1998). In steinernematids, these bacteria are held in a special vesicle in the anterior part of the gut (Boemare, 2002). In the soil, these IJs locate insect larvae and enter through natural openings (mouth, anus and spiracles) and in some species directly through the cuticle, after which the symbiotic bacteria are released in the haemolymph of the invaded insect. The bacteria proliferate and produce a wide range of toxins and hydrolytic enzymes that are responsible for the death of the insect larvae within 24–48 hours (Forst & Clarke, 2002). Species of *Steinernema* and *Heterorhabditis* are commonly referred to as entomopathogenic nematodes (EPNs) (Dillman et al., 2012). They have been recovered from soils and insects globally and they are distributed on all continents except Antarctica (Adams et al., 2006; Hominick, 2002).

The global distribution patterns of EPNs are influenced by variation in suitable habitats such as soil type and the availability of suitable hosts (Adams et al., 2006). In addition, knowledge on the distribution of EPNs has been influenced by substantial differences in scientific effort across regions. Hominick, Reid, Bohan, and Briscoe (1996) provided two explanations as to why some EPNs have a global distribution. These were, firstly, that some species were very ancient and present before continental drift. Secondly, the wide global distribution could be due to accidental human intervention through moving soil and/or infected insects. In addition to these explanations for the global distribution of EPNs, it is important to recognise that these nematodes have been widely distributed intentionally for commercial purposes (Flexner & Belnavis, 1999).

The development of mass production and consequent commercialisation of EPNs has been a major factor contributing to the global spread of these nematodes (Grewal & Peters, 2005). The utilisation of EPNs as BCAs commenced in 1931 with efforts to control the invasive Japanese beetle, *Popillia japonica*, in the USA. However, production of these nematodes on a larger scale utilising solid media or liquid culture was achieved only in the early 1980s (Ehlers, 2001, 2007). The isolation of the symbiotic bacteria of EPNs for use as a food source in liquid culture production has also enabled the large-scale production and commercialisation of a number of EPN species (Poinar & Thomas, 1966). A subsequent increase in the use of EPNs has largely been due to their wide insect pest host range and because they are viewed as an environmentally safer option than chemical pesticides. In this regard, the view of EPNs as exceptionally safe BCAs has resulted in the absence or leniency of regulatory protocols for the introduction of non-native EPNs in many countries (Ehlers, 1996; Ehlers & Hokkanen, 1996; Ehlers & Shapiro-Ilan, 2005).

In this review, we examine the contribution of commercialisation to the global distribution of EPN species. We consider issues influencing the import regulation of EPNs, including the dilemma as to whether to view EPNs as microbial or macrobial BCAs, and the likelihood of non-target effects. The use of commercial versus native EPNs and the possible interaction between these species are also discussed. Finally, we provide a view as to whether existing regulations adequately deal with the risks associated with the global movement of EPNs and we suggest future directions that should be considered for the use of EPNs as BCAs.

2. Diversity and biogeography of EPNs

The biogeography of EPNs has previously been assessed by Hominick et al. (1996) and Hominick (2002), who provided a comprehensive summary of the distribution of 34 species described at that time. By 2007, the number of described EPN species had increased to 66 (Nguyen & Hunt, 2007; Nguyen, Hunt, & Mracek, 2007). The potential use of EPNs as BCAs has also led to a recent increase in efforts to identify native EPN species, with the result that by 2015 about 107 EPN species, 88 *Steinernema* and 19 *Heterorhabditis*, had been described (Appendix 1 supplementary material). This represents a 78% increase in described species since 2000 (Table 1). The dramatic increase in the discovery of new EPN species can be explained by the fact that more extensive surveys have been conducted around the world in diverse habitats and on different host species (Campos-Herrera, 2015), and that there have been significant advances in research including that pertaining to the taxonomy of these nematodes (Nguyen & Hunt, 2007; Nguyen et al., 2007).

EPNs are distributed and described globally, although most of the currently described species are from Asia (50), with only 3 species described from Australia. It should be noted that the number of described EPN species in each continent represents original isolation for the first time from an area, thus does not necessarily represent distribution (Table 1). At the time that Hominick et al. (1996) and later Hominick (2002) summarised the distribution of EPNs, four species, namely *S. feltiae*, *S. carpocapsae*, *H. bacteriophora* and *H. indica*, were distributed globally. This is likely due to the commercialisation of these species (Ehlers, 1996), where the selection of EPN species for commercialisation mainly takes into account traits such as high virulence against the target pests, ease of culture, effectiveness against multiple insect pests and superior shelf life (Gaugler & Han, 2002). The remaining EPN species have a more restricted distribution at either the continental or local/regional level (Appendix 1 supplementary material), which would change in the future if some of these species are also traded globally as BCAs. It is also important to recognise that the global distribution of many species may be underestimated due to limited surveys, combined with the fact that many of these species have only recently been described.

3. Commercialisation of EPNs

EPNs are amongst the most successful groups of organisms used for biological control, with commercially produced products distributed globally for the management of a

Table 1. Number of described *Steinernema* and *Heterorhabditis* species by continent as of 2015 (based on lists of Appendix 1 supplementary material).

	Steinernema				*Heterorhabditis*				
	As of 1999	2000–2009	2010–2015	Sub total	As of 1999	2000–2009	2010–2015	Sub total	Total
Africa	1	2	7	10	1	1	1	3	13
Australia	0	0	0	0	2	1	0	3	3
Asia	9	26	11	45	1	3	0	4	50
Europe	4	5	3	12	0	1	0	1	12
N. America	6	3	1	10	2	2	0	4	14
S. America	5	5	1	11	0	3	1	4	14
Total	25	41	22	88	6	11	2	19	107

wide range of pests on various crop plants (Appendix 2 supplementary material). In this regard, several factors have contributed towards this successful commercialisation of EPNs. These include advances in mass-production and formulation technology (Ehlers, 2001, 2007; Ehlers & Shapiro-Ilan, 2005; Gaugler & Han, 2002), their ability to control many soil-dwelling insect pests (Koppenhöfer, 2007), the ease of application using conventional liquid application equipment and systems (Grewal, 2002) and the effect of reducing harmful chemical pesticide applications.

The commercialisation of EPNs commenced in the early 1980s and later Bedding (1984) developed a semi-artificial medium for their production. Subsequently, there has been considerable research and development to improve the production technology, formulation and storage of EPNs (Dolinski, Shapiro-Ilan, & Lewis, 2015; Georgis et al., 2006; Shapiro-Ilan & Dolinski, 2015). One of the major advances was the development of large-scale production of EPNs in liquid culture in bioreactors (Ehlers, 2007). This large-scale liquid production, coupled with the development of several formulations in order to maintain high levels of viability during storage, transport, and application, contributed greatly towards rapid commercialisation (Hiltpold, 2015).

EPNs are marketed widely across the world and they are used in a variety of crop systems (Georgis, 2002; Georgis et al., 2006). Flexner and Belnavis (1999) listed more than a dozen commercial companies that produce five *Steinernema* species (*S. carpocapsae*, *S. feltiae*, *S. glaseri*, *S. riobrave* and *S. scapterisci*) and two *Heterorhabditis* species (*H. bacteriophora* and *H. megidis*). In a later report by Kaya et al. (2006), four additional EPN species (*S. kraussei*, *H. indica*, *H. marelatus* and *Heterorhabditis* sp.) were included into the market. In a more recent report by Lacey et al. (2015), three more species (*H. downesi*, *S. kushidai* and *S. scarabaei*) have been introduced for commercialisation. However, *H. indica* and *S. kraussei* were not included in this report, though *S. kraussei* is still in production by many companies. Currently, *S. carpocapsae*, *S. feltiae*, *S. kraussei*, *S. glaseri*, *S. riobrave*, *S. scapterisci*, *H. bacteriophora* and *H. megidis* are the most commonly used and successfully applied nematodes due to the fact that they can easily be produced in liquid culture (Ravensberg, 2011; Appendix 2 supplementary material).

S. carpocapsae was first described from the former Czechoslovakia in 1955 from infected codling moth (*Cdyia pomonella*). This nematode was the first commercially mass-produced product in liquid culture, which commenced in 1962 (Ehlers, 2001). It has subsequently been produced by many companies for the control of various groups of insect pests, mostly residing in the orders Coleoptera, Lepidoptera and Orthoptera (Appendix 2 supplementary material). The other widely commercialised *Steinernema* species is *S. feltiae*, which has a wide host range and is used in varying environments including attacking leaf miner larvae within leaves, scale insects on plants, sciarid fly larvae in soil and in mushroom culture, and thrips on plants and in soil (Appendix 2 supplementary material). Both these species have a wide distribution, particularly in Europe and the USA (Figure 1; Appendix 1 supplementary material).

Although *H. bacteriophora* and *H. indica* are effective against a wide range of insect pests, they are mainly used for the control of soil-dwelling pests, including various weevils and chafer larvae. Currently, *H. indica* is not in production, potentially due to the fact that the human pathogen *Photorhabdus asymbiotica* has been found as a symbiont associated with this nematode (Kuwata, Yoshiga, Yoshida, & Kondo, 2008; Thanwisai et al., 2012). Bale (2011) suggested that risks associated with the use of *H. indica* can be

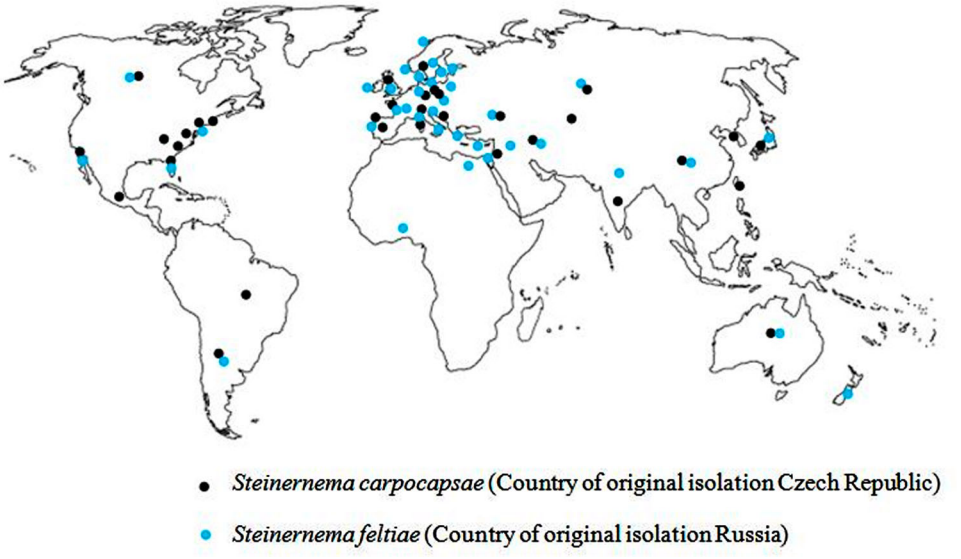

Figure 1. Global distribution of the most commercially applied *Steinernema* species.

excluded by a precise identification of its associated symbiotic bacteria. In terms of distribution, both species of *Heterorhabditis* are found on all continents except Europe, where only *H. bacteriophora* is present (Figure 2; Appendix 1 supplementary material).

One of the reasons for the success of EPNs as effective BCAs is their ease of application using conventional liquid application equipment (Grewal, 2002; Lacey et al., 2015; Shapiro-Ilan & Dolinski, 2015; Shapiro-Ilan, Han, & Dolinski, 2012). In general, the spraying technology varies from small watering cans for home garden use to aerial application over large fields (Grewal, 2002; Shapiro-Ilan, Gouge, Piggott, & Fife, 2006). In addition to conventional spraying equipment, EPNs can be applied using different irrigation systems in both the open field and greenhouse (Wright, Peters, Schroer, & Fife, 2005) as well as using infected host cadavers (Dolinski et al., 2015). These application technologies have been used in various cropping systems against soil and above-ground insect pests (Lello, Patel, Matthews, & Wright, 1996; Shapiro-Ilan et al., 2006). Improvement to the application systems of EPNs, coupled with the need to reduce harmful chemical pesticides, has contributed to the efficient transfer from chemical regimes.

Although the number of EPN-producing companies and commercially produced species has remained relatively constant during the last 15 years (Flexner & Belnavis, 1999; Georgis et al., 2006; Appendix 2 supplementary material), distribution and production levels have increased dramatically. The value of EPN market per annum in 2006 in Europe and the USA has been estimated at $6 and $8.25 million, respectively. Sale of EPNs in the Netherlands alone in 2008 was estimated at about €1 million for the control of greenhouse pests (Ravensberg, 2011). Currently, three of the largest producers of EPNs are the previous Becker Underwood (now bought by BASF), based in the USA, e-nema in Germany and Koppert Biological Systems in the Netherlands (Ravensberg, 2011). Although the current markets are mainly within the regions of production, developing countries represent growing markets for companies producing EPNs.

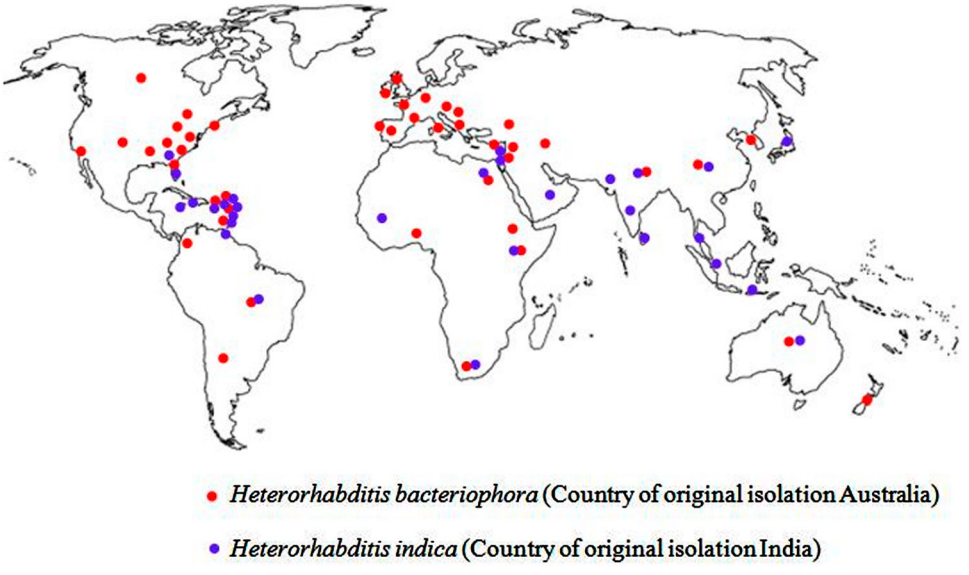

Figure 2. Global distribution of the most commercially applied *Heterorhabditis* species.

4. Import regulations for EPNs

The utilisation of BCAs for the management of pests is increasing worldwide (Bailey et al., 2010; Hajek et al., 2016; Helyer, Cattlin, & Brown, 2014). During the course of the last century, about 2000 species of classical and 150 species of augmentative BCAs have been released worldwide for the control of 265 pest species (van Lenteren, Bale, Bigler, Hokkanen, & Loomans, 2006). In Europe alone, approximately 90 species of invertebrate biological control agents (IBCAs) are currently widely used and commercialised and many more are under investigation for future release (Hunt, Loomans, & Kuhlmann, 2011). This increase in the use of BCAs has been largely due to the perception that they are more environmentally friendly, are safe in terms of human health and have minimal non-target effects compared to chemical pesticides. However, concerns began to emerge in the 1980s regarding the potential non-target effects of IBCAs of arthropods (Hajek et al., 2016; Howarth, 1983). This has lead to an increased focus on possible regulations of their use.

An important and controversial issue concerning the use of EPNs and one that influences import regulations is that regarding the biological group to which these organisms are assigned. Here, BCAs can be grouped as invertebrate biocontrol agents, microbial biocontrol agents, plant extracts or semiochemicals (Hauschild, Speiser, & Tamm, 2011). According to Bale, van Lenteren, and Bigler (2008), IBCAs can be treated in two groups: (i) macrobial agents that include predatory insects and mites, insects that parasitise other insects (parasitoids) or nematodes, and (ii) microbial agents that include bacteria, viruses, fungi and protozoa. In contrast, Bailey et al. (2010) grouped BCAs in three categories: (i) predatory insects and mites; (ii) parasitoids, which are insects with a free-living adult stage and a larval stage that is parasitic on another insect; and (iii) parasites, microbial pathogens and antagonists, such as nematodes, fungi, bacteria, viruses and

protozoa. Bailey et al. (2010) further grouped the first two as 'macrobial' and bacteria, viruses and fungi as 'microbial' control agents, but EPNs were grouped as 'biological control agents that sit halfway between the "macrobial" and "microbial" control agents' (Bailey et al., 2010).

The categorisation of EPNs as microbial or macrobial biocontrol agents is influenced by whether their symbiotic association with bacteria is considered or not. These nematodes carry, transport and release the bacteria within the target host. Once the bacteria are released into the insect haemocoel, they produce toxic substances that lead to the death of the host insect within a short period of time (Griffin, Boemare, & Lewis, 2005). Based on this mode of action, EPNs are categorised under microbial biocontrol agents (insect pathogens) together with bacteria, fungi and viruses (Flexner & Belnavis, 1999; Hajek, 2009; Ravensberg, 2011). It is evident that this categorisation focuses mainly on the symbiotic bacteria. Therefore, many biocontrol researchers, practitioners and societies, such as the Society for Invertebrate Pathology (http://www.sipweb.org; accessed 20 August 2015), tend to group nematodes with the true microbial pathogens (Bailey et al., 2010). However, others categorise EPNs as 'macrobial' or IBCAs together with beneficial arthropods, for example, insects and mites (Ehlers, 1996; Ehlers & Shapiro-Ilan, 2005). This is based on the reasoning that users of EPN products will not come into contact with the symbiotic bacteria, because the bacterial cells are embedded in the intestine of the IJ and also because the quantity of bacteria is relatively small (200–2000/IJ) (Ehlers & Shapiro-Ilan, 2005).

Prior to the 1996 Food and Agriculture Organisation (FAO, 1996) Code of Conduct for the use of BCAs, a variety of regulatory approaches had been adopted for EPNs in different countries, mainly due to the unique dual character of the nematode–bacterium complex (Ehlers, 1996). For regulation purposes, different organisations such as the European and Mediterranean Plant Protection Organisation (EPPO), Organisation for Economic Co-operation and Development (OECD) and FAO considered EPNs under IBCAs or macro-organisms (Ravensberg, 2011). Although many countries have adopted these guidelines, EPNs are commonly exempted from any form of regulation (Ehlers & Shapiro-Ilan, 2005). Recently, Piedra Buena, Lopez-Cepero, and Campos-Herrera (2015) provided a comprehensive revision on the current situation of international regulation for the production and environmental risks of EPNs. In the USA, EPNs were exempted from regulation by the Environmental Protection Agency (Gorsuch, 1982) until legislation was introduced for the regulation of exotic nematodes in 1996 (Rizvi, Hennessey, & Knott, 1996). Australia and New Zealand have, however, had a long history of regulation on the introduction of exotic EPNs (Bedding, Tyler, & Rochester, 1996). Although several attempts have been made to harmonise regulation procedures in European countries (Bale, 2011; Hunt et al., 2008), regulation varies considerably between countries. According to Ehlers (2005), some countries require regulation (e.g. Austria, Belgium, Czech Republic, Hungary, Ireland, the Netherlands, Norway, Poland, Sweden, Switzerland and UK), whereas others (Denmark, Finland, France, Greece, Italy, Portugal and Spain) do not have these requirements. In these countries, a project called regulation of BCAs in Europe (REBECA Policy Support Action) has been established, aiming at improving regulation of BCAs in Europe (Ehlers, 2011).

5. Potential risks of EPNs and their symbiotic bacteria used for biological control

Many authors have argued that EPNs and their symbiotic bacteria are safe for use as BCAs (Bathon, 1996; Boemare, Laumond, & Mauleon, 1996; Dillon, Foster, Williams, & Griffin, 2012; Ehlers, 1996; Ehlers & Hokkanen, 1996; Ehlers & Shapiro-Ilan, 2005; Georgis, Kaya, & Gaugler, 1991). The EPPO listed six EPN species on the 'positive list', which implies that they are considered as safe species. Some of the main arguments for the use of EPNs as safe BCAs are that they do not infect higher vertebrates (Akhurst & Smith, 2002; Bathon, 1996), the bacteria cannot survive outside of the nematodes or insects for a long period of time (Morgan, Kuntzelmann, Tavernor, Ousley, & Winstanley, 1997; Poinar, Thomas, Haygood, & Nealson, 1980) and the impact on non-target arthropod populations would be temporary and spatially restricted (Bathon, 1996). However, there is also opposing evidence on the host specificity, persistence and dispersal of EPNs that raises questions on their safety as BCAs.

5.1. Non-target effects

An important aspect to consider regarding the use of EPNs as BCAs is the unintended effect on non-target organisms. EPNs in general are pathogenic to a wide range of insects of the orders Diptera, Coleoptera, Blattodea, Hymenoptera, Lepidoptera, Orthoptera, Siphonaptera and Isoptera (Georgis et al., 2006; Koppenhöfer, 2007; Peters, 1996). The target insects include those from foliar, soil surface, cryptic and subterranean habitats (Lacey & Georgis, 2012). In addition, EPNs are effective against the different life stages (larvae, pupa and adults) of insects (Grewal, 2002). For most recognised EPN species, the natural hosts are unknown (Peters, 1996). This is because they have been recovered from soil using a technique known as 'insect baiting' (Bedding & Akhurst, 1975), where larvae of a highly susceptible insect such as the greater wax moth, *Galleria mellonella*, are used. EPN species that have been isolated from their natural insect host have also been shown to have broad host ranges. For example, *S. carpocapsae* was isolated from the codling moth, but was subsequently found to naturally infect more than 10 families of insects including Coleoptera (Curculionidae) and Lepidoptera under field conditions (Peters, 1996). Although EPNs are mostly considered as safe BCAs, the generalist nature of many species, including several commercially available species, is a concern.

The potential impact of EPNs on non-target arthropods has been summarised from studies in both field and laboratory by Akhurst and Smith (2002). These authors showed that non-target effects could be on beneficial predators and parasitoids or other invertebrate organisms. The effects in general could be direct, where the EPNs kill non-target arthropods, or indirect, where the EPNs parasitise the hosts of predators and parasitoids, thus reducing their food source (Akhurst & Smith, 2002). Recent laboratory studies have shown that the commercially available *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* have a negative impact on the larvae of spotted ladybird beetles and lacewings, both of which are beneficial predatory insects (Rojht, Kac, & Trdan, 2009). In another study, reduction in populations of the non-target arthropods *Forficula auricularia* and *Blapstinus discolor* was observed after the application of *S. carpocapsae*, although the impact was short term (Hodson, Siegel, & Lewis, 2012). Non-target effects have also

been reported on soil microbial communities and nutrient cycling processes (De Nardo, Grewal, Mccartney, & Stinner, 2006).

There are also concerns regarding the influence of the global distribution of commercially available EPNs on native EPN species. In this regard, Millar and Barbercheck (2001) reported a reduction in the number of native EPNs in the USA after the introduction of commercial species. Somasekhar, Grewal, De Nardo, and Stinner (2002) found that the application of *H. bacteriophora* and *H. indica* significantly reduced the abundance, species richness, diversity and maturity of the nematode community by reducing the number of genera and abundance of plant-parasitic, but not free-living nematodes. This could also be considered as a beneficial non-target effect.

One of the recent concerns of using EPNs as BCAs has been the discovery of an emerging human pathogenic bacterium, *Photorhabdus asymbiotica*, associated with the nematodes (Gerrard et al., 2006). Although the bacterium was first discovered as a human pathogen in 1989, it was discovered to be a symbiont of *Heterorhabditis gerrardi* only 20 years later (Plichta, Joyce, Clarke, Waterfield, & Stock, 2009). Thus far, clinical (infected human) and nematode isolates of *P. asymbiotica* have been found in Australia, Europe, Japan, Thailand and the USA (Thanwisai et al., 2012; Waterfield, Ciche, & Clarke, 2009). Studies have shown that *P. asymbiotica* infections represent a primary pathogen (Gerrard, Waterfield, & Sanchez-Contreeras, 2011; Waterfield et al., 2009) and not an opportunistic secondary coloniser as stated by Ehlers and Hokkanen (1996) and Ehlers (2005). This bacterium has also been found in Japan and Thailand associated with *H. indica* (Kuwata et al., 2008; Thanwisai et al., 2012), where the common symbiont of *H. indica* is *P. luminescens* subsp. *akhurstii*. Interestingly, *H. indica* has been commercialised in Florida for the control of *Diaprepes abbreviates* (El-Borai, Brentu, & Duncan, 2007) and for other insect pests (Nguyen & Hunt, 2007). However, for mass-produced nematodes, the delivery of human pathogenic bacteria with commercially produced nematodes could be excluded by strict identification of a single-strain symbiotic bacterium. In addition, a recent laboratory study confirmed that *Steinernema* species are able to harbour, multiply and disseminate the mammalian bacterial pathogen *Yersina pseudotuberculosis* for 14 weeks, but it cannot replace the common symbiotic bacterium *Xenorhabdus* sp. (Gengler, Laudisoi, Batoko, & Wattiau, 2015). If this laboratory finding is confirmed in the field, the use of EPNs for biological control would require strict regulation. These discoveries illustrate that much work is needed to better understand the non-target effects of not only the EPNs, but also their symbiotic bacteria.

There is a general lack of knowledge on the bacterial symbionts of EPNs. Of the approximately 107 known EPN species, the bacterial symbionts of only 31 have been described (Lewis & Clarkey, 2012). Importantly, there are species that have symbiotic associations with different bacterial species, and it is consequently possible that even when the bacterial symbiont has been described, there may be other bacterial symbionts of the same EPN species that are not yet known. For example, *H. bacteriophora* (a commercial species) and *S. anatoliense* can be associated with two different bacteria species (Lewis & Clarkey, 2012). In addition, it has also been found that *H. zealandica* isolated in South Africa is associated with a new bacteria species, *Photorhabdus heterorhabditis*, rather than the commonly known *P. temperata* subsp. *tasmaniensis* (Ferreira et al., 2014).

5.2. Dispersal

One of the risks of BCAs that must be assessed is their ability to move (disperse) from the areas where they are first released. The active dispersal of EPNs is in general very low. Based on foraging behaviour, EPNs are characterised as 'ambushers' that wait for potential hosts to come to them, or as 'cruisers' that move in search of sedentary hosts (Griffin, 2015; Lewis, 2002; Lewis, Campbell, Griffin, Kaya, & Peters, 2006). However, in a recent study it has been shown that 'ambusher' EPNs employed 'sprinters' foraging behaviour for long-distance dispersal (Bal, Taylor, & Grewal, 2014). A study by Shapiro-Ilan, Lewis, and Schliekelman (2014) indicated that EPNs showed an aggregated pattern behaviour described as 'follow the leader' dispersal mechanism. Environmental cues may play a role in EPN movement, where EPNs have been highly attracted to volatile compounds emitted by plants when attacked by herbivorous insects (Ali, Alborn, & Stelinski, 2011; Degenhardt et al., 2009; Rasmann et al., 2005). This implies that EPNs with 'cruiser' and 'sprinters' foraging behaviour and those attracted to environmental cues will disperse more broadly than 'ambushers' and be less responsive to cue signals. However, the distance that EPNs can move is limited and thus arguably of low risk, which has resulted in some researchers recommending that environmental risk assessments are not necessary for them (Bale, 2011; Ehlers & Shapiro-Ilan, 2005). van Lenteren et al. (2003) suggested that any BCA that moves actively or passively less than 10 metres per season should not be a concern and here, EPNs were mentioned as an example.

Passive dispersal of EPNs, namely movement facilitated by human activity, insects or water, should be considered and evaluated to assess potential risks to the environment. One means of passive dispersal is that linked to infected insects. In this regard, various studies have confirmed the infection capability of EPNs in adults of several insect pests. These include the Japanese beetle (Glaser, Farrell, & Gowen, 1935; Lacey, Bettencourt, Garrett, Simoes, & Gaugler, 1993; Lacey, Kaya, & Bettencourt, 1995); the carrot weevil, *Listronotus oregonensis* (Boivin & Belair, 1989); billbugs, *Sphenophorus parvulus*; the mint flea beetle, *Longitarsus waterhousei*; and the tawny mole cricket, *Scapteriscus vicinus* (Grewal, 2002), where many of the adult insect hosts are capable of flying after infection (Downes & Griffin, 1996; Lacey et al., 1995). For example, passive dispersal of *S. carpocapsae* (all strains) by adult beet armyworm *Spodoptera exigua* was demonstrated by Timper, Kaya, and Gaugler (1988). Likewise at some sites, *S. scapterisci* was shown to be passively dispersed by its mole cricket hosts up to 150 m, and to occupy an area of 4.2 ha, less than two years after its release (Parkman, Frank, Nguyen, & Smart, 1993). It was also detected in citrus groves by real-time qPCR, suggesting 'invasion' from close-by pastures in Florida (Campos-Herrera, El-Borai, Ebert, Schumann, & Duncan, 2014). Similarly, Lacey et al. (1995) showed the potential passive dispersal of *S. glaseri* by adult Japanese beetles. Besides insect hosts, EPNs can also be passively dispersed by water (flooding) and human activities, such as in ship ballast water (Barbercheck & Millar, 2000). In addition, it has been suggested that EPNs can move up to 25–45 m distance through soil movement by farm machinery during normal farming practices within three years (Shields, 2015; Shields & Testa, 2015). Interestingly, despite these numerous examples of passive dispersal of EPNs, this issue has been neglected in most risk assessments.

5.3. Establishment and persistence

Unlike classical biological control or inoculative releases, the establishment and persistence of exotic natural enemies in the areas of release are not desirable where inundative releases are made. This is because the risks of non-target effects on native species are increased (Boivin, Kolliker-Ott, Bale, & Bigler, 2006). The establishment of EPNs in the environment depends on several biotic (e.g. presence of susceptible host) and abiotic factors. In general, EPNs are sensitive to several abiotic factors such as high and low temperature, UV light and moisture (Glazer, 1996). These factors illustrating the limited potential of EPNs to persist at release sites have led to a view that data regarding establishment would typically not be required as part of the environmental risk assessment (Bale, 2011; Ehlers & Hokkanen, 1996; Ehlers & Shapiro-Ilan, 2005). However, this recommendation should be reconsidered, because there are tangible records of establishment at previous release sites (Dillon, Rolston, Meade, Downes, & Griffin, 2008; Jansson, Lecrone, & Gaugler, 1993; Parkman & Smart, 1996; Shields, Testa, Miller, & Flanders, 1999; Susurluk & Ehlers, 2008).

An important point to consider regarding establishment is the survival biology of IJs in the soil. IJs are the only free-living stages that have the capability to survive the harsh environment outside the insect host. Glazer (2002) summarised three morphological and physiological survival mechanisms that IJs use to escape the unfavourable environmental factors. These included (i) a lack of feeding and reliance on internal energy sources while searching for new food, (ii) closed external openings (mouth and anus) that prevent penetration by microbial antagonists and toxic chemicals and (iii) protection by two layers of external membrane cuticle that provide additional protection and prevent water loss, therefore enabling long-term survival. Understanding these characteristics are, therefore, very relevant when seeking to determine the potential of EPNs for long-term survival, persistence and establishment.

Despite its importance, there are only few studies that have investigated the long-term persistence or establishment potential of EPNs. Barbercheck and Millar (2000) summarised results of some long-term persistence studies showing that persistence varies from months to years. *Steinernema scapterisci*, originally introduced from Uruguay and applied for mole cricket control in turf grass in Florida in 1985, became established as 'natural' populations (Parkman & Smart, 1996) and is considered as an example of classical biological control using EPNs. Dillon et al. (2008) monitored the establishment of two exotic (*S. carpocapsae* and *H. megidis*) and two indigenous (*S. feltiae* and *H. downesi*) species applied for the control of pine weevil over a five-year period and showed that all species were recovered three years after application, while *S. feltiae* was also recovered in years four and five. Another study by Shields et al. (1999) evaluated the persistence of two strains of *H. bacteriophora* (strain 'NC' and 'Oswego') that were applied to control alfalfa snout beetle larvae (*Otiorhynchus ligustici*) and found that the strains NC and Oswego could survive more than one and two years, respectively. Shields (2015) showed that after a single field application of native *S. carpocapsae* 'NY 001' and *S. feltiae* 'NY 04', both species were detected after six years of the study period in different cropping systems. Jansson et al. (1993) showed that *H. bacteriophora* (HP88 strain) and *Heterorhabditis* sp. persisted for over 230 days in southern Florida after application to sweet potato fields. *Hetrorhabditis bacteriophora* was detected 23 months after release

in beans (Susurluk & Ehlers, 2008) and the North Carolina strain of *H. bacteriophora* applied to a cranberry bog in Washington to control black vine weevil was detected after one year (Shanks & Agudelo-Silva, 1990).

6. Conclusions and suggestions regarding regulation

EPNs have become one of the most widespread and successful BCAs of arthropod pests. This includes a small number of EPN species produced commercially and distributed globally, and contributing substantially to the reduction of pest populations in numerous crop systems. The success of EPNs as BCAs can at least in part be attributed to the fact that regulations for their importation have been relatively lenient, where studies to determine the environmental and economic risks, including non-target host testing, are often not required. In addition, EPNs are commonly not considered to persist in the environment and disperse long distances. However, the broad host ranges of many EPN species, their association with multiple and in many cases unknown bacterial species, and evidence of their non-target effects and persistence in and dispersal from the introduced environment suggest that there is justification for a re-evaluation of the regulations applied to the use of EPNs. This is especially true for the global use of a small number of species in contrast to the use of native species.

Retrospective analyses of biological control projects have provided quantitative data on non-target effects and they have illustrated the need for risk assessments to be fortified to ensure future safety of biological control (Bigler, Babendreier, & Kuhlmann, 2006). A classic example is the ladybird *Harmonia axyridis* which has been introduced into different parts of the world as a successful BCA. However, due to non-target effects, it is currently considered as one of the most invasive species threatening the diversity of native aphidophagous species and it has had adverse effects on the wine and fruit-growing industries (Roy & Wajnberg, 2008). Due to the wide host range of EPN species, and their commonly unknown identity or impacts of their bacterial associates, we contend that the import regulations of non-native EPN species should be subject to prior risk assessment before releases are approved.

The utilisation of native EPN species would exclude many of the risks associated with introducing exotic species into new environments. For example, *S. feltiae* is the most widespread EPN species and used for the control of several insect pests (Figure 1). It has recently been registered in South Africa for the control of false codling moth (Antoinette Malan, personal communication). However, *S. feltiae* has been shown to have non-target effects and a high establishment potential (van Lenteren et al., 2003). This is ironic considering the fact that a number of native species have been shown to be effective against false codling moth (Malan, Knoetze, & Moore, 2011) and many other newly described native species await testing. In fact, in just nine years, seven new species have been described in South Africa (Appendix 1 supplementary material) and four additional new species are currently being described (Antoinette Malan, personal communication). So far these native EPN species have been evaluated against important insect pests in the orders Coleoptera, Diptera, Hemiptera and Lepidoptera (Malan & Hatting, 2015). Similarly, countries such as Cuba have successfully used native EPN species for the control of many agricultural insect pests (Rodriguez, 2015). This illustrates a vast potential resource of native EPN species for biological control.

Native EPN species should also be considered for the control of exotic pests. One of the assumptions for the control of exotic pests is that natural enemies should be introduced from their place of origin (classical biological control), promoting the introduction of exotic BCAs (Vincent, Goettel, & Lazarovits, 2007). However, this assumption is commonly not valid, where excellent control has been obtained by releasing indigenous natural enemies against exotic pests (van Lenteren, 2000). The potential of indigenous EPNs to be used against local insect pests is often greater than that of non-native EPNs (Kaya & Gaugler, 1993). This is because they are adapted to the specific environment and as a result perform better than introduced EPN species. For example, in the USA, the native *S. glaseri* was successfully used for the control of the exotic Japanese beetle (Glaser et al., 1935). In a similar manner, native EPN species have been successfully used for the control of alfalfa snout beetle (*O. ligustici*), an important alfalfa and clover pest in Northern New York, USA (Shields & Testa, 2015). Despite this potential, many exotic BCAs have been released without considering opportunities to use native species (van Lenteren, 2000). We argue that in order to reduce the introduction of exotic EPNs and their possible unintended side effects on non-target organisms and the environment, the potential to use native/locally available species for biological control should be more actively pursued. It is understood that the small number of exotic species that are traded globally have been refined for distribution and ease of application. However, this remains a poor excuse not to promote opportunities including research and investment to utilise native EPNs for biological control. This could be achieved by engaging all stakeholders, including researchers, commercial companies and end users.

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