




Entomopathogenic nematodes: Commercial use and future perspectives[☆]

Tshimangadzo Ramakuwela^{a,*} , Eustachio Tarasco^b, Norberto Chavarría-Hernández^c, Stefan Toepfer^{d,e}

^a Department of Plant and Soil Sciences, University of Pretoria, Hatfield, Pretoria, South Africa

^b Department of Soil, Plant and Food Sciences, University of Bari "Aldo Moro", Bari, Italy

^c Food-Agrobiotechnology Group, Universidad Autónoma del Estado de Hidalgo, Tulancingo, México

^d CABI, Delemont, Switzerland

^e MARA – CABI Joint Laboratory for Biosafety, Beijing, PR China

ARTICLE INFO

Keywords:

Beneficial nematodes
Biocontrol products
Agroforestry ecosystems
Maize agroecosystem
Commercialization

ABSTRACT

The 100 years of research, development, commercialization and use of entomopathogenic nematodes (EPN) since their discovery in the 1920s have seen impressive progress. Nowadays, many EPN products, based on a relatively limited number of EPN species, are commercially available in most world regions. This paper presents a review of EPN commercialization and examples of EPN use in field crops such as maize and in agroforestry ecosystems. It demonstrates how EPN have become an important tool in biological control of insect pests offering many advantages over chemicals. These include farmer and consumer safety, minimal damage to natural enemies, no problems with pollinators, and no environmental pollution. All of these attributes are essential for an advanced and sustainable integrated pest management strategy. We explored future potential of commercialization and adoption of EPN-based biocontrol products. The future may lie in several advances: i) The ability to tailor the use of EPN to broad as well as to local agricultural needs. ii) Enhancing their shelf life and efficacy through genetic improvements. iii) Refining application technologies. iv) Improving mass production (including machine learning using production data). v) Reducing production and delivery costs, considering EPN as safe agents and therefore reducing registration burdens. vi) Finally, expanding their use to a larger market such as to new pest species and crops. With continued research and innovation, EPN could become a cornerstone of sustainable pest management strategies in an increasing number of countries.

1. Introduction

Entomopathogenic nematodes (EPN, Rhabditida) are beneficial nematodes that have gained considerable attention as biological control agents for the effective management of many different insect pests in agriculture and forestry. Belonging primarily to the families Steinernematidae and Heterorhabditidae, EPN are highly effective in controlling a broad range of belowground or otherwise hidden pests, as well as some of the aboveground insect pests (Shapiro and Lewis, 2024). In the 100 years since their discovery in the 1920s (Steiner 1923), there have been significant advances in research, development, commercialisation and use of EPN. During the last half of a century, EPN have emerged as safe and effective alternatives to chemical pesticides. This is largely due to their safety to humans, livestock, crops and the environment, as well as due to their ability to be integrated into other pest management

practices. They leave no residues on the harvest and therefore cause no problems with marketing the agricultural produce. Also, resistance development by pests to those biocontrol agents is highly unlikely due to their multiple modes of action. The biggest advantage of nematodes, however, is their ability to seek for hidden pest insects. These advantages make them an effective tool in eco-friendly pest management. The drivers behind their success are the changing global agricultural systems, such as changing consumer demands for safer food, increasing restrictions on maximum residue levels, the use of pollinators in agricultural production that limits the use of chemical insecticides, the development of resistance of insects to pesticides (Bass and Jones, 2018), and the ban of some key pesticides in a number of crops. In addition, the spread of invasive species and changing insect dynamics such as reproduction rate and longer survival during warmer climates, and further intensification of agriculture are leading to increased pest

[☆] This article is part of a special issue entitled: 'EPN 100 years' published in Journal of Invertebrate Pathology.

* Corresponding author.

E-mail address: tshimangadzo.ramakuwela@up.ac.za (T. Ramakuwela).

<https://doi.org/10.1016/j.jip.2025.108388>

Received 12 November 2024; Received in revised form 21 May 2025; Accepted 15 June 2025

Available online 16 June 2025

0022-2011/© 2025 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

problems (Skendzić et al., 2021).

Over the past several decades, EPN have gained significant attention in sustainable agriculture resulting in commercialization of a number of species, particularly in high-value crops such as in horticulture, berry production or tree nurseries, as well as in turf management (Koppenhöfer et al., 2020, CABI 2024) (Table 1). Companies and researchers across the globe are continuously developing novel EPN-based products for pest control, predominantly in developed countries (Shapiro-Ilan and Lewis, 2024). Despite successes, challenges have restricted their widespread use. Those include challenges in cost-effective mass production, their relatively short shelf-life, cool storage requirements, lack of local availability of products in some regions, and too strict registration requirement in some countries (Abate et al., 2017; Askary and Abd-Elgawad, 2021).

As the pressure of reducing chemical pesticides mounts, EPN are progressively being more recognized as an important component of future pest management strategies. To date, of more than 100 described EPN species, only about 10 (Fig. 1) to 13 species have been commercialized (CABI, 2024; Koppenhöfer et al., 2020). Entomopathogenic nematode researchers aim to improve their commercialization by enhancing nematode resilience and shelf-life and developing novel application techniques including appropriate formulations (Askary and Abd-Elgawad, 2021). Also, regulators are encouraged to simplify the registration processes for indigenous EPN, as they are macrobial biocontrol agents (MBA) which are largely considered of no-risk or very low-risk (Ehlers, 1996; Waweru et al., 2024). Looking ahead, the future of EPN likely lies in expanding their use in agriculture and agroforestry systems.

This article reviews and discusses the commercialization of EPNs, highlighting successes achieved in the examples of pest management in field crops such as maize, and in more complex agroforestry systems. Lessons learned from these two examples may help to define future perspectives and innovations needed to expand the value of EPN applications in addressing agricultural pest challenges.

2. Commercialization of EPN

We are celebrating the 100th anniversary of EPN (Toepfer, et al. 2024b) being first discovered in the 1920s (Steiner, 1923). Since then, numerous field surveys from experts across the globe isolated many new

EPN species which led to a characterization of more than 100 *Steinernema* and 16 *Heterorhabditis* spp. (Bhat et al., 2020; Tarasco et al., 2023). Commercialization attempts began in the 1980s with the invention of in vitro mass production by Bedding (1981). Cost effective high-output mass production is inevitable for sufficient and timely supply to the end-users. Applications of EPN usually require at least 2.5×10^9 infective juveniles (IJ)/ha (= 25/cm²) but often more (Shapiro et al., 2012). Most companies or facilities currently produce EPN in semi-solid in vitro culture, such as in China, Kenya, DRP Korea, Rwanda, and others. Some companies still opt for low tech in vivo mass production system such as those in India. However, significant progress was made by Lunau et al. (1993) with the introduction of monoxenic culture and liquid mass culture (Georgis et al., 1995), a method currently used by the larger EPN producers. To date, nematodes are commercially produced in bioreactors of several thousands of litres. Entomopathogenic nematodes are sold in different formulations of carriers like water-dispersible powders and sometimes granules, alginate beads, sponge and others. However, the details pertaining to product development, mass production and formulations of EPN are often hidden as trade secret by nematode producers. Nevertheless, a number of researchers are studying different aspects of in vitro liquid production of EPN to optimize and improve production (Dunn and Malan, 2023; Leite et al., 2023; Wang et al., 2023; Wang et al., 2024), while others focus on formulation technology. For example, Fallet et al. (2024), Waweru et al. (2025), (Kotliarevski et al., 2022) and (Ha et al., 2025) recently advanced with testing different formulations of *Steinernema carpocapsae*, including oils, gels or thickeners, surfactants, plant derived products and UV protectants for aboveground pest control.

As EPN research advances, most of the research focuses on evaluating efficacy of species to control various insect pests in agricultural and forestry systems (Lacey et al., 2015) while other researchers are committed to product development aspects (mass production, storage improvements, formulation, strain stabilization) – all in a bid to promote EPN as safe and environmentally friendly alternative to chemical pesticides. However, only a fraction of the described species are commercialized, this is, only nine or a few more *Steinernema* and *Heterorhabditis* spp. (CABI, 2024; Koppenhöfer et al., 2020). Entomopathogenic nematodes products are popular in USA and Europe where they are utilized in the management of various pests (Table 1) (CABI, 2024). This is supported by the countries' better economy, empowered

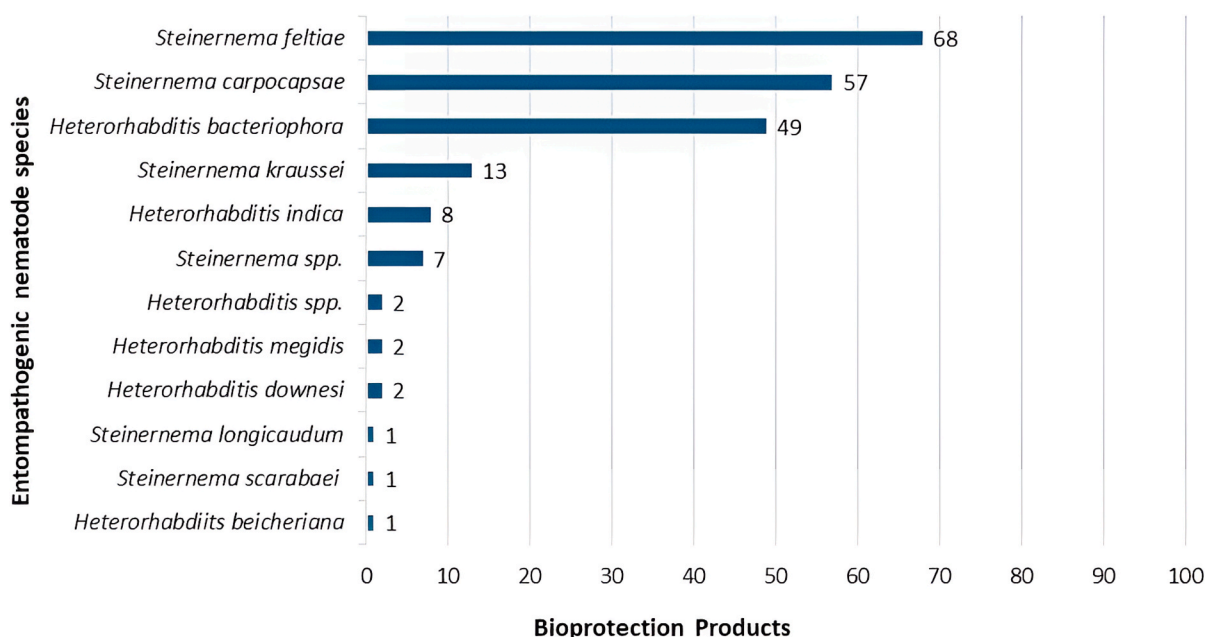


Fig. 1. Products of commercialized species of entomopathogenic nematodes from 48 countries (CABI, 2024).

Table 1

Manufacturers of commercialized species of entomopathogenic nematodes in different countries*. Information regarding distribution in other countries is available on the companies' websites or on the bioprotection portal (CABL, 2024). Several non-commercial EPN production facilities exist in a number of countries but are not listed here.

Entomopathogenic nematode species	Country	Producer	Target pests
<i>Steinernema carpocapsae</i> <i>S. kraussei</i> <i>S. feltiae</i> <i>Heterorhabditis bacteriophora</i>	Belgium	Biobest Group	Caterpillars; Codling moth; Duponchelia moth; Fungus gnats; Shore fly; Black vine weevil; Garden chafer; South American tomato moth; Thrips
<i>S. feltiae</i> <i>S. carpocapsae</i> <i>H. bacteriophora</i>	Canada	Environmental factor Inc.	Grubs; Weevil; Borers; Ants; Fungus Gnat; Thrips; Flea; Chinch; Black vine weevils; Mushroom flies; Crane flies; Sciarid larvae; Other pest larva in the soil
<i>S. feltiae</i>	Canada	Anatis Bioprotection	Fungus gnats; Thrips; Root aphids.
<i>S. scarabaei</i>	Canada	Tetra Biologicals Inc.	White grubs
<i>S. carpocapsae</i> <i>H. bacteriophora</i>	Canada	Crop defenders	Thrips
<i>S. feltiae</i> <i>S. feltiae</i>	PR China	Keyun Bio-pesticides Co., Ltd	Grubs
<i>S. longicaudum</i> <i>S. carpocapsae</i> <i>S. feltiae</i> <i>H. beicheriana</i> <i>H. indica</i> <i>S. feltiae</i> <i>H. bacteriophora</i>	PR China	Weifang Hongrun Agricultural Technology Co., Ltd	Grubs; Noctuid larvae; Leaf beetle larvae; Fungus gnats; Chive gnats; Grubs
<i>S. carpocapsae</i>	Colombia	Scientia Colombia S.A.S.	Thrips
<i>S. carpocapsae</i>	Costa Rica	Bio Control S.A.	Armyworm, Mamestra, Pineapple weevil, tomato moths
<i>S. carpocapsae</i> <i>S. kraussei</i> <i>S. feltiae</i> <i>H. bacteriophora</i>	France	Bioline Agrosiences	Black vine weevil; Strawberry root weevil; Chafer grub; Thrips
<i>S. feltiae</i> <i>S. carpocapsae</i> <i>H. bacteriophora</i> <i>H. downesi</i>	Germany	E-nema	Ants; Apple clearwing moth; Asparagus beetle; Banana moth; Black vine weevil; Box tree moth; Codling Moth; Chestnut moth; Cockchafer; Cranberry griddler; Cranberry rootworm; Cutworm; European pepper moth; Flat-headed root borer; Fungus gnat; Garden chafer; Hazelnut borer; Japanese beetle; Leaf miner; Leopard moth; Leatherjacket; March fly; Mushroom sciarid; Mole cricket; Oak processionary moth;

Table 1 (continued)

Entomopathogenic nematode species	Country	Producer	Target pests
<i>S. feltiae</i> <i>S. carpocapsae</i> <i>S. kraussei</i> <i>H. bacteriophora</i>	Germany	BASF (including Becker Underwood UK)	Oriental fruit moth; Palm moth; Pine weevil; Plum fruit moth; Red palm weevil; Shore fly; Sycamore lace bug; Tomato leafminer; Woodlice; Western flower thrips; Western corn rootworm; Welsh chafer
<i>S. feltiae</i> <i>S. carpocapsae</i> <i>S. kraussei</i> <i>H. bacteriophora</i>	Germany	BASF (including Becker Underwood UK)	Fungus gnats; Western flower thrips; Furrowed weevil; Californian flower thrip; Leaf miners; Codling moth; Ants; Oak processionary moth, <i>Tuta absoluta</i> ; Meadow gnats; Salt flies; Shore flies; Caterpillars; Spodoptera spp.; <i>Chrysodeixis chalcites</i> ; <i>Mamestra brassicae</i> ; <i>Duponchelia fovealis</i> ; Spruce weevil; Garden leaf beetle; June beetle; Somersault beetle
<i>H. indica</i>	India	Multiplex, Bangalore	Sugarcane pests
<i>H. indica</i>	India	KNBiosciences, Hyd	Sugarcane pests
<i>H. indica</i>	India	T. Stanes, Coimbatore	Sugarcane pests
<i>H. indica</i>	India	Anshul, Bangalore	Sugarcane pests
<i>H. indica</i>	India	Ponalab biogrowth, Bangalore	Sugarcane pests
<i>H. indica</i>	India	Kerala Biocontrol Farmroot, Bengalore	Sugarcane pests
<i>S. carpocapsae</i> <i>H. bacteriophora</i> <i>S. feltiae</i>	Kenya	Dudutec Bioline AgroSciences Africa	Leafminers; Sciarid flies; Thrips; Beetles; Fungus gnats; White grubs; Cutworms
<i>S. carpocapsae</i> <i>H. bacteriophora</i>	DPR Korea	17 Provincial and county Plant Protection Stations	Cutworms; Grubs
<i>S. feltiae</i> <i>S. carpocapsae</i> <i>H. bacteriophora</i>	The Netherlands	Koppert Biological Systems	Box tree moth; Shore Flies; Crane fly; Tomato looper; Cabbage looper; European pepper moth; Diamond back moth; Crane fly; June beetles; European chafer; Japanese beetles; Black vine weevil; Asparagus beetle; Fungus gnats; thrips
<i>S. carpocapsae</i> <i>H. bacteriophora</i>	New Zealand	Bioforce Ltd	Black vine weevils; Grass grubs; Beetles; Fleas; Cutworms; Other moths in the soil

(continued on next page)

Table 1 (continued)

Entomopathogenic nematode species	Country	Producer	Target pests
<i>S. feltiae</i>	Spain	Bgreen Biological Systems	Leaf miners
<i>H. bacteriophora</i> <i>S. carpocapsae</i> <i>S. feltiae</i>	Spain	Bioplanet Ibérica	Grubs; Weevils; <i>Cydia</i> spp., <i>Capnodis</i> spp. Sciaridae, Phoridae, Agromizyidae, Muscidae, Noctuidae, Cossidae, Sessidae
<i>S. carpocapsae</i>	Spain	Geiplag	African cotton leaf worm; Summer fruit tortrix; Navel orange worm; Cutworms; Apple brown tortrix; Weevils
<i>S. feltiae</i> <i>H. bacteriophora</i> <i>S. carpocapsae</i> <i>S. feltiae</i>	Spain Spain	Idebio Saniveg	Weevils Different <i>Spodoptera</i> spp.; <i>Autographa</i> spp.; Thrips; <i>Helicoverpa armigera</i> ; <i>Heliothis</i> spp.; Leafminers
<i>S. feltiae</i> <i>S. carpocapsae</i> <i>H. bacteriophora</i>	Switzerland	Syngenta Group AG	Red palm weevils; Codling moth larvae; Mole crickets; Crane flies; Cutworms; Southern Europe marshland pyralid; Black vine weevil; Garden chafer; Welsh chafer; Dung beetle; Leatherjackets; Chafer grubs; Western corn rootworm; Hazelnut borer; Chestnut moth; Beech moth; Sciarid flies; Thrips; Leaf miners
<i>H. bacteriophora</i> <i>H. downesi</i> <i>S. carpocapsae</i> <i>S. feltiae</i> <i>S. feltiae</i> <i>S. carpocapsae</i> <i>H. bacteriophora</i>	UK USA	Bionema ARBICO Organics BioLogic Company, Inc.	Soil pests Fungus gnat Control; Grub control; Thrips; Leaf miner; Armyworms; Banana root borers; Black current borers; Black vine weevils; Cabbage root; Maggots; Carpenter worms; Codling moth larvae; Corn earworms; Corn rootworms; Crane fly larvae; Cucumber beetles; Cutworms; Dogwood borers; Fire ants; Beetles; Flea larvae; Gypsy moth larvae; Iris borers; Japanese beetle grubs; Mole crickets; Pine weevils; Poplar clearwing borers; Peach tree borers; Raspberry crown

Table 1 (continued)

Entomopathogenic nematode species	Country	Producer	Target pests
			borers; Root knot nematodes; Shore flies; Sod webworms; Strawberry weevils; Sweet potato Weevils; Tobacco budworms; White grubs; Wireworms; Strawberry root weevils; Chinch bugs; Cranberry girdlers; Giant palmetto weevils; Mint root borers; Navel orange worms; Rotten sugarcane borers; Banana moths; Billbugs; Citrus root weevils; Grape root borers; Small hive beetles

* Headquarters.

consumers in their demands for safe food, and in some country's regulations that exclude native EPN from registration requirements or have fast-track and less-costly registration procedures for such safe agents in place. The majority of described EPN species in many countries are still in the development phase for commercialization.

Africa and many other developing regions still have very few EPN products developed and commercialized. In Africa, South Africa and Kenya have expertise in EPN product development, commercialization, and use. Other African countries, such as Egypt, Tanzania, Rwanda, Ethiopia, and Benin are also exploring the potential of EPN as biocontrol agents (Fallet et al., 2022). Research is directed towards efficacy trials (Lacey et al., 2015), mass production (Dunn et al., 2020; Ramakuwela et al., 2016), formulation (Fallet et al., 2024; Kagimu et al., 2017; Toepfer et al., 2024a; Waweru et al 2025) and some more innovative application strategies (Fallet 2024). The focus is on the management of pests affecting field crops, fruit orchards, vineyards, vegetables and sometimes forest pests.

3. EPN use in maize agroecosystems

Maize (*Zea mays*, corn) is a major carbohydrate provider and therefore crucial for food security. Numerous coleopteran and lepidopteran key pests, as well as some minor pests attack maize in most agricultural regions. Nevertheless, there are by far less EPN products available and used against insect pests in field crops such as maize than in cash crops such as vegetables, berries, or tree nurseries (Toepfer et al., 2024a). Although there has been plenty of research on the use of EPN against maize pests, only few products have reached the market for pest management in maize. About 1/3rd of countries has EPN-based plant protection products registered for use in all crops (CABI, 2024). Therefore, those products are, in principle, also allowed for pest management in maize those include six EPN species, i.e., *H. bacteriophora*, *H. downesi*, *H. megidis*, *S. carpocapsae*, *S. kraussei*, and *S. feltiae*. About 1/10th of countries have EPN-based plant protection products registered specifically for use in maize (CABI, 2024). Those products include only three EPN species, namely *H. bacteriophora*, *S. carpocapsae*, and *S. feltiae*, which are registered for a number of other pests, of which only few of those are key pests in maize. *Heterorhabditis bacteriophora* is, for example, registered for use against soil pests such as *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), *Melolontha* spp. (Coleoptera: Scarabaeidae) and several other white grubs, as well as against

aboveground pests such as *Autographa gamma* (Lepidoptera: Noctuidae), *Chrysodeixis chalcites* (Lepidoptera: Noctuidae), *Helicoverpa armigera* (Lepidoptera: Noctuidae), *Heliothis peltigera* (Lepidoptera: Noctuidae), *Heliothis* spp. (Lepidoptera: Noctuidae), and finally against *Spodoptera* spp., such as *S. exigua* (Lepidoptera: Noctuidae), or *S. littoralis* (Lepidoptera: Noctuidae). Efficacies reached by *H. bacteriophora* against soil pests are similar or better than for granular soil insecticides and slightly below the use of seed coatings by systemic insecticides (Toth et al. 2022). *Steinernema carpocapsae* is, for example registered against soil pests such as *Agrotis* spp. such as *A. segetum*, *A. gamma*, *D. v. virgifera* or *Gryllotalpa* spp., as well as against aboveground pests such as *C. chalcites*, *H. armigera*, *Sphenophorus parvulus* or *Ostrinia furnacalis*, *Heliothis* species such as *H. peltigera*, and *Spodoptera* spp. such as *S. exigua* or *S. littoralis*. *Steinernema carpocapsae* is particularly useful for aboveground use as it is among the strongest EPN species with regard to surviving harsh climate conditions and UV light. It has proven to achieve higher efficacies against aboveground lepidopteran pests than synthetic insecticides, if properly formulated (Fallet et al. 2024). *Steinernema feltiae* is registered for soil pests such as *A. gamma*, and against aboveground pests such as *C. chalcites*, *H. armigera*, *Heliothis* spp. such as sp. *peltigera*, *Heliothis* spp., *Spodoptera* spp. such as *S. exigua*, or *S. littoralis*, but is less frequently used in field crops. A currently investigated key target is *S. frugiperda*, that may, because of its economic importance and the connected increasing use of insecticides in maize, warrant commercialization of biocontrol products such as EPN. Destructive in invasive pests, such as *S. frugiperda* or *D. v. virgifera* can lead to an enormous influx of pesticides into agroecosystems, such as maize, that has seen little or no treatment in the past. This may lead to environmental concerns, and to some extent to human safety concerns.

It also needs to be noted that a number of countries, such as Germany or China, do not require any specific registration for native macrobial biocontrol agents (MBAs), such as EPN, predators or parasitoids (Ehlers, 1996; Ehlers and Hokkanen, 1996). Also, at European Union level, native macrobial biocontrol agents are not regulated, and registration is handled differently by the EU member countries. Practical or non-requirements of registration of macrobials has heavily eased the commercialization and success of EPN. Nevertheless, even if registered and locally available, this does not necessarily guarantee that EPN are widely used. For example, the use of EPN has been limited in field crops including maize. However, soil and aboveground application techniques for EPN have largely improved and are becoming less costly for field crops. Those application techniques have been recently reviewed in Toepfer et al. (2024a). For example, Shapiro-Ilan and Goolsby (2021) concluded that fire gels can enhance the effectiveness of *S. riobrave* and other EPN by minimizing desiccation and lowering IJs' mortality despite exposure to UV. Also, Fallet et al (2204) and (Metwally et al., 2025) propose novel gels or fluid gel formulations. Finally (Kotliarevski et al., 2022) propose titanoxide and Ha et al. (2025) proposed plant-derived products as novel UV protectant.

Moreover, recent bans of several soil insecticides and insecticidal seed coatings in various countries have increased the demand for alternative solutions, and may lead to a wider use of EPN in maize. For example, neonicotinoid seed coating has been banned from maize and other crops due to their bee toxicity, and classical soil insecticides such as chlorpyrifos and teluthrin are more and more banned due to their high toxicity (Toepfer et al. 2024a).

4. EPN use in agroforestry ecosystems

Agroforestry systems are major crop production systems that can on one hand produce food for humans and livestock, but can on the other hand prevent soil erosion, provide wood, and provide ecosystems services. In agroforestry systems, products based on EPNs like *S. carpocapsae*, *S. feltiae*, *H. bacteriophora* and *H. megidis*, have been successfully used to control various pests of Heteroptera, Lepidoptera, Diptera, Coleoptera and Hymenoptera in agroforestry ecosystems (De

Luca et al., 2015; Tarasco et al. 2023). Taking Italy as an example, EPN formulations have been used (Danishiar et al., 2023) to control the bark beetle *Tomicus piniperda* (Coleoptera: Scolytidae) (Triggiani, 1983) and the pine processionary caterpillars *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) on pines (Triggiani and Tarasco, 2002) with a larval mortality of up to 60% by a *S. feltiae* native strain injected in gelatinous suspension inside the overwintering nest. Entomopathogenic nematodes have also been used against tortrix lepidopterans like *Pamene fasciana* (Lepidoptera: Tortricidae), *Cydia splendana* (Lepidoptera: Tortricidae) and *C. fagiglandana* in fruit trees, as well as against the coleopterans *Curculio elephas* (Coleoptera: Curculionidae) and *C. glandium* on chestnut trees (Curto et al., 2009; Vinciguerra and Clausi, 2006; Clausi et al., 2014) achieving larval mortality between 80 and 100 % with native strains of *H. bacteriophora* and *H. megidis*. Further targets are the tingid *Corythucha ciliata* (Hemiptera: Tingidae) on sycamores (Tarasco and Triggiani, 2006); the elm-leaf beetle *Xanthogaleruca luteola* (Coleoptera Chrysomelidae) on elm trees (Triggiani and Tarasco, 2007) controlled with a native strain of *S. carpocapsae*. In the Mediterranean region, the spread of the invasive palm weevil *Rynchophorus ferrugineus* (Coleoptera: Dryophthoridae) is sought to be managed by several local and commercial *Heterorhabditis* and *Steinernema* spp. in several countries, such as Egypt, Spain and Italy (Triggiani and Tarasco, 2011). Other applications of EPNs have been effective in controlling sciarid dipteran in ornamental nurseries and the weevil curculionid *Curculio nucum* on hazels (Batalla-Carrera et al., 2013); EPN have also been found to be effective against xylophagous insect larvae residing in cryptic habitats, such as the flatheaded woodborer *Capnodis tenebrionis* (Coleoptera: Buprestidae), the cerambycid *Arhopalus syriacus* (Coleoptera: Cerambycidae), the goat moth *Cossus cossus* (Lepidoptera: Cossidae) (Tarasco et al., 2016; El Khoury et al., 2020) controlled by native strains of *S. feltiae*. Recently, these biological control agents have found broad application in integrated control programs of the Japanese beetle *Popillia japonica* (Coleoptera: Scarabaeidae) in large cultivated and non-cultivated areas of Lombardy and Piedmont Regions (El Khoury et al., 2020; Glazer et al., 2022; Torrini et al., 2020) with good results obtained with open field EPN applications.

There are a few highly successful cases of use of EPNs for biological control of agroforestry pests on a wider more global scale. For example, the larvae of Coleoptera Curculionidae of the genus *Otiorhynchus* represent the classic example of a target pest of EPNs, to the extent that this type of biological control is widely used around the world. The most suitable nematodes against *Otiorhynchus* larvae belong to the genus *Heterorhabditis*, although satisfactory results have also been achieved with formulations based on *S. feltiae* and, to a lesser extent, on *S. carpocapsae* (Backhaus, 1984; Corradi et al., 2023; Long et al., 2000). Formulations based on *H. bacteriophora* are marketed in Italy in packages containing 50 to 250 million IJs. The doses used correspond to 200,000 to 400,000 nematodes per m², or 25,000 to 40,000 nematodes per plant (De Luca et al., 2015). The best time for application is late season in order to target the early larval stages of the insects, which are more sensitive and are at the beginning of their phytophagous action under optimum soil temperatures of 18 °C to 22 °C (El Khoury et al., 2018). Early season treatments of dose of 500,000 EPN per m² is used on larger overwintering larvae and newly formed pupae are also somewhat effective if soil temperatures reach above 15 °C (De Luca et al., 2015). The crops targeted for this type of application are typically ornamental plants in nurseries.

Another example of EPN's application, whose use has expanded in recent years to cover a surface area of 1,500 ha throughout northern Italy, is that of the late season treatments used against overwintering larvae of *C. pomonella* on pear and apple orchards (De Luca et al., 2015). The nematodes used belong to the species *S. feltiae* and *S. carpocapsae*, which are applied to the trunks and lower branches of the trees, where the larvae overwinter in bark crevices, protected in a light cocoon that the nematodes can perforate (Tarasco et al., 2017). The application dose corresponds to 1.5 × 10⁹ IJs diluted in 15 hl of water per ha, distributed

by an atomizer device, for which it is necessary to close the highest nozzles and remove the filters (Tarasco et al., 2017). Thorough moistening of the soil before and after the application of EPN result in good efficacy of the EPN treatment as it ensures the moving of IJs to the insect. If water availability is limited; it is advisable to inject the EPN suspension into the soil around the trees.

Xylophagous insects, such as Lepidoptera Cossidae (*Cossus cossus* and *Zeuzera pyrina*), Coleoptera Cerambycidae (*Saperda carcharias*) and Buprestidae (*Capnodis tenebrionis*) can also be effectively controlled with injections of EPN suspensions into the penetration holes or by the obstruction of these openings with pieces of sponge soaked in nematodes. Entomopathogenic nematodes are typically applied to culture systems and substrates that are regularly treated with other chemicals, including natural soil improvers and fertilizers. *Heterorhabditis bacteriophora*, *S. carpocapsae* and *S. feltiae* are in most cases compatible with plant protection products, but can interact with such substances, in some cases producing synergistic effects (El-Ashry and El-Marzoky, 2018), calling for more compatibility testing.

5. Future prospects

The future prospects of EPNs in commercial use are promising, driven by the growing demand for environmentally friendly pest management solutions and consumer demand for safe food (Dara, 2019). Unfortunately, EPNs are sometimes at disadvantage to their chemical counterparts in terms of accessibility, practicality, knowledge intensity, and sometimes costs in the market. There are several key areas where advances could further broaden their applications. For example, EPN are being developed for use against a broader range of pests, beyond traditional targets like soil-dwelling insects. With ongoing research, EPNs could be formulated to combat pests affecting different crops and in various environments, such as foliar applications and cryptic environments (Dito et al., 2016). Since EPNs are compatible with most chemical pesticides and other entomopathogens (Sharma et al., 2011), nematodes show great potential as a contributor in integrated pest management programmes.

Currently, major constraints to widespread adoption and commercialization of EPN are their limited shelf life, and express shipment costs to the agri-input dealer or farmer, and the lack of products in some regions. Moreover, EPN have, although recently reduced, a still relatively high cost of production, involving a careful balance of energy requirements, equipment, labour, raw materials, waste disposal, IJ-recovery processes, formulation, storage, and transportation (Liu, 2020). Efforts are being made to reduce the cost of EPN's production by advances in mass production techniques and bioreactor technology (Cortes-Martinez and Chavarria-Hernandez, 2020), as well as improving in vitro semi solid production (Chairin et al., 2022). It is currently accepted that the most efficient and productive method for the production of EPNs is through the in vitro cultivation of EPNs and its symbiotic bacteria (i.e., monoxenic culture) in liquid media (Cortes-Martinez and Chavarria-Hernandez, 2020). Moreover, other aspects that determine the production of EPNs, include the species improvement (i.e., strain crossing, mutations), metabolism, transcription processes, and role of pheromones, among others, which have been addressed by some groups and pose undeniable cause-effect relationships with the obtained results in bioprocesses to produce EPNs (Lulamba et al., 2021; Susurluk et al., 2013; J. Wang et al., 2022; Wang et al., 2023). Fig. 2 presents the main aspects addressed in recent years in the field of mass production of nematodes for biological control purposes, mainly through liquid culture, and published as research articles indexed in ScopusTM (Elsevier 2024) and Web of ScienceTM, WoS (Clarivate Analytics 2024).

Research on the mass production of nematodes for biological control purposes has focused mainly on the liquid culture, using different configurations of bioreactors, producing heterorhabditis and steinernematids (although there are incipient efforts with *Oscheius* sp. and *Phasmarhabditis* sp., the last being a slug-parasitic nematode). Focus is also on seeking better conditions for the recovery of IJ stages in the initial stages of fermentation, as well as exploring alternative production methods, among other aspects, all of which are undoubtedly important for the EPN production technology. More experimental data and methods are required for the mass production of EPN concerning physicochemical properties, operating conditions, and geometry factors

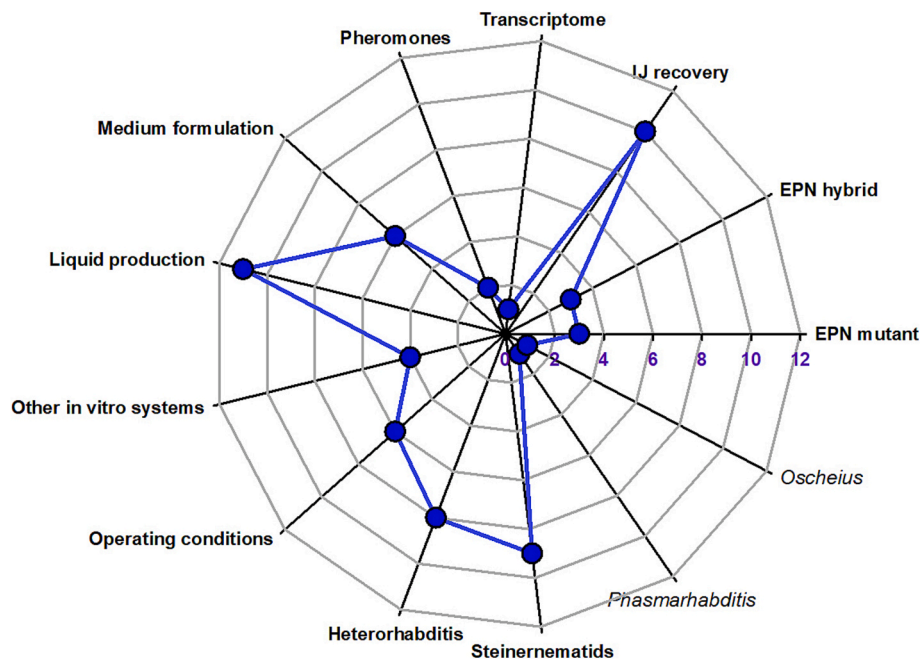


Fig. 2. The most relevant aspects considered in the last four years by research groups in the mass production of nematodes for biological pest control purposes and published as original research articles in journals indexed in ScopusTM (Elsevier 2024), and WoSTM (Clarivate analytics 2024) (Chairin et al., 2022; Cortés-Martínez et al., 2023a; Cortés-Martínez et al., 2023b; Dunn and Malan, 2023a; Dunn and Malan, 2023b; Dunn, Belur and Malan, 2021; Dunn, Belur and Malan, 2022; Kong et al., 2022; Leite et al., 2023; Matlhabe et al., 2022; Pieterse et al., 2022; Tumialis et al., 2021; Ulu and Susurluk, 2024; Wang et al., 2022; Wang et al., 2023a; Wang et al., 2023b; Wang et al., 2024).

prevailing in the production systems. In this sense, we can take advantage of artificial intelligence (AI) to improve these processes, mainly through machine learning (ML), which can be applied to hydrodynamics, transport, and biochemical reactions in multiphase systems within bioreactors (Zhu et al., 2022). There are incipient efforts in the field, for instance, using supervised ML by nonlinear regression to characterize the population growth of *Steinernema feltiae* in liquid culture (Chavarría-Hernández and de-la-Torre, 2001) for the estimation of the kinetical parameters lag phase (λ , day), maximal specific growth rate (μ_{\max} , day⁻¹), al multiplication factor $[(C/C_0)_{\max} (-)]$. Providing enough data, including thermodynamic and transport properties (Kontogeorgis et al., 2021), the possibilities are vast and can involve the use of supervised ML (i.e., Neural networks, Naive Bayesian model, etc.), semi supervised ML (i.e., Q-learning, Generative adversarial networks, etc.) and unsupervised ML (i.e., Principal component analysis), as well as proper orthogonal decomposition to develop reliable EPN-mass production bioprocesses at the industrial scale.

The future of EPNs may lie in the ability to tailor them to a wider diversity of agricultural needs, while breeding EPNs or improving carriers for longer shelf life, increasing their efficiency through genetic improvements, refining application technologies, reducing production and delivery costs, expanding their use to a wider range of pest species, crops, and regions. As the market expands, cost will decline. In addition, there is potential for regulatory frameworks to further encourage the adoption of EPNs. With continued research and innovation, EPNs could become a cornerstone of sustainable pest management strategies.

CRedit authorship contribution statement

Tshimangadzo Ramakuwela: Conceptualization; writing and review (Abstract, Introduction, Commercialization of EPN and Future Prospects). **Eustachio Tarasco:** Writing and review (Use in agroforestry ecosystems). **Norberto Chavarría-Hernández:** Writing and review (Future prospects). **Stefan Toepfer:** Writing and review (Commercialization of EPN and EPN use in maize agroecosystems). **All authors** have jointly revised and finalised the manuscript.

Funding

We acknowledge funding from the SOR4D project of Swiss National Science Foundation and SDC (400440_217975), CABI's Plantwise Plus Programme funded by DFID, DGIS and SDC. CABI is also supported through its core donors (<https://www.cabi.org/about-cabi/who-we-work-with/key-donors/>). We acknowledge travel grant from the University of Pretoria (Department of Plant and Soil Sciences, as well as the Vice Principal's Conference Funding) for Tshima Ramakuwela to participate in the 100 years of 1st EPN discovery celebration, held in Logrono, Spain from 10 to 12 April 2024.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We'd like to thank Raquel Campos-Herrera and the organizing committee for organizing the conference for the 100 years of discovery of entomopathogenic nematodes, held in Logrono, Spain from 10 to 12 April 2024. We'd like to thank Xun Yan (Zhongkai University of Agriculture and Engineering Guangzhou, China), and Kishore Nalam (Arthrobio Ltd, India) for providing information from their respective countries. We thank Frankie Downs (CABI) for help with the analyses of commercial nematode products.

References

- Abate, B.A., Wingfield, M.J., Slippers, B., Hurley, B.P., 2017. Commercialisation of entomopathogenic nematodes: should import regulations be revised? *Biocontrol Sci. Technol.* 27 (2), 149–168. <https://doi.org/10.1080/09583157.2016.1278200>.
- Askary, T.H., Abd-Elgawad, M.M.M., 2021. Opportunities and challenges of entomopathogenic nematodes as biocontrol agents in their tripartite interactions. *Egypt J Biol Pest Control* 31, 42. <https://doi.org/10.1186/s41938-021-00391-9>.
- Backhaus, G.F., 1994. Biological control of *Otiorynchus sulcatus* F. by use of entomopathogenic nematodes of the genus *Heterorhabditis*. *Acta Hort.* 364, 131–142. <https://doi.org/10.17660/ActaHortic.1994.364.16>.
- Bass, C., Jones, C., 2018. editorial overview: pests and resistance: resistance to pesticides in arthropod crop pests and disease vectors: mechanisms, models and tools. *Curr. Opin. Insect Sci.* 27, iv–vii. <https://doi.org/10.1016/j.cois.2018.04.009>.
- Batalla-Carrera, L., Morton, A., Garcia-del-Pino, F., 2013. Field efficacy against the hazelnut weevil, *Curculio nucum* and short-term persistence of entomopathogenic nematodes. *Spanish J Agr Res* 11, 1112–1119. <https://doi.org/10.5424/sjar/20131114-4210>.
- Bedding, R.A., 1981. Low cost in vitro mass production of *Neoplectana* and *Heterorhabditis* species (Nematoda) for field control of insect pests. *Nematologic* 27, 109–114.
- Bhat, A.H., Chaubey, A.K., Askary, T.H., 2020. Global distribution of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*. *Egypt J Biol Pest Control* 30, 31. <https://doi.org/10.1186/s41938-020-0212-y>.
- Cabi, 2024. *Bioprotection Portal*. CABI Publishing, Wallingford, UK <https://bioprotectionportal.com/>.
- Chairin, T., Piromkarn, N., Wandee, N., Chaiyadit, K., Anothai, J., 2022. The use of soybean residue, an alternative low-cost substrate, for culturing entomopathogenic nematode. *Biol. Control* 105021. <https://doi.org/10.1016/j.biocontrol.2022.105021>.
- Chavarría-Hernández, N., de-la-Torre, M., 2001. Population growth kinetics of the nematode, *Steinernema feltiae*, in submerged monoxenic culture. *Biotechnol. Lett* 23, 311–315. <https://doi.org/10.1023/A:1005694709366>.
- Clausi, M., Leone, D., Vinciguerra, M., T., Rappazzo, G., Tarasco, E. 2014. Laboratory tests on the biocontrol of Chestnut insect pests of Etna (Sicily, Italy) by means of entomopathogenic nematodes. *Redia*, XCvii: 133-136.
- Corradi, M., Fezza, E., Hawthorne, B., Salisbury, A., Harris, E., Roberts, J., Pope, T., 2023. Efficacy of entomopathogenic nematodes in peat alternative growing media. *IOBC-WPRS Bulletin* 167, 33–34.
- Cortes-Martinez, C.I., Chavarría-Hernandez, N., 2020. Production of entomopathogenic nematodes in submerged monoxenic culture: a review. *Biotechnol. Bioeng.* 117 (12), 3968–3985. <https://doi.org/10.1002/bit.27515>.
- Cortés-Martínez, C.I., Rodríguez-Hernández, A.I., Ávila-López, M.B., Chavarría-Hernández, N., 2023a. Impact of egg yolk type in liquid culture production of *Steinernema carpocapsae* JAP1 and their virulence. *Biol. Control* 184. <https://doi.org/10.1016/j.biocontrol.2023.105279>.
- Cortés-Martínez, C.I., Rodríguez-Hernández, A.I., del Rocío López-Cuellar, M., Chavarría-Hernández, N., De los Santos Romero, R., 2023b. Culture of *Steinernema glaseri* on three solid media and their virulence against *Galleria mellonella* larvae. *Plant Prot. Sci.* <https://doi.org/10.17221/1/2023-pps>.
- Curto, G., Caruso, S., Reggiani, A., Vergnani, S., 2009. Efficacia dei nematodi entomopatogeni nel contenimento delle larve di *Cydia pomonella* L. nell'Italia Settentrionale. *Frutticoltura* 3, 45–49.
- Danishiar, S.Q., Falahzadah, M.H., Tarasco, E., 2023. A study of Entomopathogenic nematodes and their role in microbial control of pests. *J Natl Sci Rev* 1 (1), 52–63. <http://kujnsr.com/index.php/JNSR>.
- Dara, S.K., 2019. The new integrated pest management paradigm for the modern age. *J Integr Pest Manag* 10 (1), 12. <https://doi.org/10.1093/jipm/pmz010>.
- De Luca, F., Clausi, M., Troccoli, A., Curto, G., Rappazzo, G., Tarasco, E., 2015. Entomopathogenic Nematodes in Italy: Occurrence and use in microbial control strategies. In: Campos Herrera, R. (Ed.), *Nematode Pathogenesis of Insects and Other Pests*. Springer International Publishing, Switzerland, pp. 431–449.
- Dito, D.F., Shapiro-Ilan, D.I., Dunlap, C.A., Behle, R.W., Lewis, E.E., 2016. Enhanced biological control potential of the entomopathogenic nematode, *Steinernema carpocapsae*, applied with a protective gel formulation. *Biocontrol Sci. Technol.* 26 (6), 835–848. <https://doi.org/10.1080/09583157.2016.1159659>.
- Dunn, M.D., Belur, P.D., Malan, A.P., 2020. A review of the in vitro liquid mass culture of entomopathogenic nematodes. *Biocontrol Sci. Technol.* 31 (1), 1–21. <https://doi.org/10.1080/09583157.2020.1837072>.
- Dunn, M., Malan, A., 2023a. In vitro liquid mass production of a South african isolate of *Heterorhabditis zealandica*. *African Entomology* 31. <https://doi.org/10.17159/2254-8854/2023/a16150>.
- Dunn, M.D., Belur, P.D., Malan, A.P., 2021. Effect of Glucose, Agar Supplementation and Bacterial Cell Density on the in vitro Liquid Culture of *Steinernema jeffreyense*. *African Entomology* 29 (2). <https://doi.org/10.4001/003.029.0423>.
- Dunn, M.D., Belur, P.D., Malan, A.P., 2022. Development of cost-effective media for the in vitro liquid culture of entomopathogenic nematodes. *Nematology* 24 (7), 763–775. <https://doi.org/10.1163/15685411-bja10166>.
- Dunn, M.D., Malan, A.P., 2023b. *Steinernema yirgalemense* inoculum size and timing impact on the population dynamics of in vitro liquid culture shake flask production. *Nematology* 25 (10), 1079–1093. <https://doi.org/10.1163/15685411-bja10278>.
- Ehlers, R.-U., 1996. Current and future use of nematodes in biocontrol: Practice and commercial aspects with regard to regulatory policy issues. *Biocontrol Sci. Technol.* 6, 303–316.
- Ehlers, R.-U., Hokkanen, H.M.T., 1996. Insect biocontrol with non-endemic entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.): Conclusions

- and recommendations of a combined OECD and COST workshop on scientific and regulatory policy issues. *Biocontrol Sci. Technol.* 6, 295–302.
- El Khoury, Y., Noujeim, E., Ravlic, J., Oreste, M., Addante, R., Nemer, N., 2020. The effect of entomopathogenic nematodes and fungi against four xylophagous pests. *Biocontrol Sci Technol* 30, 983–995. <https://doi.org/10.1080/09583157.2020.1781059>.
- El Khoury, Y., Oreste, M., Noujeim, E., Nemer, N., Tarasco, E., 2018. Effect of temperature on the pathogenicity of Mediterranean native entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from natural ecosystems. *Redia* 101, 123–127. <https://doi.org/10.19263/REDIA-101.18.16>.
- El-Ashry, R.M., El-Marzoky, A.M., 2018. Compatibility of entomopathogenic nematodes, *Heterorhabditis bacteriophora* Poinar and *Steinernema carpocapsae* Weiser with some chemical and pesticides. *Zagazig J Agric Res* 45, 905–916. <https://doi.org/10.21608/zjar.2018.49129>.
- Fallet, P., Bazagwira, D., Ruzzante, L., Ingabire, G., Levivier, S., Bustos-Segura, C., Kajuga, J., Toepfer, S., Turlings, T.C.J., 2024. Entomopathogenic nematodes as an effective and sustainable alternative to control the fall armyworm in Africa. *PNAS Nexus*. <https://doi.org/10.1093/pnasnexus/pgae122>.
- Fallet, P., Gianni, L., Machado, R.A.R., Bruno, P., Bernal, J.S., Karangwa, P., Kajuga, J., Waweru, B., Bazagwira, D., Degen, T.H., Toepfer, S., Turlings, T.C.J. 2022. Comparative screening of Mexican, Rwandan and commercial entomopathogenic nematodes to be used against invasive fall armyworm, *Spodoptera frugiperda*. *Special Issue Entomopathogenic Nematodes: Lethal Parasites of Insects*. *MDPI Insects* 13(2), 205. doi: 10.3390/insects13020205 IF 3.1.
- Georgis, R., Dunlop, D.B., Grewal, P.S., 1995. Formulation of entomopathogenic nematodes. In: Hall, F.R., Barry, J.W. (Eds.), *Biorational Pest Control Agents: Formulation and Delivery*. American Chemical Society, Washington, DC, pp. 197–205.
- Glazer, I., Santoiemma, G., Battisti, A., De Luca, F., Fanelli, E., Troccoli, A., Tarasco, E., Sacchi, S., Bianchi, A., Gilioli, G., Gherardo, P., 2022. Invasion of *Popillia japonica* in Lombardy, Italy: Interactions with soil entomopathogenic nematodes and native grubs. *Agric. For. Entomol.* 24 (4), 600–608.
- Ha, B., Wei, X., Lu, P., Qing, H., Guo, J., Zhang, R., Chen, L., Li, X., Hu, B., Wang, S., Xu, Y., Fu, Z., Shapiro-Ilan, D., Ruan, W., 2025. Natural UV protectants and humectants to improve the efficiency of *Steinernema carpocapsae* in controlling foliar pests. *Pest Manag. Sci.* 81, 1422–1431. <https://doi.org/10.1002/ps.8544>.
- Kagimu, N., Ferreira, T., and Malan, A.P. (2017). The attributes of survival in the formulation of entomopathogenic nematodes utilised as insect biocontrol agents. *Afr. Entomol.* 25, 275–291. doi: 10.4001/003.025.0275.
- Kong, X., Huang, Z., Gu, X., Cui, Y., Li, J., Han, R., Cao, L., 2022. Dimethyl sulfoxide and ascarosides improve the growth and yields of entomopathogenic nematodes in liquid cultures. *J. Invertebr. Pathol.* 193, 107800. <https://doi.org/10.1016/j.jip.2022.107800>.
- Kontogeorgis, G.M., Dohrn, R., Economou, I.G., de Hemptinne, J.C., Ten Kate, A., Kuitunen, S., Vesovic, V., 2021. Industrial requirements for thermodynamic and transport properties. *Ind. Eng. Chem. Res.* 60 (13), 4987–5013. <https://doi.org/10.1021/acs.iecr.0c05356>.
- Koppenhöfer, A.M., Shapiro-Ilan, D.I., Hiltbold, I., 2020. Entomopathogenic nematodes in sustainable food production. *Front Sustain Food Syst* 4, 125. <https://doi.org/10.3389/fsufs.2020.00125>.
- Kotliarevski, L., Cohen, R., Ramakrishnan, J., Wu, S., Mani, K.A., Amar-Feldbaum, R., Yaakov, N., Zelinger, E., Belausov, E., Shapiro-Ilan, D., Glazer, I., Ment, D., Mechrez, G., 2022. Individual coating of entomopathogenic nematodes with titania (TiO₂) nanoparticles based on oil-in-water pickering emulsion: a new formulation for biopesticides. *J. Agric. Food Chem.* 70, 13518–13527. <https://doi.org/10.1021/acs.jafc.2c04424>.
- Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., Goettel, M.S., 2015. Insect pathogens as biological control agents: Back to the future. *J. Invertebr. Pathol.* 132, 1–41.
- Leite, L.G., Chacon-Orozco, J.G., Shapiro-Ilan, D.I., Baldo, F.B., Cardoso, J.F.M., 2023. Effects of temperature for optimizing production and storage of *Steinernema rarum* in a novel biphasic process, and efficacy of the nematode against *Sphenophorus levis*. *Biol. Control* 187. <https://doi.org/10.1016/j.biocontrol.2023.105381>.
- Liu, S., 2020. *Bioprocess engineering, kinetics, sustainability, and reactor design*, 3rd ed. Elsevier, Netherlands.
- Long, S.J., Richardson, P.N., Fenlon, J.S., 2000. Influence of temperature on the infectivity of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) to larvae and pupae of the vine weevil *Otiorynchus sulcatus* (Coleoptera: Curculionidae). *Nematol* 2, 309–317. <https://doi.org/10.1163/156854100509187>.
- Lulamba, T.E., Green, E., Serepa-Dlamini, M.H., 2021. Genome assembly and annotation of *Photorhabdus heterorhabditis* strain ETL reveals genetic features involved in pathogenicity with its associated entomopathogenic nematode and anti-host effectors with biocontrol potential applications. *Gene* 795. <https://doi.org/10.1016/j.gene.2021.145780>.
- Lunau, S., Stoessel, S., Schmidt-Peisker, J.A., Ehlers, R.-U., 1993. Establishment of monoxenic inocula for scaling up in vitro cultures of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis* spp. *Nematologica* 39, 385–399.
- Mathhabe, O.D., Lepphoto, T.E., Gray, V.M., 2022. Monitoring life cycle stages of axenic cultures of In vitro produced *Oscheius* L8 MCB in semi-solid state and liquid state and comparative efficacy studies between in vitro and in vivo nematode production against *Galleria mellonella*. *Biol. Control* 170. <https://doi.org/10.1016/j.biocontrol.2022.104904>.
- Metwally, H.M.S., Saleh, M.M.E., Abonaem, M., 2025. Formulation for foliar and soil application of entomopathogenic nematodes for controlling the onion thrips *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). *Egypt J Biol Pest Control* 35. <https://doi.org/10.1186/s41938-025-00841-8>.
- Pieterse, A., Haukeland, S., Puza, V., Ross, J.L., Malan, A.P., 2022. In vitro liquid culture of the mollusc-parasitic nematode *Phasmarhabditis* (Rhabditida: Rhabditidae). *J. Helminthol.* 96. <https://doi.org/10.1017/S0022149X22000736>.
- Ramakulwela, T., Hatting, J., Laing, M.D., Hazir, S., Thiebaut, N., 2016. In vitro solid-state production of *Steinernema innovationi* with cost analysis. *Biocontrol Sci. Technol.* 26 (6), 792–808.
- Shapiro-Ilan, D.I., Han, R., Dolinski, C., 2012. Entomopathogenic nematode production and application technology. *J of Nematol* 44, 206–217.
- Shapiro-Ilan, D.I., Goolsby, J.A., 2021. Evaluation of Barricade® to enhance survival of entomopathogenic nematodes on cowhide. *J. Invertebr. Pathol.* 184, 107592.
- Shapiro-Ilan, D., Lewis, E., 2024. Entomopathogenic nematodes as biological control agents. CABI Publishing, Wallingford, UK.
- Sharma, M.P., Sharma, A.N., Hussaini, S.S., 2011. Entomopathogenic nematodes, a potential microbial biopesticide: mass production and commercialisation status – a mini review. *Arch Phytopathol Pflanzenschutz* 44 (9), 855–870. <https://doi.org/10.1080/03235400903345315>.
- Skendžić, S., Zovko, M., Živković, I.P., Lešić, V., Lemić, D., 2021. The impact of climate change on agricultural insect pests. *Insects* 12 (5), 440. <https://doi.org/10.3390/insects12050440>.
- Steiner, G., 1923. *Aplectana kraussae* n. sp., eine in der blattwespe *lyda* sp. parasitierende nematodenform, nebst bemerkungen über das seitenorgan der parasitischen nematoden. *Zbl Bakt II Natur* 2 (59), 14–18.
- Susurluk, I., Ulu, T., Kongu, Y., 2013. Tolerances of hybridized entomopathogenic nematode *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) strains to heat and desiccation. *Turkish. J. Entomol.* 37.
- Tarasco, E., Fanelli, E., Salvemini, C., El-Khoury, Y., Troccoli, A., Vovlas, A., De Luca, F., 2023. Entomopathogenic nematodes and their symbiotic bacteria: from genes to field uses. *Front. Insect Sci.* 3, 1195254. <https://doi.org/10.3389/finsc.2023.1195254>.
- Tarasco, E., Ragni, A., Curto, G., 2017. Status of entomopathogenic nematodes in integrated pest management strategies in Italy. In: Abd-Elgawad, M.M.M., Askary, T. H., Coupland, J. (Eds.), *Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes*. CABI International, pp. 429–444. <https://doi.org/10.1079/9781786390004.0429>.
- Tarasco, E., Bubic, G., Oreste, M., 2016. Biological notes on *Parahyppota caestrum* and first microbiological control assays. *Bull. Insectol.* 69 (2), 259–262.
- Tarasco, E., Triggiani, O., 2006. Evaluation and comparison of entomopathogenic nematodes and fungi to control *Corythucha ciliata* say (Rhynchota Tingidae). *Redia* 89, 51–54.
- Toepfer, S., Vandenbossche, B., Yan, X. 2024a. Applications of Entomopathogenic Nematodes for Insect Pest Control in Maize In: Shapiro-Ilan D.I., Lewis, E. (2024). Entomopathogenic nematodes as biological control agents. CABI Publishing, Wallingford, UK.
- Toepfer, S., Coles, W. 2024b. CABI helps celebrate 100 years since the discovery of entomopathogenic nematodes. CABI Blog, CABI Publishing, Wallingford, UK. <https://blog.cabi.org/2024/04/18/cabi-helps-celebrate-100-years-since-the-discovery-of-entomopathogenic-nematodes/>.
- Torrini, G., Paoli, F., Mazza, G., Simoncini, S., Benvenuti, C., Strangi, A., Tarasco, E., Barzanti, G., Bosio, G., Cutino, I., Roversi, P.F., Marianelli, L., 2020. Evaluation of indigenous entomopathogenic nematodes as potential biocontrol agents against *Popillia japonica* (Coleoptera: Scarabaeidae) in Northern Italy. *Insects* 11 (804), 1–15.
- Toth, Sz, Toepfer, S., Szalai, M., Kiss J. 2022. Limited influence of abiotic and biotic factors on the efficacy of soil insecticides and entomopathogenic nematodes when managing the maize pest *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae). *MDPI Agronomy*. 12, 2697. doi: 10.3390/agronomy12112697 IF 3.9.
- Triggiani, O., Tarasco, E., 2002. Efficacy and persistence of entomopathogenic nematodes in controlling larval populations of *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae). *Biocontrol Sci Technol* 12, 747–752. <https://doi.org/10.1080/0958315021000039923>.
- Triggiani, O., Tarasco, E., 2007. Applying entomopathogenic nematode to *Xanthogaleruca luteola* (Coleoptera Chrysomelidae) infested foliage. *Redia* 90, 29–31.
- Triggiani, O., Tarasco, E., 2011. Evaluation of the effects of autochthonous and commercial isolates of Steinernematidae and Heterorhabditidae on *Rhynchophorus ferrugineus*. *Bull. Insectol.* 64 (2), 175–180.
- Triggiani, O., 1983. Sensibilità del *Tomicus (Blastophagus) piniperda* L. (Coleoptera: Scolytidae) a nematodi della famiglia Steinernematidae e Heterorhabditidae. *Entomologica* 18, 215–223.
- Tumialis, D., Mazurkiewicz, A., Skrzec, I., 2021. Effect of agitation speed on the density of bacteria *Photorhabdus luminescens* and the population dynamics of nematodes *Heterorhabditis megidis* in liquid culture. *J. Helminthol.* 95, e54.
- Ulu, T.C., Susurluk, I.A., 2024. In vitro liquid culture production and post-production pathogenicity of the hybrid *Heterorhabditis bacteriophora* HBH strain. *Crop Prot.* 175. <https://doi.org/10.1016/j.cropro.2023.106443>.
- Vinciaguerra, M.T., Clausi, M., 2006. Biological control of chestnut insect pests by means of entomopathogenic nematodes. *Adv Horticult Sci* 20, 40–44.
- Wang, J., Cao, L., Huang, Z.H., Gu, X.H., Cui, Y.H., Li, J.Z., Han, R.C., 2022. Influence of the ascarosides on the recovery, yield and dispersal of entomopathogenic nematodes. *J. Invertebr. Pathol.* 188. <https://doi.org/10.1016/j.jip.2022.107717>.
- Wang, Z., Dhakal, M., Vandenbossche, B., Dorfler, V., Barg, M., Strauch, O., Molina, C., 2023a. Enhancing mass production of *Heterorhabditis bacteriophora*: influence of different bacterial symbionts (*Photorhabdus* spp.) and inoculum age on dauer juvenile recovery. *World J. Microbiol. Biotechnol.* 40 (1), 13. <https://doi.org/10.1007/s11274-023-03803-0>.
- Wang, Z., Garcia, F., Ehlers, R.U., Molina, C., 2024. Dauer juvenile recovery transcriptome of two contrasting EMS mutants of the entomopathogenic nematode

- Heterorhabditis bacteriophora*. World J. Microbiol. Biotechnol. 40 (4). <https://doi.org/10.1007/s11274-024-03902-6>.
- Wang, Z., Ogaya, C., Dorfler, V., Barg, M., Ehlers, R.U., Molina, C., 2023b. Pheno- and genotyping in vitro dauer juvenile recovery in the nematode *Heterorhabditis bacteriophora*. Appl. Microbiol. Biotechnol. 107 (23), 7181–7196. <https://doi.org/10.1007/s00253-023-12775-y>.
- Waweru, B.W., Kajuga, J.N., Hategekimana, A., Ndereyimana, A., Kankundiye, L., Umulisa, C., Nyombayire, A., Mutumwinka, M., Ishimwe, P.M., Bazagwira, D., Mukundiyabo, G.C., Bigirimana, V.D.P., Yan, X., Kiss, J., Toepfer, S., 2025. Formulation of entomopathogenic nematodes for aboveground use against tomato leaf miner. *Phthorimaea Absoluta*. Insects 16, 189.
- Waweru, B.W., Kajuga, J.N., Hategekimana, A., Toepfer, S., and Taremwa, K.N. 2024. Incorporating beneficial nematodes based biocontrol agents into an integrated pest management strategy of Rwanda: A POLICY BRIEF. EAJSTI, (in press).
- Zhu, L.-T., Chen, X.-Z., Ouyang, B., Yan, W.-C., Lei, H., Chen, Z., Luo, Z.-H., 2022. Review of machine learning for hydrodynamics, transport, and reactions in multiphase flows and reactors. Ind. Eng. Chem. Res. 61 (28), 9901–9949. <https://doi.org/10.1021/acs.iecr.2c01036>.