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Ralf-Udo Elhers

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Entomopathogenic Nematodes – Save Biocontrol Agents for Sustainable Systems

Dr. Ralf-Udo Ehlers

Institute for Phytopathology, Christian-Albrechts-University Kiel, Dept. Biotechnology & Biol. Control, Klausdorfer Str. 28-36, 24223 Raisdorf, Germany

BIOLOGY

Steinernema and *Heterorhabditis* (Rhabditida) are symbiotically associated with bacteria of the genera *Xenorhabdus* and *Photorhabdus* within the Enterobacteriaceae in the gamma subdivision of purple bacteria (Ehlers *et al.*, 1988), respectively. Each nematode species has a specific association with one bacterium species (Akhurst & Boemare, 1990; Ehlers & Niemann, in press). Like other nematodes of the order Rhabditida, *Steinernema* and *Heterorhabditis* spp. form dauer (enduring) juveniles (DJs), which are morphologically and physiologically adapted for long term survival in the soil environment (Womersley, 1993; Glazer, 1996). DJs can be isolated from almost all habitats where soil insects occur. Whereas the few *Heterorhabditis* species are distributed all around the world, the majority of the *Steinernema* species seem to be restricted to certain geografical regions and other species, e.g. *S. feltiae*, are widly distributed (Hominick *et al.*, 1996). The nematode dauer juveniles (DJ) carry between 0 to 250 cells of their symbiont in the anterior part of the intestine. The symbiotic bacteria are released into the haemolymph after penetration of the DJ into a suitable host insect. Inside the DJ the bacteria are well protected against detrimental conditions in the soil. Neither *Xenorhabdus* nor *Photorhabdus* spp. have ever been isolated from soil environments (Akhurst & Boemare, 1990; Poinar, 1990). Accordingly, this phoretic relation seems to be of vital necessity for the associated bacteria. They totally depend on transmission by the DJ into a sterile environment like the insect's haemocoel, as they lack any means for survival in the soil environment (Morgan *et al.*, 1997) or invasion of insect's haemocoel without the help of the DJ. Whereas *Steinernema* spp. can kill insects even without their symbiotic bacteria (Ehlers et al., 1997), *Heterorhabditis* spp. lack insect pathogenicity in the absence of *P. luminescens*. Bowen & Ensign (1998) identified insecticidal proteins produced by *P. luminescens* with potential to substitute Bt toxins in transgenic plants.

Host-finding behaviour of DJs can differ within a population and can also be species specific. "Hunters" are highly mobile and a large proportion of their population tends to actively seek for suitable hosts. In populations of *S. glaseri* and *Heterorhabditis* spp. the majority of the individuals show this character. The sit-and wait "ambushers" often attach to soil particles and nictate, waiting for an insect to pass by and then attack. *S. carpocapsae* is a species with this behaviour (Gaugler, 1993). Penetration of the host insect occurs via natural openings or directly through the insect cuticle (Bedding & Molyneux, 1982; Peters & Ehlers, 1994). There are indications that the penetration process is supported by proteolytic factors produced by the exsheathed dauer juvenile (Roque *et al.*, 1994). Upon reaching the haemocoel the DJ is recognized as non-self and insect defense mechanisms can eliminate the DJs through encapsulation (Peters & Ehlers, 1997). Defence mechanisms against the bacteria also

have been described (Götz *et al.*, 1981). Providing the insect's defense mechanisms fail to eliminate the nematode-bacterium complex, the insect dies 2-4 days after infection. Akhurst & Dunphy (1993) and Simoes & Rosa (1996) have summarized the current knowledge on the pathogenicity mechanisms of the nematode-bacterium complex and the interactions with the defense system of host insects. Once established inside the cadaver, the bacteria proliferate and produce suitable conditions for the nematode to grow and reproduce. The nematodes feed on cells of their symbiont and host tissue. Without the presence of the symbiotic bacteria in the insect cadaver the nematodes are unable to reproduce (Poinar & Thomas, 1966). Infective DJs of *Steinernema* develop to amphimictic adults and *Heterorhabditis* spp. to self-fertilizing hermaphrodites. Their offspring either develop to DJs or to a F1 adult generation. Another adult generation (F2) is usually not developed. Instead, in response to depleting food resources, DJs are formed. Two to three weeks after colonisation of the host insect, the DJs leave the cadaver searching for new target insects in the soil.

SAFETY AND REGISTRATION

Entomopathogenic nematodes and their symbionts are environmentally safe and show no evidence of mammalian pathogenicity (Boemare *et al.*, 1996, Ehlers & Hokkanen, 1996). Besides what has been published in the scientifc literature, safety tests have been conducted by the Pasteur Institute (Boemare *et al.*, 1996) and by commercial companies. Although these results have not been made public, the documentation was provided to the US agency APHIS. No attributes of the nematodes could be identified which would prohibit their use in biocontrol (R. Georgis, personal communication; Parkman *et al.*, 1992).

The consensus view of the participants of a combined OECD and COST 819 workshop on introduction and commercial use of non-endemic nematodes for insect biological control (Ehlers & Hokkanen, 1996) was that entomopathogenic nematodes (EPNs) possess specific biological and ecological features, which make their use in biological control exceptionally safe. All of the scientific evidence available supports the conclusion that EPNs are safe to the environment, as well as to the production and application personnel, to the general public, and to the consumers of agricultural products treated with EPNs. Only a few potential, but very remote risks could be identified (Tab. 1 & 2). Therefore it was recommended that EPNs should not be subject to any kind of registration.

The introduction of non-endemic nematode species, however, should be regulated. Species should be accurately identified, details of the origin, known distribution, probable host range and

Table 1. Possible risks to human health as identified by the expert group. Scale: 0 = no risk at all, 1 = remote, 2 = slight, 3 = moderate, 4 = high, 5 = very high risk.

	Production & Application Personnel	General Public
1. Toxicity	1	0
2. Allergenicity	2	0
3. Infectivity		
nematodes	0	0
bacteria	1	0
4. Carcinogenicity	0	0
5. Teratogenicity	0	0
6. Food and Feed	0	0
7. Pathogenicity of hot-adapted strains	?	?

Table 2: Possible environmental risks of using EPNs, as identified by the experts. Risk rating as in Table 1. NTO = Non-Target Organsims

Possible environmental risks	Rating
1. Non-target organisms (NTOs)	0
In untreated fields	2
In treated fields	3
- in the soil	2
- in other cryptic environments	1
- on foliage	
Vertebrates	0
- warm blodded	2
- cold blooded	
Invertebrates	
Arthropods	
- Predators	2
- Parasitoids	2
- Pollinators	1
- Rare or endangered species	1
- Others	1
Non Arthropods	
- Earthworms	0
- Others	1
Plants	0
2. Competitive displacement of native EPN in treated fields	
- Temporary	2
- Permanent	0
3. Changes in ecosystem balance	
- Local temporary suppression of NTOs	2
- Permanent suppression of NTOs	0
4. Contamination of ground water	1
5. Gene transfer from exotic symbiotic bacteria to other soil bacteria	2
6. Biological "pollution" with new EPN species	4
7. General biodiversity	1

its safety to the user must be provided together with an expert opinion based on available information of the possible impact on non-target organisms. Nematodes are beneficial animals and although symbiotically associated with bacteria, in most countries they are not placed in the category of microorganisms for pest control. As such they are usually exempted from registration requirements, as documented for the United States by Gorsuch (1982). This makes the commercial development of nematode products even more attractive for industry as large costs related with registration can be avoided.

COMMERCIAL USE OF EPNs

Nematodes can be mass produced in *in vitro* culture. Their symbiotic bacteria can convert protein-rich media into suitable resources for the nematodes. A significant breakthrough in nematode biotechnology was the discovery of the symbiotic bacterium by Poinar & Thomas (1965) and the development of *in vitro* production techniques on solid substrates applicable for nematodes of both genera (Bedding, 1981, 1984). Since then the commercial use of nematodes in high value crops in horticulture started. In industrilized countries

the labour-intensive solid state production is often substituted by liquid culture in bioreactors. Liquid mass production started with *S. carpocapsae*, which was scaled up to 80.000 ltr. (Friedman, 1990). Yields of *Heterorhabditis* spp. liquid cultures are still highly variable (Ehlers *et al.*, 1998). In the future, commercial development will rely solely on liquid culture technology as this is the only means for a development of nematode-based products for agricultural markets at economically reasonable prices.

The art of nematode formulation technology is to define and maintain environmental conditions which transfer the DJ into a quiescent state from which it can immediately recover when placed into the spraying tank. Formulation compounds, like clays or biopolymers, have the function to immobilize the nematodes, and all kinds of adjuvants and additives preserve favourable conditions for the nematode. Sufficient gas exchange and a stable moisture content have to be preserved. Shelf life of nematode based products of a few months is nowadays achieved at ambient temperatures not exceeding 30°C. The DJ is resistant to shear forces and can bear pressure of 2,000 kPa (Georgis, 1990), which is why DJs can be sprayed with conventional spraying equipment.

In the overall turnover in the biocontrol market, EPNs have reached the second position after Bacillus *thuringiensis* based products. Currently seven companies in Europe produce EPNs for commercial application. In 1994 world-wide nematode sales surpassed $US 10 million. In Europe and the USA, larvae of the black vine weevil *Otiorhynchus sulcatus* are controlled in strawberries, craneberries, ornamentals and tree nurseries with *Heterorhabditis* spp., *S. feltiae* or *S. carpocapsae* (Georgis, 1992). Treatments in citrus plantations reduce root weevils (Curculionidae) by 50-65% (Downing *et al.*, 1991; Schroeder, 1990, 1992). Sciarid flies (Sciaridae) in mushroom cultures are reduced by *S. feltiae* strains by 51-94% (Grewal & Richardson, 1993; Grewal *et al.*, 1993). Turf insect pests can be successfully controlled. The mole cricket *Scapteriscus vicinus* is controlled with *S. scapterisci* in Florida and Georgia (Redmond & Georgis, 1993). White grubs (Scarabaeidae) in turf can be managed by application of *Heterorhabditis* spp. (e.g., Georgis & Gaugler, 1991; Klein & Georgis, 1992, Downing, 1994, Sulistyanto & Ehlers, 1996). In China the peach borer (*Carposina nipponensus*) is an important pest of apples and pears (Jinxian, 1993), and several hundred hectares are already treated with *S. carpocapsae* (Doeleman, 1990). Also in China, this nematode is successfully introduced to control the cossid carpenterworm *Holocerus insularis* in shade trees (Huaiwen *et al.*, 1993; Jinxian, 1993). *S. carpocapsae* is also used against flea larvae and pupae in soil in the USA.

EPNs IN SUSTAINABLE SYSTEMS

Little is known about the impact of naturally occuring EPN populations. The observations reported so far can be divided into relatively balanced host-nematode associations and epizootics. The association of *S. kraussei* with the false spruce webworm, *Cephalcia abietis*, in Central Europe is an example for a balanced system with a cumulative annual control of approximately 25% (Mracek, 1986; Eichhorn, 1988). In Austria Führer & Fischer (1991) used lime to increase the soil pH in forest systems in order to enhance the host finding of the natural nematode popuation and they could increase the nematode infestation of *C. abietis*. Another example for an attempt to increase the naturally occurring EPN population by cultural methods is reported by Brust (1991). No-tillage and less intense weed control resulted in higher levels of nematode infestation, significantly higher corn yields and less root damage by the corn rootworm *Diabrotica undecimpunctata howardi*.

Epizootics have been observed in grub populations infested with *Heterorhabditis* sp. reaching 71% control in a sugarcane field (Akhurst *et al.*, 1992)

and 80% in the population of the garden chafer *Phyllopertha horticola* (Peters, 1996). In New Zealand an increasing infestation reaching 56% after a one-time release of *H. bacteriophora* was reported in the second year (Jackson & Wouts, 1987). Since four years *H. bacteriophora* is used inundatively in Germany to control grubs (*P. horticola*) in turf. On many plots treated with nematodes the grub population was below the damage threshold in following years (Ehlers & Peters, 1998). These observation encourage further research to investigate the conditions necessary to obtain long-term effects through nematode inoculation.

The distribution of EPNs in the field seems to be related to the distribution of potential host insects. As the distribution of soil insect populations are usually patchy, the same is recorded for nematodes. Those species with a wide host range, e.g. *S. feltiae,* are found in almost every second soil sample, whereas *Heterorhabditis* spp. are seldomly encounted. The latter seem to have the potential to cause epizootics and thus have a potential for inoculative release. Their potential for long term survival seems to be poor. In contrast, *S. feltiae* is able to survive for long periodes without hosts available and thus is more abundant, but has not been observed to control more than 20% of a potential host population.

Nowadays, EPNs are used inundatively, however, the reports of natural occurance, their potential for long-term control and possible cultaral means of stabilizing their population should encourage research to investigate their potential as antagonists in sustainable systems.

FUTURE PERSPECTIVES

Currently, the market size in the different countries seldomly surpasses treatments of 500 hectares. The use in outdoor crops would involve much larger areas and consequently a an increase in liquid culture capacity. Considering the short shelf life of nematodes and the seasonal variation in the demand of agriculture markets, the production also becomes a logistic problem. The capacity and timing of mass production needs to be adapted to the market's seasonal demand (von Reibnitz & Backhaus, 1994). To make available such quantities of entomopathogenic nematodes, the technology needs to be reproducible, liquid culture yields need to be further increased and DJ quality must be stabilized.

Significant progress also is needed in the improvement of the post harvest and application technology. Large nematode quantaties can only be handled when storage technology can guarantee a survival of the DJs at high quality. The objective of formulation improvement is to guarantee a shelf life of approximately 6 months at ambient temperature. This is already achieved with some, but not all nematode species. For field use, low volume application technology and the lack of irrigation facilities must also be considered. Currently, nematodes are applied with high volumes of water. Future efforts will have to consider adjuvants and additives in order to decrease the amount of water necessary for nematode application in the field. Research and development in application technology will also focus on an overall reduction of the nematode application density. Many of the nematodes applied never reach a target host (Curran, 1993). The improvement of the application technology and of the nematode's control potential by genetic means (Burnell & Dowds, 1996) are possible measures to reduce the application density in the field. Together with progress in the liquid culture technology this will significantly contribute to lower application costs.

In conventional agriculture systems biocontrol products can not compete with chemical control measures. Therefore biologicals are only used in situation were chemicals fail, either due to resistance of the insect population or to survival in cryptic environments, or when the chemical control is restricted by legislation. Chemical compounds are to be degraded in the environment within two weeks after application,

persistent pesticides have been banned. In contrast, the impact of biologicals can be long lasting. Sustainable agriculture and ecological farming practice will rely on the potential of biologicals. They can either develop cultural methods to increase and maintain the potential of antagonistic populations or, in the case where the antagonist is absent, they will rely on inundative release. Sustainability will largely depend on biological regulation of pest populations and EPNs contribute to the overall antagonistic potential in soil environments.

EPNs have many advantages over other control agents. They can be applied with conventional spraying equipment, chemical compounds cannot interfere with their control potential and they are usually exempted from registration (Ehlers & Peters, 1995). Like microbial agents nematodes can easily be mass produced in liquid culture and stored maintaining their control potential for considerable time (Ehlers, 1996). They have a potential in inundative and inoculative release and have insignificant effects on non-target organisms (Bathon, 1996). They are mobile in the soil environment and can persist for years.

Other microbials need registration and the costs related with the registration cannot be justified taking into consideration the size of the potential markets. Transgenic plants met the same conditions as their development is equally expensive and they will be available only in the large scale markets. As long as registration policy is not changed, marketing of microbial control agents is prevented and nematodes will probably be the number one agents for insect control in soil environments.

- The question is whether long-term sustainable effects can be measured? Quantification of nematode populations is almost impossible and usually we cannot distinguish between released and endemic populations. Increases in yields of 5% or more are within the confidentiality range of our data.
- Another question is whether societies are willing to support the development of biocontrol agents and will subsidies costs related to safety tests?
- Biocontrol in sustainable systems will rely on a spectrum of possible agents. Are our politicians prepared to reduce the costly hurdles which are currently related with registration of microbial agents?

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