Safety and regulation of entomopathogenic nematodes

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Safety and potential effects on non-target organisms

Entomopathogenic nematodes (EPN) are exceptionally safe biological control agents. They are certainly more specific and are less of a threat to the environment than chemical insecticides (Ehlers and Peters, 1995). Since the first use of the entomopathogenic nematode *Steinernema glaseri* against the white grub *Popillia japonica* in New Jersey (USA) (Glaser and Farrell, 1935), not even inferior damages or hazards caused by the use of EPN to the environment have been recorded. The use of EPN is safe for the user. EPN and their associated bacteria cause no detrimental effect to mammals or plants (Poinar et al., 1982; Boemare et al., 1996; Bathon, 1996; Akhurst and Smith, 2002). A joint workshop supported by the EU COST Action 819 "Entomopathogenic Nematodes" and the OECD (Organisation for Economic Co-operation and Development) Research Programme "Biological Resource Management for Sustainable Agriculture Systems", which met in 1995 to discuss potential risks related with the use of EPN in biological control, concluded that EPN are safe to production and application personnel and the consumers of agriculture products treated with EPN (Ehlers and Hokkanen, 1996). The expert group could not identify any risk for the general public related to the use of EPN.

No reports exist that document any effect on humans by the symbiotic bacteria. A related non-symbiotic species, *Photorhabdus asymbiotica*, was reported five times from humans in the USA (Farmer *et al.*, 1989). Another group of non-symbiotic *Photorhabdus* is reported from five patients in Australia (Peel *et al.*, 1999). From most of the patients other human-pathogenic bacteria were also recorded, thus the *Photorhabdus* spp. were opportunistic. The route of the infections was not established. Three infections might have been related with spider bites. Both clinical groups lack symbiotic relations with nematodes and strains within each group have a high level of within-group relatedness but do not cluster in groups containing the nematode symbionts (Szállás *et al.*, 1997; Akhurst and Smith, 2002). The existence of bacterial species with and without pathogenic effects on humans within one genus is common (e.g. Bacillus). No action is therefore required and no conclusions should be drawn from the reports of pathogenic effects on humans by non-symbiotic *Photorhabdus* spp. on potential risks related with the use of EPN and their symbiotic bacteria.

The COST-OECD expert group evaluated possible risks for the environment. Long-term effects on non-target organisms (NTOs) or other environmental impacts following the application of indigenous or exotic EPN have not been reported. Even after release of an exotic nematode species, no detrimental effects were observed (Parkman and Smart, 1996). The possible short-term environmental risks of using EPN are effects on predators and parasitoids of the target pest and effects on NTO in the soil or cryptic environments. These risks were classified as remote to moderate and temporary (Ehlers and Hokkanen, 1996).

Much scientific information on the safety and possible impacts of entomopathogenic nematodes on NTOs and the environment is available. Significant effects on foliage inhabiting NTOs can be excluded as EPN cannot survive for long time above the soil (Glazer, 2002). Bathon (1996) summarized available results on non-target effects on soil inhabiting insects and concluded that mortality caused by released EPN among non-target arthropod populations can occur, but will only be temporary, will be spatially restricted and will effect only part of the population. The potential wide host range of > 200 species recorded from laboratory assays (Poinar, 1986) could not be supported in field trails (Georgis et al., 1991; Buck and Bathon, 1993; Koch and Bathon, 1993, Bathon 1996). Bathon (1996) summarized results of extensive field studies performed over a period of 3 years with several 100 m² plots in different environments. A total of approximately 400.000 specimens were evaluated. EPN application never resulted in the extinction of a local population. The density of few species was reduced (some increased) after EPN application, however, the reduction was temporary and spatially restricted. In general, the impact on the non-target populations was negligible.

The affect nematodes can have on NTOs are transient. Several environmental factors limit survival of EPN in the soil (Glazer, 1996). The half-time of EPN is between a few days and one month (Strong, 2002). After inundative release with 0.5 million nematodes/m², EPN population density rapidly declines followed by a period of about 2 weeks with lower rates of decline, after which the population reaches background levels of about 10,000/m² (Smits, 1996). Consequently, EPN need to reproduce in order to establish and have long term effects on an insect population. Their population density is always correlated with the occurrence and density of potential host insect populations, which, on the other hand, is a result of available food resources supporting these insect host populations (Strong, 2002). Density and distribution of EPN in a field thus depends on recycling in hosts and is a consequence of the distribution of host insects. Likewise the distribution of host insect population, EPN populations are typically patchy and aggregated (Stuart and Gaugler, 1994; Spiridonov and Voronov, 1995). The polyphagous nature of EPN antagonists in the soil (Kaya and Koppenhöfer, 1996) is another factor limiting EPN population density and dispersal. Considering the low overall density, the high patchiness and a reduced mobility of nematodes, the risk for large impacts on NTO population is negligible.

A high risk was rated by the experts for the possible "biological pollution" with exotic EPN species. Although one could also argue that it is beneficial to the agro-ecosystem when an additional antagonist has been successfully established, others think that the original species structure should not be disturbed. Although this is largely an ethical problem, possible hazards can be related with an introduction. Barbercheck and Millar (2000) introduced exotic *S. riobrave* from Texas on plots in North Carolina with an endemic population of *S. carpocapsae* and *H. bacteriophora*. The introduction resulted in a reduction of insect mortality caused by the endemic species when soil samples were baited with *G. mellonella*. Data suggest that coexistence of the three species in the field was possible and that the risk for local extinction of the native nematodes was minimal. However, the results indicate that the application of the exotic species can cause a reduction of endemic species populations.

Coexistence is facilitated by highly aggregated populations. The relatively low mobility of EPN are likely to result in fragmented populations. The highly aggregated distribution (Taylor, 1998) will ensure parts of the population to survive while other parts might be transiently extincted by introduction of exotic populations. Survivors can later re-colonize locally extincted populations. These metapopulation dynamics are of major importance for the survival and coexistence of species (Harrison and Taylor, 1997).

Naturally occurring nematode populations cause sustainable effects on pest populations (Ehlers, 1998). These effects have not been very well exploited also because we understand little of EPN population dynamics and possibilities to enhance EPN populations by culture methods (Brust, 1991; Fischer and Führer, 1990). At present we cannot evaluate the economic benefits of sustainable effects. The economic effect of introducing an exotic species is more easy to assess. In the case of a pest population surpassing the economic threshold the use of an exotic nematode might be economically reasonable. It is often argued that prior to the release of exotic species it should be tested whether an endemic population might also be the solution to a problem. However, the naturally occurring species, even if superior in its control potential, might not be commercially available. Waiting until the endemic population has increased and reached an even distribution to significantly reduce the pest population will result in economic losses. The benefit from introducing the exotic species will overwhelm the damage caused by a reduction of the population of the endemic EPN species. Should the exotic species persist, we have a case of "biological pollution". However, is this a damage or is this a benefit for the farmer? As exotic species have not been recorded to eliminate the endemic EPN species, no real hazard has yet been identified with the introduction of the exotic species and the "biological pollution".

Registration

In biocontrol science, EPN are assigned to the group of beneficial invertebrate parasites and predators. However, they are also classified as pathogens or microbial control agents because of their mutualistic relation with their symbiotic bacteria. In regard to registration policy, EPN are usually covered within the macroorganisms together with beneficial arthropods. For that reason they have been exempted from registration in many countries. There are strong arguments why nematodes should be considered as macroorganisms and, if necessary, be registered as such. Users of EPN products do not get into contact with the symbiotic bacteria as the bacterial cells are embedded in the intestine of the DJ. On the other hand, the number of bacteria is relatively small (200-2000 per DJ). Should EPN be registered as microbial agents due to their symbiotic relation with *Xenorhabdus* and *Photorhabdus* spp., then decision makers in regulatory offices will face a serious problem. They would have to also consider endosymbionts of insects, e.g. *Wolbachia* spp. or virus symbionts, which often contribute to insect death. Harwood and Beckage (1994), for instance, identified a polydnavirus associated with eggs of the parasitoid *Cotesia congregata*. During deposition of the parasitoid egg, the virus is also injected into the haemocoel of the lepidopteran host. The virus suppresses the immune response of host (*Manduca sexta*), which otherwise would encapsulate the eggs of the parasitoid in the haemolymph. Should *Cotesia* spp. now be considered as microbial control agents and be registered as such? Besides, any beneficial arthropod is grown under non-sterile conditions and hence carries a large variety of microorganisms in the intestine.

In most countries EPN are exempted from registration requirements. Only few countries have developed some kind of requirements for registration, which are usually not comparable with the data requirements needed for the registration of chemical compounds or microbial agents. The only exemption from this practice is Japan. The company SDS Biotech had to provide data files for *S. carpocapsae* and *S. glaseri* comparable to those required for chemical pesticides and costs reached comparable amounts (> 200.000 US$).

Table 1: Requirements for registration of EPN in different countries. Denmark, Finland, France, Greece, germany, Italy, Portugal and Spain do not require registration.

Country	**Requirements**
Australia	No, importation and release of exotic species requires permits of a series of authorities (see Bedding *et al.*, 1996)
Austria	Yes, although following the requirements for chemical pesticides, the time-consuming procedure for EPN is limited to data which are in a reasonable context with biocontrol agent
Belgium	Required for new EPN species not marketed yet
Brasil	Registration required already for field testing of indigenous species
Canada	No, but guidelines for registration are being developed
Czech Republic	Yes, requirements include efficacy data from field trails
European Union	No, Directive 91/414/EEC distinguishes between chemical pesticides and micro-organisms and viruses. Nematodes and macro-organisms are not mentioned; EU tries to avoid implementation of registration for low risk products
Hungary	Yes, requirements include efficacy data from field trails
Ireland	Yes, new law recently implemented
Japan	Yes, data requirements are not different from those required for chemical compounds, costs are enormously high
Netherlands	Required for new EPN species not marketed yet
New Zealand	Yes, although other macro-organisms do not require registration, nematodes must be registered (see Bedding *et al.*, 1996)
Norway	Yes, requirements follow recommendations of the OECD guidelines, except that the assessment of the environmental risk is not necessary
Poland	Yes, efficacy data from field trails in Poland requested
Sweden	Yes, EPN must be approved under the Act on Preliminary Examination of Biological Pesticides, limited data requirement
Switzerland	Yes, but rarely more than a paperwork exercise
United Kingdom	No, indigenous EPN do not need registration, but the introduction of non-indigenous species or strains is controlled through the Wildlife and Countryside Act (see Richardson, 1996).
United States	No, but any import of living material must be accompanied by shipment permits, release of exotic species is regulated by the Animal and Plant Health Inspection Service (APHIS) and other federal organisations (see Rizvi *et al.*, 1996; Akhurst and Smith, 2002)

In most European countries no registration is required. The exemption from registration requirement aided the commercial development of EPN based products. Those countries that require registration, usually ask for information which is freely available in the scientific literature. In Switzerland, for instance, all biocontrol agents need to be registered, however, the requirements are not comparable with those for chemical compounds. Even microbial agents undergo a reduced procedure in Switzerland, which is not comparable to EU requirements. The registration of EPN is based on published efficacy data and safety information, accompanied by descriptions of the production and quality control procedures. In Austria, Sweden and Norway the requirements are similar. Eastern European countries ask for data from field trials performed within their borders (Poland, Czech Republic and Hungary). The complete procedure is required for every new product. Although other EPN products exist in these markets already, which contain the same species or even strains, authorities go through the whole bureaucratic process again for every new product. The problems related to registration are high costs and loss of time. The registration requires at least two years until a product can be marketed. Fortunately, companies were able to sell their products in other EU markets in the meantime until a registration was granted. Many small and medium-sized enterprises would not have been able to start commercializing their EPN products, if registration were required in all EU countries and the USA.

Attempts to control the use of invertebrate biocontrol agents are underway. The Netherlands and Belgium implemented a registration procedure recently for all nematode-based products, which are not yet marketed. Germany wants to implement a similar procedure to avoid uncontrolled release of exotic species. Products, which are already in the market will be covered on a positive list and will not need registration. The Pesticide Steering Committee of the OECD (Organisation for Economic Cooperation and Development) produced guidelines for the regulation of invertebrate biocontrol agents. This document exaggerated the risks involved with the use of biocontrol organisms and therefore implementation of the requirements would result in severe negative impacts on the development and marketing of EPN based products. It is most unfortunate that the OECD steering committee spent much time in producing this recommendation instead of working on a consensus document including a positive list of invertebrate biocontrol agents, which have a history of safe use. This approach was taken by the EPPO (European and Mediterranean Plant Protection Organisation), which has produced the document PM 6/3(2), containing a positive list (EPPO, 2002). EPPO states that “There is extensive previous knowledge and experience of the use of introduced biological control agents in a number of countries in the EPPO region, sufficient to indicate the absence of significant risks, or the availability of reliable risk management measures, for many individual organisms. This list accordingly specifies indigenous, introduced and established biological control agents which are recognized by the EPPO Panel on Safe Use of Biological Control to have been widely used in several EPPO countries. Other EPPO countries may therefore presume with some confidence that these agents can be introduced and used safely.” The list includes five nematode species used in biocontrol.

Should EPN be regulated?

In risk analysis the major hazard is the loss of human lives. Never in the past has there been a loss of human lives related with the use of EPN and the environmental damage caused by biological control are of much less magnitude than hazards related with alternative control measures (chemical pesticides). A particular problem is that people think that products or activities are either “safe” or “unsafe”. But the ideal situation is not a risk-free existence. Everyone analyses risks many times during the daily life and decides, which risks he is able to accept and which he needs to avoid. As everything else, also biological control causes hazards. But these risks outweigh risks related with alternative control measures and can be accepted by users and consumers. We must be aware that regulation of EPN causes risks as well. Risks are on both sides of the equation and regulation guards against one set of risks while ignoring tradeoffs related with regulation. Regulation might keep older, riskier technology like pesticides in use. If we exaggerate the risks of EPN and as a consequence governments implement major regulation of biological control, we transfer potential risks to the environment and to other groups in society. If, as a consequence of regulation, insecticides have to be used, farmers can be harmed, particularly those who apply pesticides in glasshouse environments, where they are highly exposed to chemical compound.

Governments should attempt to use effective and inexpensive tools to regulate EPN. If we take costly steps to address all risks, however improbable they are, we will quickly impoverish ourselves. The search for cheaper and more effective tools to achieve the basic goal is of major importance and might produce creative solutions for risk assessment. Tradeoffs of regulation must be considered and evaluated. Weighing the costs related with the assessment of risks of EPN and adding the costs related with countervailing risks, our societies should search for more effective possibilities to regulate risks related with the use of IBCAs than implementing registration procedures following the rules used to register chemical compounds and microbials. Biological control currently needs less regulation instead of more bureaucratic hurdles. Therefore, as a first principal, any kind of regulation of indigenous IBCAs should be avoided. Regulating the use of indigenous EPN is over-regulation without valid foundation concerning ecological risks (Blum *et al.*, 2003). If our base-line concept for cost-effective regulation of EPN is driven by the fact that beneficials have a long history of safe use than we can waive any kind of regulations for those agents, which have already been used for many years without any problems, including exotic EPN species.

The COST-OECD expert committee concluded that the use of exotic EPN, which have never been used in biological control in an ecosystem or country needs some regulation. Species should be accurately identified and specimens should be deposited. Expert opinions based on available information on origin, natural distribution, biology, host range and safety for the user are desirable to assess possible risks related with the release of exotic species (Ehlers and Hokkanen, 1996). These data should be evaluated by expert committees with the final goal of listing the exotic species on a positive list if no major risks can be identified related with the use of the exotic species. This committee would also have to consider costs related with the risk assessment and perform a risk trade-off analysis in order to weigh costs of regulation with benefits and tradeoffs. If further risk assessments are necessary before the experts can make a decision, these should be supported by the public. In order to reduce the costs for risk assessments, public-private partnerships are one possibility to gather necessary information on potential risks. Unfortunately, many countries adapt the precaution principle " better safe than sorry" and do not allow the use of exotic species at all (e.g. Norway). The consequence is that much lower numbers of biocontrol products are on the market with all related effects.

Any kind of regulation of the use of EPN in biological control should always consider the tremendous benefits to the environment related with the use of EPN. Biocontrol nematodes are exceptionally safe for users and the environment and the benefits outweigh possible risks to non-target organisms.

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