Koppert invests in quality results: from scientific research to utilization

Foliar application of Koppert nematodes offers growers a new perspective

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Entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) are way more versatile than people think. EPNs are soft-bodied, non-segmented roundworms that live in the soil and are specialized in parasitizing soil borne insects. The use of entomopathogenic nematodes to control soil pests has been a common agricultural practice for many decades. Extensive literature reports that they can also infect foliar insects, but foliar application of nematodes does not yet appear to be a popular option. For some time now, Koppert Biological Systems has invested in high quality research to explore EPNs and results show they can be highly effective, especially to control caterpillars (Figure 1). That's good news for growers, as they now have more effective options for biological control that make them less dependent on chemical products.

Figure 1. Foliar insects parasitized by Koppert nematode products (Entonem and Capsanem).
Tomato leaf miner Tuta absoluta, leaf chewer Spodoptera exigua and the oak processionary caterpillar Thaumetopoea processionea, left to right. Note the nematodes inside the heads of Spodoptera and Thaumetopoea (photos M. Aragon and L. Tonino).

It all starts in the soil

It is not surprising that the use of entomopathogenic nematodes (EPNs) has focused on controlling soil pests, as nematodes occur naturally in the soil and thrive extremely well there. Species of the *Heterorhabditidae* and *Steinernematidae* families have been effectively used as biological control agents for decades. Since they are easy to apply using the same equipment as for applying chemicals, and relatively specific to insects and non-toxic for humans, they fit well into biological control programs. For more than 3 decades, Koppert has delivered EPN products (Figure 2) that cover a wide range of soil insect pests from diverse orders such as Coleptera (grubs), Diptera (sciarid flies), Thysanoptera (thrips), and others.

When used to control soil pests, the nematodes are diluted in water and applied to the soil. Once they have found a pest/host insect, they penetrate the body and secrete the pathogenic bacteria they carry, turning host tissue into food for the nematodes and causing the larvae to die within a few days. 'The fact that EPNs can also infest foliar insects is well documented, but this hasn't been much exploited in practice yet,' Roxina Soler, lead of the Agronomical Development NL team at Koppert, explains. 'It is often assumed that nematodes would die minutes after being applied on the foliage, once the water dries off the leaf surface.'

Figure 2. Koppert nematode products

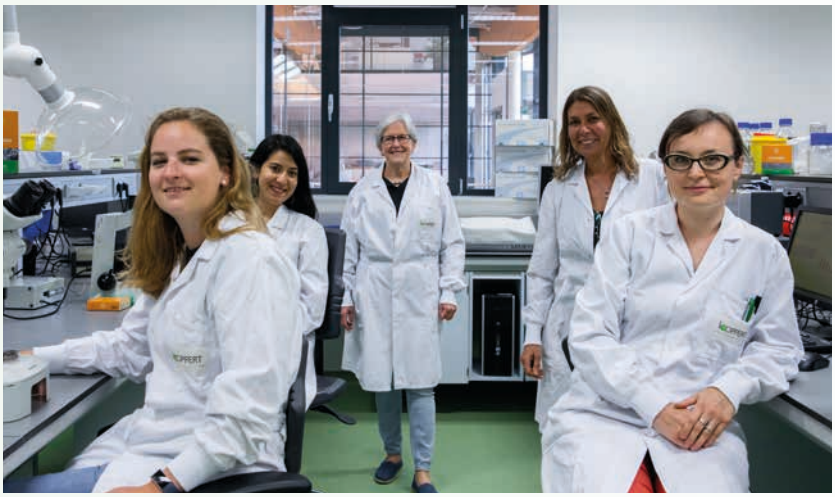
Check your strain! Ask for Entonem, Capsanem and Larvanem

Steinernema and Heterorhabditis EPNs have been extensively studied and occur worldwide. You will find them in almost every soil you dig into. But it is becoming more and more clear that EPNs can differ a great deal between populations (strains) of the same species. For example, a population of Steinernema feltiae (strain) from one location/product can differ from the Sf from another location/ product as much as with another nematode species or genera. For this reason, it is crucial to carry out solid scientific studies on the strains of interest. It is the only way to provide solid advice to growers, so this is what Koppert is doing.

Koppert invests in solid research

In recent years, Koppert's Agronomical Development teams in the Netherlands and Spain (Figure 3) have carried out extensive in-depth research into the possibilities of controlling foliar pests with the Koppert EPNs. We work with qualified scientists who develop and adjust the bioassays and carry out laboratory and climate-cell/greenhouse experiments and coordinate the field research. A specialised scientist with extended experience in experimental design and data analysis supports the design and plan of effective experiments and sound results, and scientists with experience in research and agronomy that lead the Dutch and Spanish teams.

The reason for the research into foliar application actually started with a different purpose; that of label extension. 'Koppert has been conservative in their labels, so we started studying the potential of our 3 EPNs to control more pests than the ones labelled. These included not only soil pests of economic relevance but also foliar insects,' Soler explains. The project started by developing sound protocols, to deliver high quality and reliable results. Based on scientific literature, protocols were adapted for the Koppert laboratories. 'Thereafter, the potential effectiveness of our nematodes on many different pests was studied' Soler adds.



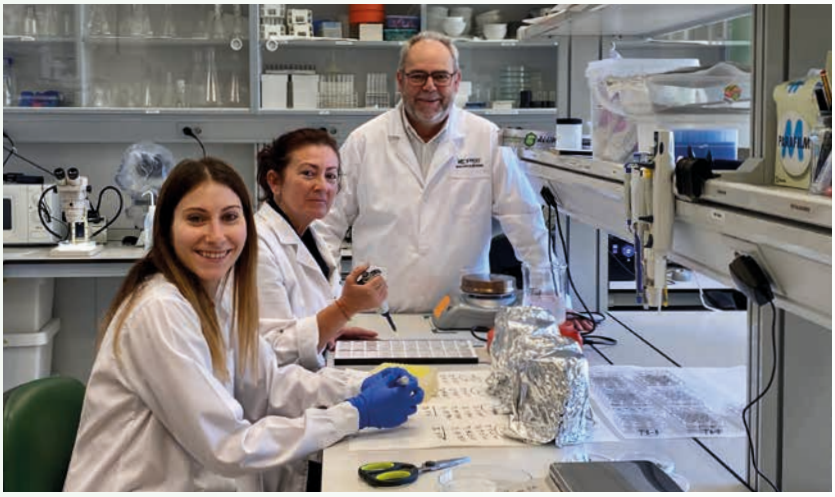


Figure 3. The scientific teams.
Agronomical Development teams working on EPNs at The Netherlands (top) and Spain (bottom), June 2020.

Surprising results

Laboratory and climate-cell tests with large plants show that Entonem and Capsanem are significantly effective against diverse foliar insects, particularly caterpillars which are one of the largest foliar pest groups (Figure 4). Interestingly, the protection that leaves offer leaf miners is not a barrier for our nematodes. Both the leaf chewer *Spodoptera exigua* (beet armyworm) and the tomato leaf miner *Tuta absoluta* can be successfully controlled by Entonem and Capsanem sprayed over the plants, as can be seen in the figure below.

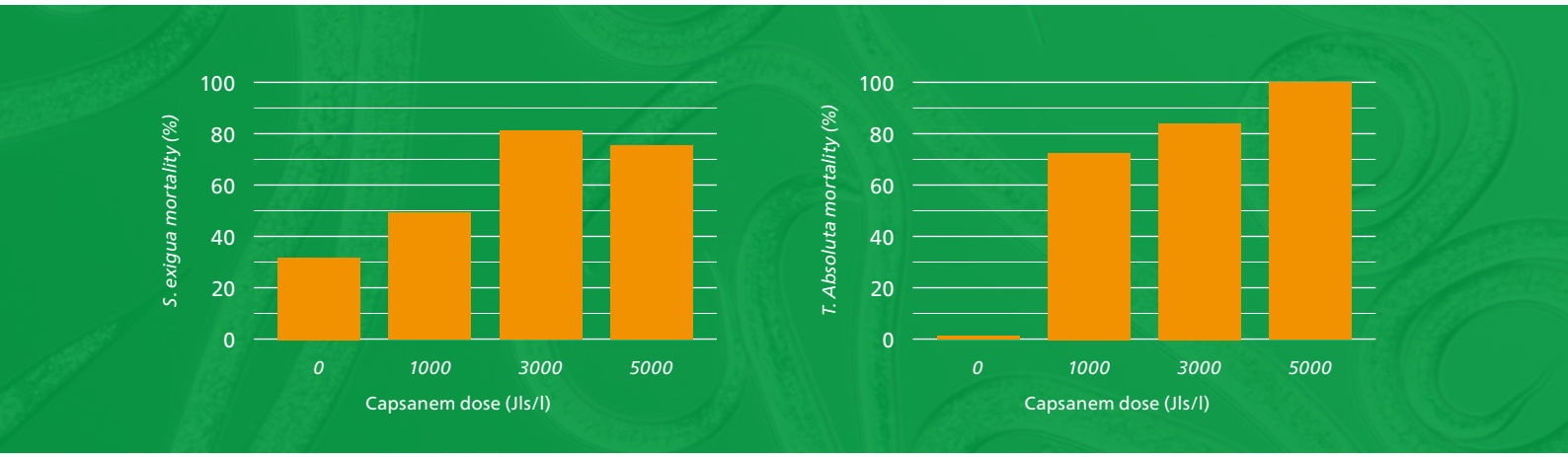


Figure 4. Control of Foliar insects by Koppert nematode products (Entonem and Capsanem). *The graph illustrates effect of Capsanem at a range of doses on Spodoptera exigua (left panel) and Tuta absoluta (right panel) larvae mortality. Orange bars show mean percentage mortality, black lines are standard errors, and black dots are raw data points (each point being a replicate). Similar results are obtained with Entonem (example not shown)!*

Over the past year, Koppert has also investigated how long nematodes can survive on leaves after being sprayed. General expectation was that EPNs would die after a few hours, soon after being sprayed and once the water dries off. These results were therefore surprising and indicated that, under favourable environmental conditions (RH > 75%), they can survive on leaves long after the water has evaporated; so much longer than expected (Figure 5). While in the soil the survival of our EPNs is relatively similar -10 days with 70 to 100% efficacy- (Figure 5, left), on the leaves survival differs significantly between Larvanem -1 day- and Entonem and Capsanem -4 to 7 days- (Figure 5, right).

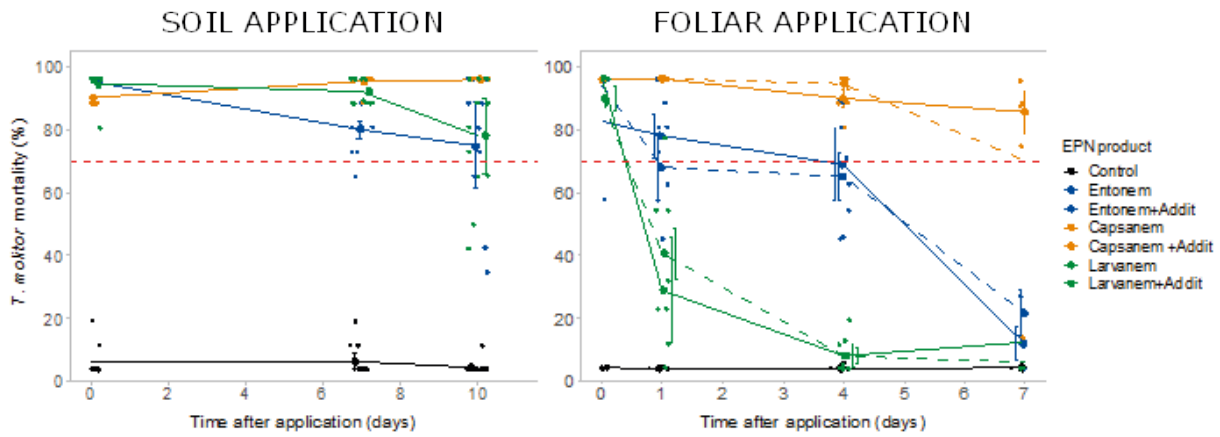


Figure 5. Mortality of model pest species (mealworms) in soil and on plant leaves (caterpillars) at different times after application of Koppert nematode products (Larvanem, Capsanem and Entonem). *The graphs show mean (large dots) ± standard errors percentage mortality, and raw data points around the mean (small dots). For foliar application, the EPN products were applied with (dashed lines) and without (soild lines) Addit.*

These results together with results on potential efficacy on foliar pests position **Entonem and Capsanem for foliar applications.**

The right nematode for the job: temperature matters

Temperature has a great influence on the efficacy of EPNs. In recent years, Koppert has carried out extensive research to define the temperatures at which Koppert's nematode products thrive best. EPNs are typically able to survive over the whole continuum of tested temperatures from 5 (storage temperature) to over 30 degrees Celsius, but can infect a host and reproduce over a more restricted range, and reach high level of infectivity within an even narrower band. 'We have studied to what extent temperatures affect our EPNs,' Soler explains. 'Our first results show that Entonem (blue) response curve shifts to the lower temperatures (cold tolerant nematode), whereas the Capsanem (orange) and Larvanem (green) response curves are very similar and shift more to the higher temperatures (Figure 6).

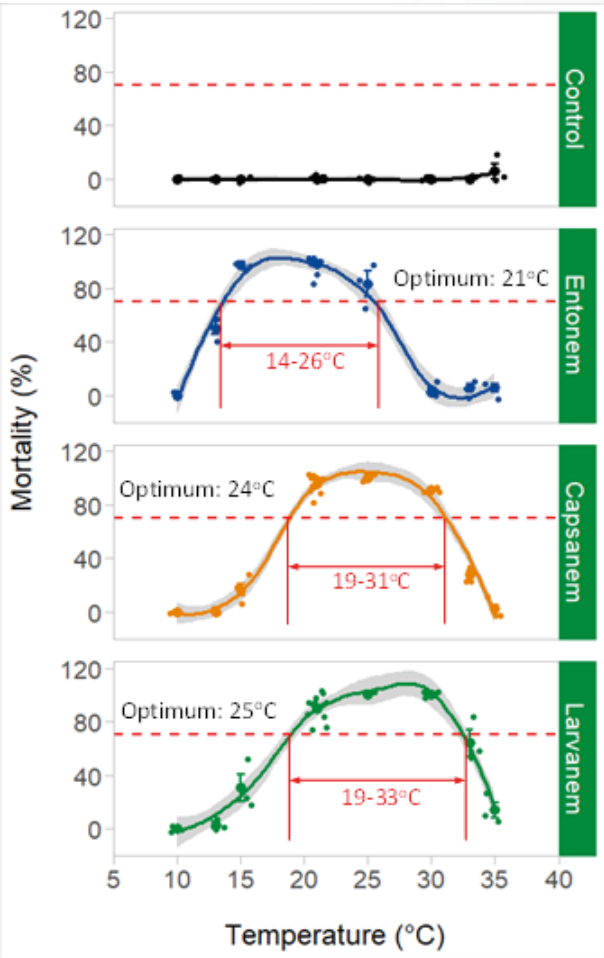
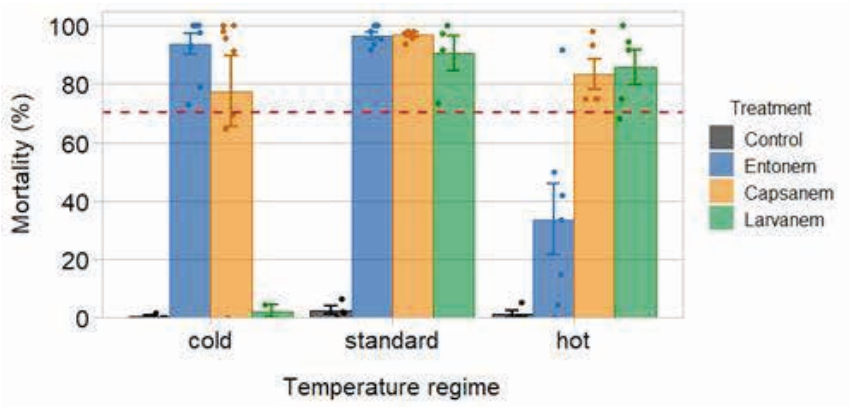


Figure 6. Mortality of model pest species (mealworms) in sand column bioassay at different temperatures.
Large dots show mean ± SE percentage mortality. Small dots represent the raw data points. Temperature is presented as continuous variable. The lines are local polynomial regression lines with 0.95 confidence intervals. The optimal range of infectivity (i.e. >= LD -lethal dose- 70%) is indicated in red (solid) lines. The optimum of the temperature is based on the weighted averaging procedure.

But this is when using constant exposure to the different temperatures tested, so does not take into account the cold/hot extremes experienced during the days. Temperature is not constant but varies along the day, even in cold or warm days the extreme temperature does not last the entire day but is often for a number of hours only. Taking this into account, hot and cold extremes were mimicked and further tested (Figure 7).

Figure 7. Mortality of model pest species (mealworms) after application of EPN products (Larvanem, Capsanem and Entonem) at "extreme" temperature regimes. *"Cold" regime (periods of low 5oC), "standard" (24 hours at 21oC) and "hot" (periods of high 35oC). The bars show mean ± SE percentage mortality. Dots represent the raw data points from multiple trials. The red dashed line corresponds to the 70% mortality. In blue Entonem, in orange Capsanem and in green Larvanem.*



This different approach confirms that Entonem's (blue) performance is better/more stable in cold days, in line with the previous results, but allows further distinction with the other two products. Capsanem (orange), despite more variation as the spread data points shows, closely follows Entonem in the clod end. This, together with the results that shows that it also performs well at hot temperatures, makes Capsanem what we call 'our versatile nematode', ideal for spring days when relatively cold and warm temperature can be reached along the day. Larvanem (green), in line with previous results, performs well on hot days. Back to foliar application, this makes Entonem a very good candidate for foliar pests in cooler days (cold tolerant Koppert strain), and Capsanem a plastic nematode to be used when temperatures may fluctuate significantly during the day, like in early spring.

Conditions for effective application

In addition to choosing the right nematode, creating the most optimal conditions is essential to maximize the efficacy of the foliar application of our products. Humidity is a key factor and it should be as high as possible. Applying the product at the end of the day is therefore highly recommended as it will be less dry, while UV will also be lower. Dosage also plays an important role. 'Our average recommended foliar dose is 3 million per litre. But for pests that are difficult to control and/or adverse environmental conditions, such as dry conditions, we advise a dose of up to 5 million if possible. While for pests that are easier to control and/or optimal environmental conditions, such as humidity above 75%, 1,5 million can be sufficient,' Soler recommends. Together with the dosage, it is essential to cover the entire leaf surface with water, and so with the EPNs. 'Nematodes can only move about 10 cm per day in optimal soil conditions, so we can't expect them to carry out a long search for the pests along the plant'. Therefore, try to apply them at the end of the day. Use the right dosage and cover the plant with water. And if possible, use an adjuvant for a better distribution of the water on the leaves. All these application tips will facilitate the job of our nematodes, so efficacy/control will be maximized,' Soler advises 'Your technical Koppert consultant can help you take the best decision for your specific situations.'

Multiple benefits

In summary, the substantial investments that Koppert keeps making in research, in this case in the foliar application of nematodes, are bearing fruit. Based on the results of our research, we can provide growers with reliable well-founded advice. Koppert EPNs can help growers to make their business operations more sustainable and offer new effective biological control options for foliar pests that are becoming increasingly difficult to control, even with chemical products. Successful results point to Entonem and Capsanem as two very promising solutions for foliar pests, especially caterpillars!

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