Experiences with entomopathogenic nematodes for the control of overwintering codling moth larvae in Germany

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Abstract

Entomopathogenic nematodes were tested for their potential as tool in resistance management of codling moth Cydia pomonella *L. in organic fruit growing. In two field tests, the adults emerging from stems treated with nematodes were monitored. In one trial, 90 % of efficacy could be shown. On-farm trials with favourable weather conditions showed an efficacy about 50-60 % on fruit infestation by codling moth in the year following the application. On-farm trials with unfavourable weather conditions showed no results. The favourable weather conditions for the application are discussed with reference to German conditions.*

Keywords: *Cydia pomonella*, entomopathogenic nematodes, *Steinernema feltiae, Steinernema carpocapsae*

Introduction

The codling moth *Cydia pomonella* L. (CM) is an important pest in fruit growing in whole Europe. Some years ago, CM control in organic fruit growing seemed to be well established with a combined strategy of mating disruption and the *Cydia pomonella* granulovirus CpGV. In 2004, the first case of resistance against CpGV was proven in two organic orchards in Germany (Fritsch et al., 2005). Since then, resistance management became important also for organic farming and new tools for combination strategies in CM control should be found. In the orchards with CpGV-resistant CM populations the level of infestation was very high and exceeded rapidly an unmanageable level (level of no control). Thus, there was a great interest in methods for the reduction of populations. The efficacy of entomophagous nematodes (EPN) reported by Lacey et al. (1998, 2005, 2006) was shown under German conditions first time by Elias (2006). In a project financed by Deutsche Bundesstiftung Umwelt several methods of CM control are tested for their potential and should be possibly improved. In this context, the potential of EPN as tool for resistance management in CM control in organic fruit growing should be examined.

Material and Methods

To get more information about **overwintering sites** of CM larvae, 28 trees of an orchard with high infestation of CM were removed. The trees were separated into four parts: roots, stem, middle part, treetop. Each part was stored separately in big boxes. In spring, before hatching of CM, the boxes were wrapped in dark plastic material and kept in a barn out of the sun. At each side of the box, glued honey glasses were applied to catch the hatching moth (figure 1). The number of moths were assessed at 16. July 2007.

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For an exact evaluation of the efficacy of the EPN on the overwintering population of CM, two **field trials** with randomised block design and 4 repeats (10 trees per repeat, 1 border tree) were conducted in orchards with high infestation of CM at the locations A (near Stuttgart) and B (near Lake Constance). At location A, the trees of cv. Elstar were grafted on M9 (ca. 10 years old) with a pile of pine wood at an intertree distance of 1.50 m. The CM infestation in 2006 was about 5-7 %. At location B, the orchard was rather old with cv. Boskoop. Not all trees had piles. The infestation at harvest in 2006 exceeded 10 %.

In location A, the application was done with a motorised knapsack sprayer (SOLO) until runoff. Only the stem until 1 m height was treated. In location B, a herbicide sprayer with two flatfan nozzles with >0.5 mm orifice was used for the treatment of the stem (1 m). Per m tree height, 500 l of water per ha were applied with 4 bar pressure. The amount of nematodes was calculated per m tree height. During applications, the rows were passed always twice in opposite direction to ensure a good wetting even of the inner part of the stem. In both trials, 0,25 l of Trifolio-S forte per ha and m tree height was added as wetting agent.

In location A, the EPN were applied at 17.10.2006. The treatment was done in the evening when the stems and the grass were humid with dew and the RH reached 100 %. For the following day, rainfall was forecasted but did not occur. The temperature during the treatment was about 15 °C, during the night it decreased reaching 12 °C, the following day it oscillated from 13 to 19 °C.

In location B, the application was done at 12.10.2006 late in the evening when fog was setting in. The treated stems did not dry. During the night and the morning it was foggy, the temperature in the evening at 18.00 during application was 15°C and discended to 9,5 °C at 8.00 a.m. at the following morning. The stems started drying about 11.00 a.m. next morning, the temperature at this time was about 12°C. The forecasted rain did not fall on the trial site.

Figure 1: Eclectors for assessment of overwintering CM in the field trials and in the boxes

For the assessment, at the stem of each tree shortly before the beginning of CM flight, an eclector with dark fleece material and rubber foam was applied (figure 1). At the top of this eclector a glued honey glass was installed to capture the adult moths flying against the light. It seemed, that the hatching was remarkably retarded by the eclector. Furthermore, not all moths went into the glass. Thus, when the eclector was removed, the stem was examined carefully for remainings of hatched pupae. It could happen, that adult moth were found and no remainings of pupae and also pupae and no adult moth.

Thus, for each moth found in the glass, the number of pupae remainings was subtracted for the number of moth found to avoid double counting.

A certain mistake is possible since evidently not all pupae were found and not all moths went into the glass. Nevertheless, since this mistake was distributed equally over all repetitions, the data can be used.

The eclectors were applied on 23./24./27.4.07 in location A and on 25./26.4.07 in location B. In both orchards, CM flight was monitored with pheromone traps and first moth were found in the week following the application of the eclectors. The eclectors were removed the 13.7.07 at location A and at 16.7.07 at location B.

The **on-farm trials** were conducted in the German regions Rhineland-Palatinate (trial 1 and 2) and Baden-Wuerttemberg (trial 3).

Since the experimental design requires rather big plots and the orchards in these regions are rather small, repetitions were not possible. The orchards were just divided in two parts transverse to the tree rows. Before harvest in 2006, the CM infestation was assessed. In all orchards, there were only marginal differences in fruit infestation between the plots used for the trials.

If only the stems were treated (1 m tree height), the application was carried out with a herbicide sprayers with flatfan nozzles and a special construction (figure 2). If the whole tree was treated, a normal sprayer with flatfan nozzles was used.

Figure 2: herbicide sprayer with special arrangement for treatment of stems

In all trials, 0,25 l of Trifolio-S forte per ha and m tree height was added as wetting agent.

In 2007, in trial 1 and 2 the main assessment was done during harvest. In each treatment, a complete big box (ca 500 kg) of fruits was collected from marked trees, which were spared when infested fruits were removed before harvest and CM infestation assessed. Thus, the economical damage for the grower can be estimated very good.

In trial 3, the infestation of fruits by first CM generation was assessed on 1500 randomly selected fruits per plot and variety. The statistical analysis was done by analysis of variance and Tukey-Test ($p<0.05$).

Results

The study of the **overwintering of CM** presented some surprising results: From the boxes with the middle part of the trees nothing emerged. One moth emerged from the box with the roots, 8 moth from the stems and in the eclectors of the treetop box 15 moth were found. These results seemed surprising since the treetop seemed to present no hiding places for the larvae. Thus, the content of the box was examined very carefully to find the exuviae of the pupae. It was found, that in the treetop several fruits damaged by fungal diseases like *Monilia* spec. had remained from the year before 2006. These fruits were used evidently by the larvae as hiding places. It can be excluded that the fruits or the larvae were originary from fruits CM infested fruits stored in the boxes before since these were not used 2006.

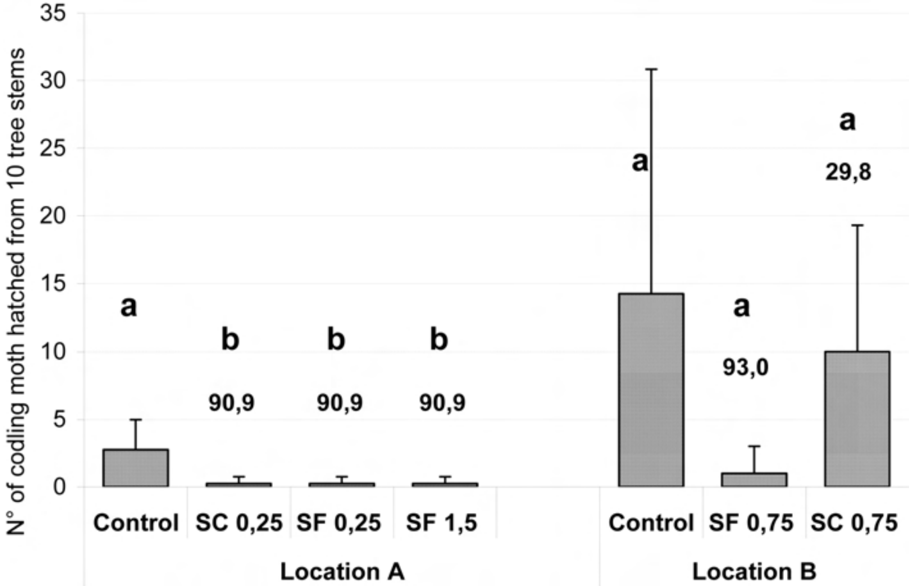

Figure 3: Number of codling moth hatched and degree of efficacy (ABBOTT) in % in the different treatments with *Steinernema feltiae* (SF) and *S. carpocapsae* (SC) in the trials at the two locations A and B. The amount of EPN per ha and m tree height is given in billions (10^9). Different letters indicate a significant difference (TUKEY-test, $p<0.05$).

Table 1: Results of on-farm trials in the season 2006/2007: Control of CM infested fruits in 2007

Tr. Nr.	Nematode species	N° of EPN per ha and m tree height	Tree height (part of the tree treated)	Amount of water per ha	Date of application	Weather conditions	Degree of efficacy (ABBOTT) in %
1	*S. feltiae*	1,5 x 10^9	1 m (stem)	500 l	19.10.06	14 °C, 100 % RH, rain after treatment	Gala: 68.2 Elstar: 65.8
	S. feltiae	2 x 10^9	2 m (whole tree)	1800 l	19.10.06		Gala: 49.9 Elstar: 68.2
2	*S. carpo-capsae*	0,75 x 10^9	1 m (stem)	500 l	20.10.06	16 °C, 80 % RH, rain before treatment	Gala: -66.3 Elstar: -2.2
	S. feltiae	2 x 10^9	2 m (whole tree)	1800 l	20.10.06		Gala: -28.5 Elstar: 4.2
3	*S. carpo-capsae*	0,75 x 10^9	1 m	500 l	27.10.06	12-16 °C rainfall after evening treatment wet during following period	Idared: 19.4 Sommer-regent: 12.7

In location A of the **field trial**, all EPN treatments showed a significant reduction of the surviving adults on the stems with high degrees of efficacy. There was no difference between the high and the low amount of *S. feltiae.* In location B, the CM population was very high. Here the distribution between the different plots was not so homogeneous and there were some single trees that seemed to be preferred hideaways for the diapausing larvae. There was one tree with a very high number of larvae in the control so that the differences between the treatments were not statistically significant. Nevertheless, the treatment with *S. feltiae* showed a considerable reduction of the infestation whilst there was few effect shown of *S. carpocapsae* (figure 3).

In the on-farm trial 1 the results were rather good for stem and whole tree treatment. There was only marginal difference between these two treatments. In trial 2 with worse weather conditions both treatment showed no efficacy. In trial 3 only stem was treated. At the assessment it could be observed that the upper and untreated part of the trees had aerial roots and some other hiding places for diapausing larvae.

Discussion

These trials confirm in principle the findings of Elias et al (2006) and Curto et al (2008) that EPN application on diapausing CM larvae can reduce the fruit infestation in the following year about 50 – 70 %. The results from assessment of the adults emerging from treated stems in the field trials suggest better efficacies. On of the reasons might be that in this case the direct efficacy of EPN on the emerging adults was assessed. When the infestation of fruits in the following year is the assessment criterium, orchards with high CM populations are chosen to achieve reliable data. If the emerging adults of a very high population is reduced in the EPN plot, there may be less competition in this plot in comparison to the control plot. This could lead to a certain compensation of the efficacy of EPN on the emergence of adults by more successful oviposition of the remaining adults. Thus, even high efficacies of EPN on adult emergence may result in medium efficacies in fruit damage in the orchard.

This effect could reverse if the population is not as high as in these trials and in the year after the treatment mating disruption is applied. In this case, the EPN treatment can reduce the population to a level where mating disruption is more efficient. Thus, in orchards with medium or rather low population levels, EPN application should also tested. In combination with mating disruption it might prove to be an even more valuable tool in maintaining low population levels than it seems to be in the reduction of high populations.

The on-farm trial 3 showed no result although the weather conditions were favourable. A possible reason might be in this case, that the upper part of the tree remained untreated whilst it presented hiding places for diapausing larvae. In trial 1, with different and younger trees, the stem and the whole tree-applications showed no difference even if the amount of EPN applied per m tree height is not really comparable.

This indicates, that the decision if only stem or whole tree is treated depends of the orchard and must be decided in each special conditions. More research should be done to find out the overwintering places of the diapausing larvae. Our findings with the *Monilia*-infested fruits show, that “surprises” cannot be excluded and that an orchard has to be examined very carefully before deciding to treat only the stem.

The difference in efficacy between the on-farm trial 1 and 2 can be explained by less favourable weather conditions in trial 2. On the other hand, in the field trials in both locations, there were considerable results even if the EPN were applied without rainfall before or after the treatment, just using the humidity of dewfall during the night. Elias also had good efficacy with lower umidity, applying a very high amount of water.

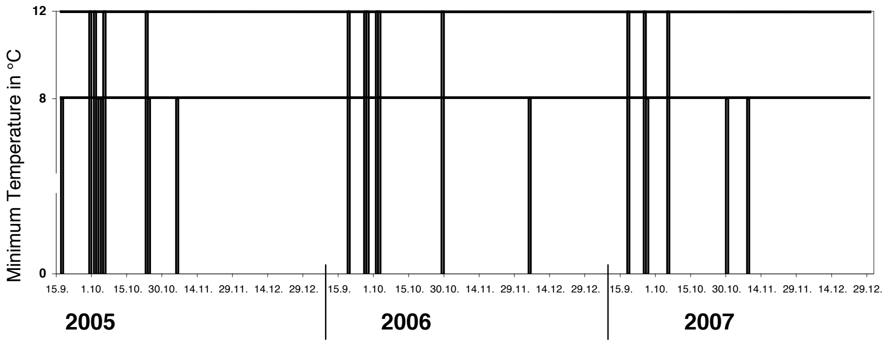

Figure 4: Number of days with favourable weather conditions for EPN treatments (stems humid or wet, rain or RH > 95 % during almost 9 h, minimum temperature > 8 (*S. feltiae*) resp. 12 °C (*S. carpocapsae*); when the temperature discended under the minimum and the humidity remained high, the period was not considered as interrupted)

In the trial in location A, the lower efficacy of *S. carpocapsae* might be due to the lower temperatures which were not always higher than 12 °C during the period after application. For most regions in Germany it must be considered that farmers have no possibility of irrigation. Main experiences with EPN derive from Lacey et al. (1998, 2005, 2006). In his country it is possible to irrigate, and the optimal conditions are easy to achieve with pre-application irrigation. In our conditions, the weather conditions necessary for a good efficacy of the treatment turn out to be a key question for the successful use of EPN. Since there is evidence for efficacy problems in some cases of not really favourable weather conditions, the recommendation for practice actually must be to treat only with really favourable conditions. There is a certain consense that EPN should not be applied just at harvest time of the fruits.

In most organic orchards there is also need of a mechanical soil cultivation to remove weeds from the bottom part of the stem before EPN are applied. This can be done only after harvest when no fruits are damaged by passing with the machine.

Thus, the period when EPN can be applied starts in mid of September (often also mid of October depending of the end of harvest). It is assumed that in January the diapausing larvae start to reinforce their cell for pupation and then it might be more difficult for EPN to penetrate the web. Results with spring applications of EPN indicate a low efficacy (Curto et al., 2008) so that it seems justified to fix the end of the possible period for EPN application at the end of December.

In figure 4 for the years 2005-2007 in a location near Lake Constance the days are indicated when good weather conditions for treatments were given for the two EPN species *S. feltiae* and *S. carpocapsae*. Especially after October there are few possible application dates, still fewer if *S. carpocapsae* is used which requires a higher minimum temperature (12 °C, (Grewal *et al.*, 1994). This situation is not really comfortable for an application.

Unfortunately, none of the laboratory and semi-field trials carried out in 2006 and 2007 in the frame of this project to clarify the climatic conditions necessary for a reliable and repeatable efficacy gave really sustainable results (Kienzle, Jung et al., unpublished results). Since the conditions refer not only to the surface of the stem but mainly to the hiding places of the larvae they are very difficult to define.

Further research is necessary but it is possible that to ensure efficacy practice must always rely on the weather conditions mentioned above. Actually, application technique, which can be improved since it is clear that higher pressures can be used ((Wright *et al.*, 2005)) and additives are tested with the aim to improve efficacy, especially with not so favourable weather conditions.

Nonetheless the questions still to solve EPN seem to have the potential to become a valuable tool for resistance management in CM control in organic fruit growing. If they become part of the common strategy, it is important to understand also the effects on the ecosystem in the orchard. It is well known that EPN are harmless for the common earwig (*Forficula auricularia* L.) which is most exposed to the stem applications (Nachtigall, 1991; Georgis et al., 1991, Grewal et al., 1993). A potential effect on the whole arthropod system is difficult to monitor. In each case, the treated plots should be carefully observed during the introduction of the EPN in practice.

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