Three years experience with entomopathogenic nematodes for the control of overwintering codling moth larvae in different regions of Germany

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Abstract

The potential of entomopathogenic nematodes (EPN) for control of overwintering larvae of Cydia pomonella *L. was tested during three years in numerous field experiments in three regions of Germany (Lake Constance, Rhineland Palatinate, Saxony). In all experiments,* Steinernema feltiae *(Filipjev) with 0,75 x 10^9 EPN per ha and m stem height was used. The long term efficacy on the infestation in the following year in relation to weather conditions 12 h after application is reported. If conditions were favourable, the efficacy of the treatments ranged about 40 to 50 % on average.*

An optimal application technique was developed with a high amount of water (750 l/ha and m tree height) and special nozzles. Investigations on the overwintering sites of the Codling moth larvae revealed that not only the trees but also the posts are important hideouts for the larvae. From bamboo and pinewood posts removed from the orchards a considerable number of adult emerged. Recommendations are given for application of EPN in organic fruit growing.

Keywords: Codling moth, *Steinernema feltiae*, nematodes

Introduction

The efficacy of entomopathogenic nematodes (EPN) against overwintering larvae of the Codling moth was reported by Lacey et al. (1998, 2005, 2006). In a project financed by Deutsche Bundesstiftung Umwelt several methods of CM control were tested for their potential as tools in a combined strategy for Codling moth biocontrol. The aims of this project were to elaborate optimal recommendations for fruit growers to use these biological agents.

In three German regions (Lake Constance, Rhineland Palatinate, Saxony)and different environments field experiments were carried out from 2006 to 2009. Based on the reliable and repeatable results obtained and due to the high number of experiments an even reliable recommendation is possible.

Material and Methods

Experimental design

Since the assessment of overwintering CM larvae directly in the orchard is not really possible, it is always difficult to determine the efficacy of control of these larvae directly. Thus, the determination of effects is possible only after adult emergence.

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For this reason, the experiments were conducted with two methods:

1. Field experiments, large plots

In these experiments, nematodes were applied in autumn. Control efficacy was assessed as fruit infestation in the following year. Since adults disperse within the orchard, the plots had to be chosen rather large. In each case, fruit infestation of the first CM generation was assessed on 1,500 to 4,000 fruits per plot. When plots were large enough and when it seemed reasonable, infestation by the second generation was also assessed.

This experimental design needs very large plots. Thus, in many experiments there was no possibility for repeats or randomized block design. If the orchard was large enough to allow repeats, often the infestation was not distributed homogenously in the whole orchard and repeats were not considered. With an efficacy about 50 % usually achievable with entomopathogenic nematodes, natural variability of infestation may reach the same values and render impossible any conclusions from such experiments To counterbalance this technical problem, many experiments with one or two repeats were designed in different orchards, i.e. representing different environments, and the orchardswere put into the rank as repeat.

A conclusion to be drawn from results is also difficult if the infestation is low. Thus, in the summary of results only experiments with an infestation of 2 % in the first or 4 % in the second generation were considered. Usually, the infestation in the orchards used for the experiments was assessed at harvest in the years before. If plots varied too much in the level of infestation, the orchard was also not considered. In one year, large plot experiments with repeats but without assessment of infestation in each single plot in the preceeding year were not recorded because differences in infestation between single repeats of one treatment exceeded 50 %.

One experiment in Saxony had two repeats. In one repeat, bamboo posts were removed in the year following the application, stored and emerged adults were assessed in the following summer. In the other repeat, the posts remained in the orchard. With this experiment it was possible to estimate the importance of cracked bamboo posts as hibernation sites for CM larvae. In this experiments, all fruits on 40 trees per plot were assessed at the end of the first generation.

In all experiments, except that in Saxony, regular applications of Codling moth Granulovirus were done during the season.

2. Trials with trees uprooted in the winter season following application

These experiments could be carried out only in orchards with high natural infestation which were uprooted in the winter season following the application in autumn. In this case, it was possible to treat small plots and to work with two repeats. The experiments could also be used for the determination of overwintering sites of CM larvae.

In trial A the trees were uprooted in spring after application and stored in big boxes until adult emergence. The boxes were covered with nettings. Adults were collected in eclectors lined with glue and put under a light source. In this trial, the trees were divided in three parts: stem, middle part, crown. These parts were assessed separately to get more informations about the overwintering places of the larvae. In each repetition, 8 trees (16 trees per treatment) were assessed. The posts were assessed separately.

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Trees in experiment B were uprooted in winter. In spring, 30 stems per plot were wrapped in fibrous web. Posts were treated in the same way. The web was opened two times in the season to get access to emerged adults. The weather conditions after application were favourable (i.e. high humditiy and T > 8 °C) for 24 h after application.

Nematodes

In all experiments, *Steinernema feltiae* was used. The preference for *Steinernema feltiae* is due to the fact that this nematode species is active even at lower temperatures. Under German climate, in autumn it is difficult to find days in autumn with a high relative humidity correlated with higher temperatures (Kienzle et al., 2008). The amount of EPN used was $0{,}75 \times 10^9$. In all treatments, Trifolio-S forte, a formulation agent based on plant oils, was added to the treatments (2,5 l per ha and m stem height). In experiment B, additionally to Trifolio-S forte, the polysaccharide Xanthan was added (1 % w/v). This formulation is tested actually to delay drying of the stems after application.

Application technique

Whole tree treatment:

In 12 of the experiments shown in figure 2, a special technique developed for EPN application was used: Based on a normal sprayer with nozzles Lechler ID 90-06 grey and pressure 8-10 bar, the bottom nozzle was replaced with a TWIN Spray CAP with two nozzles. The cap must be adjusted to ensure optimal treatment of the bottom part of the stem (figure 1).

In one experiment in 2006 flatfan nozzles were used (signed with 1 in figure 2).

Treatment of the bottom part of the stems only (1 m tree height):

In one experiment an herbicide sprayer with flatfan nozzles and a special construction (not depicted here) was used to treat only bottom part of the stems (signed with 2 in figure 2).

For all applications all filters of the sprayer were removed. In all experiments, the amount of water applied was 750 l/ha and m tree height.

Figure 1: TWIN SPRAY CAP with nozzles and optimal direction of the twin cap towards the bottom part of the stem

Results

Overwintering places of CM larvae

The most important place for overwintering CM larvae on trees was the bottom part of the stem. On normal trees on M 9, few or no larvae were found in the crown and the middle part (table 1). On elder trees with aerial roots and cracks, larvae could also be found in the upper part. Even fruit mummies were accepted (Kienzle et al., 2008). Many larvae, however, were found not on the tree but on the posts. Bamboo posts cracked open or pinewood posts with cracks were the preferred hiding places of the larvae. In the experiments A and B more larvae were found in the posts than on the trees (table 1 and 2). The larvae were distributed over the whole height of the posts. This means in practice, that in orchards with these conditions, the whole height of posts must be treated even if the trees do not offer hiding places in the middle part or the crown.

In the plot in Saxony where bamboo posts were removed in combination with EPN treatment, efficacy on the first generation in the following year was very high. Efficacy of EPN were lower in the plot with bamboo posts (table 1).

Table 1: Results of a field experiment in Saxony in the year 2007/2008: Comparison of efficacy of EPN on infestation by CM in the first generation (assessed on 40 trees per plot) in two plots with and without removing of bamboo posts.

Treatments		Infested fruits per tree	Efficacy in relation to untreated control of the same plot	Efficacy in relation to untreated control of the plot where posts remained	Adults hatched from 100 bamboo posts
Plot where bamboo posts were removed	EPN-treated	1,2	50 %	**82,9 %**	283
	Untreated control	2,5	--	65,9 %	418
Plot where bamboo posts remained	EPN-treated	5,0	31 %	31 %	--
	Untreated control	7,2	--	--	--

The number of adults emerged from stored bamboo posts points out the importance of these posts as overwintering places. The efficacy of EPN treatment on the larvae in the posts only reached 31,3 %. This corresponds to the results in the field. Infestation in the plot with bamboo posts removed was much lower. The efficacy of the treatment with EPN, however, was as high as usual: about 50 %.

The combination of removing bamboo posts and application of EPN resulted in an control efficacy of 82,9 % in terms of Codling moth infestation in the first generation.

Table 2: Number of adults emerged during summer 2008 from different parts of trees and of posts treated with *Steinernema feltiae* (0,75 x 10^9) in autumn 2007 in comparison to trees from the untreated control plots.

Treatment	stem	Middle part of the tree	Crown	Posts
Treated with EPN	3	0	0	4
Untreated	10	0	0	20

Efficacy

In figure 2 the efficacy of 14 field experiments with large plots is summarized in relation to weather conditions. Most experiments with favourable weather conditions for 12 to 24 h after application showed an efficacy of about 50 % on fruit infestation in the year after application. If the weather conditions were not favourable during a period of about 12 hours after application, efficacy of the treatment was decreasing. In two cases the efficacy was low even if the conditions were favourable.

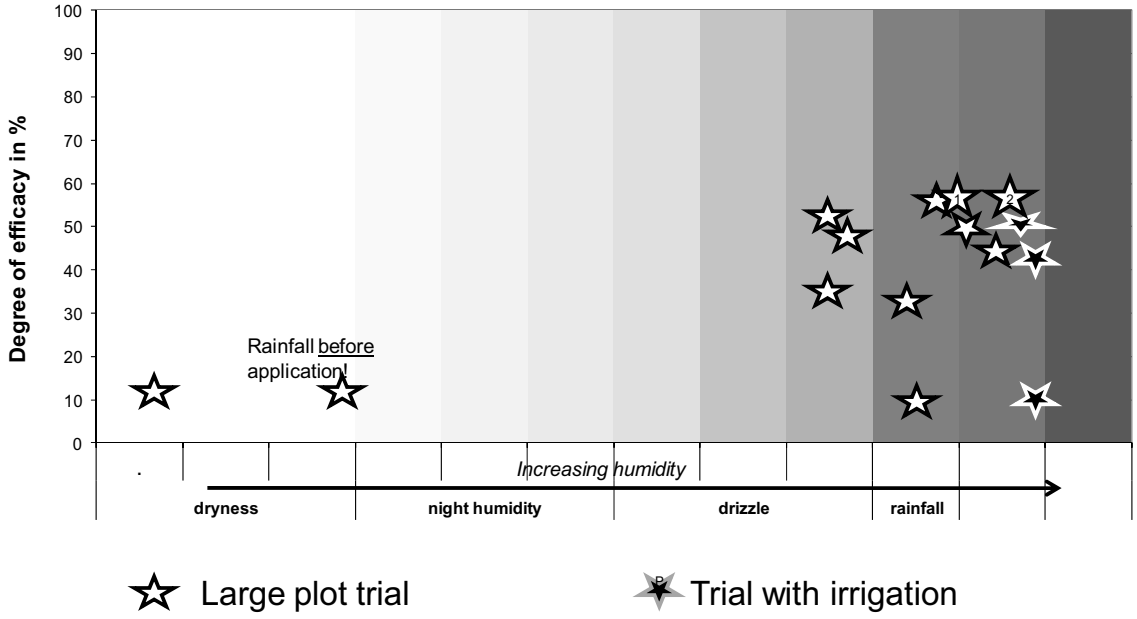

Figure 2: Efficacy of different large plot trials with *S. feltiae*, 750 x 106 EPN/ha and m tree height in relation to weather conditions 12 hours after application. Numbers 1 and 2 indicate that flatfan nozzles were used for application.

Table 3: Number of adults hatched during summer 2009 from tree stems and posts treated with *Steinernema feltiae* in autumn 2008 in comparison to trees from the untreated control plots in Experiment B.

Plot	Assessment 17.7.09	Assessment 2.9.09	Total number
Treated stems	35	10	45
Treated posts	65	7	72
Total treated plot	**100**	**17**	**117**
Untreated stems	108	15	123
Untreated posts	118	14	132
Total untreated plot	**226**	**29**	**255**

In experiment B the assessment of the adults emerged from the treated and untreated plots showed an efficacy of the treatment of 54 % (Table 3).

Conclusions

For the decision whether the whole trees or the stems only have to be treated, it is important to know the overwintering sites of the CM larvae. >In this context, not only the trees but also the posts have to be considered If the orchard has posts that are favourable for overwintering larvae, the whole tree must be treated even if the trees themselves offer few overwintering places beyond the bottom part of the stem. For new plantations in regions with high infestation of CM, it is recommended to abandon the use of bamboo posts and possibly also of pinewood posts since these structures offer too many hideouts for the CM larvae.

For the efficacy of the application of EPN, it is concluded:

- The application of *Steinernema feltiae* with a rate of 0,75 x 10^9 per ha and m tree height reduced in most cases the infestation in the field in the year following application. Efficacy was about 40 to 50 % when weather conditions were favourable.
- Timing of the application is the dominant factor to obtain a high efficacy. If the weather conditions are not favourable during a period of about 12 hours after application, efficacy of the treatment is decreasing.
- In Germany, except Northern Germany, usually there are only few days with weather conditions favourable for EPN application. Since timing is much more easier if lower temperatures are required, only *Steinernema feltiae* is recommended – except for orchards with irrigation system. This can be different in other regions (Curto et al., 2008)

Further research is done on the development of formulations reducing the risk of low efficacy if weather conditions change unexpectedly.

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