Effectiveness of entomopathogenic nematodes in the control of *Cydia pomonella* larvae in Northern Italy

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Abstract

Since 2006, a large scale research on the effectiveness of entomopathogenic nematodes (EPN) in the control of codling moth (CM), Cydia pomonella, *overwintering larvae has been performed on about 35 ha of pear orchards per year in Emilia-Romagna, Northern Italy.* Steinernema carpocapsae *and* Steinernema feltiae *activity was checked after spray applications of EPNs to the trunk and branches. In 2006, the treatment was applied either in spring or in autumn at different doses, before CM pupation; in 2007 the EPN treatment was applied only in autumn at 1.5 x* 10^9 *I.J. ha* $^{-1}$*. Every year it was distributed by means of a conventional mist blower. The larval mortality was assessed directly on sentinel larvae in card boards on the trunks and indirectly on the eggs laid by the females of the first CM generation in spring. Moreover, each year, a trial was performed applying only* S. carpocapsae *on sentinel larvae with the aim of testing this nematode at suitable temperatures but at different water volumes.*

The CM sentinel larvae were effectively parasitized after autumnal EPN application. Moreover, the egg assessment demonstrated a good decrease in CM population in spring 2007, when EPNs had been applied at the best weather conditions (t° 12-14 °C and rain) in the previous autumn.

Keywords: Codling moth, *Cydia pomonella*, biological control, entomopathogenic nematodes, *Steinernema*.

Introduction

The codling moth (CM), *Cydia pomonella*, is a key pest in pear and apple orchards. It completes 3 generations in a year and overwinters as mature larva in the bark shelters. The damage is inflicted to the fruit, caused by the larvae.

Large scale trials have been performed in pear orchards, with the aim of evaluating the effectiveness of entomopathogenic nematodes (EPN) in the CM control, applying either *Steinernema carpocapsae* or *Steinernema feltiae* to the trunks, in autumn or spring (Lacey & Unruh, 1998; Lacey & Unruh, 2005; Lacey *et al*., 2006; Shapiro-Ilan *et al*., 2005), as continuation of some previous trials carried out in plots against either CM larvae (Reggiani *et al*., in press) or other target pests as saw flies (Curto *et al*., 2007).

Moreover, each year, a trial was performed applying only *S. carpocapsae* on sentinel larvae, with the aim of testing this nematode at suitable temperatures, but at different water volumes.

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Materials and methods

In 2006 the characteristics of the chosen orchards were settled as high CM pressure and ascertained resistance to the insecticides.

2006: Spring trials

Locations: Emilia-Romagna, Po Valley, Northern Italy; 3 sites in Modena (Castelfranco Emilia) and Bologna (S. Matteo della Decima and Galliera) provinces.
Orchards: pear cvs. William and Abate Fetel.
Experimental design: randomised block, with 3 repetitions each 1 ha.
The EPN suspension was applied in the late afternoon, by means of a conventional mist blower, at 5% larval pupation, according to a simulation model (Table 1).

Table 1: 2006. Spring trials on wide surface. Treatments and doses.

Orchards (ha)	Timing	Weather conditions	Treatment	Commercial product	Dose (I.J. ha^{-1})	Water volume (hl ha^{-1})
3 pear orchards 3 ha	28/03/06	Mean t° = 12-14 °C R.H. = 83%	*Steinernema carpocapsae*	Nematac C ®	2.5×10^9	15
	28/03/06	Mean t° = 12-14 °C R.H. = 83%	*Steinernema feltiae*	Nemaplus ®	1.25×10^9	15
	28/03/06	Mean t° = 12-14 °C R.H. = 83%	*Steinernema carpocapsae*	Nematac C ®	2.5×10^9	15
	28/03/06	Mean t° = 12-14 °C R.H. = 83%	*Steinernema feltiae*	Nemaplus ®	1.25×10^9	15

2006 – 1: Autumnal trial on large surface

Location: Emilia-Romagna, Po Valley, Northern Italy; provinces of Ferrara, Modena, Bologna and Ravenna.
Orchards: total surface of 33 ha, 15 pear orchards (cvs. William and Abate Fetel) with high CM damage (more than 50% worm-eaten fruits at the 2006 harvest).
Experimental design: randomised block, with 3 repetitions each formed by 1 ha. The treatment was carried out in the afternoon, by means of a conventional mist blower (Table 2).

Table 2: 2006. Autumnal trials on wide surface. Field conditions, treatments and doses.

Orchards (ha)	Timing	Weather conditions	Treatment	Commercial product	Dose (I.J. ha^{-1})	Water volume (hl ha^{-1})
6 pear orchards 14.5 ha	21/10/06	Mean t° = 12-14 °C Rainfall = 10 mm	*Steinernema carpocapsae*	Nemasys C ®	1.5×10^9	15
4 pear orchards 8.5 ha	21/10/06	Mean t° = 12-14 °C Rainfall = 10 mm	*Steinernema feltiae*	Nemasys ®	1.5×10^9	15
3 pear orchards 5 ha	19/10/06; or 7/11/06	Mean t° = 5-10 °C Rainfall = 1-2 mm	*Steinernema carpocapsae*	Nematac C ®	1.5×10^9	15
3 pear orchards 5 ha	19/10/06; or 7/11/06	Mean t° = 5-10 °C Rainfall = 1-2 mm	*Steinernema feltiae*	Nemaplus ®	1.5×10^9	15

2006 - 2: Autumnal trial on sentinel larvae in a pear orchard

Location: Emilia-Romagna, Po Valley, Northern Italy; province of Modena.

Orchards: 1 pear orchard.

Experimental design: randomised block, with 4 repetitions, each formed by 15 sentinel larvae in card boards.

The treatment was carried out in the afternoon, by means of a motor pump. Before and after the EPN treatment, 25 hl/ha of water were sprayed on the trees (Table 3).

Table 3: 2006. Autumnal trial on sentinel larvae in a pear orchard. Field conditions, treatments and doses.

Target	Timing	Weather conditions	Treatment	Commercial product	Dose (I.J. ha^{-1})	Water volume (hl ha^{-1})
1 pear orchards 60 sentinel larvae	13/10/06	Mean t° = 14 °C Rainfall = 1-2 mm	*Steinernema carpocapsae*	Nemasys C ®	1.0 x 10^9	15
	13/10/06	Mean t° = 14 °C Rainfall = 1-2 mm	*Steinernema carpocapsae*	Nemasys C ®	1.5 x 10^9	15
	13/10/06	Mean t° = 14 °C Rainfall = 1-2 mm	*Steinernema carpocapsae*	Nemasys C ®	2.5 x 10^9	15

2007 -1: Autumnal trial on large surface

Location: Emilia-Romagna, Po Valley, Northern Italy; provinces of Ferrara, Modena, Bologna and Forlì.

Orchards: total surface more than 40 ha, belonging to 20 farms. Pear orchards.

Experimental design: randomised block, with 3 replications each formed by 1 ha.

The treatment was carried out in the afternoon, by means of a conventional mist blower (Table 4).

Table 4: 2007. Autumnal trials on wide surface. Field conditions, treatments and doses.

Orchards (ha)	Timing	Weather conditions	Treatment	Commercial product	Dose (I.J. ha^{-1})	Water volume (hl ha^{-1})
10 pear orchards, 20 ha	24-27/10/07	Mean t° = 9-11 °C Rainfall = 51 mm in three days	*Steinernema carpocapsae*	Nemasys C ®	1.5 x 10^9	15
10 pear orchards, 20 ha	24-27/10/07	Mean t° = 9-11 °C Rainfall = 51 mm in three days	*Steinernema feltiae*	Nemaplus ®	1.5 x 10^9	15

2007 - 2: Autumnal trial on sentinel larvae in pear orchards

Location: Emilia-Romagna, Po Valley, Northern Italy; provinces of Ravenna and Forlì.

Orchards: 3 pear orchards.

Experimental design: randomised block, with 4 repetitions; the checked sentinel larvae in each orchard were totally 160 and 344 in Forlì farms and 153 in Ravenna farm.

The treatment was carried out in the afternoon, by means of a motor pump (Table 5).

Table 5: 2007. Autumnal trial on sentinel larvae in a pear orchard. Field conditions, treatments and doses.

Target	Timing	Weather conditions	Treatment	Commercial product	Dose (I.J. ha^{-1})	Water volume (hl ha^{-1})
1 pear orchards 160 sentinel larvae (Forlì 1)	06/10/07	Mean t° = 19 °C Rainfall = 18 mm	*Steinernema carpocapsae*	Nemasys C ®	1. 5 x 10^9	20
1 pear orchards 344 sentinel larvae (Forlì 2)	23/10/07	Mean t° = 11.6 °C R.H. = 80-100%	*Steinernema carpocapsae*	Nemasys C ®	1.25 x 10^9	20
1 pear orchards 153 sentinel larvae (Ravenna)	06/10/07	Mean t° = 16.4 °C Rainfall = 15.6 mm	*Steinernema carpocapsae*	Nemasys C ®	1.25 x 10^9	15

In the 2006 and 2007 autumnal trials on large surface, the EPN persistence in the soil was evaluated burying some net envelopes, containing *Galleria mellonella* larvae, beneath the trees and assessing them every weeks during 22 days.
In these trials, the EPN effectiveness was checked directly on the mortality of sentinel larvae in the card boards and indirectly on the eggs laid by CM first generation females, sampling 100 fruitful bunches in the central area of each plot.

In the trials 2006 -2 and 2007 – 2 only the sentinel larvae were assessed as percentage of CM mortality due to EPNs and as percentage of EPNs efficacy vs. untreated control.

Results

2006: Spring trials
The EPN application in spring 2006, did not achieve any effect on the sentinel larvae parasitization. Almost all the CM larvae were found alive: 128 living larvae and 14 living chrysalis vs. 14 dead larvae.
The spring applications at any EPN rate did not achieve any noticeable effect in the control of the CM first generation; in fact similar results were recorded in the three different locations. The percentage of fruit clusters infested by CM (eggs and worm-eaten fruits) reached 66% in the EPN treated plots and 40% in the untreated control at Castelfranco Emilia (MO), 9% in the EPN applied plots and 6% in the untreated control at S. Matteo della Decima (BO), 28% in the EPN treated plots and 16% in the untreated control at Galliera (BO).

2006 - 1: Autumnal trial on large surface
On October 21st, the EPN treatments were applied in 10 orchards (23 ha) at best weather conditions (Table 2). The CM mortality in the sentinel larvae achieved 73%, while in the quince moth (QM), *Euzophera bigella* larvae, naturally cocooned in the card boards, the mortality achieved 93%. In 6 orchards (14.5 ha) treated with *S. carpocapsae*, the larval mortality achieved 64% in CM and 90% in QM, while in 4 orchards (8.5 ha) applied with *S. feltiae*, the CM mortality reached 85% and the QM mortality 96%. No parasitized insect larvae were found in the untreated control of each orchard.

In 5 orchards (10 ha) the EPNs were applied on days, with non optimal weather conditions (mean temperatures of 5-10 °C, rainfall of 1-2 mm). The mortality of sentinel larvae placed in the card boards was, in general, poor: 27% in CM and 22% in QM. No parasitized insect larvae were found in the untreated control of each orchard.
However, the effectiveness of *S. feltiae* (51% in CM mortality and 31% in QM mortality in 2 orchards of total 7 ha) was significantly higher than *S. carpocapsae* (4% in CM mortality and no QM dead larvae in 3 orchards of total 3 ha) at the same weather conditions.
In spring 2007, the assessment of the first CM generation eggs, showed that their number was significantly reduced to 1-7 eggs in the orchards treated at the best weather conditions, while in the untreated control it reached 4-24 eggs. Only in one farm, where the treatment was distributed 36 hours after the rain, on trees which were going to dry, the EPNs were ineffective and the number of eggs laid in the treated orchard (27 eggs) was higher than in the untreated control (24 eggs).

2006 - 2: Autumnal trial on sentinel larvae in a pear orchard
The mortality of the sentinel larvae treated with *S. carpocapsae* was 72% at 2.5 x 10^9 I.J. ha $^{-1}$, 79% at 1.5 x 10^9 I.J. ha $^{-1}$ and 62% at 1.0 x 10^9 I.J. ha $^{-1}$ (1% in the untreated control). The effectiveness in the treated plots was: 83.1%, 87.1% and 71.9%, respectively, compared with the untreated control.

2007 -1: Autumnal trial on large surface
The advice for applying EPNs was given at the forecast of the first useful rain, which altogether lasted 4 days; so the EPN application was carried out during the rainfall or in the breaks between 2 or more rainfalls. In those days the temperature was quite low (9-11 °C), not completely suitable for EPN survival (Table 4). Even so, the EPN effectiveness was very satisfactory, *S. feltiae* giving better result than *S. carpocapsae* because of its higher resistance to low temperatures: the mortality of CM sentinel larvae reached 76% in the trees treated with *S. feltiae* and 34% in those applied with *S. carpocapsae*.
Next spring (2008) the eggs of the first CM generation will be assessed for checking the decrease in CM populations in consequence of the EPN autumnal treatment.

2007 - 2: Autumnal trial on sentinel larvae in pear orchards
In these trials, EPNs were applied during a rainfall of 16 mm (Ravenna) or 18 mm (Forlì). In the second orchard in Forlì province it didn't rain, neither during nor after the treatment. In all trials a high R.H. (from 80% to 100%) was recorded in October, while the temperature remained around 16 °C (Table 5). These climatic features demonstrated to be very suitable for boosting the *S. carpocapsae* infectivity, reaching a mortality in sentinel larvae of 80% with 18 mm rainfall and temperature of 19 °C, and 56% with 16 mm rainfall and 16 °C. Even in the orchard where it didn't rain, at a temperature of 12 °C, the sentinel larvae parasitization reached 52%, probably because of the high R.H.

EPN persistence in the soil
Regarding the EPN persistence in the soil, every year the end of their activity was observed about two weeks after the application, because of the temperature decline. In 2006, the *G. mellonella* mortality in net trap in the soil, amounted to 95.5 % two days after the EPN application, to 63.2 % after 9 days, to 2.6% after 16 days and to 0 % after 22 days, while in the untreated control only living larvae were observed.

Discussion

The trials on large scale have demonstrated the possibility for an effective control of CM populations by *Steinernema* species, applied with common farm equipments. The farmers and the extension service technicians have easily learned the correct EPN storage and the methods for spraying the nematode suspension on the orchard.
These trials have ascertained that: (1) the EPNs are able to penetrate the CM overwintering larvae in the cocoon; (2) their effectiveness is exalted by autumnal applications on the branches and trunks; (3) the choice of the application timing is based on the intense tree wetting; (4) it is better to apply EPNs at the beginning of a rainfall (as soon as the trunks are wetted) rather than at its end, because the plant can rapidly dry and the EPNs die; (5) *S. feltiae* seems to be more effective than *S. carpocapsae* at lower temperatures; and (6) EPNs flushed to the soil by the rain are active for two weeks after their application.

Conclusions

The EPN treatments can effectively be applied in an IPM strategy, with the aim of reducing the CM population in an orchard. Their effectiveness depends on three prerequisites at the same time: (1) presence of host as mature larvae in the cocoon, (2) suitable temperature (>10-14 °C), and (3) either rainfall or high R.H. for the most of 24 hours.

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