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# Toxicity and residual activity of spinetoram to neonate larvae of *Grapholita molesta* (Busck) and *Cydia pomonella* (L.) (Lepidoptera: Tortricidae): Semi-field and laboratory trials

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A B S T R A C T

Spinetoram is a fermentation insecticide, derived from the actinomycete *Saccharopolyspora spinosa*. It works by disrupting the GABA-gated chloride channels and by causing persistent activation of insect nicotinic acetylcholine receptors. This study aimed to evaluate the efficacy of spinetoram for control of neonate larvae of both oriental fruit moth (OFM) *Grapholita molesta* (Busck) and codling moth (CM) *Cydia pomonella* (L.) in semi-field and laboratory trials. OFM and CM neonate larvae responded similarly to spinetoram, which showed high efficacy on both species. In semi-field experiments, regression analysis of the percentage of damaged fruits as a function of days after treatment showed a better performance of the highest spinetoram dose (10 g a.i./hl) in comparison with the maximum recommended field dose of the reference product emamectin benzoate (2.85 g a.i./hl). Surface-treated diet assays revealed $LC_{50}$ values of 6.59 and 8.44 ng a.i./$cm^2$ for neonate larvae of OFM and CM larvae, respectively. High percentages of mortality were recorded on both species after 24-h exposure to treated diet. For these reasons spinetoram could be considered a valuable tool in IPM strategies for OFM and CM control.


## 1. Introduction

The codling moth – *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) – is a key pest of pome fruit and walnut in all temperate regions of the world, except Japan, Korea and Brazil. The oriental fruit moth – *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) – is a worldwide key pest of stone fruits, but occurs widely also on pome fruits (Kirk et al., 2013; Lu et al., 2014; Myers et al., 2007; Najar-Rodriguez et al., 2013; Natale et al., 2003; Rothschild and Vickers, 1991; Wearing et al., 2001).

Although mating disruption is a useful management tool (Carde and Minks, 1995; Il'ichev et al., 2007; Witzgall et al., 2008), the control of these tortricids largely relies on insecticide sprays (Giner et al., 2012; Knight and Light, 2013). Notwithstanding, the control of CM by means of insecticides is threatened by the widespread development of resistance (Ioriatti et al., 2007; Knight, 2010; Reyes et al., 2007; Rodríguez et al., 2011). Moreover, the current revision of directive on pesticides in the European Community (Directive 128/2009/CE in EU) is strongly reducing the number of active ingredients allowed in many European countries and could enhance the resistance problems.

Spinetoram is a semi-synthetic spinosyn insecticide derived from a fermentation product of the actinomycete *Saccharopolyspora spinosa* Mertz et Yao (Mertz and Yao, 1990). The mechanism of action (IRAC MoA Group 5) involves the disruption of nicotinic acetylcholine receptors (nAChR) and gamma amino butyric acid GABA-gated chloride channels (Dripps et al., 2008; Kirst, 2010). Spinetoram is a broad-spectrum insecticide active against several insect pests in the orders Coleoptera, Diptera, Hemiptera, Lepidoptera, and Thysanoptera (Bacci et al., 2016). Spinetoram is undergoing registration in Europe for its use in fruit and olive orchards. In 2015 the Spanish Ministry of Agriculture granted an exceptional authorisation for using spinetoram to control *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) on cherries and against Psyllidae in pear orchards.

This study aimed to evaluate the efficacy and the residual activity of spinetoram on CM and OFM neonate larvae in semi-field experiments. Laboratory trials were also carried out to assess the

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dose-mortality relationships and speed of action. These data are necessary to indicate an appropriate range of doses to be used in the field to control the two pests on stone and pome fruits.

## 2. Materials and methods

### 2.1. Insects

The populations of *G. molesta* and *C. pomonella* used in the experiments were provided by University of Lleida (Spain) and were reared at the Department of Agricultural Science (University of Bologna, Italy). Up to one hundred generations of both species have been continuously reared on the same artificial diet (Pons et al., 1994) in laboratory conditions (25 ± 1 °C, 70–80% RH and 16/8 L/D). Only active neonate larvae (<24-h old) were used for all the experiments.

### 2.2. Insecticides

A commercial formulation of spinetoram (Delegate® 25 WG) was provided by Dow AgroScience Indianapolis, IN. Emamectin benzoate was chosen as a chemical reference because this insecticide is used against Lepidoptera larvae and recommended in IPM of both CM and OFM (Ioriatti et al., 2009). A commercial formulation of emamectin benzoate 0.95% (Affirm® Syngenta Crop Protection Inc.) was purchased. Spinetoram and emamectin benzoate were evaluated both in laboratory and in semi-field trials as commercial wettable granules (WG) formulation (Table 1).

### 2.3. Semi-field trials

The insecticides were applied by a backpacked sprayer until run off using a water volume of 12 hl/ha. The nectarine orchard was sprayed on July 9th, 2013, while the apple orchard was sprayed on July 14th, 2014. Spinetoram was tested at three concentrations ranging from 2.5 g a.i./hl to 10 g a.i./hl, emamectin benzoate was applied at the maximum recommended field dose (2.85 g a.i./hl) (Table 1). The control plots were sprayed with tap water. OFM and CM larval mortality was assessed on nectarines and apples collected from treated orchards. The nectarine orchard was located in the Ravenna district (44° 14′ 19″N, 11° 56′ 16″ E), its main features were: cv. August Red, 11-yr old, palmette trained, 4.0 × 1.4 m planted, N/S oriented, plants were approximately 4 m high. The apple orchard was located in Bologna district (44° 42′ 44″ N, 11° 33′ 47″ E) and its main features were: cv. Imperatore, 10-yr old, palmette trained, 3.5 × 1.0 m planted, N/S oriented, plants were approximately 3 m high. Each treatment was applied to three plots randomly assigned within the orchard; the plots consisted of three and five plants for nectarine and apple, respectively. Samples of 10 fruits were collected from plots 3, 7, 14 and 21 days after treatment (DAT). Fruits were carefully inspected for OFM or CM eggs and larvae, before being taken to the laboratory. The main weather conditions during the field trials were: T (mean) = 25.1 °C; RH = 61.4% and 22 mm of rain in 2013 and T = 23.2 °C; RH = 70.2% and 78.80 mm of precipitations in 2014. The highest amounts of rain were recorded at 12 DAT (July 26th, 22.8 mm), at 16 DAT (July 30th, 19.6 mm) and at 20 DAT (August 3rd 14.0 mm).

In the laboratory, fruits were separately placed in plastic cups. A neonate larva was then transferred onto each fruit using a fine paintbrush, and cups were closed with lids allowing air circulation. The cups were kept in a climatic chamber at 25 ± 1 °C, 75% RH and 16/8 h L/D photoperiod. The fruits were examined and dissected 10–14 days later, thus allowing the larvae to produce detectable damage. The number of damaged fruits was recorded, and treatments were compared taking into account the percentage of damaged fruits.

### 2.4. Laboratory trials

Laboratory trials were carried out by the surface-treated diet method (Bosch et al., 2007; Reyes and Sauphanor, 2008; Rodríguez et al., 2011). The same meridic diet containing, wheat germ, cereal flours, brewer's yeast, dried apples, agar-agar and preservatives (Pons et al., 1994) was used for both OFM and CM larvae.

The insecticide doses used in the lab corresponded to a ten-fold dilution of the doses applied in the field. Spinetoram was tested at concentrations ranging from 0.125 to 1 g a.i./hl to investigate the dose response relationship. Emamectin benzoate was applied at only one rate (0.285 g a.i./hl) as a chemical reference in the trials for the assessment of the speed of action (Table 1).

The insecticide dilutions were homogeneously distributed on the surface of the diet with a previously humidified paintbrush at a dose of 2 μl/cm$^2$. After drying the diet was cut in 1-cm$^3$ pieces that were individually placed in plastic jars, then a neonate larva was transferred on the diet surface. Larvae were isolated in a gelatine capsule (Ø 0.5 cm) inserted into the diet, to ensure the contact with the treatment and to avoid escaping. The gelatine capsule was removed 24 h after treatment. Insects were kept at 25 ± 1 °C, 70–80% RH and 16/8 h L/D photoperiod.

Mortality was tallied at 24 h and 96 h after treatments and at adult emergence. Given that larvae usually burrow in the diet, it was necessary to dig into the diet pieces to find them for the assessment of mortality. This implies an alteration of the surface test procedure. Therefore, an independent destructive sampling design was used, testing each replicate only once. Four independent replicates with 10 larvae each were carried out for each treatment and for each mortality checking time.

Larvae unable to respond to probing with a fine paintbrush were counted as dead.

### 2.5. Statistical analysis

In semi-field trials, linear regression was used to examine the percentage of damaged fruits as a function of DATs for each treatment.

The concentration-mortality relationships were analysed by

**Table 1**
Insecticides and concentrations used in semi-field and laboratory experiments on neonate larvae of *Grapholita molesta* and *Cydia pomonella*. WG = Wettable Granules.

| Commercial name | Active ingredient | Semi-field trial doses (g a.i./hl) | Laboratory trial doses (g a.i./hl) |
|---|---|---|---|
| Delegate 25 WG | Spinetoram | | 0.125 |
| | | 2.5 | 0.25 |
| | | 5 | 0.5 |
| | | 10 | 1 |
| Affirm 95 WG | Emamectin benzoate | 2.85[a] | 0.285 |
| Untreated control | – | Tap water | Distilled water |

[a] Maximum recommended field dose.