

RESEARCH PAPER

## Side effects of the natural pesticide Spinosad (GF-120 Formulation) on *Eretmocerus paulistus* (Hymenoptera: Aphelinidae), a parasitoid of the whitefly *Aleurothrixus floccosus* (Hemiptera: Aleyrodidae), under laboratory conditions

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### Abstract

**V. Tello, L. Díaz, and M. Sánchez. 2013. Side effects of the natural pesticide Spinosad (GF-120 Formulation) on *Eretmocerus paulistus* (Hymenoptera: Aphelinidae), a parasitoid of the whitefly *Aleurothrixus floccosus* (Hemiptera: Aleyrodidae), under laboratory conditions. Cien. Inv. Agr. 40(2):407-417.** The side effects of the insecticide GF-120 NF 0.02 Naturalyte CB® were evaluated on adults of *Eretmocerus paulistus* Hempel, a parasitoid of *Aleurothrixus floccosus* (Maskell) in citrus orchards of northern Chile.  $LC_{50}$  and  $LC_{90}$  were determined by applying the insecticide using a Potter tower in decreasing doses from 0.96 to 0.1 mg a.i.  $L^{-1}$  (corresponding to 1% to 0.1% of the recommended dose, respectively). After 24 h of exposure, the results were fitted to a probit model, and the  $LC_{50}$  and  $LC_{90}$  were estimated at 0.21 and 0.79 mg a.i.  $L^{-1}$ , respectively. In feeding tests and evaluation of the toxic effect of dry residues of GF-120, it was determined that a high percentage of mortality (100%) was obtained using a combination of 24.0 mg a.i.  $L^{-1}$  x residues at 1 hour drying time. Residues at 96 h (4 days) drying time with concentrations of 38.4 or 24.0 mg a.i.  $L^{-1}$  caused mortalities over 60%. According to the classification of the IOBC (International Organization for Biological and Integrated Control of Noxious Animals and Plants) the manufacturer's recommended concentration of 96 mg a.i.  $L^{-1}$  was level 4, which is harmful (> 99%), as GF-120 concentrations can range from 0.77 to 72 mg a.i.  $L^{-1}$ . The GF-120 residues applied on inert surfaces were harmful to *E. paulistus* with a high mortality rate under laboratory conditions. Field studies are needed to validate these results by evaluating parasitoid populations in insecticide-treated and untreated areas.

**Key words:** Bioassays, insect parasitoids, natural pesticide, residual effect, side effects, toxicity.

### Introduction

Pesticides are designed to be toxic to target noxious organisms that cause damage to crops and economic losses. However, some active ingredients can be harmful to the environment and to

non-target organisms (Delaplane, 2000) despite the efforts of research to develop and promote more selective and ecologically safer molecules (Schmutterer 1990; James *et al.*, 1993; Thomson *et al.*, 2000; Ishaaya *et al.*, 2011).

The use of pesticides to control pests is validated by its many contributions to society. These contributions include the following: habitats

previously uninhabitable because of vector-borne diseases are now habitable; crops are grown in large monocultures with minimal contamination by weeds and/or destruction by insects; and household pests can be easily eliminated (Rose *et al.*, 1999; Waterfield and Zilberman, 2012). However, by their very nature, pesticides can also have deleterious effects. The indiscriminate use of synthetic pesticides in agriculture can adversely affect beneficial organisms, such as biocontrol agents and pollinators. This harmful effect on the activity of natural enemies may cause a resurgence of pests considered secondary or under natural control (*e.g.*, Suma *et al.*, 2009). These compounds contribute to the contamination of soil and aquatic environments (Martínez *et al.*, 2004) and are also responsible for damaging human health, both in its preparation and during its application (Périte *et al.*, 2009).

This situation has forced the introduction of regulations for the production and application of these products, which have restricted the supply of pesticides and promoted research and development of pesticides based on natural products, such as spinosyns, avermectins, neem, rotenone and natural derivatives of pyrethrins (Dayan *et al.*, 2009).

Spinosad is a natural insecticide derived by fermentation of the Actinomycete bacterium *Saccharopolyspora spinosa* Mertz & Yao (Kirst, 2010; Miles *et al.*, 2011). The active ingredient is composed of two metabolites, 85% spinosyn A and 15% spinosyn D (Orr *et al.*, 2009). A bait was formulated to attract multiple fruit fly species and to use the minimum concentration of an environmentally compatible toxicant for ultra-low volume (2-4 L ha<sup>-1</sup>) application (Mangan *et al.*, 2006). Spinosad has a novel mechanism of activity on nicotinic acetylcholine receptors, which would be the primary cause of death, most likely acting as an antagonist at the post-synaptic cholinergic ion channels and GABA-gated ion channels (Young *et al.*, 2003). Spinosad is highly active when ingested or through contact and causes quick death

in a wide range of insect pests, *e.g.*, lepidoptera, diptera, thrips and foliage-feeding beetles (Biondi *et al.*, 2012). Despite these promising experiences, spinosad has exhibited negative effects on the survival, longevity and fecundity of beneficial organisms under laboratory (Williams *et al.*, 2003; Wang *et al.*, 2005), greenhouse (Studebaker and Kring, 2003) and field conditions (Cisneros *et al.*, 2002; Thomas and Mangan, 2005; Ruiz *et al.*, 2008). There is a large amount of information about spinosad characteristics and their effects on beneficial organisms; however, in Chile, this information is very limited. The Chilean studies include control of the following: California thrips, *Frankliniella occidentalis* (Pergande) (Vargas and Ubillo, 2005); *Tuta absoluta* (Meyrick) (Pozo, 2010); yellowjacket wasp, *Vespa germanica* (F.) (Ulloa *et al.*, 2006); *Cryptolaemus montrouzieri* Mulsant and *Acerophagus* (= *Pseudaphycus flavidulus*) (Brethés) (Rojas, 2011); and *Aphidius ervi* (Haliday) (Araya *et al.*, 2010). Considering this problem, the objective of this research was to evaluate, under laboratory conditions, the potential toxic effects of spinosad on *Eretmocerus paulistus* Hempel (Hymenoptera: Aphelinidae), a parasitoid of the woolly whitefly parasitoid in Pica, Tarapacá Region, Chile.

## Materials and methods

The present study tested the insecticidal action of GF-120 NF Naturalyte 0.02 CB® (Dow AgroSciences), which is a mixture of the toxicant spinosad (spinosyn A and D), at a concentration of 240 mg a.i. L<sup>-1</sup>, and a feeding attractant. A hydrolyzed protein is the lure component that attracts and induces feeding.

All tests were implemented during the months of April and May 2010. The determination of the LC<sub>50</sub> and LC<sub>90</sub> values was conducted at the Laboratory of Plant Health of Universidad Arturo Prat, located in Estación Experimental Canchones, Tamarugal Province, Tarapacá Region, Chile (20°16'15.3" S, 70°07'46.6" W), under experimental conditions

of  $25 \pm 5$  °C,  $30 \pm 10\%$  RH and a photoperiod of 16:8 (light:darkness).

For all studies, we used parasitoids collected from branches infested with *Aleurothrixus floccosus* (Maskell); the vegetal material was obtained from chemically untreated citrus orchards in Pica ( $20^{\circ}29'12.40''$  S,  $69^{\circ}19'34.14''$  W), Tamarugal Province, Tarapacá Region, Chile. The insecticide used for all tests corresponded to concentrate bait GF-120.

#### *Lethal concentrations ( $LC_{50}$ and $LC_{90}$ )*

Dilutions of GF-120 were applied using a Potter tower (Makers Burkards Manufacturing Co. Ltd., Rickmansworth, England) with a pressure of 55 KPa (Vargas and Ubillo, 2001). The insects were exposed to a dry pesticide film applied on the internal surface of glass cages ( $578 \text{ cm}^3$ ) made of six plates, which were joined externally using transparent adhesive tape to form a cube. The components of this container were attached externally using clear tape and parafilm (Viggiani and Tranfaglia, 1978; Suma *et al.*, 2009).

The dose was based on manufacturer recommendations for the control of fruit flies (Mangan *et al.*, 2006) but with further decreases to the concentration. The concentrations tested were 0.96, 0.77, 0.67, 0.48, 0.10, and 0.00 mg a.i.  $L^{-1}$ , which corresponded to 1%, 0.8%, 0.7%, 0.5%, 0.1%, and 0.0% of the dose recommended by the manufacturer (DRM), respectively. Distilled water was used to dilute the insecticide treatment and the control. After applying the solution to the plates, the solution was allowed to dry for 1 h at room temperature to prevent mortality due to adherence of specimens to the surface (Iannacone and Lamas, 2003).

Branches were collected from citrus orchards, taken to the laboratory in plastic boxes at low temperature and placed in rearing chambers for the emergence of parasitoids. The emerged insects

were aspirated to a glass vial and selected under a stereoscopic microscope at 40X magnification (Carl Zeiss Stemi SV6 model, Germany) inside the same container. Once selected, the specimens were released into the cubes through a hole of 3 cm diameter in one of its faces, and the hole was then sealed using absorbent paper and fixed with tape. The age of the insects was less than 24 hours since emergence; they were not given food or water.

The exposure time of the insects to the treatments, including the control, was 24 h, and the evaluations were performed at 12 and 24 h from the introduction of the insect to the arenas for 30 s under the microscope. Parasitoids were considered dead if they could not walk when probed (Iannacone and Lamas, 2003; Yee and Alston, 2012).

#### *Adult mortality feeding by *E. paulistus* (Non-choice and residual tests)*

To evaluate the combined effect of GF-120 concentrations [96 mg a.i.  $L^{-1}$  (DRM), 38.4 mg a.i.  $L^{-1}$  (40% DRM) and 24.0 mg a.i.  $L^{-1}$  (25% DRM)] and the time on dry residues, nine treatments were applied with a factorial structure. For treatment application, the internal surfaces of glass vials were wet with a cotton swab containing the insecticide (Ruiz *et al.*, 2008). The vials were allowed to dry for 1, 48 and 96 hours before introducing between 10 and 30 adults of *E. paulistus* less than 24 h old. The exposure time of insects to treatment was 12 h. Parasitoids were considered dead if they could not walk when probed.

#### *Design and statistical analysis*

*Lethal concentrations.* To determine the lethal concentrations, a completely randomized design was used with four replicates of the six treatments, including the control. A glass cube containing between 10 and 30 specimens was considered an experimental unit. To calculate the  $LC_{50}$  and

LC<sub>90</sub>, the results were processed by probit analysis following the methodology used by Salazar and Araya (2001). The slopes of the regression between mortality (probit) and concentration (log) were analyzed using PROC PROBIT in SAS (SAS Institute Inc., Cary NC, USA, 2000) and applied according to Flores *et al.* (2007).

*Feeding by E. paulistus.* For feeding and residual tests, a completely randomized design was applied with a factorial structure, with nine treatments and four replicates, including the control. Treatments were formed by the interaction of two factors: GF-120 concentration and the pre-exposure time (dry residues).

Mortality percentages were corrected using Abbott's formula (Abbott, 1925) if mortality in the controls was greater than 5% (Lagunes and Villanueva, 1999). The percentages were transformed using an arcsine transformation before evaluation (Zar, 2006). Tukey's test was used to determine which means ( $P < 0.05$ ) differed significantly from one another using PROC GLM (SAS Institute Inc., Cary NC, USA, 2000).

## Results and discussion

### Determination of lethal concentrations

At 1.0% (0.96 mg a.i. L<sup>-1</sup>) of the recommended dose, the insecticide had an effect of 100% mortality on individuals after 12 and 24 hours of exposure ( $F=71.60$ ; d.f.=5, 17;  $P \leq 0.001$ ) (Table 1). After 24 h, significant differences between treatments remained ( $F=113.63$ ; d.f. = 5, 17;  $P \leq 0.001$ ), registering mortality approaching 100% in the 0.67 mg a.i. L<sup>-1</sup> treatment. A dose corresponding to 0.096 mg of a.i. L<sup>-1</sup> was not significantly different from the control in both tests. Mortality at 36 hours was 100% for all treatments (data not shown). According to Probit analysis (Table 2), the LC<sub>50</sub> and LC<sub>90</sub> for adults of *E. paulistus* were 0.49 (fiducial limits of 0.35 and 0.85) and 2.25 (fiducial limits of 1.17 and 10.27) mg a.i. L<sup>-1</sup>, respectively.

According to the classification of the IOBC (Hassan *et al.*, 1994), GF-120 was placed in level 4, harmful (>99%) at concentrations of 100, 75, 50, 25, 5, 1 and 0.8% of DRM (96, 72, 48, 24, 4.8, 0.96, and 0.77 mg a.i. L<sup>-1</sup>, respectively). GF-120 was assigned to level

**Table 1.** Adult mortality of *Eretmocerus paulistus* Hempel at 12 and 24 hours after application of different concentrations of GL-120 under laboratory experimental conditions of 25±5 °C, 30±10% RH and a photoperiod of 16:8 L:D.

Concentration (mg a.i. L <sup>-1</sup> )	Mortality (% of dead adults) <sup>1</sup>		Category (24 h) <sup>2</sup>
	12 hours	24 hours	
96.00	100.00±0.00 a	100.00±0.00 a	4
72.00	100.00±0.00 a	100.00±0.00 a	4
48.00	100.00±0.00 a	100.00±0.00 a	4
24.00	100.00±0.00 a	100.00±0.00 a	4
4.80	100.00±0.00 a	100.00±0.00 a	4
0.96	100.00±0.00 a	100.00±0.00 a	4
0.77	93.17±2.93 a	100.00±0.00 a	4
0.67	68.50±7.31 b	97.06±2.94 a	3
0.48	29.46±4.19 c	53.04±3.58 b	2
0.10	4.78±3.01 d	30.66±4.77 c	2
Control (distilled water)	4.20±2.40 d	20.13±2.78 c	1

<sup>1</sup>Means followed by different letters within each column are significantly different according to Tukey's test ( $P \leq 0.05$ ). Data are reported as the means ± standard error.

<sup>2</sup>Categories of IOBC/WPRS (Boller according to mortality: Level 1, harmless (< 30%); level 2, slightly harmful (30-79%); level 3, moderately harmful (80-99%); level 4, harmful (>99%).

**Table 2.** Toxicity of spinosad on adults of *Eretmocerus paulistus* Hempel under laboratory experimental conditions of 25±5 °C, 30±10% RH and a photoperiod of 16:8 L:D.

CL50	IC 95%	CL90	CI 95%	± S.E.	slope X <sup>2</sup>	d.f.
0.21	0.16-0.26	0.79	0.63-1.08	3.83±0.45	72.23	1

CI: confidence intervals. SE: Standard error. d.f.: degrees of freedom.

3, moderately harmful (80-99%), at a concentration of 0.7% of the DRM (0.67 mg a.i. L<sup>-1</sup>), and to level 2, slightly harmful (30-79%), at the concentrations of 0.50 and 0.10% of the DRM (0.48 and 0.1 mg a.i. L<sup>-1</sup>, respectively). The control, distilled water, was level 1, harmless (<30%).

Spinosad has been reported to be toxic to many hymenopteran parasitoids (Haseeb *et al.*, 2004; Jones *et al.*, 2005; Penagos *et al.*, 2005; Schneider *et al.*, 2004; Williams and Price, 2004; Williams *et al.*, 2003a,b). The results obtained for the lethal concentrations are consistent with those found by Hill and Foster (2000), who determined LC<sub>50</sub>=0.3 mg a.i. L<sup>-1</sup> in adults of *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), a parasitoid of the col moth (*Plutella xylostella* L.) (Lepidoptera: Plutellidae) subjected to different doses of spinosad. Suh *et al.* (2000) obtained LC<sub>50</sub> and LC<sub>90</sub> values of 3.3 and 10.3 mg a.i. L<sup>-1</sup>, respectively, for adults of *Trichogramma exiguum* Pinto & Platner (Hymenoptera: Trichogrammatidae), an effective parasitoid of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) exposed to spinosad. Penagos *et al.* (2005), comparing their results with other works related to the susceptibility of parasitoids to spinosad, indicated that the response to this active ingredient depends largely on the size of the insect, as those of smaller sizes were clearly more susceptible to this product. The responses to spinosad can also be variable according to the sex of individuals. The results obtained by Wang *et al.* (2005) confirm this finding; these authors determined LC<sub>50</sub> values of 8.3 and 10.0 mg a.i. L<sup>-1</sup> for females and males, respectively, of *Dia-chasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae) and also studied parasitoid fruit flies of the genera *Anastrepha* Schiner and *Ceratitidis* MacLeay (Diptera: Tephritidae). In the case of female and male *Pysttalia fletcheri* (Silvestri)

(Hymenoptera: Braconidae), a parasitoid of the melon fly [*Bactrocera cucurbitae* (Coquillet) (Diptera: Tephritidae)], the LC<sub>50</sub> values were 17.5 and 10.0 mg a.i. L<sup>-1</sup>, respectively.

Rogers *et al.* (2011) found mortalities of 12, 29, and 72% at concentrations of 12, 24, and 48 mg i.a. L<sup>-1</sup> of spinosad, respectively, in *Aphelinus mali* (Hald.) (Hymenoptera: Aphelinidae) and the parasitoid of woolly apple aphid [*Eriosoma lanigerum* (Hausmann)] (Hemiptera: Aphididae). These authors indicated that, at label rate (0.048 g a.i. L<sup>-1</sup>), spinosad was “moderately to highly toxic” for adults of *A. mali* as categorized by the rating system developed by Croft (1982). The exposure of the parasitoids to one-half and one-quarter of the label rate showed a rate response, where mortality increased with increasing insecticide concentration.

Cordero *et al.* (2007) determined for adults of *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) LC50 values of 0.346 mg a.i. L<sup>-1</sup> (fiducial limits 0.034-0.904) and 4.938 (fiducial limits 2.593-8.348), respectively, only a fraction (<2%) of the actual field rate concentration. Field rate concentrations resulted in 100% mortality to both *D. insulare* and *O. sokolowskii*.

According to Luna-Cruz *et al.* (2011), spinosad would be located mostly in IOBC category 3 (moderately toxic, 80-99% mortality) when tested on *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae), a parasitoid of *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) at doses of 600 mg i.a. L<sup>-1</sup> deposited on the leaves of tomato plants located in entomological cages.

Michaud (2003) also found high mortality for *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) and



*Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) exposed to dried residues of baited spinosad (1  $\mu\text{L}$  a.i. per five insects) on inert materials, and Stark *et al.* (2004) exposed two parasitoids, *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) and *Psytalia fletcheri* (Silvestri) (Hymenoptera: Braconidae), to 15  $\mu\text{L}$  of dried residue in glass vials. A dose-mortality response of topical treatment was demonstrated by Ruiz *et al.* (2008) for the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), which was also greatly affected in contact with dried residues and when the field rate was ingested.

Spinosad was classified, based on the IOBC toxicity ratings, as class 4 (harmful, >99% reduction) because of its high direct mortality on the parasitized mummies of *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae) when sprayed with a concentration of 120.0 mg a.i.  $\text{L}^{-1}$  (Fernández *et al.*, 2010).

#### *E. paulistus* Feeding (no-choice and residual tests)

The analysis of variance showed significant effects of the concentration of GF-120 ( $F=93.40$ ,  $d.f.=2, 27$ ,  $P\leq 0.001$ ), in times of dry residues

( $F=33.24$ ,  $d.f.=2, 27$ ,  $P\leq 0.001$ ) and in the interaction between both factors ( $F=18.47$ ,  $d.f.=4, 27$ ,  $P\leq 0.001$ ). The highest mortalities (>90%) were obtained with residues of 1 hour drying time with concentrations of 25% or 40% of the recommended dose (Table 3). When the drying time was longer (48 and 96 h), mortalities were between 60 and 87%.

According to IOBC classification, GF-120 was level 4, harmful (> 99%) in the combination of 25% DRM (24 mg a.i.  $\text{L}^{-1}$ ) x 1 h (residue time). Level 3, moderately harmful (80-99%), was achieved in the combinations of 40% DRM (38.4 mg a.i.  $\text{L}^{-1}$ ) x 1 h and 40% D.R.M. x 48 h, and level 2, slightly harmful (30-79%), was achieved in the combinations of 25% DRM x 48 h, 25% DRM x 96 h and 40% DRM x 96 h. Level 1, harmless (<30%), was displayed by the control (distilled water) at all residue times.

Studies on spinosad intake, using GF-120 in doses recommended for the control of fruit flies as a way to study the effect on beneficial organisms, have shown considerable variation according to the species and doses tested. Ruiz *et al.* (2008) subjected adults of the braconid *Diachasmimorpha longicaudata* (Ashmead) to mixtures of GF-120

**Table 3.** Categorization of mortality on *Eretmocerus paulistus* adults according to the IOBC after 12 h of exposure to different doses of GF-120 and periods of persistence.

Treatments	Residue time (hours)	Mortality <sup>1</sup> (%) $\pm$ S.E.	Category <sup>2</sup>
GF 120 at 25%	1	100.00 $\pm$ 0.00 a	4
GF 120 at 40%	1	91.61 $\pm$ 2.80 b	3
GF 120 at 25%	48	73.82 $\pm$ 3.68 cd	2
GF 120 at 40%	48	87.02 $\pm$ 0.77 bc	3
GF 120 at 25%	96	68.55 $\pm$ 5.09 cd	2
GF 120 at 40%	96	60.77 $\pm$ 0.72 d	2
Control (distilled water)	1	5.64 $\pm$ 2.26 e	1
Control (distilled water)	48	2.28 $\pm$ 1.45 e	1
Control (distilled water)	96	9.40 $\pm$ 1.04 e	1

<sup>1</sup>Means followed by the same letter in each column are not significantly different according to Tukey's Test ( $P\leq 0.05$ ).

<sup>2</sup>Categories of IOBC according to mortality obtained: Level 1, harmless (<30%); level 2, slightly harmful (30-79%); level 3, moderately harmful (80-99%); level 4, harmful (99%).

at 80 mg a.i. kg<sup>-1</sup> and honey (v/v), a simulation of hemipteran honeydew contamination with attractant. The mortality and fertility results obtained indicate that this route of entry is detrimental to this species. However, these authors noted that parasitoids were rarely observed feeding directly on the bait mixed with honey, which suggests that some components would be repellent to this species, but there was an incidence by way of contact. Similar results were obtained by Michaud (2003), who exposed adults of *A. melinus* and *L. testaceipes* to applications of 1 µL of GF-120 and honey. The author obtained significant mortality within the first 24 h of evaluation, reaching between 88 and 80% for *A. melinus* and *L. testaceipes*, respectively.

Vargas *et al.* (2002) determined that *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), a parasitoid of fruit flies, does not feed on mixtures of protein baits of GF-120 intended for the control of tephritids, which provide an efficient control for the pest, with minimal effect on biological control. Wang *et al.* (2005) conducted feeding trials on the same braconid; these tests included three different types of food: GF-120 at 80 ppm, 33% honey solution and distilled water. These authors concluded that this species does not feed directly on GF-120, suggesting that mortality obtained was a result of a direct contact between the insect and the product.

#### Residual test

In laboratory tests, Suma *et al.* (2009) determined that fresh residues of spinosyn at 480 g L<sup>-1</sup> active ingredient caused 100% mortality in *A. melinus*, *Coccophagus lycimnia* Walker (Hymenoptera: Aphelinidae), and *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) in 24 h. These same authors, in semi-field trials with *L. dactylopii*, observed a reduction in mortality, while the rate of parasitism and survival in females was not affected.

According to Ruiz *et al.* (2008), mortality in parasitoids of considerable size, such as *D. longicaudata*, is directly related to the dose of

this product. However, the effect of walking on spinosyn-treated surfaces for long periods of time, regardless of dose (20, 40 and 80 mg of a.i. kg<sup>-1</sup>), resulted in high mortality and a reduction of survival time for this species compared with the control, demonstrating the contact toxicity of this insecticide. In addition, the authors noted that exposures longer than 10 d of this braconid to the spinosyn residues on mango leaves caused a reduction in the progeny and net fertility of females.

Medina *et al.* (2008) evaluated the toxicity and kinetics of spinosad in adults of *Hyposoter didymator* (Thunberg) (Hymenoptera: Ichneumonidae). This study found that insects accumulate relatively small amounts of insecticide in the body but that half of the active ingredient is found in the ovary. This finding is explained by the large amount of hemolymph that is directed to that zone for the production of eggs and has a direct relation to the sub-lethal effects of this insecticide on the reproductive parameters of this species.

The survival of female wasps of *Anaphes iole* Girault (Hymenoptera: Mymaridae) exposed to field-weathered residues of spinosad on cotton leaves resulted in <3% survival (its persistence was >11 days) at a concentration of 0.1 kg a.i. ha<sup>-1</sup> (Williams *et al.*, 2003a).

While spinosad proved highly harmful to adults of *E. paulistus* under the conditions of this study, there is a need to develop new studies in more adverse conditions, such as semi-field and field conditions, less susceptible life stages of the parasitoid, or under the action of light and temperature on bait residues, to understand the insect-pesticide relationship more extensively.

Spinosad concentrations of 0.48 mg a.i. L<sup>-1</sup> induced significant mortality of *E. paulistus* adults at 12 and 24 h under laboratory conditions. The route of exposure to spinosad by surface contact caused significant mortality of *E. paulistus*. Spinosad residues with 1, 48 and 96 h of preparation resulted in high mortality of *E. paulistus* adults. It

should be considered that the impact in relation to the behavior of this insecticide on biocontrol agents, both in different weather conditions and at different application methods (spray on patch, baited tablets and bait stations), are still unknown in most places where is applied. Therefore, the

development of field experience is essential to obtain more information about the effects of this natural active ingredient on populations of biocontrol agents without confinement and its relationship with the eventual resurgence of pest species populations.

### Resumen

**V. Tello, L. Días y M. Sánchez. 2013. Side effects of natural pesticide spinosad (GF-120 formulation) on *Eretmocerus paulistus* parasitoid of the whitefly *Aleurothrix floccosus* under laboratory conditions. Cien. Inv. Agr. 40(2): 407-417.** Se evaluó el efecto colateral del insecticida GF-120 NF Naturalyte 0.02 CB® sobre adultos de *Eretmocerus paulistus*, parasitoide de *Aleurothrix floccosus* en cítricos en el norte de Chile. Se determinó la CL50 y CL90, aplicando el insecticida mediante torre Potter, en dosis decreciente desde 0,96 hasta 0,1 mg a.i. L<sup>-1</sup> (correspondientes al 1% y al 0,1% de la dosis recomendada, respectivamente). Los resultados correspondientes a las 24 h se ajustaron a un modelo Probit y se estimaron en 0,21 y 0,79 mg i.a. L<sup>-1</sup> (CL50 y CL90, respectivamente). En ensayos de alimentación y evaluación del efecto tóxico de residuos secos de GF-120, se determinó que la mayor mortalidad (100%) se obtuvo con una combinación de 24,0 mg i.a. L<sup>-1</sup> × residuos de 1 hora. Residuos de 96 h (4 días), con concentraciones de 38,4 ó 24,0 mg i.a. L<sup>-1</sup>, produjeron mortalidades superiores al 60%. De acuerdo a la clasificación de la IOBC (International Organization for Biological and Integrated Control of Noxious Animals and Plants), la concentración recomendada por fabricante de 96 mg i.a. L<sup>-1</sup> fue de nivel 4 (perjudicial, >99%), lo mismo ocurrió con concentraciones de GF-120 que variaron entre 0,77 a 72 mg i.a. L<sup>-1</sup>. Los residuos de GF-120, aplicados sobre superficies inertes fueron dañino para *E. paulistus*, con altas tasa de mortalidad bajo condiciones de laboratorio. Se requieren estudios de campo para validar estos resultados evaluando poblaciones del parasitoide en áreas tratadas y no tratadas con este insecticida.

**Palabras clave:** bioensayos, efectos colaterales, efecto residual, insectos parasitoides, pesticida natural, toxicidad.

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