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Insecticidal action of five allyl esters on eggs and larvae of three tortricid fruit pests: laboratory tests

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Abstract

Several substances containing the allyl ester group in their structure have shown insecticidal properties. In the search for new insecticide molecules, five allyl esters from aromatic and heterocyclic acids (allyl 1-naphthoate, allyl 2-thiophenecarboxylate, allyl 2-furoate, allyl salicylate, and allyl cinnamate) were synthesized. Their toxicity on *Cydia pomonella* (L.), *Grapholita molesta* (Busck) and *Lobesia botrana* (Denis et Schiffermuller) (Lepidoptera Tortricidae) eggs of different age, by topical application, and on neonate larvae, by exposition to topically treated diet, has been assessed.

When applied to less than 24-h old eggs, the most active compounds on the three species were allyl cinnamate, allyl 1-naphtoate, and allyl 2-thiophenecarboxylate (LC_{50} range: 2.4 - 7.9 mg/mL), while allyl salicylate was the least active one (LC_{50} range: 13.4 - 17.5 mg/mL). The most active ones killed the eggs in the earlier phases of development (white egg and red ring). In contrast to the action of allyl esters of fatty acids, none of the tested allyl esters affected the duration of the development of treated eggs. The mortality and the duration of development of surviving larvae were not affected. Allyl cinnamate was also the most active compound on neonate larvae (LC_{50} range: 3.1 - 6.1 mg/mL) of three species.

As conclusion, allyl cinnamate is suggested to be the best insecticide candidate of this series of compounds.

Key words: allyl esters, ovicidal action, larvicidal action, *Cydia pomonella*, *Grapholita molesta*, *Lobesia botrana*.

Introduction

Cydia pomonella (L.), *Grapholita molesta* (Busck) and *Lobesia botrana* (Denis et Schiffermuller) (Lepidoptera Tortricidae) are important pests of many fruit crops worldwide. *C. pomonella* is a key pest of apple, pear and walnut orchards; *G. molesta* is a key pest mainly of stone and pome fruit orchards; and *L. botrana* is a key pest of vineyards (ISPI, 2009).

Because these species are direct pests of high-value crops, several control methods have been developed against them (Charmillot *et al.*, 2001; Kovanci *et al.*, 2004; Angeli *et al.*, 2007; Dunkelblum, 2007; Srivostava *et al.*, 2009; Ioriatti *et al.*, 2009a; 2009b). Due to their economic importance and low tolerance levels, several insecticide treatments, either alone or in combination with other control methods, are usually needed each season to keep their populations below economic threshold levels.

Larvae of *C. pomonella* develop in fruits, those of *G. molesta* develop in shoots and fruits and those of *L. botrana* feed on fruits, so the larvae remain protected against insecticides inside the fruit or by clusters during most of their development. As the eggs of the three species are laid on leaves and fruits, chemical treatments against these pests target eggs and neonate larvae.

Organophosphates, carbamates, pyrethroids and other insecticides are successfully used to control these pests. However, many of these chemicals are harmful to humans and beneficial organisms, and can cause ecological disturbances (Devine and Furlong, 2007). Furthermore, the development of resistant populations (Pree *et al.*, 1998; Rodríguez *et al.*, 2011; http://www.irac-online.org/) implies the study of new compounds and more ecologically acceptable methods for controlling insect pests as part of integrated pest management (IPM) programs. Research on new insecticides includes the use of extracts from plants (Isman, 2006), that are usually a mixture of several compounds. Some of them, including several esters, have shown insecticidal properties (Park *et al.*, 2003). Moreover, some substances containing an allyl group are toxic, antifeedant or repellent against insects (Ojimelukwe and Adler, 1999; Peterson *et al.*, 2000; Huang *et al.*, 2002; Leelaja *et al.*, 2007).

Allyl esters can be synthesized from bio-diesel industry wastes with low economical value (Escribà *et al.*, 2011). In a previous study, some allyl esters of several fatty acids were synthesized (Escribà *et al.*, 2009) and their action on *C. pomonella* eggs was assessed. The action of the allyl esters was related to the length of the fatty acid alkyl chain. The most active ones produced 100% mortality at 10 mg/mL and increased the duration of the development of the eggs (Escribà *et al.*, 2009). As continuation of this research, the aim of this work was to know the action of five allyl esters from aromatic and heterocyclic acids (allyl 1-naphthoate, allyl 2-thiophenecarboxylate, allyl 2-furoate, allyl salicylate, and allyl cinnamate) on *C. pomonella*, *G. molesta* and *L. botrana* eggs and neonate larvae.

Materials and methods

Insects

A *C. pomonella* population was collected from unsprayed apple tree orchards in 1993 in Lleida (northeast Spain). It has been kept since then as a laboratory population at the UdL-IRTA Center for R + D.

G. molesta and *L. botrana* populations were obtained from mass-reared laboratory cultures from IEPVFA (Piacenza, Italy) and INRA (Bordeaux, France), respectively. Both have been reared for more than 10 years in their laboratories of origin and they have been reared at the laboratory of the UdL-IRTA Center for R + D since 2005 and 2007, respectively. Each species was reared on agar-based semisynthetic diets (Ivaldi-Sender, 1974, for *G. molesta*; Pons *et al.*, 1994, for *C. pomonella* and *L. botrana*) at room temperature (22 ± 2 ºC) under a 16:8 (L:D) h photoperiod. Adults were kept in cylindrical rearing cages using wax paper as the egg-laying substrate.

Allyl esters

Allyl 1-naphthoate (**1**), allyl 2-thiophenecarboxylate (**2**) and allyl salicylate (**4**) (figure 1) were synthesized following the general procedure described by Escribà *et al*. (2009) and identified by NMR spectroscopy using a VARIAN® AS400 spectrometer (400 MHz for ^{1}H). Allyl 2-furoate (**3**) and allyl cinnamate (**5**) (figure 1) were purchased from Sigma-Aldrich (Madrid, Spain) (purity 98% and 99%, respectively).

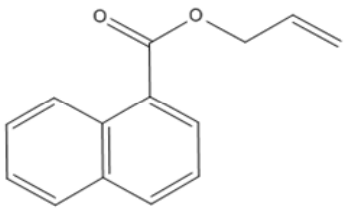
Mortality on less than 24-h old eggs

C. pomonella, *G. molesta* and *L. botrana* adults were allowed to oviposit during less than 24 h on wax paper. The eggs were then topically treated with 0.1 µL of the allyl ester dissolved in pure acetone (JR Baker, Deventer, the Netherlands). An injection pump (Harvard Apparatus Model 11, Holliston, MA) equipped with 10 µL syringe (Hamilton, Reno, NV, USA) with a fused silica needle was used for the application. Five concentrations of each allyl ester, in the interval needed to obtain mortality between 5 and 100%, were tested. Three replicates (30 eggs per replicate) were carried out at each concentration. A control (no treated eggs) and an acetone-control (treated with 0.1 µL of pure acetone) were also done. For a replicate to be validated, the mortality of both controls had to be < 20%. Each replicate was kept in plastic Petri dishes (9 cm diameter, 2 cm height) with a wet filter paper on the bottom, sealed with Parafilm to prevent egg drying, and kept in the same conditions as the insect stock culture. Eggs were checked daily for 10 days and egg mortality and egg developmental phase at death (white egg, red ring or black head) (Richardson *et al.*, 1982) was recorded.

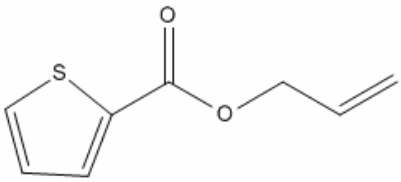
A probit analysis of the mortality vs. concentration was carried out using the Polo Plus® program, version 1.0 (Robertson *et al.*, 2003). Lethal concentrations (LC_{50} and LC_{90}) and their confidence intervals (CI) were calculated (95%). The comparison of LC_{50} and LC_{90} was done using the overlapping CI as a criterion. Hypothesis of equality (equal slopes, equal intercepts) and hypothesis of parallelism (equal slopes) were tested using the same software to compare concentration-response lines among compounds and among species.

Duration of egg development

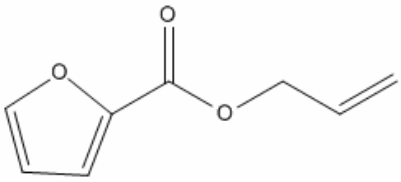
Less than 24-h old *C. pomonella*, *G. molesta* and *L. botrana* eggs were treated with species and ester respective LC_{50}, calculated in previous experiment. If the LC_{50} was higher than 10 mg/mL, a concentration of 10 mg/mL was used. Acetone-treated eggs were used as controls. Treated larvae were kept at 15 ± 0.5 ºC, to allow the enhancement of possible differences in the duration of egg development (Rock and Shaffer, 1983; Chaudhry, 1956; Tobin *et al*., 2001). For each allyl ester and species, three replicates of 30 eggs each were carried out. The eggs were checked daily to record the duration of their development. The mean development time of allyl ester-treated and acetone-treated eggs were compared by a Student *t*-test ($P < 0.05$) (SAS® Version 8; SAS Institute, Cary, NC, USA).



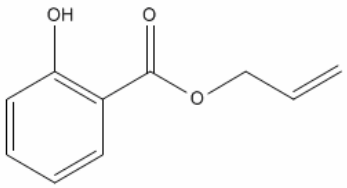
Allyl 1-naphthoate **[1]**



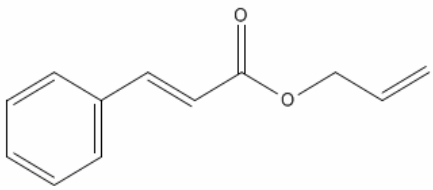
Allyl 2-thiophenecarboxylate **[2]**



Allyl 2-furoate **[3]**



Allyl salicylate **[4]**



Allyl cinnamate **[5]**

Figure 1. Chemical structures of the allyl esters tested.

Delayed effects

A minimum of 20 neonate larvae that had emerged from eggs treated with the LC_{50} or 10 mg/mL and from the respective control were transferred into plastic boxes (5 cm diameter, 2.5 cm height) using a fine paintbrush and maintained on a piece of semisynthetic diet in the same conditions as insect stock culture. Elapsed time until adult emergence and percent of adults emerging from allyl ester-treated eggs were compared with those of the controls by a Student *t*-test ($P < 0.05$) (SAS® Version 8; SAS Institute, Cary, NC).

Mortality on eggs of different phases

Aforementioned methodology was used to apply 0.1 µL of each allyl ester at a concentration of 10 mg/ml to *C. pomonella*, *G. molesta* or *L. botrana* eggs in three different egg developmental phases (white egg, red ring and black head). Three replicates of 30 eggs each were used for each allyl ester and egg phase for the three tested species. A binocular microscope was used to recognize the egg phases. Acetone-treated eggs were used as controls. Corrected mortality (Abbot, 1925) was analyzed by means of an ANOVA followed by Duncan's Multiple Range Test ($P < 0.05$) (SAS® Version 8; SAS Institute, Cary, NC).

Mortality on less than 24-h old larvae

U-shaped 0.5 ml wells of 96-well plates were half filled with solidified diet. A metal tube was used to press on the diet to eliminate crevices between the diet and the wall of the well. Five µL of a solution of each allyl ester was applied on the top of the diet using a micropipette. Plates were kept at room temperature during 2 h for solvent evaporation. Then, a less than 24-h old larva was put in each well, the plate was sealed with Parafilm and kept in the same conditions as the insect stock cultures. Three replicates with 12 larvae per replicate were used for each allyl ester and species. A minimum of five concentrations were tested for each product. Wells treated with 5 µL of acetone were used as controls and the mortality recorded on them had to be less than 20% to validate the replicate. Mortality was checked 24 h and three days after treatment. A probit analysis was performed following the aforementioned procedure.

Results

Synthesis of allyl esters

Yields obtained in the synthesis of allyl esters **1**, **2** and **4** were between 72 and 86%. The structures were confirmed by their NMR spectra [**1:** ^{1}H-NMR (400 MHz, $CDCl_3$): 4.84 (dt, J = 5.86 Hz, J = 1.56 Hz, 2H, O–C**H**$_2$), 5.25 (d, J = 10.55 Hz, 1H, **H**–C= *trans*), 5.38 (d, J = 17.2 Hz, J = 1.56 Hz, 1H, **H**–C= *cis*, C16), 6.03 (m, 1H, **H**C= *gem*, C15), 7.52 (m, J = 8.2 Hz, J = 6.6 Hz, 2H, **H**–C arom), 7.55 (m, J = 6.6 Hz, J = 1.56 Hz, 1H, **H**–C arom), 7.8 (d, J = 8.2 Hz, 1H, **H**–C arom), 7.94 (d, J = 8.2 Hz, 1H, **H**–C arom), 8.14 (dd, J = 7.03 Hz, J = 1.56 Hz, 1H, **H**–C arom), 8.85 (d, J = 8.6 Hz, 1H, **H**–C arom). **2**: ^{1}H-NMR (400 MHz, $CDCl_3$): 4.72 (d, J = 5.47 Hz, 2H, O–C**H**$_2$), 5.22 (d, J = 10.55 Hz, 1H, **H**–C= *cis*), 5.34 (d, J = 17.2 Hz, 1.56 Hz, 1H, **H**–C= *trans*), 5.95 (m, 1H, **H**–C= *gem*), 7.04 (t, J = 3.9 Hz, 1H, **H**–C ring), 7.49 (d, J = 5.1 Hz, 1H, **H**–C ring), 7.76 (d, J = 2.3 Hz, 1H, **H**–C ring). **4**: ^{1}H-NMR (400 MHz, $CDCl_3$): 4.78 (dt, J = 5.86 Hz, J = 1.56 Hz, J = 1.17 Hz, 2H, O–C**H**$_2$), 5.25 (d, J = 10.16 Hz, 1H, **H**–C= *cis*), 5.36 (d, J = 17.2 Hz, J = 1.56 Hz, 1H, **H**–C= *trans*), 5.96 (m, 1H, **H**–C= *gem*), 6.81 (t, J = 7.03 Hz, 1H, **H**–C arom, *para* to OH), 6.91 (d, J = 7.03 Hz, 1H, **H**–C arom, *ortho* to OH), 7.38 (m, J = 5.47 Hz, 1H, **H**–C arom, *meta* to OH), 7.81 (dd, J = 6.25 Hz, J = 1.56 Hz, 1H, **H**–C arom, *meta* to OH), 10.66 (s, 1H, OH)].

Mortality on less than 24-h old eggs

For each species, the hypotheses of equality and parallelism of the probit lines were rejected [*C. pomonella* ($\chi^2 = 585$, df = 8, $P < 0.001$ and $\chi^2 = 38.67$, df = 4, $P < 0.001$, for the hypotheses of equality and of parallelism, respectively); *G. molesta* ($\chi^2 = 577$, df = 6, $P < 0.001$ and $\chi^2 = 92.21$, df = 3, $P < 0.001$); and *L. botrana* ($\chi^2 = 491$, df = 8, $P < 0.001$ and $\chi^2 = 30.62$, df = 4, $P < 0.001$)]. For each allyl ester, the hypotheses of equality and parallelism of the probit lines were also rejected (allyl 1-naphthoate: $\chi^2 = 102$, df = 4, $P < 0.001$ and $\chi^2 = 55.96$, df = 2, $P < 0.001$; allyl 2-thiophenecarboxylate: $\chi^2 = 66.49$, df = 4, $P < 0.001$ and $\chi^2 = 11.57$, df = 2, $P = 0.003$; allyl 2-furoate: $\chi^2 = 39.11$, df = 4, $P < 0.001$ and $\chi^2 = 44.57$, df = 2, $P < 0.001$; allyl salicylate: $\chi^2 = 13.97$, df = 4, $P = 0.007$ and $\chi^2 = 9.01$, df = 2, $P = 0.011$); and allyl cinnamate: $\chi^2 = 59.13$, df = 4, $P < 0.001$ and $\chi^2 = 10.01$, df = 2, $P = 0.007$).

Table 1 shows the results of all the probit analyses carried out. With only one exception, allyl cinnamate (**5**) and allyl 1-naphthoate (**1**) were the most active allyl esters tested, followed by allyl 2-thiophenecarboxylate (**2**). Allyl 2-furoate (**3**) and allyl salicylate (**4**) were the least active ones.

Table 2 shows the mortality of less than 24-h old eggs treated with 10 mg/mL of each allyl ester and the percentage of eggs that died at each developmental phase. Untreated eggs, acetone-treated eggs and eggs treated with the least active compound (allyl salicylate, **4**) died mostly in the black head stage. By contrast, the most active allyl esters (allyl cinnamate, **5**, and allyl 1-naphthoate, **1**) caused egg death in an earlier developmental phase (white egg or red ring phase).

Duration of egg development

A significant increase in the duration of egg development respect to acetone-treated control was only observed in *C. pomonella* eggs treated with allyl 2-thiophenecarboxylate [control: 13.0 ± 0.4 d; allyl 2-thiophenecarboxylate: 14.5 ± 0.1 d ($t = 2.74$, df = 4, $P = 0.01$)]. No significant differences in duration of egg development of treated eggs compared with control (*G. molesta* = 10.8 ± 0.2 d, *L. botrana* = 10.5 ± 0.4 d) were observed in the rest of the cases ($P > 0.05$).

Delayed Effects

A significant reduction in percentage of adults emerging from surviving eggs was only observed when

Table 1. Results of the probit analyses for < 24-h-old *C. pomonella*, *G. molesta* and *L. botrana* eggs topically treated with 0.1 µL of allyl ester solutions. For the numbering of allyl esters see figure 1.

Species	Allyl ester	n	Slope ± SE	LC_{50} (CI) mg/ml	LC_{90} (CI) mg/ml	χ^2	HF
C. pomonella	1	630	5.5 ± 0.5	4.7 (4.4 - 5.1) a	8.1 (7.4 -9.1) a	16.5	0.8
	2	540	4.4 ± 0.5	7.9 (7.1 - 8.7) b	15.4 (13.4 - 19.0) bc	13.8	0.9
	3	540	8.6 ± 1.3	14.7 (13.3 - 16.0) c	20.6 (18.5 - 25.5) c	18.3	1.5
	4	450	3.6 ± 0.5	17.5 (14.4 - 25.8) c	47.1 (30.1 - 138.6) d	10.1	0.8
	5	450	3.3 ±0.3	3.8 (2.7 - 4.9) a	8.5 (6.4 - 15.5) ab	47.4	3.9
G. molesta	1	630	2.9 ± 0.3	4.4 (3.6 - 5.2) a	11.9 (8.9 - 20.8) ab	50.2	2.6
	2	720	3.5 ± 0.3	6.2 (5.5 - 6.8) b	14.3 (12.1 - 18.1) a	20.3	1.6
	3	450	8.9 ± 1.3	15.3 (14.2 - 16.6) c	21.4 (19.2 - 26.0) bc	11.2	1.1
	4	450	5.0 ± 0.6	14.1 (12.9 - 15.6) c	26.8 (21.1 - 38.2) b	10.3	0.9
	5	540	2.5 ± 0.2	3.3 (2.6 - 4.2) a	10.9 (8.4 - 15.9) a	33.2	2.2
L. botrana	1	630	2.1 ± 0.2	6.8 (5.9 - 7.9) c	27.2 (20.7 - 41) b	7.0	0.4
	2	450	2.4 ± 0.3	4.1 (3.2 - 5.0) b	13.8 (10.4 - 21.6) b	17.6	0.4
	3	450	4.4 ± 0.5	11.0 (8.7 - 15.2) d	21.6 (15.9 - 66.9) b	77.9	5.9
	4	450	4.0 ± 0.7	13.4 (11.2 - 17.8) d	28.0 (20.2 - 63.6) b	12.3	1.4
	5	450	3.5 ± 0.3	2.4 (2.1 - 2.7) a	5.6 (4.9 - 6.8) a	6.8	0.5

n = total number of treated eggs; CI = confidence intervals (95% probability); HF = heterogeneity factor.
For each species, values followed by the same letter in the same column are not significantly different because of the overlapping of the confidence intervals ($P < 0.05$).

Table 2. Mortality (%) and percent distribution of the developmental phase at death of < 24-h-old *C. pomonella*, *G. molesta* and *L. botrana* eggs topically treated with 0.1 µl of a 10 mg/mL solution of allyl esters. For the numbering of allyl esters see figure 1.

Species	Treatment	Mortality	Egg developmental phase at death		
			White egg	Red ring	Black head
C. pomonella	Control	1.5 ± 0.5	15.0 ± 8.1	10.0 ± 4.3	75.0 ± 9.7
	Acetone	10.1 ± 0.6	20.8 ± 6.6	16.2 ± 6.1	63.0 ± 8.0
	1	95.5 ± 1.3	0.0 ± 0.0	58.0 ± 3.0	42.0 ± 8.0
	2	69.4 ± 4.0	0.0 ± 0.0	45. 3 ± 11.1	54.7 ± 11.1
	3	13.3 ± 1.1	17.9 ± 9.5	71.0 ± 17.0	11.1 ± 7.5
	4	15.1 ± 7.6	0.0 ± 0.0	10.0 ± 0.0	90.0 ± 0.0
	5	100.0 ± 0.0	29.6 ± 10.4	64.3 ± 7.1	6.1 ± 3.8
G. molesta	Control	4.4 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0
	Acetone	7.6 ± 0.8	18.2 ± 12.5	7.6 ± 5.5	74.2 ± 12.7
	1	96.6 ± 0.1	27.5 ± 4.0	72.5 ± 4.6	0.0 ± 0.0
	2	65.3 ± 7.7	54.9 ± 17.5	23.6 ± 12.1	54.7 ± 11.1
	3	10.0 ± 2.9	0.0 ± 0.0	42.7 ± 26.0	58.3 ± 26.0
	4	24.2 ± 5.8	4.2 ± 2.0	15.0 ± 7.0	80.8 ± 10.8
	5	100.0 ± 0.0	91.3 ± 2.5	7.6 ± 3.4	1.1 ± 1.0
L. botrana	Control	5.0 ± 1.0	0.0 ± 0.0	1.0 ± 1.0	99.0 ± 4.9
	Acetone	5.2 ± 1.2	15.5 ± 12.1	1.5 ± 1.9	83.0 ± 12.0
	1	52.1 ± 3.1	24.2 ± 10.2	44.1 ± 14.2	31.6 ± 8.9
	2	51.3 ± 9.2	19.3 ± 7.4	40.4 ± 4.5	40.3 ± 6.4
	3	25.6 ± 1.7	44.8 ± 12.4	32.4 ± 3.8	22.9 ± 8.6
	4	42.5 ± 5.3	0.0 ± 0.0	3.3 ± 3.3	96.7 ± 3.3
	5	100.0 ± 0.0	77.6 ± 5.5	16.7 ± 2.6	5.7 ± 3.8

Three replicates of 30 eggs each one

L. botrana eggs were treated with allyl 2-furoate (acetone-treated control: 78.5.5 ± 2.4 %; allyl 2-furoate: 58.0 ± 3.5 %; $t = 3.93$, df = 15, $P = 0.001$). No significant differences were observed in the rest of the cases ($P \geq 0.05$, percent of emergence in the controls: *C. pomonella* = 77.2 ± 1.2 %; *G. molesta* = 69.3± 1.7 %).

A significant reduction in time until adult emergence was observed only in *G. molesta* when treated with allyl 2-furoate [control: 23.9 ± 0.2 days; allyl 2-furoate: 21.6 ± 0.1 days ($t = 4.57$, df = 28, $P < 0.001$)], whereas no significant differences were observed in the rest of the cases ($P > 0.05$, time to adult emergence: *C. pomonella*: 31.5 ± 0.4 d; *L. botrana*: 29.4 ± 0.8 d).

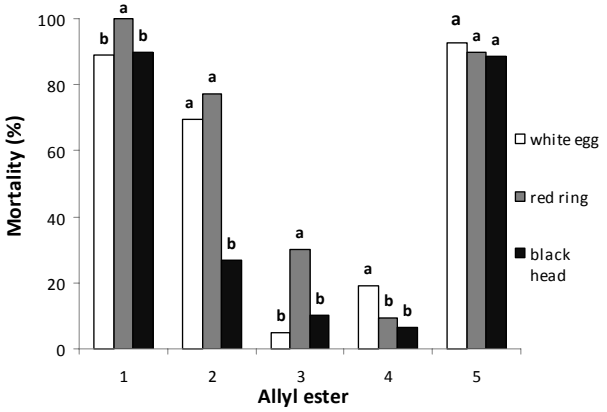


Figure 2. Mean corrected mortality of *C. pomonella* eggs treated in different developmental phases with 0.1 µL of a 10 mg/mL solution of allyl esters. (3 replicates, n = 30 eggs). For each ally ester, columns with the same letter were not significantly different (Duncan's multiple range test, $P < 0.05$). For the numbering of allyl esters see figure 1.

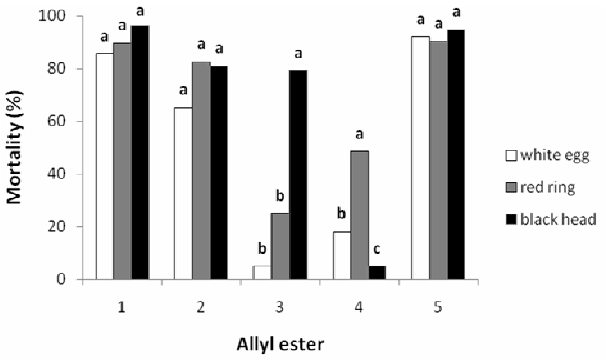


Figure 3. Mean corrected mortality of *G. molesta* eggs treated in different developmental phases with 0.1 µL of a 10 mg/mL solution of allyl esters. (3 replicates, n = 30 eggs). For each ally ester, columns with the same letter were not significantly different (Duncan's multiple range test, $P < 0.05$). For the numbering of allyl esters see figure 1.

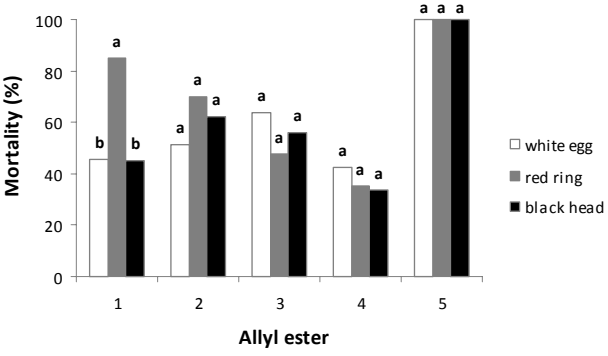


Figure 4. Mean corrected mortality of *L. botrana* eggs treated in different developmental phases with 0.1 µL of a 10 mg/mL solution of allyl esters. (3 replicates, n = 30 eggs). For each ally ester, columns with the same letter were not significantly different (Duncan's multiple range test, $P < 0.05$). For the numbering of allyl esters see figure 1.

Mortality on eggs of different phases

No general trend was observed in mortality produced by allyl esters when eggs were treated at different egg phases (figures 2, 3 and 4). *C. pomonella* egg mortality was independent from the egg phase at which the allyl esters were applied only in the case of compound **5**; mortality was significantly higher in the white egg phase for compound **4,** and in the red ring phase for compounds **1** and **3**. The activity of compound **2** decreased significantly when it was applied in the black head phase (figure 2). *G. molesta* egg mortality was also independent from the egg phase in the case of compounds **1**, **2** and **5**, but the action of compound **4** increased significantly when it was applied in the red ring phase, and the action of compound **3** increased significantly when it was applied in the black head phase (figure 3). *L. botrana* egg mortality was independent from the egg phase in the case of compounds **2**, **3**, **4** and **5,** but the action of compound **1** increased significantly if applied in the red ring phase (figure 4).

Mortality on less than 24-h old larvae

Table 3 shows the results of the probit analysis when mortality was recorded at 24 h from treatment. The most active allyl ester was allyl cinnamate, whose LC_{50}'s for the three species were significantly lower than those of the other allyl esters tested, with the exception of the LC_{50} of allyl 2-thiophenecarboxylate for *L. botrana*. The probit line of allyl salicylate was not calculated, as it did not did produce a significant mortality when tested at 10 mg/mL.

Hypotheses of equality and parallelism were rejected when all concentration-response lines of active allyl esters were compared ($\chi^2 = 495.0$, df = 24, $P < 0.001$ and $\chi^2 = 88.36$, df = 12, $P < 0.001$).

When species concentration-response lines were compared for each allyl ester, the hypothesis of equality was rejected in all cases (**1**: $\chi^2 = 33.17$, df = 4, $P < 0.001$; **2**: $\chi^2 = 30.49$, df = 4, $P < 0.001$; **3**: $\chi^2 = 78.96$, df = 4, $P < 0.001$; **5**: $\chi^2 = 37.86$, df = 4, $P < 0.001$). The hypothesis of parallelism was not rejected for compounds **1** and **5** ($\chi^2 = 2.04$, df = 2, $P = 0.361$; $\chi^2 = 0.78$, df = 2, $P = 0.676$, respectively) but it was rejected for compounds **2** and **3** ($\chi^2 = 22.76$, df = 2, $P < 0.001$ and $\chi^2 = 16.44$, df = 2, $P < 0.001$, respectively). When the comparisons were done per species, the hypotheses of equality and parallelism were rejected in *C. pomonella* ($\chi^2 = 196.0$, df = 6, $P < 0.001$ and $\chi^2 = 23.32$, df = 3, $P < 0.001$) and in *L. botrana* ($\chi^2 = 66.13$, df = 8, $P < 0.001$ and $\chi^2 = 16.31$, df = 4, $P = 0.003$) but not parallelism in *G. molesta* ($\chi^2 = 20.88$, df = 6, $P = 0.002$ and $\chi^2 = 4.54$, df = 3, $P = 0.209$).

No significant differences were observed when comparing LC values recorded 24 h and three days after the treatment, with the exception of allyl furoate on *C. pomonella*, where a significant reduction on LC values at 3 days respect 1 day was observed.

Discussion

The allyl esters of aromatic or heterocyclic acids had not been previously tested as pesticides. As a whole, the ovicidal activity of the tested allyl esters followed the

Table 3. Results of the probit analysis for < 24-h-old *C. pomonella*, *G. molesta* and *L. botrana* larvae exposed to diet topically treated with 5 µL of allyl esters. For the numbering of allyl esters see figure 1.

Species	Allyl ester	n	Slope ± SE	LC_{50} (CI) mg/ml	LC_{90} (CI) mg/ml	χ^2	HF
C. pomonella	1	250	2.5 ± 0.4	12.5 (10.1 - 16.1) b	41.6 (28.2 -88.8) b	22.3	1.2
	2	280	3.2 ± 0.5	9.4 (7.3 - 11.9) b	23.6 (16.8 - 55.2) ab	62.6	2.8
	3	360	7.6 ± 1.1	17.9 (16.3 - 19.5) c	26.4 (23.3 - 34.0) b	43.4	1.5
	5	320	3.4 ±0.4	6.1 (5.2 - 7.0) a	14.6 (12.5 - 18.2) a	20.9	0.8
G. molesta	1	180	3.0 ± 0.5	5.7 (4.2 - 7.3) b	15.1 (11.2 - 25.8) b	14.1	1.1
	2	180	3.1 ± 0.6	9.3 (7.5 - 11.5) c	23.9 (17.7 - 41.4) b	10.5	0.9
	3	280	5.1 ± 1.1	11.5 (9.0 - 13.1) c	20.6 (17.2 - 33.8) b	32.2	1.5
	5	210	3.3 ± 0.5	3.1 (2.5 - 3.7) a	7.5 (6.1 - 10.3) a	16.4	0.9
L. botrana	1	180	2.2 ± 0.6	12.5 (10.0 - 27.4)c	48.7 (29.3 - 184.1) a	4.4	0.6
	2	210	1.3 ± 0.2	5.9 (3.5 - 9.5) ab	13.8 (10.4 - 21.6) a	17.0	1.2
	3	252	2.4 ± 0.4	10.8 (7.7 - 15.6) bc	31.8 (19.5 - 70.0) a	69.2	3.1
	5	250	2.7 ± 0.4	4.8 (3.7 - 6.0) a	14.1 (9.9 - 29.8) a	41.3	2.1

n = total number of treated larvae; CI = confidence intervals (95% probability); HF = heterogeneity factor.
For each species, values followed by the same letter in the same column are not significantly different because of the overlapping of the confidence intervals ($P < 0.05$).

same trend in the three pest species tested: allyl cinnamate and allyl 1-naphthoate were more effective than the other three allyl esters. As the probit lines of all the allyl esters for the same species were not parallel, the results of the comparison of their toxicity depend on the concentration. At the LC_{50} level, the toxicity of allyl cinnamate was 5 times higher than the toxicity of the less active one (allyl salicylate). The comparison of the slopes of the probit lines also suggests that the mode of action of the compounds may differ, what would be explained by differences in chemical structure of tested compounds. When compared to allyl esters of fatty acids, the substitution in the structure of the molecule of alkyl chains for aromatic or heterocyclic moieties entails decreased the activity on *C. pomonella* eggs, as the LC_{50} of the most active allyl esters of fatty acids was 0.71 mg/mL (Escribà *et al*., 2009). The less active compound also took longer to kill the eggs than the most active ones did, as the death occurred at the black head phase in the former ones, and in the earlier phases (white egg or red ring) in the latter ones. The same trend was observed in the case of allyl esters from fatty acids (Escribà *et al*., 2009). For the three species, the toxicity of the allyl esters was not clearly produced in a specific phase suggesting that the tested compounds do not act as IGR, as IGR compounds mainly kill eggs in the red ring or black head phase (Canela *et al.*, 2000; Charmillot *et al.*, 2001; Sáenz-de-Cabezón *et al.*, 2005; 2006).

In general, all compounds had a similar activity on the three developmental egg phases tested. This fact would eventually facilitate its application in the field. It was more evident in the case of the most active compound, allyl cinnamate (figures 2, 3 and 4), suggesting a kind of suffocation produced in few time from application of allyl ester, as observed by other authors when using horticultural oils (Wins-Purdy *et al*., 2009).

In general, the duration of the development of treated eggs was not significantly different to that of the control ones, while allyl esters of fatty acids increased egg developmental time on *C. pomonella* (Escribà *et al.*, 2009). However, in the case of fatty acids allyl esters, although significantly different, the observed differences were < 1 d, and they had not any biological importance. No delayed effects in the surviving larvae from treated eggs were observed, reinforcing the hypothesis that allyl esters have not any IGR action.

The larvicidal effect of the tested allyl esters showed the same trend discussed for the ovicidal effect: allyl cinnamate were the most active one, and allyl 2-furoate and allyl salicylate the least active ones for the three species. LC_{50} of allyl cinnamate to eggs or larvae was very similar, what would facilitate its use in the field. The methodology used simulated field conditions, because only the surface of the diet was treated, as the leaf and fruit surface would be, so the amount of compound needed to act by ingestion had to be low. Only significant increase of LC_{50} of allyl naphthoate larvicidal action respect ovicidal action on *C. pomonella* and *L. botrana* were recorded suggesting that main action on larvae took place by contact.

Concentrations needed to kill eggs and larvae of the three species, even in the case of the most active compounds, indicate that high amounts of compound are needed in all cases. So, the possibility of synthesizing this family of products from biodiesel industry wastes with low economical value, such as glycerol, could be an advantage (Escribà *et al*., 2011). Yields obtained in the synthesis were high, and in the same range of those reported for other allyl esters (Escribà *et al.*, 2009; Eras *et al.*, 2009). Additionally, most of the tested allyl esters have been described as food aromas and fragrances (Bhatia *et al.*, 2004; Burdock, 2010), so low toxic effects on vertebrates may be expected. Another possible advantage would be the lack of cross-resistance. In a preliminary experiment, we tested the toxicity of allyl cinnamate on a *C. pomonella* population selected for resistance to Chlorpyrifos-ethyl, and it was not significantly different from its toxicity to the laboratory population, what suggests that cross-resistance is not probable to occur.

In conclusion, allyl cinnamate was the best candidate of the serie of allyl esters assessed against the three tor-

tricid pests studied. It acted as ovicide and larvicide, at similar concentrations on the three species, what could be an advantage in areas where the species cohabit. However, more assays were required about allyl ester fate in environmental conditions.

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