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Effects of methoxyfenozide on *Lobesia botrana* Den & Schiff (Lepidoptera: Tortricidae) egg, larval and adult stages

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Abstract: The effect of the non-steroidal ecdysone agonist methoxyfenozide was evaluated against different developmental stages of the grape berry moth, *Lobesia botrana* Dennis & Schiffermuller (Lep, Tortricidae). Methoxyfenozide administered orally reduced the fecundity and fertility of adults treated with 1, 5 and 10 mg litre^{-1}; longevity was not affected. An LC_{50} value of 4.5 mg litre^{-1} was obtained when applied to eggs of less than 1 day old. Surface treatment was more effective than when applied by spraying. Administered into the diet, methoxyfenozide had a larvicidal effect; older larvae were more susceptible than younger larvae, with LC_{50} values of 0.1 mg litre^{-1} for L_1, 0.04 for L_3 and 0.02 for L_5. Larvae treated with sub-lethal doses throughout their lives did not emerge as adults at the highest doses (0.08, 0.04, 0.02 and 0.01 mg litre^{-1}), with 65% and 40% emergence occurring for the lowest (0.005 and 0.0025 mg litre^{-1}). Mortality occurred only in the larval stage.

Keywords: *Lobesia botrana*; grape berry moth; methoxyfenozide; larval mortality; egg mortality; adult sterility

1 INTRODUCTION

The grape berry moth *Lobesia botrana* Dennis & Schiffermuller, is a major pest of European and Mediterranean vineyards. The yield reductions caused by the insect are due both to direct damage by the larva and to subsequent invasion by fungi. Most growers control this pest with traditional chemical pesticides; however, mating disruption and microbiological insecticides are used as alternatives in a few areas.[1] Considerable effort is being directed towards reducing the use of traditional pesticides with increased use of integrated pest management (IPM) techniques, emphasizing the joint use of natural enemies and selective pesticides, an alternative compatible with the protection of non-target organisms and the environment. Methoxyfenozide (RH-2485) is an ecdysone agonist that represents a new group of insect growth regulators.[2] Wing[3] and Wing *et al*[4] showed that RH-5849 and other diacylhydrazines caused insect larvae to moult prematurely and die. Many authors have demonstrated that these compounds have ovicidal effects.[5–8] Several effects that are related to changes of ecdysone (20E) homeostasis include alterations to development, behaviour and reproduction.[9] Methoxyfenozide is highly toxic to a wide range of lepidopteran larvae and has no effect on other arthropods orders.[9–13]

In the only published study of methoxyfenozide on *L botrana*, Pasquier and Charmillot[7] dipped eggs laid over 2 days on a grape cluster, obtaining a LC_{50} of 2 mg litre^{-1}. Additional studies are needed to determine the most efficient usage against this important pest.

2 MATERIALS AND METHODS

2.1 Insects

A stock culture of *L botrana* was established from larvae collected in an ecological vineyard in La Rioja (Spain) during May, 2000, and augmented with new individuals once a year. The colony was maintained in a growth chamber at 24 (±1)°C, 60 (±10)% RH and 16:8 h light:dark photoperiod, following the method described by Del Tío[14] modified by Sáenz-de-Cabezón Irigaray.[15] All bioassays were conducted under these uniform conditions.

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2.2 Chemicals
Methoxyfenozide 240 g litre^{-1} SC was obtained in Spain from Rohm & Haas Corp.

2.3 Effect on adult fecundity, fertility and longevity
To assess the activity of methoxyfenozide on *L botrana* adults, three pairs of recently emerged adults were introduced into an enclosure consisting of a 33-ml plastic container placed upside-down on the base of a 90-mm diameter Petri dish. Methoxyfenozide was administered orally by means of a water trough which contained 10, 5 or 1 mg litre^{-1} of methoxyfenozide mixed in a 10% honey solution. Water troughs were changed every 3 days to avoid fungal proliferation. Every day, the container onto which the eggs had been laid was replaced and the eggs were then transferred to plastic boxes (12 cm diameter, 5 cm high) until larval emergence. Fecundity and fertility were determined daily during the period of oviposition. Adult mortality was recorded daily. Five replicates, each replicate consisting of one rearing cage with three pairs of adults, were used for the treatment and the control (10% honey solution). Embryonic development was observed under a stereoscopic lupe (Olympus SZH-10).

2.4 Ovicidal bioassays
To test contact ovicidal effects, ten pairs of *L botrana* adults were introduced into an oviposition chamber consisting of a cylindrical plastic body (9 cm diameter, 13 cm high) covered inside with a transparency film on which females laid their eggs. The bottom was covered with a Petri dish, and the top with a filter paper. Moths were provided with a water trough. Eggs laid during the next 24 h were treated using a manually loaded Potter spray tower, with 5.5 ml in the tank at 20 kPa pressure producing a deposit of 0.05(±0.005)ml cm^{-2}. Dosages employed were obtained by preliminary assays that ranged from 0.39 to 25 mg litre^{-1}. Eggs of different age classes were collected and treated in the same manner. Treatments were made for the following age groups: 0–24, 24–48, 48–72, 72–96 h old. The dose employed was the LC_{50} obtained in the previous assay. Egg hatch was recorded 5 days after treatment. There were five replicates per dose/treatment, in addition to a water-treated control.

Surfaces were treated by dipping four plastic glasses in 1 litre solution of the LC_{50} contact dosage, and then air-drying for 5 min. Five pairs of adults were introduced and allowed to lay eggs for 24 h. Larval emergence from eggs was checked on the sixth day following treatment. Four replicates were used for the treatment and the control (dipped in distilled water).

2.5 Larvicidal bioassays
To determine the effect of methoxyfenozide on first, third and fifth instars, ten larvae were introduced into a 5-cm diameter Petri dish containing treated diet at concentrations ranging from 0.031 to 0.177 mg litre^{-1} for first, 0.0125 to 0.075 mg litre^{-1} for third and 0.00625 to 0.025 mg litre^{-1} for fifth instars (established by preliminary assays); five replicates were used for each dose and control. Mortality was measured when control larvae reached the next stadium (at the fourth, third and eighth day after treatment respectively for first, third, and fifth instars). The effects of sub-lethal doses were measured during the larval stage by providing, *ad libitum*, contaminated diet containing low concentrations of methoxyfenozide: 0.0025, 0.005, 0.01, 0.02, 0.04 and 0.08 mg litre^{-1}. Mortality and sublethal effects on treated individuals (such as ulcerations, malformations) were checked daily, and adult emergence recorded. We used five replicates of 10 individuals for each dose and the control.

2.6 Statistical analysis
Estimates of LC_{50} and LC_{90} for different stage mortalities and their 95% confidence limits were obtained using the POLO program[16] based on Finney.[17] The significance of results (fecundity, fertility, egg mortality, and larval mortality) was tested by ANOVA and means separated by a LSD multiple range test at $P < 0.05$ using SPSS.[18]

Abbott's formula[19] was used to correct mortality values. Percentage sterility was calculated using the formula of Toppozada *et al*.[20]

3 RESULTS
3.1 Effects on adults
Adult *L botrana* pairs treated *ad libitum* with methoxyfenozide experienced a decline in fecundity at each dose tested (Table 1). Total eggs laid per female [8.3 (±7.2), 0.6 (±0.3), 2.3 (±2.3) for 1, 5 and 10 mg litre^{-1} respectively] were significantly fewer than in the control [112.8 (±16.7)]. Nevertheless, *ad libitum* treatment did not decrease fertility in *L botrana* adults (Table 1). Eggs laid by methoxyfenozide-treated females did not produce embryos with the toxic symptoms arising from spray-treated eggs (see Sections 3.2 and 3.3). Longevity was not affected by treatment with any dose of methoxyfenozide.

3.2 Effects on eggs
The parameters obtained for the probit-log dose regression line on eggs treated with methoxyfenozide

Table 1. Fecundity and fertility parameters of adult *Lobesia botrana* pairs treated *ad libitum* with different doses of methoxyfenozide: each mean is the result of three pairs [a]

Dose (mg litre^{-1})	*n*	Fecundity (±SD)	Fertility (%) (±SD)	Corrected fertility[19] (%)	Sterility (%)[20]
0	5	112.8 (±16.74) a	95.1 (±0.64)	—	—
1	5	8.3 (±7.23) b	96.9 (±3.06)	0	92.5
5	5	0.6 (±0.33) b	100	0	99.4
10	5	2.3 (±2.33) b	100	0	97.9

[a] Means followed by different letters are significantly different at $\alpha = 0.05$. (ANOVA and LSD).

Table 2. Probit-log dose-response regression line parameters of different stages of *Lobesia botrana* treated with methoxyfenozide

Stage/ stadia	Slope (±SE)	Intercept (±SE)	χ^2	*G*	LC_{50} (mg litre^{-1}) (95% CL)	LC_{90} (mg litre^{-1}) (95% CL)
Egg	1.023 (±0.307)	4.328 (±0.162)	0.4	0.089	4.537 (2.694; 7.126)	81.240 (40.086; 270.008)
L_1	1.679 (±0.248)	6.618 (±0.364)	2.1	0.151	0.109 (0.087; 0.147)	0.630 (0.350; 2.300)
L_3	2.902 (±0.380)	6.618 (±0.364)	2.0	0.066	0.040 (0.035; 0.048)	0.112 (0.086; 0.170)
L_5	3.378 (±0.503)	10.960 (±0.914)	2.4	0.085	0.017 (0.015; 0.020)	0.041 (0.032; 0.063)

Table 3. Hatching percentage and egg mortality between treatments, for 0- to 24-h-old eggs treated with 4.5 mg litre^{-1} of methoxyfenozide[a]

Treatment	*n*	Hatching (%) (±SE)	Corrected mortality[19] (%) (±SE)
Control	5	90.7 (±1.3)a	—
Surface treatment	5	14.6 (±2.9)b	83.9 (±3.5)
Contact	5	54.2 (±4.7)c	39.8 (±4.6)

[a] Means followed by different letters are significantly different at $\alpha = 0.05$. (ANOVA and LSD).

Table 4. Hatching percentage and corrected mortality of four age classes of *Lobesia botrana* eggs treated with 4.5 mg litre^{-1} of methoxyfenozide[a]

Egg classes	*n*	Hatching (%) (±SE)		Corrected mortality[19] (%) (±SE)	
		Control	Treated	Control	Treated
0–1	5	90.7 (±1.3)	54.2 (±4.7)	—	40.2 (±4.6) a
1–2	5	92.5 (±2.4)	57.0 (±3.5)	—	38.4 (±3.2) a
2–3	5	96.4 (±3.1)	70.3 (±2.6)	—	27.1 (±3.0) b
3–4	5	93.7 (±2.6)	74.3 (±2.6)	—	20.7 (±3.1) b

[a] Within the columns, data followed by the same letter do not differ significantly ($\alpha = 0.05$).

and aged less than 24 h are given in Table 2. As shown by the results in Table 3, surface treatment was more effective than spray treatment. Methoxyfenozide significantly reduced emergence on all egg classes. The reduction was higher for eggs less than 48 h old than for the other egg classes (Table 4). Methoxyfenozide interrupted normal egg development, producing deformities in the body, cephalic capsule and mandibles upon reaching the final black-head stage.

3.3 Effects on larvae

Table 2 shows probit-log dose regression line parameters obtained for the different stages tested. Older larvae were more susceptible than younger larvae. Phenotypic effects included inability to shed the old cuticle and cephalic capsule. Larvae that reached the next moult showed mouth part deformities, ulcers and malformations. When treated with sub-lethal doses of methoxyfenozide, there was a high percentage of larval mortality in the third stadium with the highest doses tested. Almost 100% of the individuals died without reaching adulthood at doses above 0.01 mg litre^{-1}. Lower doses reduced adult emergence up to 60%. Mortality occurred only during the larval stage (Fig 1).

4 DISCUSSION

Given these results, we can affirm that the ecdysteroid agonist methoxyfenozide had larvicidal and ovicidal effects, and significantly affected the reproduction of *L botrana*. Only one previous study relative to methoxyfenozide and *L botrana* has been published. In

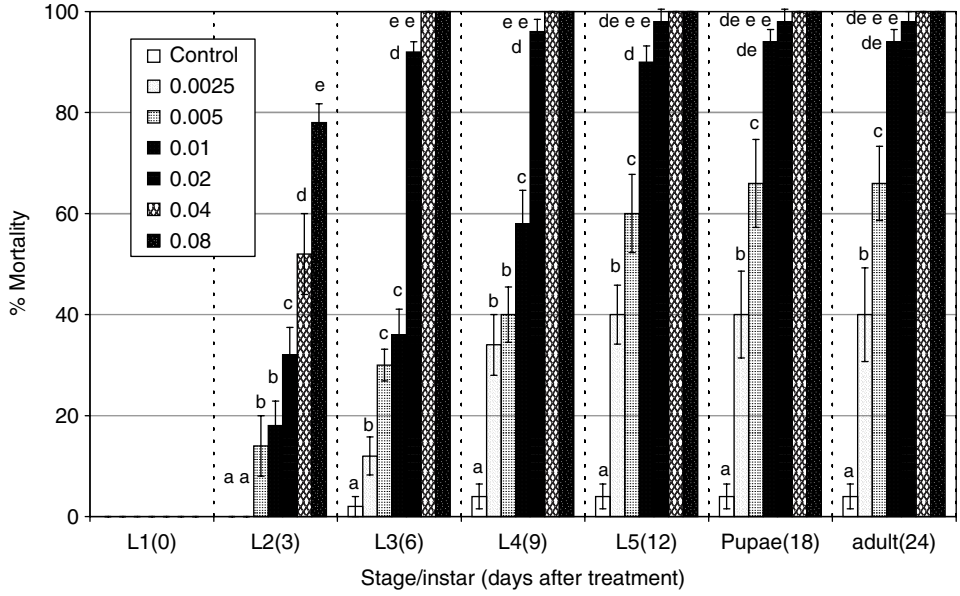

Figure 1. Stage-instar mortality when larvae are treated *ad libitum* with sub-lethal doses of methoxyfenozide (mg litre^{-1}).

their study, Pasquier and Charmillot[7] treated eggs laid for 2 days on grape clusters by immersion, obtaining a LC_{50} of 2 mg litre^{-1}. In this study, we have assessed the compound's effects on *L botrana* adults, eggs and larvae, emphasizing the time and mode of treatment.

4.1 Effects on adult fertility, fecundity and longevity

Methoxyfenozide strongly affects the reproduction of *L botrana*, producing a high percentage of sterility at the doses tested. Fecundity was severely reduced, and almost no eggs were laid at each dose tested. Fertility was not affected by methoxyfenozide, but due to the low number of eggs laid, these results are not conclusive. It has been demonstrated that adult exposure to nonsteroidal agonists by topical, surface or ingestion treatments, reduces fecundity and/or fertility among different lepidopterans.[9,21–28] In contrast, Ishaaya *et al*[29] observed a significant increase in fecundity when *Spodoptera littoralis* (Boisduval) larvae were fed on leaves treated with sub-lethal doses of methoxyfenozide. Smagghe and Degheele[23] and Smagghe *et al*[24] suggest that these results may be due to interference with ovulation by the pesticide as a result of ovariole reabsorption. It is known for *Drosophila* spp, and *Cydia pomonella* L that 20E levels play a important role during oogenesis and fertility,[28,30] and the regulatory cascade is needed for oogenesis. Tebufenozide is also believed to interfere with spermatogenesis in lepidopteran males.[9]

Eggs laid by females did not show embriocidal effects, which could be due to pesticide transference through the gravid female.[25,27]

Given the mode of action of ecdysone agonists, it is not surprising that there is no effect on adult longevity.

4.2 Ovicidal effects

Methoxyfenozide showed a high degree of activity against eggs of *L botrana*. As reported by other authors with similar compounds, younger eggs are more susceptible than older ones.[8,9] Methoxyfenozide had the same effect on eggs up to 48 h, and was less effective against older ones. Trisyono and Chippendale[5,6] treated *Ostrinia nubilalis* (Hübner) and *Diatraea grandiosella* Dyar 0- to 72-h-old eggs, dipping them in concentrations from 1 to 200 mg litre^{-1} and showing ovicidal activity (mortality went from 40 to 100%). Charmillot *et al*[8] obtained little effect and could find no relationship between dose and mortality when treating 1-day-old *C pomonella* eggs by dipping in methoxyfenozide. We found that surface treatment was more effective than treatment by spraying.

Eggs that reach the 'black-head' stage develop into embryos that have abnormal head capsules, weak mandibular attachment and other diverses malformities.

4.3 Larvicidal effects

Lobesia botrana larvae were highly susceptible to diet containing methoxyfenozide, with older stages being more susceptible than younger ones.

Larvicidal activity was proportional to age, but, as shown by other authors with other lepidopteran species,[9] younger ones are more susceptible than older ones. Therefore, our results differed from previous studies. 20E response was determined by the quantity of EcR (ecdysone receptor complex) and USP (Ultraspiracle) available;[31] USP levels are constant but EcR level could vary. It could be that EcR levels are greater in older stages than in younger stages, triggering the response at lower levels of 20E.

Sub-lethal doses of methoxyfenozide resulted in high mortality of the first stages, and progressively less mortality at lower doses. Smagghe and Degheele[22] found that for *S exempta* (Walker) and *S exigua* (Hübner) larvae, excretion rate was very fast, with a half life of only 2–3 h inside the insect body. However, because the elimination of 20E is given in the active form by means of a bomb of sodium,[32] it appears that the insect increases its capacity for elimination, clearing major quantities of product from older instars.

Larvae treated with methoxyfenozide show the typical symptoms of ecdysone agonists: extra cephalic capsule, feeding inhibition, etc.[9]

4.4 Integrated pest management

More laboratory, small plot and field testing is required before the insecticidal potential of methoxyfenozide against *L botrana* will be understood completely, but the activity observed in our studies suggest that the combination of the acutely toxic and sub-lethal doses could lead to the incorporation of this compound in IPM programmes against the grape berry moth. Sprays for the second and third generations should be applied during the peak flight, to cover oviposition period and egg hatching.

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