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Residual activity of chitin synthesis inhibitors on *Lobesia botrana* larvae reared in the laboratory on field collected grape berries

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Abstract

The residual activity of two chitin synthesis inhibitors (flufenoxuron and lufenuron) was compared to the neurotoxic insecticide (fenitrothion) against *Lobesia botrana* (Denis and Schiffermüller) larvae. Applications of insecticides against the 1st generation of *L. botrana* to reduce the 2nd generation were considered unnecessary and contrary to IPM programs. Recently, it has been demonstrated that the use of chitin synthesis inhibitors against the 1st generation, unlike neurotoxic insecticides, reduces the 2nd generation to the same extent as treatments aimed specifically at that generation. To ascertain if this effect was due to the residual activity of these compounds, larvae of *L. botrana* were reared under laboratory conditions on grape berries treated with insecticides in the field and collected at the beginning of the 2nd generation. Larvae reared on berries treated with chitin synthesis inhibitors against the 1st generation (before or at the end of flowering) or against the 2nd generation (berries pea-sized) showed the same level of mortality, irrespectively whether the berries had been treated 9 or 45 days earlier. In contrast, mortality due to fenitrothion decreased significantly the second week after treatment. Therefore the IGRs residual activity can explain how applications made during the 1st generation can also control European vine moth 2nd generation.

Key words: Lepidoptera, Tortricidae, *Lobesia botrana*, European vine moth, chemical control, chitin synthesis inhibitor.

Introduction

The European vine moth, *Lobesia botrana* (Denis *et* Schiffermüller) (Lepidoptera Tortricidae), and the European grape berry moth, *Eupoecilia ambiguella* (Hübner) (Lepidoptera Cochylidae), are the principal insect pests in European vineyards. The larvae of the 1st generation of both species feed on flowers and normally cause harvest loss only if infestation levels exceed one larval nest per cluster (Coscollá *et al*., 1982; Roehrich and Boller, 1991). This population level is rarely exceeded in northern Italy (Pavan *et al*., 1989). The larvae of the 2nd and 3rd generations feed on berries, causing yield loss and the spread of rots (e.g. *Botrytis cinerea* Persoon). Thus the economic injury levels are much lower than for the attacks of 1st generation (Roehrich and Boller, 1991; Pavan *et al*., 1998).

The application of neurotoxic insecticides against the 1st generation of grape berry moths to reduce the 2nd generation was not considered in IPM programs since there is only a weak or modest correlation between the infestation level of the two generations (Coscollá, 1997; Emery and Schmid, 2001; Pavan, unpublished data). In any case, the 2nd generation is better controlled by specific treatments (Bressan *et al*., 2002). Moreover, treatments against the 1st generation are frequently associated with spider mite outbreaks (Duso *et al*., 1989) and can cause the death of honeybees (Bolchi Serini *et al*., 1985; Wallner, 1997; Sgolastra *et al*., 2005).

Recently, the application of insect growth regulators (IGRs), chitin synthesis inhibitors (e.g. flufenoxuron) and moulting accelerating compounds (e.g. tebufenozide) (Charmillot, 1989; Charmillot *et al*., 1994), against the 1st generation gave the opportunity to control the 2nd generation with the same efficacy as specific treatments (Barbieri, 1997; Boselli *et al*., 2000; Bressan *et al*., 2002).

Barbieri (1997) hypothesised that IGRs applied against the 1st generation affect the subsequent generations because they have a high efficacy on larvae, reduce reproductive capacity of survived population and are little toxic to natural enemies. In a later study, flufenoxuron used against the 1st generation of both *L. botrana* and *E. ambiguella* moths, controlled the 2nd generation with the same effectiveness irrespective of plot size (0.5 hectare or 36 m^2), showing that the efficacy of the treatment on the subsequent generation was not reduced when the risk of colonization by adults moving from surrounding untreated plots is higher (Bressan *et al*., 2002).

The aim of this research was to ascertain whether the residual activity of chitin synthesis inhibitors used in the field against the 1st generation of *L. botrana* could explain the reduction in the 2nd generation population.

Materials and methods

Two trials were carried out in north eastern Italy (Friuli Venezia Giulia region) in 2004 testing the residual activity of insecticides in the laboratory by rearing larvae on berries collected in the field from previously treated vineyards.

Field treatments

The first trial was conducted in a vineyard of the cultivar “Chardonnay”. The treatments are described in table 1. Flufenoxuron and lufenuron were applied, using an air-assisted sprayer, on a plot of about 5000 m^2. Sixteen clusters, chosen in advance according to a an a priori scheme (Pavan *et al*., 1998), were collected from each plot on June 27, when it was expected that *L. botrana* eggs of the 2nd generation would begin hatching in the field (22 days after the first application and 12 days after the second).

The second trial was conducted in a vineyard of the cultivar “Tocai friulano”. The treatments are described in table 1. Each insecticide was applied, using a back-mounted mistblower, on 4 plots of about 50 m^2. Before both the first and the second application with flufenoxuron, 10 clusters per plot were enclosed in polyethylene bags. These were removed 2 hours later, when the unbagged clusters had dried. Sixteen clusters, chosen in advance according to an a priori scheme, were collected from each treatment (four per plot) on July 2, when the earliest 2nd generation larvae were observed inside the berries in the field. The fenitrothion treatment was also applied on this date. Flufenoxuron had been sprayed respectively 34 and 7 days earlier.

Laboratory assays

The activity of insecticides applied in the field was tested in the laboratory by enclosing two berries and one egg of *L. botrana* in cylindrical polystyrene capsules (r = 2.5 cm; h = 1.8 cm). The berries were randomly removed from the clusters previously collected in the field.

In the first trial, 35 capsules per treatment were prepared on June 27. In the second trial, to simulate egg laying in the field, 40 capsules per treatment were prepared on four different dates (10 on July 2, 5 on July 3, 10 on July 4, 10 on July 5 and 5 on July 7). In the latter trial, the clusters, collected on July 2, were kept in polyethylene bags until July 7 at 12 ± 1 °C.

The capsules were then kept in a climatic chamber (16:8 L:D; 70 ± 5 RH; T = 21 ± 1 °C) and checked daily until egg hatch.

Penetration of larvae into berries was checked on July 13 in the first trial and on July 23 in the second. When the egg had hatched but the larva had died outside the berries, whether it had or had not fed was visually determined.

On August 25 in the first trial and on August 13 in the second the infested berries were manually dissected to check if the larvae were alive or dead. Thereafter the berries were examined weekly. At each check the live larvae were left in the berries, whereas dead ones, together with head capsules of previous instars, were mounted on microscope slides. If pupae were found, they were left in the capsules until the adult emergence.

Observation of dead larvae under dissection microscope and measurement of mandible length allowed determining the larval instar (head-capsule is black in 1st instar and brown in the others). In a preliminary study the mandible length (mean ± SD) of different instars had been determined (91.6 ± 5.3 μm for the 2nd instar, 134.3 ± 8.2 μm for the 3rd instar, 192.7 ± 12.1 μm for the 4th instar).

For each trial and treatment the individual larvae (except those that had died before feeding on berries) were classified as: (1) died as 1st instar or during moulting

Table 1. Treatments compared in trial 1 on cultivar Chardonnay and in trial 2 on cultivar Tocai friulano in 2004 (a. i. = active ingredients; bag = bagged clusters).

Trial	Treatment	Active ingredient	Commercial formulation (% a. i.)	Dosage (g a. i. / ha)	Application date (growth stage)	Target generation
1	Flu I	flufenoxuron	Cascade DC (4.7%)	47	June 5 (end of flowering)	First
	Luf I	lufenuron	Match EC (5.32%)	53		
	Flu II	flufenoxuron	Cascade DC (4.7%)	47	June 15 (berries pea-sized)	Second
	Luf II	lufenuron	Match EC (5.32%)	53		
	Untreated					
2	Flu I	flufenoxuron	Cascade DC (4.7%)	47	May 29 (inflorescences fully developed)	First
	Flu I + bag	flufenoxuron	Cascade DC (4.7%)	47		
	Flu II	flufenoxuron	Cascade DC (4.7%)	47	June 25 (berries pea-sized)	Second
	Flu II + bag	flufenoxuron	Cascade DC (4.7%)	47		
	Fen II	fenitrothion	Sumifene EC (47.5%)	710	July 2 (beginning of berry touch)	
	Untreated					

from 1st to 2nd instar larva, (2) died as 2nd instar larva, (3) died while moulting from 2nd to 3rd instar larva or as 3rd instar larva, (4) died as 4th or more advanced instar larvae, (5) died as pupa, (6) reaching adult stage. Larvae were considered as moulting when the mandibles of the next instar were visible under the dissection microscope.

Larvae from each trial and treatment were further subdivided into two groups depending on the egg-hatching period (July 2-3 and July 4-5 in the first trial and July 4-9 and July 10-14 in the second trial).

To compare proportions the χ^2 test was used.

Results

Trial 1

In the different treatments a number of larvae varying from 29 to 33 began feeding on berries after hatching (table 2). Only one of the larvae died without feeding.

All the eggs hatched in the four days after they were enclosed in capsules (table 3). About 70% of the eggs hatched on July 2 or 3 and the rest on July 4 or 5 (27 to 30 days after the first insecticide application in the field and 17 to 20 days after the second).

The percentage of larvae that died young (L1-L3 instars) was significantly higher on berries treated with the two IGRs than on untreated berries considering both the larvae that died before the second moulting (total A) and all those that died before the third moulting (total B) (table 2). In the untreated control larvae always reached at least the 4th instar and 21 out of 32 pupated.

The percentage of dead larvae before the second moulting (total A), over the total number of the larvae dead before the third moulting (total B), did not differ among the treatments (table 2).

For both IGRs the treatments applied at the end of flowering (Flu I and Luf I) were significantly more effective than when applied 10 days later (Flu II and Luf II) (table 2). In the second-application period, flufenoxuron caused a higher rate of mortality than lufenuron ($p < 0.05$).

The mortality of larvae was not significantly different in relation to the egg-hatching period (table 3).

Among those larvae that survived beyond the 3rd in-

Table 2. Development stages reached by each individual in trial 1 (L1 = 1st instar larvae; L1-2 = larvae moulting from 1st to 2nd instar; L2 = 2nd instar larva; L2-3 = larvae moulting from 2nd to 3rd instar; L3 = 3rd instar larva).

Treatment		Total individuals (a)	Total A (L1 + L1-2 + L2)	χ^2 (b)	Total B (Total A+ L2-3 + L3)	χ^2 (b)	>L3	Pupa	Adult
Flu I	No.	29	24	dD	25	dD	2	1	1
	%	100	83		86		7	3	3
Luf I	No.	33	19	cCD	22	cdCD	10	1	0
	%	100	58		67		30	3	0
Flu II	No.	30	14	bcBC	16	cC	14	0	0
	%	100	47		53		47	0	0
Luf II	No.	32	8	bB	9	bBC	20	0	3
	%	100	25		28		63	0	9
Untreated	No.	32	0	aA	0	aA	11	10	11
	%	100	0		0		34	31	34

(a) newly hatched larvae and feeding on berries.
(b) differences in small letters and capital letters indicate respectively 5% and 1% levels of significance at the χ^2 test.

Table 3. Percentage of dead larvae (dead/total hatched) in trial 1 and 2 in two hatching periods.

Trial	Treatment	Hatching period	
		July 2 to 3	July 4 to 5
1	Flu I	80% (16/20)	89% (8/9)
	Luf I	62% (18/29)	50% (1/4)
	Flu II	43% (9/21)	44% (4/9)
	Luf II	30% (7/23)	44% (4/9)
	Untreated	0% (0/18)	0% (0/14)
		July 4 to 9	July 10 to 14
2	Flu I	92% (13/14)	100% (22/22)
	Flu I + bag	33% (4/12)	37% (7/19)
	Flu II	100% (19/19)	100% (12/12)
	Flu II + bag	0% (0/6)	6% (2/31)
	Fen II	80% (12/15)	45% (13/22)
	Untreated	7% (1/15)	0% (0/23)

star, a significantly lower number reached the adult stage in Flu II, Luf I and Luf II treatments than in the untreated group (table 2).

Trial 2

In the different treatments a number of larvae varying from 31 to 37 began feeding on berries after hatching (table 4). Ten eggs did not hatch and twenty-one of the live larvae died without feeding on berries. The proportion between the unhatched eggs plus larvae that died before feeding and the total number of eggs (40 per treatment) was not significantly different among the treatments.

The eggs hatched gradually from July 4 to July 14 (40-50 days after the first-application period, 9-19 days after the second-application period and 2-12 days after the third-application period) (table 3). About 40% of eggs hatched from July 4 to 9 and the rest from July 10 to 14.

The percentage of larvae that died young (L1-L3 instars) was always significantly higher on berries sprayed with insecticides (whose clusters had not been enclosed in bags) than on untreated berries considering both those that died before the second moulting (total A) and all those that died before the third moulting (total B) (table 4). In the untreated control and in Fen II all the larvae that survived the 1st instar always reached at least the 4th instar and 35 out of 49 pupated.

Flufenoxuron treatment, when flowering had not yet begun (Flu I), was just as effective as when applied to pea-sized berries (Flu II) (table 4). Nevertheless, the latter treatment showed a faster action, since the proportion of larvae that died before the second moulting (total A) was significantly higher in Flu II (94%) than in Flu I (72%). For both the treatments the larval mortality was significantly lower in bagged than unbagged clusters (table 4). The berries collected from clusters bagged during the first-application period, in contrast to those from clusters bagged during the second-application period, showed a larval mortality significantly higher than that observed on untreated control berries. In the first-application period the percentage of dead larvae before the second moulting (total A), over the total number of the larvae dead before the third moulting (total B), was higher on berries of unbagged clusters (74%) than on the bagged ones (55%) (table 4).

The mortality of larvae on berries treated with fenitrothion, unlike those treated with flufenoxuron, was significantly higher in the early-hatched group (eggs hatched 2-7 days after application in the field) than in the late-hatched group (eggs hatched 8-12 days after application in the field), showing a reduction in activity of the organophosphate during the time (table 3).

Discussion and conclusions

The laboratory data showed that the hypothesised persistence does explain the effectiveness of treatments using flufenoxuron and lufenuron against the 1st generation larvae in the control of the 2nd generation larvae. The high mortality observed indicates that the residual bioactivity is a deciding factor that explains the field data.

Flufenoxuron gives a high level of larval mortality only when the treatment directly hits the clusters. However, slight mortality was also observed on berries of clusters bagged during an application carried out before the beginning of flowering (trial 2). Redistribution of the product due to rain or other causes in the days following treatment could explain this.

Flufenoxuron applied before the beginning of flowering, when ovaries are covered by flowerhoods, is able to cause a significant mortality at least up to 45 days following its application in the field. The strong penetrative activity and persistence of flufenoxuron on flowers was indirectly confirmed by the same effectiveness of treatments carried out respectively before flowering and one month after (see trial 2), even if a faster action was observed in the second case. The high degree of pene-

Table 4. Development stages reached by each individual in trial 2 (L1 = 1st instar larvae; L1-2 = larvae moulting from 1st to 2nd instar; L2 = 2nd instar larva; L2-3 = larvae moulting from 2nd to 3rd instar; L3 = 3rd instar larva).

Treatment		Total individuals (a)	Total A (L1 + L1-2 + L2)	χ^2 (b)	Total B (Total A+ L2-3 + L3)	χ^2 (b)	>L3	Pupa	Adult
Flu I	No.	36	26	cB	35	dD	0	0	1
	%	100	72		97		0	0	3
Flu I + bag	No.	31	6	bA	11	bC	7	6	7
	%	100	19		35		23	19	23
Flu II	No.	31	29	dC	31	dD	0	0	0
	%	100	94		100		0	0	0
Flu II + bag	No.	37	2	abA	2	aA	16	8	11
	%	100	5		5		43	22	30
Fen II	No.	37	25	cB	25	cC	4	2	6
	%	100	68		68		11	5	16
Untreated	No.	37	0	aA	0	aA	10	12	15
	%	100	0		0		27	32	41

(a) newly hatched larvae and feeding on berries.
(b) differences in small letters and capital letters indicate respectively 5% and 1% levels of significance at the χ^2 test.

tration of flufenoxuron into berries during the earlier growing period could explain the fact that, when applied at fruit set, it was more effective than when applied 10 days later (berries pea-sized) (see trial 1). IGRs long lasting effect could influence also *E. ambiguella* attacks.

Persistence of flufenoxuron for at least two months was observed against *Empoasca vitis* (Göthe) on grapevine (Stefanelli *et al*., 2000). Residual activities of 16 months against leaf-chewing lepidopterans on pine and of a year against *Sitophilus oryzae* (L.) in stored wheat grain were respectively noticed (Skatulla and Kellner, 1989; Eisa and Ammar, 1992). Therefore, it is possible that even the control of the 3rd generation of *L. botrana*, observed in plots treated against the 1st generation with flufenoxuron in Italy (Boselli *et al*., 2000), is due to persistence. Strong residual activity of flufenoxuron must be evaluated also in relation to the risk of selecting resistant pest populations and harmful side effects on beneficial insects.

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