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Residual activity of insecticides applied against *Lobesia botrana* and its influence on resistance management strategies

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Abstract

Some insect growth disruptors, in particular chitin synthesis inhibitors (benzoylphenyl ureas) and ecdysone agonists (bisacylhydrazines), show high efficacy against *Lobesia botrana* (Denis et Schiffermuller) (Lepidoptera Tortricidae) and are characterized by a longer residual activity than the traditional organophosphate active ingredients. Previous research had shown that the persistence of some active ingredients applied against the first generation of *L. botrana* also ensured control of the second generation. In this study, the residual activity of insecticides applied against the second generation was evaluated on the third generation in field trials and laboratory bioassays. Methoxyfenozide controlled the third generation at the same efficacy level (more than 90%) when applied against the second or the third generations. Some residual activity was observed in the field also for indoxacarb (efficacy 75%). In contrast, a very low residual activity (efficacy lower than 30%) was recorded for chlorpyrifos. Because a longer residual activity is associated with a higher risk of selecting insecticide resistant populations, operational resistance management strategies are discussed to ensure a longer usable life span of these insecticides.

Key words: European grapevine moth, insect growth disruptors, neurotoxic insecticides, insecticide persistence, insecticide resistance.

Introduction

The European grapevine moth, *Lobesia botrana* (Denis et Schiffermuller) (Lepidoptera Tortricidae), is the major pest in European vineyards and recently it was found in the Americas (Ioriatti *et al.*, 2012). The species completes from two to four generations per year depending on climate and annual meteorological conditions (Coscollá, 1997; Roehrich and Boller, 1991; Martin-Vertedor *et al.*, 2010; Pavan *et al.*, 2013). The larvae of the first generation feed on flowers, whereas those of the subsequent generations feed on berries in different phases of their development. In Italy, the number of insecticide applications per year against this moth varies from 1-2 in northern grape-growing areas to at almost 3 in southern areas (Guario *et al.*, 2005; Scannavini *et al.*, 2006). Economic damage is normally associated with the carpophagous generations that cause yield losses and qualitative damage due to a higher spread of bunch rots (Pavan *et al.*, 1987; 1998; 2014; Moschos, 2006). Thus, until the last years of the 20th century, insecticide applications against *L. botrana* were aimed at controlling the second and third generations. Moreover, the use of neurotoxic insecticides against the first generation was not considered useful for a better control of the carpophagous generations (Coscollá, 1997; Emery and Schmid, 2001) and could be associated with spider mite outbreaks (Duso *et al.*, 1989). Since chitin synthesis inhibitors (benzoylphenyl ureas as flufenoxuron and lufenuron) and moulting accelerating compounds (bisacylhydrazines as tebufenozide) applied against the first generation controlled the second generation as well as applications targeted directly against this latter generation (Pavan *et al.*, 2005), from the late twentieth century many farmers have applied these insecticides against the

first generation. At first it was thought that the prolonged control of the *L. botrana* population, resulting from the application of these active ingredients, was due to their high effectiveness, often near to 100%, and selectivity towards natural enemies (Barbieri, 1997; Boselli *et al.*, 2000). Later it was shown that the control of two consecutive generations (i.e. first and second generations) was due to the high persistence of these insecticides (Pavan *et al.*, 2005). These results confirm the long-term residual activity of some benzoylphenyl ureas and bisacylhydrazines reported in literature (Pener and Dhadialla, 2012). More recently, also methoxyfenozide, an ecdysone agonist insecticide very effective against *L. botrana* (Sàenz-de-Cabezón Irigaray *et al.*, 2005; Charmillot *et al.*, 2006; Smagghe *et al.*, 2012), was shown to control at low population levels the second generation when applied against the first generation (Scannavini *et al.*, 2006).

The aim of this research was to determine if applications of methoxyfenozide against the second generation of *L. botrana* would also control the third generation. In fact, if methoxyfenozide applications made at flowering time are effective against second-generation larvae developing on green berries, it can be expected that applications made on green berries would be effective against third-generation larvae developing on ripening berries. In North America, methoxyfenozide significantly decreased infestation of the grape berry moth *Paralobesia viteana* (Clemens) for more than two months (Teixeira *et al.*, 2009). Moreover, considering that neurotoxic indoxacarb applied against the first generation was also effective in controlling the second generation (Scannavini *et al.*, 2006), the residual activity of this active ingredient applied against the second generation was also tested.

Table 1. Active ingredients, commercial products, application rates of active ingredients and treatments compared in the two years against *L. botrana*. II = insecticide applied only against the second generation; III = insecticide applied only against the third generation.

Active ingredient (a.i.)	Commercial product	% a.i. in commercial product	Field rate (a.i. / ha)	Target generation	
				2008	2011
Methoxyfenozide	Prodigy (Bayer)	22.5	90 mL	II III	II III
Chlorpyrifos	Dursban 75 WG (DOW Agrosience)	75.0	525 g	II III	II III
Indoxacarb	Steward WG (DuPont)	30.0	45 g	II	-
-	-	-	-	Untreated control	Untreated control

Materials and methods

The study was carried out in 2008 and 2011 in a vineyard located in northeastern Italy (locality Cormons, Gorizia province, 45°57'N 13°27'E, 55 m a.s.l., cultivar Chardonnay). In this grape-growing area *L. botrana* has three generations per year and severe damage is often observed (Pavan *et al.*, 2006). The grapevines were grown using the Guyot training system with distances between and along rows of 3.0 m and 0.5 m respectively. No insecticides were applied in addition to those used in the trials.

Field trials

In 2008 and 2011 the efficacy of single insecticide applications against the second or the third generations of *L. botrana* was evaluated in field trials on the third generation (table 1). In both years, timings of insecticide application were established on the basis of the flight of *L. botrana* recorded with pheromone traps (Traptest®, Isagro, Novara, Italy) (figures 1 and 2). The traps were checked every single day till the first male captures and then twice a week. The application timings were the beginning of moth egg laying (about five days from the

first male captures) for methoxyfenozide and the expected egg hatching time (about eight days from the first male captures) for chlorpyrifos and indoxacarb.

Experimental design was randomized complete blocks (grapevine rows) with four replicates (OEPP/EPP0, 2012). On each row treatments and untreated control were randomized. To avoid drift, the four rows were separated each other by a border row not treated with insecticides. Each replicate comprised 12 grapevines. The insecticides were applied with a backpack sprayer (Oleo-Mac Sp-126, Emak S.p.A, Bagnolo in Piano, Italy) at the rate reported in table 1 using a spray volume equal to 10 hL per hectare. Spray was directed from the top down and with an angle of 45° with respect to the line of the rows. At application timings the BBCH-scale of phenological growth stages of grapevines (Lorenz *et al.*, 1994) and berry weight (100 berries collected at random) were recorded. Considering that the berry density is constant and about equal to 1 g/mL, the berry surface area and volume at application timings were estimated on the basis of berry weight. Average air temperature and precipitation during June to August were provided by OSMER of the Friuli Venezia Giulia region (<http://www.osmer.fvg.it>).

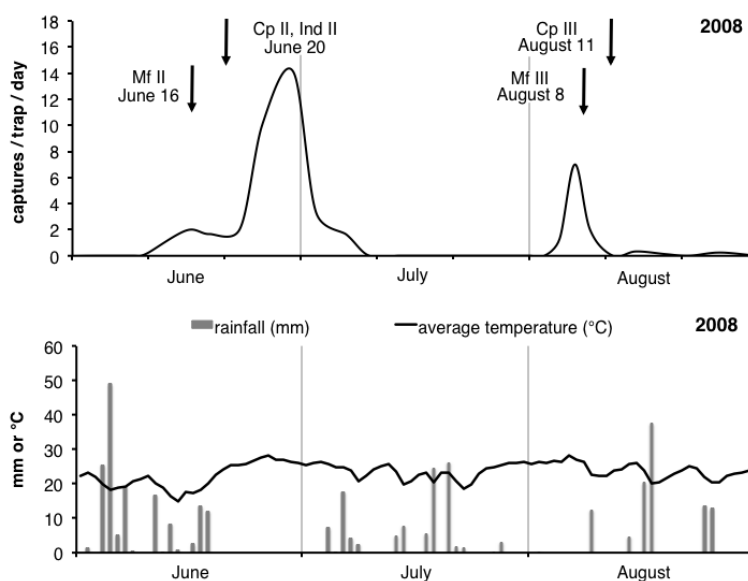


Figure 1. *L. botrana* flights and meteorological data recorded in 2008. The dates of insecticide applications are also reported. Mf = Methoxyfenozide; Cp = Chlorpyrifos; Ind = Indoxacarb.

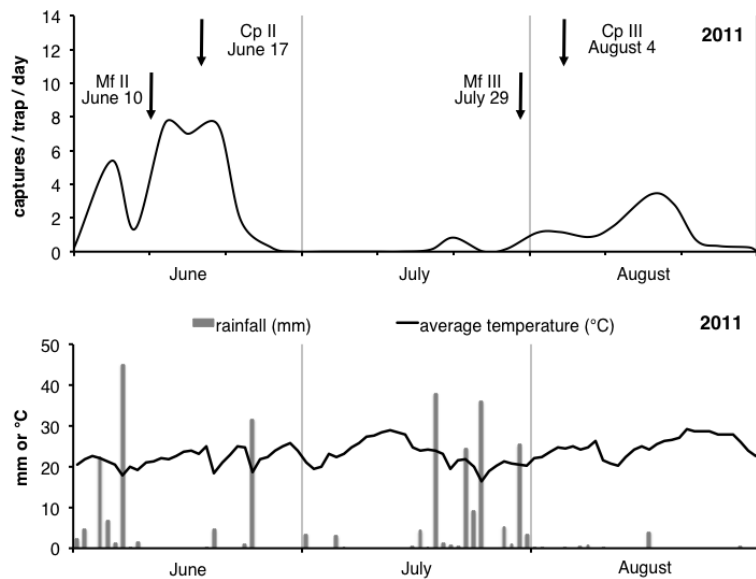


Figure 2. *L. botrana* flights and meteorological data recorded in 2011. The dates of insecticide applications are also reported. Mf = Methoxyfenozide; Cp = Chlorpyrifos.

Sampling was carried out at harvest time on fifty bunches per replicate. Bunches were taken in the ten central grapevines of each replicate (five bunches per grapevine). The five bunches of each grapevine were collected on the basis of an *a priori* scheme that determines the position of shoots along canes and of bunches along shoots (Pavan *et al.*, 1998). On each bunch the number of third-generation larval nests was counted. At harvest time the berries damaged by the two carpophagous generations are distinguishable because those affected by the second-generation are shrivelled, whereas those affected by the third-generation are still turgid and larvae can be often observed among the berries.

Count data were log transformed and submitted to ANOVA and Tukey's post test. The statistical analysis was performed with GraphPad 3.1 for Macintosh.

Laboratory bioassays

Bioassays were conducted with newly-hatched larvae obtained in laboratory conditions. The grandparents of these larvae had been collected as first-generation larvae in the experimental vineyard and reared to adult stage on an artificial diet (Rapagnani *et al.*, 1990) in a climatic chamber (Sanyo Versatile Environmental Test Chamber) at constant RH ($70 \pm 5\%$) and temperature (24 ± 0.3 °C), and a photoperiod with day length of 16 h.

For bioassays berries collected in the field (trials 2008 and 2011) were used. Few hours after the spraying with methoxyfenozide against the third generation (August 8, 2008 and July 29, 2011, respectively), four bunches per replicate were collected from untreated control, and methoxyfenozide II, methoxyfenozide III and chlorpyrifos II treatments. Bunches were taken on the basis of a fixed scheme (the proximal bunch of the distal shoot of the four central plants belonging to each replicate) and were kept in the refrigerator (4-6 °C) for one day.

In the laboratory for each treatment, 33 (2008) and 24 (2011) pairs of berries jointed together at their pedicels were collected at random from bunches. Each pair was

put in a cylindrical box ($d = 5.0$ cm; $h = 1.8$ cm) of polystyrene with a newly hatched first-instar larva. The boxes were maintained in the same climatic chamber in which field-collected larvae had been reared. The boxes were checked daily to note the following: (1) larva visible and alive; (2) larva visible and dead; (3) larva not visible, but presence of brown frass extruded from berry entrance holes; (4) larva not visible and absence of brown frass extruded from berry entrance holes. After one and two weeks, in boxes in which evidence of larval activity was no longer observed, the berries were dissected to collect dead larvae. After 40 days, in the remaining boxes in which pupating had not yet occurred the berries were dissected to detect larvae. All dead larvae were mounted on slides in Berlese's medium to identify the instars on the basis of mandible length (Pavan *et al.*, 2010). Then, for each treatment the larvae were classified as: i) dead as first instar (L1) or during moulting from first to second instar (L1-L2), ii) dead as second instar (L2) or before reaching the last instar. To assess if dead first-instar larvae had begun to feed, the berry surface was observed under a dissection microscope for larval borings. The death of larvae before feeding on berries was not attributed to insecticide activity.

The proportions were compared with the χ^2 -test followed by Ryan's multiple comparison test (Ryan, 1960).

Residual analyses

In both 2008 and 2011 and in both the methoxyfenozide II and methoxyfenozide III treatments, the day after the methoxyfenozide application against the third generation four bunches per replicate were collected on the basis of an *a priori* scheme. The 16 bunches belonging to each treatment were taken to the laboratory and kept in a freezer at -20 °C until residue determination. Residues of methoxyfenozide were determined according to food standard UNI EN 15662:2009 by Chelab S.r.l. (Resana, TV, Italy).

Table 2. Comparison of the number of larval nests of third-generation *L. botrana* recorded at harvest time on 100 bunches from the 2008 and 2011 treatments. For each year different capital letters among treatments indicate significant differences at the 0.01 level (Tukey's test). The efficacy of insecticides in comparison with the untreated control according to Abbott (1925) is also reported.

Treatment	2 0 0 8		2 0 1 1	
	Larval nests	Efficacy %	Larval nests	Efficacy %
Methoxyfenozide II	10.5 A	95.1	15.5 A	91.9
Methoxyfenozide III	10.0 A	95.3	10.0 A	94.8
Chlorpyrifos II	150.5 C	29.2	141.0 B	26.4
Chlorpyrifos III	18.0 AB	91.5	17.0 A	91.1
Indoxacarb II	54.0 B	74.6	-	-
Untreated control	212.5 D	-	191.5 B	-
ANOVA	$F_{5,18} = 62.39; P < 0.0001$		$F_{4,14} = 20.82; P < 0.0001$	

Results

BBCH phenological growth stages of grapevines and meteorological conditions

In both years the interval between the two insecticide applications, i.e. against the second and third generations, was about 50 days (figures 1 and 2).

At the two insecticide-application dates the BBCH phenological growth stages of grapevines were 75 (berries pea-sized) and 83 (berries developing colour) in 2008 and 77 (berries beginning to touch) and 83 in 2011. During the interval between the two applications the average surface area of berries increased from 1.5 to 4.5 cm² in 2008 and from 1.75 to 4.5 cm² in 2011, and the average volume increased from 0.18 to 0.90 cm³ in 2008 and from 0.22 to 0.90 cm³ in 2011. Therefore, across these growth intervals, the surface area increased about 2.5-3 times and the volume about 4-5 times.

Between the two insecticide applications, 119 mm (2008) and 197 mm (2011) of rain fell (figures 1 and 2).

Field trials

In both years methoxyfenozide II and methoxyfenozide III reduced significantly the third-generation larval nests with a efficacy more than 90% (table 2). The effectiveness of the chlorpyrifos III treatment did not differ from the methoxyfenozide II and methoxyfenozide III treatments. In contrast, chlorpyrifos II treatment was always significantly less effective than the methoxyfenozide II and methoxyfenozide III treatments, and only in 2008 it differed significantly from the untreated control. Indoxacarb II treatment reduced the third generation at levels significantly less than methoxyfenozide II treatment and more than chlorpyrifos II treatment.

Laboratory bioassays

Only a few larvae reared on untreated control berries (four out of 33 in 2008 and three out of 24 in 2011) failed to reach the last larval instar (figure 3). In 2008, two larvae, reared on berries belonging to the methoxyfenozide II and methoxyfenozide III treatments respectively, died before feeding on berries and thus their mortality was not attributed to the insecticide. In both

years, all the larvae reared on berries from the methoxyfenozide III treatment died before reaching the last larval instar. Also, the larvae reared on berries belonging to the methoxyfenozide II treatment died before reaching the last larval instar, except three larvae in 2008 bioassay. However, the mortality was faster for larvae reared on berries belonging to the methoxyfenozide III than to the methoxyfenozide II treatments. In both years the mortality of larvae feeding on berries belonging to the chlorpyrifos II treatment was intermediate between that recorded on berries belonging to the methoxyfenozide (II and III treatments) and the untreated control, and was significantly different from both.

The proportion of dead larvae was significantly lower in the untreated control than in the methoxyfenozide II, methoxyfenozide III and chlorpyrifos II treatments, in which most of larvae did not reach the second instar (table 3). The highest and lowest percentage of larval mortality was observed in the methoxyfenozide III and chlorpyrifos II treatments, respectively. Concerning the remaining dead larvae, whose mortality occurred between the second and the last larval instars, a significantly higher mortality (calculated on the number of larvae surviving after the first moulting) was observed in the methoxyfenozide II and methoxyfenozide III treatments than in control and chlorpyrifos II treatments. Therefore, methoxyfenozide II and methoxyfenozide III caused the mortality of some larvae after reaching the second instar.

Residual analyses

The presence of methoxyfenozide was detected not only in berries with insecticide applied the day before (methoxyfenozide III) but also in those treated about 50 days before (methoxyfenozide II), despite the increase in berry size and exposure to over 100 mm of rainfall (figures 1 and 2). The residues in berries (mg/kg) in 2008 were 0.175 in methoxyfenozide II and 0.52 in methoxyfenozide III, and in 2011 0.07 in methoxyfenozide II and 0.357 in methoxyfenozide III. The amount of residue in the methoxyfenozide II treatments was 3 and 5 times smaller than in the methoxyfenozide III treatments in 2008 and 2011, respectively.

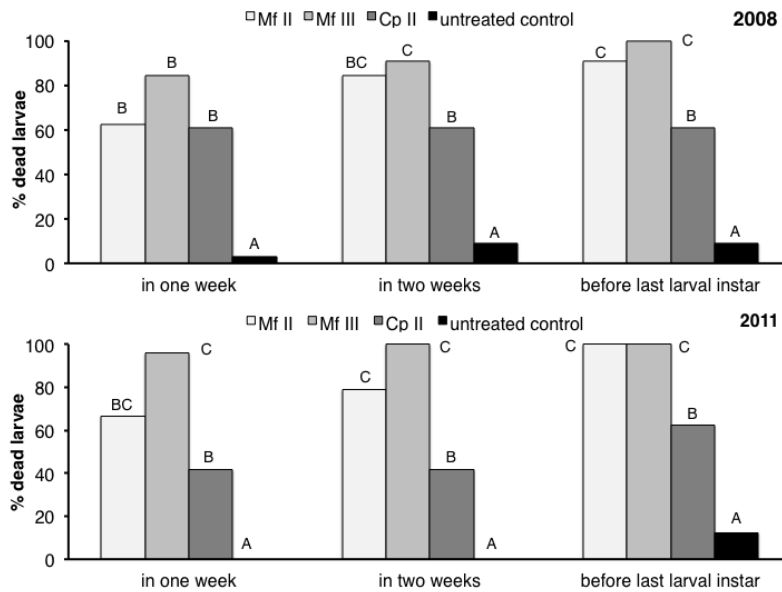


Figure 3. Mortality of *L. botrana* larvae, feeding on berries following different treatments, recorded over time in laboratory bioassays conducted in 2008 and 2011. In all six cases the χ^2 (50.9, 67.8, 93.9 in 2008 and 49.5, 60.6, 72.2 in 2011) was significant at $P < 0.0001$. Different capital letters between treatments within the same period indicate significant differences at the 0.01 level (Ryan's multiple comparison test). Mf = Methoxyfenozide; Cp = Chlorpyrifos.

Table 3. Number of *L. botrana* larvae feeding on berries following different treatments in laboratory bioassays conducted in 2008 and 2011. The percentages of dead larvae before completing the first moult, calculated from total larvae, and after the first moult, calculated from larvae still alive after the first moult, are reported. Different capital letters indicate significant differences at the 0.01 level (Ryan's multiple comparison test).

Year / treatment	Total larvae	Dead larvae before completing the first moult	Survived larvae after the first moult	Dead larvae after the first moult among those survived
	No.	%	No.	%
2008				
Methoxyfenozide II	32	71.9 BC	9	66.6 B
Methoxyfenozide III	32	90.6 C	3	100.0 B
Chlorpyrifos II	33	60.6 B	13	0.0 A
Untreated control	33	9.1 A	30	3.3 A
		$\chi^2 = 56.9; df = 3; P < 0.0001$		$\chi^2 = 36.9; df = 3; P < 0.0001$
2011				
Methoxyfenozide II	24	87.5 C	3	100.0 B
Methoxyfenozide III	24	100.0 C	0	-
Chlorpyrifos II	24	45.8 B	13	30.8 AB
Untreated control	24	0.0 A	24	12.5 A
		$\chi^2 = 66.7; df = 3; P < 0.0001$		$\chi^2 = 14.9; df = 3; P < 0.001$

Discussion

Residual activity of tested insecticides

Methoxyfenozide applied against the second generation of *L. botrana* controlled the third generation at the same level of applications targeted directly against this latter generation. This agrees with the effectiveness of applications against the first generation in controlling the second generation (Scannavini *et al.*, 2006). The laboratory data reported in the present study demonstrate that the toxicity on third-generation larvae is due to the residual activity of methoxyfenozide. In the laboratory the residual activity of methoxyfenozide was observed on newly-hatched larvae, but in the field conditions it may involve also the eggs due to the ovicidal

activity of this active ingredient. A long residual activity of methoxyfenozide against tortricid moths was reported in literature on grape berries (Teixeira *et al.*, 2009) and apple fruits (Borchert *et al.*, 2004; Magalhaes and Walgenbach, 2011; Cormier *et al.*, 2013). Residues of methoxyfenozide found after 50 days in berries sprayed in coincidence with second-generation egg laying showed values that could be explained on the basis of the increase in berry volume, in agreement with Smagge *et al.* (2012). The concentration of methoxyfenozide present 50 days after treatment still allowed an optimal control of the *L. botrana* third-generation. However, in this case an important proportion of the larvae died after completing at least one moulting, whereas a few moulting larvae were observed with the

treatments targeted directly against the third generation. This suggests that the insecticide doses are suboptimal and then associated with the possibility to select individuals with a lower susceptibility to this active ingredient. The slight decrease of efficacy of methoxyfenozide on *L. botrana* recently observed in an Italian grape-growing area (Civolani *et al.*, 2014) could be a clue that resistant populations are being selected.

Chlorpyrifos applied against the second generation only slightly reduced the third one (significant differences only in 2008) and this is in agreement with a slight residual activity observed in laboratory bioassays. Organophosphates applied against the first generation have not previously resulted in effective control of the second generation in the field (Bressan *et al.*, 2002) and have not shown any residual activity in laboratory bioassays (Pavan *et al.*, 2005). The occurrence of residual activity only for applications against the second generations could be explained by the larger increase in berry size in the interval between the first two generations of *L. botrana* than between the two carpophagous generations. This could have resulted in a different dilution of insecticide residues. However, it should be noted that in both field trials and laboratory bioassays, which were carried out to evaluate the effectiveness on the second generation of organophosphates applied against the first generation, the active ingredients used (i.e. fenitrothion and chlorpyrifos methyl) were thought to be less persistent than chlorpyrifos (Bressan *et al.*, 2002; Pavan *et al.*, 2005).

Indoxacarb applied against the second generation significantly also affected the third generation, although at a level lower than methoxyfenozide. Similar results were obtained previously when the activity of applications targeting the first generation was evaluated on the second generation (Scannavini *et al.*, 2006). The toxicity of indoxacarb on two consecutive generations was significantly higher than for chlorpyrifos, showing that this active ingredient has a higher residual activity than the organophosphates. In the case of grapevines, indoxacarb has shown a high rainfastness and residual activity against the scarabeid beetle *Popillia japonica* Newman unlike the organophosphate phosmet which has poor residual qualities (Hulbert *et al.*, 2011). On apple, the residual activity of indoxacarb against larvae of the tortricid moth *Pandemis heparana* (Denis et Schiffermüller) was equal to 100% at 22 days after application and similar to that of chitin synthesis inhibitors and ecdysone agonists (Ioriatti *et al.*, 2006). The high residual activity of indoxacarb on fruits could be one of the factors involved in high resistance levels observed for this active ingredient before on *Cydia pomonella* (L.) in USA (Mota-Sanchez *et al.*, 2008) and recently on *L. botrana* in Italy (Civolani *et al.*, 2014).

Among insecticides effective against *L. botrana*, the neurotoxic spinetoram and emamectin benzoate, and chlorantraniliprole, a ryanodine receptor modulator, sprayed on apple leaves in the field caused a mortality of 100% in the tortricid moth *Choristoneura rosaceana* (Harris) larvae up to 59, 10 and 38 days after treatment, respectively (Sial and Brunner, 2010). However, the residual activity of chlorantraniliprole on grape berries against *L. botrana* (Ioriatti *et al.*, 2009b), and that of

emamectin benzoate on apple fruits against the tortricid moths *C. pomonella* and *Grapholita molesta* (Busck) (Ioriatti *et al.*, 2009a) decreased significantly seven and 14 days after insecticide application, respectively. Therefore, chlorantraniliprole and emamectin benzoate should not be associated with selection pressure against two subsequent generations of *L. botrana*.

Resistance management strategies on grapevine for methoxyfenozide and highly persistent insecticides

A long residual activity of insecticides can be a useful tool for pest management, but it is also a risk for resistance development in multivoltine species due to exposure to low-residue levels (Georghiou and Taylor, 1977; 1986; OEPP/EPPO, 2003; Onstad, 2008; Yu, 2008; FAO, 2012). Insecticides more recently introduced against tortricid moths infesting fruit crops, in particular chitin synthesis inhibitors (benzoylphenyl ureas) and ecdysone agonists (bisacylhydrazines) (Doucet and Retnakaran, 2012; Pener and Dhadialla, 2012; Smagghe *et al.*, 2012), often have a longer residual activity than traditional organophosphate active ingredients (Borchert *et al.*, 2004; Smirle *et al.*, 2004; Pavan *et al.*, 2005; Charmillot and Pasquier, 2006; Teixeira *et al.*, 2009). This could be one explanation why resistance phenomena in *C. pomonella* emerged more rapidly with benzoylphenyl ureas and bisacylhydrazines than with organophosphates (Waldner 1993; Sauphanor *et al.*, 1994; Sauphanor and Bouvier, 1995; Charmillot *et al.*, 2003; Reyes *et al.*, 2007; Mota-Sanchez *et al.*, 2008).

The insecticides that exert a long lasting effect against *L. botrana* have a selective pressure not only on the moth target generation but also on the next generation, if it exists. In analogy with what was observed in fruit crops, this feature may promote the onset of resistance phenomena. This high residual activity could also have a high selection pressure on other insect pests that are not the target of the treatment.

According to Georghiou and Taylor (1986), the greater the population density subjected to selective pressure, the greater the risk of selecting resistant populations. This could occur when the *L. botrana* generation subsequent to the target generation (i) is not treated with any insecticide, (ii) or it is treated with an active ingredient of little effect. Indeed, in both cases a larger number of *L. botrana* individuals are submitted to selection pressure from the insecticide applied against the previous generation. The first possibility occurs in grape-growing areas where a third generation has appeared in recent years as a result of climate warming (Pavan *et al.*, 2006). In fact, in these areas this carpophagous generation is not yet considered harmful so it is not treated. The second possibility occurs when insecticides with relatively low efficacy (e.g. *Bacillus thuringiensis* toxin) are applied so as to comply with the pre-harvest interval.

To maintain the effectiveness of active ingredients against *L. botrana* for as long as possible, resistance management strategies should follow the rules below:

1. In compliance with the pre-harvest interval, long residual insecticides should be used against the last

generation of the year so to not expose a subsequent larval generation to low residual levels;

2. In alternative, if an insecticide with long residual activity is applied against a non-overwintering generation, an active ingredient without cross resistance must be applied against the following generation, giving priority to those that ensure the highest effectiveness in compliance with the pre-harvest interval;
3. Do not apply the same active ingredient (or others characterized by cross resistance) against the first generation as was applied against the last or penultimate generations of the previous year if it has a high residual activity. In the latter case, four generations of *L. botrana* would be subjected to selection pressure from the same active ingredient (i.e. the last two of a year and the first two of the subsequent year).

We can conclude that high insecticide persistence can be considered positive because treatment can cover all of the egg-laying period of pests with prolonged deposition (Teixeira *et al.*, 2009) or of two pests with not completely synchronous oviposition (Borchert *et al.*, 2004). Alternatively, the persistence can be considered negative because it exerts a selective pressure over at least two consecutive generations of an insect pest, and particularly if the second one is subjected to a low insecticide dose.

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