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Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* and grey mould

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Abstract

Bunch-zone leaf removal in vineyards has been reported as an effective cultural practice to prevent damage by *Lobesia botrana* (Denis et Schiffermuller) (Lepidoptera Tortricidae) or *Botrytis cinerea* Pers. Fr. *L. botrana* larval activity is well known as a factor favouring the spread of *B. cinerea*. In 2007-2014, trials were carried out in a number of vineyards in north-eastern Italy to study the effects of leaf removal on the two carpophagous generations of the moth and on grey mould, simultaneously, and on the spatial distribution in relation to sunlight exposure of *L. botrana* larval nests within the grapevine canopy and eggs on bunches. Bunch-zone leaf removal applied at the pea-sized berry stage or a little later, during the *L. botrana* second-flight, reduced the infestation of both carpophagous generations by about 50% as well as *B. cinerea* infection at harvest time. This latter effect was partially due to moth control. In one of the two vineyards where distribution of larval infestation was studied, bunches not covered by leaves were significantly less infested than those covered. Females confined on bunches facing south and exposed to sunlight preferred to lay eggs on the sun-exposed side of the bunch. The lower *L. botrana* infestation observed both on plots subjected to bunch-zone leaf removal and on bunches facing south could be due to a higher egg/larval mortality caused by the very high temperatures reported for berries exposed to sunlight.

Key words: European grapevine moth, *Botrytis*, green pruning, cultural control, egg-laying preference, sunlight exposure.

Introduction

The European grapevine moth, *Lobesia botrana* (Denis et Schiffermuller) (Lepidoptera Tortricidae), is one of the most destructive grapevine pests with economic importance in the Palearctic region and, recently, it has expanded its geographical distribution to the Americas (Ioriatti *et al*., 2012). It is a polivoltine species that performs, depending on the geographical areas and microclimates, from two to four generations per year (Caffarra *et al*., 2012; Gilioli *et al*., 2016; Martin-Vertedor *et al*., 2010; Pavan *et al*., 2013).

The larvae of the second and third generations are carpophagous and can cause yield losses (Moschos, 2006; Pavan *et al*., 1998; Pavan and Sbrissa, 1994; Roehrich, 1978) and favour the spread of bunch rots such as grey mould *Botrytis cinerea* Pers. Fr. (Fermaud and Giboulot, 1992; Pavan *et al*., 2014a; Roehrich, 1978), black aspergilli rot *Aspergillus* Section *Nigri* (Cozzi *et al*., 2009) and sour rot (Bisiach *et al*., 1986).

In areas where *L. botrana* completes three generations per year, some studies reported that both carpophagous generations can favour the spread of grey mould at harvest time (Fermaud and Giboulot, 1992), whereas others showed a greater role of the third generation (Pavan *et al*., 2014a; Roehrich 1978). These latter authors explained their results with the fact that at harvest time the berries bored by the second-generation larvae are mostly shrivelled or fallen, whereas those bored by third-generation larvae are often rotten and still turgid (Pavan and Sbrissa, 1994). Moreover, because the amount of rotten berries non-contiguous to larval nests is not correlated with larval infestation (Pavan *et al*., 2014a), it can be assumed that the development of grey mould in these berries is independent of larval activity.

The spread of grey mould at harvest time is also favoured by plant vigour (Mundy, 2008; Valdés-Gómez *et al*., 2008), leaf density around bunches (Fermaud *et al*., 2001), berry susceptibility and bunch compactness (Vail and Marois, 1991). Some of these factors can be modified by cultural practices (Muckensturm and Decoin, 2000; Hed *et al*., 2011; R'Houma *et al*., 1998; Valdés-Gómez *et al*., 2008). Leaf density around bunches can be managed by manual or mechanical leaf removal, applied from pre-bloom to veraison, with reductions in bunch rots that can even approach 100% (Diago *et al*., 2010; Duncan *et al*., 1995; English *et al*., 1993; Gubler *et al*., 1987; Percival *et al*., 1994; Sivilotti *et al*., 2011).

L. botrana carpophagous generations are usually controlled by pesticides, *Bacillus thuringiensis* Berliner (Ifoulis and Savopoulou-Soultani, 2004) and mating disruption techniques (Ioriatti *et al*., 2011), but grapevine cultivar and cultural practices can contribute to the moth control (Fermaud, 1998; Vartholomaiou *et al*., 2008; Villani *et al*., 1997). In an Italian grape-growing area where *L. botrana* develops only two generations per year, Villani *et al*. (1997) showed that bunch-zone leaf removal applied at the "berries pea-sized" (beginning of *L. botrana* second-flight) or "berries beginning to touch" (about 50% of *L. botrana* second-flight) stages can reduce the second-generation larval infestation by more than 70%. In a Greek grape-growing area where *L. botrana* completes three generations per year (Ifoulis and Savopoulou-Soultani, 2004), Vartholomaiou *et al*. (2008) showed that leaf removal undertaken in June reduced the percentage of infested bunches at harvest time by about 15%. Based on these data, it cannot be excluded that the effect of some cultural practices in reducing bunch rots is partially due to *L. botrana* control.

Currently, it is necessary to understand how bunch-zone leaf removal affects the level of *L. botrana* infestation. From the theoretical point of view this practice could reduce egg laying and/or cause a higher egg/larval mortality on sunlight exposed berries. The first hypothesis is based on the supposed preference of *L. botrana* females for bunches placed in a denser canopy, but to our knowledge there is no rigorous demonstration of this assumption. Moreover, according to this hypothesis, the effect would be evident only if females can choose between plots characterized by different leaf density, unless it is proved that bunch-zone leaf removal reduces female fecundity. The second hypothesis is based on the possibility that eggs and newly-hatched larvae on berries not protected by leaves are more susceptible to meteorological factors (i.e., sunlight, relative humidity and rain). In fact, high temperatures, mostly associated with low relative humidities, cause *L. botrana* egg and larval mortality (Coscollá *et al*., 1986; Rapagnani *et al*., 1988) and berries exposed to sunlight reach higher temperatures than those not exposed (Kliewer and Lider, 1968; Tarara *et al*., 2008). This hypothesis might only be plausible if the females lay eggs on berries independently from the berries previous exposure to sunlight because oviposition occurs after sunset. Regarding this, Zahavi *et al*. (2003) observed that: (i) in a vineyard with north-south oriented rows, the bunches exposed to sunlight in the afternoon (west-facing) were much less infested than non-exposed ones (east-facing); and (ii) in the laboratory, females had a slight preference for laying eggs on bunches collected on the eastern side.

The first aim of this study was to verify the influence of bunch-zone leaf removal on *L. botrana* and grey mould attacks, and to extend it with two additional purposes: (i) to distinguish the direct effect of leaf removal on grey mould spread from the indirect effect associated with *L. botrana* control; and (ii) to evaluate the effect of leaf removal on the two carpophagous generations of *L. botrana*, separately. The second aim was to study the spatial distribution of *L. botrana* in relation to sunlight exposure by comparing in the field: (i) the larval infestation within the grapevine canopy considering row side and leaf coverage; and (ii) the female egg-laying preference for different bunch sides.

Materials and methods

Influence of bunch-zone leaf removal on *L. botrana* and grey mould

To study the effect of bunch-zone leaf removal (LR) on *L. botrana* and grey mould, four trials were carried out in north-eastern Italy (Trial 2007, Trial 2008, Trial 2011 and Trial 2013; table 1). In the locality of Trials 2007, 2008 and 2011, *L. botrana* normally has three generations per year, whereas in the locality of Trial 2013 third-generation larvae are detected on bunches only in the warmest years.

Six and two treatments were compared in Trials 2007 and 2008, and in Trials 2011 and 2013, respectively (table 2). Experimental design was randomized blocks (grapevine rows) with four replicates. The plots within each replicate consisted of at least 16 grapevines. No insecticides were applied other than those used in the trials. In all plots the same fungicides against grapevine downy mildew and grapevine powdery mildew were applied with a trailed air blast sprayer. In Trials 2007 and 2008, to avoid pesticide drift interference, the four blocks (rows) were separated from each other by a border row that was not treated with either the fungicides against grey mould or the insecticides against *L. botrana*.

Insecticides and anti-grey mould products were distributed with a backpack sprayer [Oleo-Mac Sp-126, Emak S.p.A, Bagnolo in Piano (RE), Italy]. The male flights of *L. botrana* were recorded with pheromone traps (Traptest®, Isagro, Novara, Italy) (figure 1). Two traps per vineyard were placed from late April to late September. The traps were checked daily coinciding with the expected beginning of the second and third flights and twice a week up to the end of each flight.

Table 1. Studies carried out during 2007–2014 in four experimental vineyards of north-eastern Italy. LR = Trials on the influence of bunch-zone leaf removal on *L. botrana* and grey mould; LSD = Trials on the spatial distribution of *L. botrana* larval infestation; ESD = Experiment on the spatial distribution of *L. botrana* eggs.

Trial or Experiment	Locality, district Coordinates Altitude (*)	Cultivar Training system Distances between and along rows	Row orientation
Trial 2007 (LR) Trial 2008 (LR)	Cormòns, Gorizia 45°56'N 13°27'E 39 m a.s.l.	Chardonnay Guyot 1.5 m and 0.5 m	N30°W
Trial 2011 (LR)	Cormòns, Gorizia 45°57'N 13°26'E 50 m a.s.l.	Chardonnay Guyot 2.8 m and 1.0 m	N25°W
Trial 2013 (LR) Trial 2014a (LSD) Experiment 2013 (ESD)	Romans d'Isonzo, Gorizia 45°54'N 13°27'E 24 m a.s.l.	Chardonnay Guyot 2.7 m and 0.9 m	N65°W
Trial 2014b (LSD)	Buttrio, Udine 46°00'N 13°20'E 83 m a.s.l.	Chardonnay Guyot 2.5 m and 0.9 m	N80°W

(*) All the vineyards were on the plains, except for Buttrio which was on the south slope of a hill.

Table 2. Treatments considered in the four trials on the influence of bunch-zone leaf removal (LR) on *L. botrana* and grey mould. BBCH stages (Lorenz *et al*., 1995): 75, “berries pea-sized, bunches hang”; 79, “majority of berries touching”; 81, “beginning of ripening: berries begin to develop variety-specific colour”; 83, “berries developing colour”; 85, “softening of berries”.

Treatments	Trial	Cultural practices, active ingredients (a.i) and products	a.i. per hectare	Number of applications (timing)
Untreated control	All Trials		-	-
LR (leaf removal)	Trial 2007	Manual bunch-zone leaf removal	-	1 (BBCH stage 79)
	Trial 2008	"	-	1 (BBCH stage 75)
	Trial 2011	"	-	1 (BBCH stage 79)
	Trial 2013	Bunch-zone leaf removal with pneumatic machine Mod. “con 2 testate”, Olmi, Castiglione d’Asti (AT), Italy	-	1 (BBCH stage 79)
LR + T2 (leaf removal + insecticide application against *L. botrana* 2nd generation)	Trial 2007	Chlorpyriphos Dursban, DOW Agroscience, 44.5% a.i.	490 mL	1 (7 days after beginning of egg hatching)
	Trial 2008	Indoxacarb Steward, Dupont, 30% a.i.	45 g	1 (beginning of egg hatching)
LR + T3 (leaf removal + insecticide application against *L. botrana* 3rd generation)	Trial 2007	Methoxyfenozide Prodigy, Bayer, 22.5% a.i.	90 mL	1 (beginning of egg laying)
	Trial 2008	*Bacillus thuringiensis* Berliner DiPel DF, Valent BioSciences Corporation, *B. thuringiensis* subsp. *kurstaki*, strain ABTS-351	1000 g	2 (egg hatching and one week later)
LR + AM (leaf removal + anti-grey mould applications)	Trial 2007	Cyprodinil + Fludioxonil Switch, Syngenta, 37.5% and 25% a.i., respectively	300 + 200 g	3 (BBCH stages 79, 81, 83)
	Trial 2008	Cyprodinil + Fludioxonil Switch, Syngenta, 37.5% and 25% a.i., respectively	300 + 200 g	3 (BBCH stages 75, 81, 85)
LR + T2 + T3 + AM	Trial 2007	See above	See above	See above
	Trial 2008	See above	See above	See above

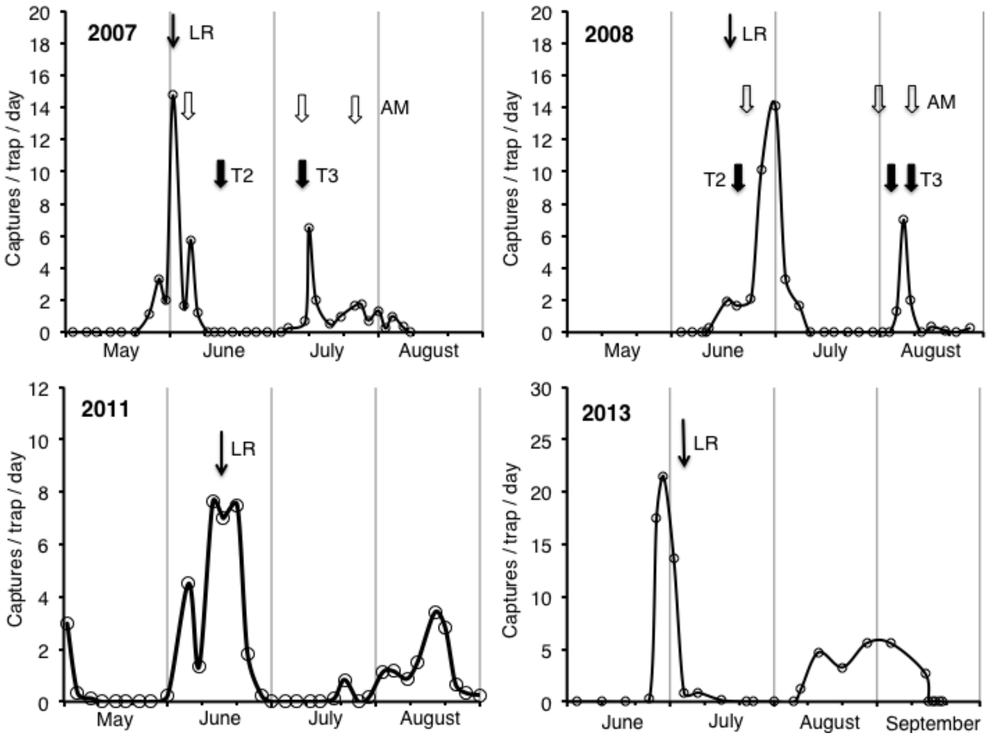

Figure 1. Second and third flights of *L. botrana* males recorded in the four trials of table 2. The timings of bunch-zone leaf removal (LR), fungicides against grey mould (AM) and insecticides against moths (T2 = second generation, T3 = third generation) are indicated.

Bunch-zone leaf removal consisted of removing all the leaves that covered the bunches. When leaf removal was undertaken in Trials 2007, 2011 and 2013 the *L. botrana* second-flight had started 10 days before, whereas in Trial 2008 five days before.

The second-generation infestation of *L. botrana* was estimated at about 40 days after the beginning of the second flight, whereas the third-generation infestation and bunch rots were estimated at harvest time. In all the trials, 50 bunches per plot (i.e., per replicate of each treatment) were sampled. Bunches were examined directly in the field on 10 grapevines per plot, excluding edge plants, with 4 and 6 bunches collected alternately from each grapevine based on an *a priori* scheme to avoid subjective choice (Pavan *et al*., 1998). The number of larval nests of the second generation was counted without dissecting the bunches. For this generation also the damaged berries were counted except in Trial 2007 because bunches were too compact to accurately count the bored berries without removing them. The number of larval nests of the third generation was counted by dissecting the bunches. These nests can be easily recognized from those of the second generation because the damaged berries are still turgid and larvae are normally present. At harvest time, the number of rotten berries was counted, separating those contiguous to *L. botrana* third-generation larval nests (groups of rotten berries in contact with nests) from those that were non-contiguous (groups of rotten berries not in contact with nests).

Count data were square root transformed and submitted to a t-test when the treatments in the comparison were two, or an ANOVA and Tukey's post test when the treatments in the comparison were more than two. Statistical analysis was performed with GraphPad InStat 3 for Macintosh.

Spatial distribution of *L. botrana* in relation to sunlight exposure

In 2014 the spatial distribution of larval infestation (LSD) within the grapevine canopy was studied in two vineyards with east-west oriented rows (Trials 2014a and 2014b; table 1). Larval infestation on bunches located on north and south sides of rows and subjected or not to leaf removal was compared. Experimental design was randomized blocks (grapevine rows) with four replicates. Within each replicate two plots of 16 grapevines were considered. At the beginning of the moths' second flight (June 12th, BBCH 75 "berries pea-sized, bunches hang") one plot per row was subjected to manual bunch-zone leaf removal on both sides (i.e., north and south facing). Therefore, bunches were classified in four different groups in relation to row side and leaf coverage: (i) located on the north side of rows and covered by leaves; (ii) located on the north side of rows and not covered by leaves; (iii) located on the south side of rows and covered by leaves; (iv) located on the south side of rows and not covered by leaves.

At harvest time the second-generation larval nests were counted because in 2014 the third generation did not develop in the studied grape-growing area. In each plot, with or without leaf removal, all north- and south-facing bunches were sampled up to a total of 50 per row side.

Count data were square root transformed and submitted to a two-way ANOVA with row side and leaf coverage as factors and Tukey's post test. Statistical analysis was performed with GraphPad InStat 3 for Macintosh.

At mid August 2013 the spatial distribution of eggs laid by *L. botrana* females (ESD) on south-facing bunches was studied in the field (Experiment 2013; table 1). For this purpose, two-day-old *L. botrana* females that had mated and had started to lay eggs in the laboratory were confined at sunset by tulle bags on shoots located on the south side of a grapevine row. On each of 21 shoots with two bunches not covered by leaves, two females were confined for 36 h, in order to allow two egg-laying days. Berries of south-facing bunches were distinguished in two groups: (i) berries facing sun during late morning and afternoon (named "sun-exposed side"); and (ii) berries facing the interior part of the canopy (named "shaded side"). At the end of the experiment the bags were removed and eggs laid on the berries of the sun-exposed and shaded side of bunches separately counted.

To compare the number of eggs laid on berries between the two sides of bunches, data were submitted to the Wilcoxon matched-pairs signed-ranks test. Statistical analysis was performed with GraphPad InStat 3 for Macintosh.

Results

Influence of bunch-zone leaf removal on *L. botrana* and grey mould

In the four Trials only *B. cinerea* was found among bunch rots.

In Trial 2007, even though leaf removal (LR) had reduced the second-generation infestation of *L. botrana* by 50% on average, the differences were not significant in comparison to the untreated control due to the high variability among replicates (table 3). A significant reduction in infestation was observed with the insecticide addition (LR + T2). The third generation of *L. botrana* was significantly reduced by LR (50% of efficacy; table 3). A further significant reduction in infestation compared with LR was determined by the insecticide application against the third generation (LR + T3). The total amount of rotten berries by grey mould was significantly reduced in the two treatments with fungicide applications (LR + AM and LR + T2 + T3 + AM) and by the insecticide application against the third generation (LR + T3) (table 3). The rotten berries contiguous to larval nests were significantly reduced only when insecticides and/or fungicides against grey mould were added to LR, even if the insecticide against the second generation (LR + T2) did not significantly differ from LR. The rotten berries non-contiguous to *L. botrana* larval nests were significantly reduced only in the two treatments with fungicide applications (LR + AM and LR + T2 + T3 + AM). In this year there were four days of rainfall during the six days before sampling on August 14th (data from http://www.osmer.fvg.it/OSMER).

In Trial 2008, LR significantly reduced the second-generation infestation of *L. botrana* (56% of efficacy;

table 3). A further significant reduction in infestation was determined by the addition of the insecticide application (LR + T2). The third-generation infestation of *L. botrana* was significantly reduced by LR (59% of efficacy; table 3). Only the addition of all pesticides together (LR + T2 + T3 + AM) significantly increased moth control compared to LR. *B. thuringiensis* against the third generation (LR + T3) did not significantly improve the control of this generation and was tendentially less effective than indoxacarb against the second generation (LR + T2), since only this latter treatment did not differ from LR + T2 + T3 + AM. The total number of rotten berries and the number of rotten berries contiguous to *L. botrana* larval nests were significantly reduced by LR (about 75% of efficacy) and only the addition of all pesticides together (LR + T2 + T3 + AM) resulted in a further significant reduction in infected berries (table 3). The number of non-contiguous rotten berries was significantly reduced by LR (84% of efficacy) but there was no further significant reduction following fungicide and insecticide applications. Thus, the efficacy of leaf removal was higher for non-contiguous than contiguous berries. The number of contiguous rotten berries was reduced by leaf removal in higher proportion than the number of third-generation larval nests (75% vs 59%), due to a lower number of contiguous rotten berries per larval nest (2.3 in the untreated control and 1.7 in LR). In this year, the global radiation, to which the UV radiation is positively correlated, was very high and rains were absent during the eight days before the sampling carried out on September 2nd (data from http://www.osmer.fvg.it/OSMER).

In Trial 2011, LR significantly reduced the second-generation infestation of *L. botrana*, even though a high variability among replicates was observed (74% of efficacy; table 3). At harvest time, neither *L. botrana* larval nests of the third generation nor grey mould were recorded.

In Trial 2013, LR significantly reduced both the second- and third-generation infestation of *L. botrana* (33% and 63% of efficacy, respectively; table 3). The berries bored by third-generation larvae were not rotten. The amount of rotten berries non-contiguous to larval nests was not significantly different between treatments.

The control of the second generation by bunch-zone leaf removal was effective both when leaf removal was carried out 10 days after the beginning of the *L. botrana* second-flight (i.e., Trials 2007, 2011 and 2013) and when it was carried out 5 days after the beginning of the flight (i.e., Trial 2008). In Trials 2007, 2011 and 2013 females had already laid many eggs, but larval hatching had not yet started, whereas in Trial 2008 females had just started to lay eggs (data not reported).

Spatial distribution of *L. botrana* in relation to sunlight exposure

In Trial 2014a, there was no difference in the second-generation infestation between the north- and south-facing bunches ($F_{1,12}$ = 2.96, P = 0.11), whereas in Trial 2014b the south-facing bunches were significantly more infested than the north-facing bunches ($F_{1,12}$ = 10.87, P = 0.006) (figure 2). In Trial 2014a, there was no difference in the infestation between the covered and not-covered bunches ($F_{1,12}$ = 1.56, P = 0.24), whereas in Trial 2014b

Table 3. Number per 100 bunches ± standard deviation of *L. botrana* larval nests (l.n.), *L. botrana* damaged berries (d.b.), *B. cinerea* rotten berries at harvest time (r.b.) observed in the treatments in the four trials. Different small letters among treatments indicate significant differences at 0.05 (Tukey post-test or t-test). 2nd = second generation; 3rd = third generation; tot. = total; con. = contiguous to larval nests; non con. = non contiguous to larval nests. LR = bunch-zone leaf removal at "berry pea-sized" or "majority of berries touching" stages; T2 and T3 = one insecticide application against the second and the third generations of *L. botrana*, respectively; AM = three fungicide applications against grey mould.

Trial 2007	LR	LR + T2	LR + T3	LR + AM	LR + T2 + T3 + AM	Untreated control	ANOVA or t-test
l.n. - 2nd	27.5±13.3 ab	5.5±4.4 a	–	17.5±8.7 ab	7.0±2.6 a	61.0±47.3 b	$F_{4,15}$=6.837; P=0.0024
l.n. - 3rd	81.5±20.7 c	64.5±19.8 c	25.5±13.7 ab	61.0±30.6 bc	16.0±5.2 a	162.5±38.9 d	$F_{5,18}$=22.656, P<0.0001
r.b. - tot.	403.0±152.0 bc	367.0±133.6 bc	225.0±57.4 ab	136.0±80.2 a	80.0±40.8 a	579.5±227.9 c	$F_{5,18}$=11.341, P<0.0001
r.b. - con	155.0±96.3 cd	86.0±47.5 bc	22.5±15.9 ab	29.0±16.8 ab	7.5±10.0 a	286.0±100.4 d	$F_{5,18}$=17.487, P<0.0001
r.b. - non con.	248.0±99.6 b	281.0±100.8 b	202.5±56.2 ab	107.0±83.8 a	72.5±45.1 a	293.5±130.3 b	$F_{5,18}$=5.226, P=0.0039
Trial 2008							
l.n. - 2nd	92.3±28.0 b	2.5±3.0 a	–	72.5±27.92 b	5.5±9.71 a	209.0±60.1 c	$F_{4,15}$=26.860, P<0.0001
d.b. - 2nd	333.8±139.5 b	7.5±9.0 a	–	257.5±126.2 b	16.5±27.9 a	804.0±260.25 c	$F_{4,15}$=35.380, P<0.0001
l.n. - 3rd	20.5±5.3 b	12.5±9.6 ab	13.0±9.9 b	19.0±8.4 b	1.5±1.9 a	50.0±6.9 c	$F_{5,18}$=13.612, P<0.0001
r.b. - tot.	55.5±21.4 b	29.5±9.2 ab	46.5±23.1 b	26.0±13.6 ab	6.0±6.7 a	243.5±106.3 c	$F_{5,18}$=13.451, P<0.0001
r.b. - con	35.5±12.8 b	15.5±13.4 b	18.5±15.2 b	23.0±12.9 b	0.0±0.0 a	116.5±36.5 c	$F_{5,18}$=20.043, P<0.0001
r.b. - non con.	20.0±12.0 a	14.0±10.2 a	28.0±17.5 a	3.0±2.6 a	6.0±6.7 a	127.0±72.5 b	$F_{5,18}$=13.450, P<0.0001
Trial 2011							
l.n. - 2nd	7.0±3.8 a					27.0±18.7 b	t_6=2.463, P=0.049
d.b. - 2nd	15.0±8.4 a					79.0±47.9 b	t_6=3.301, P=0.016
Trial 2013							
l.n. - 2nd	48.0±5.7 a					71.5±8.9 b	t_6=4.543, P=0.0039
d.b. - 2nd	117.0±22.3 a					189.0±24.9 b	t_6=4.207, P=0.0060
l.n. - 3rd	27.0±15.9 a					73.0±31.2 b	t_6=2.951, P=0.0026
d.b. - 3rd	48.0±31.9 a					146.0±61.9 b	t_6=3.062, P=0.0022
r.b. – non con.	117.3±112.9 a					241.8±207.9 a	t_6=1.152, P=0.29

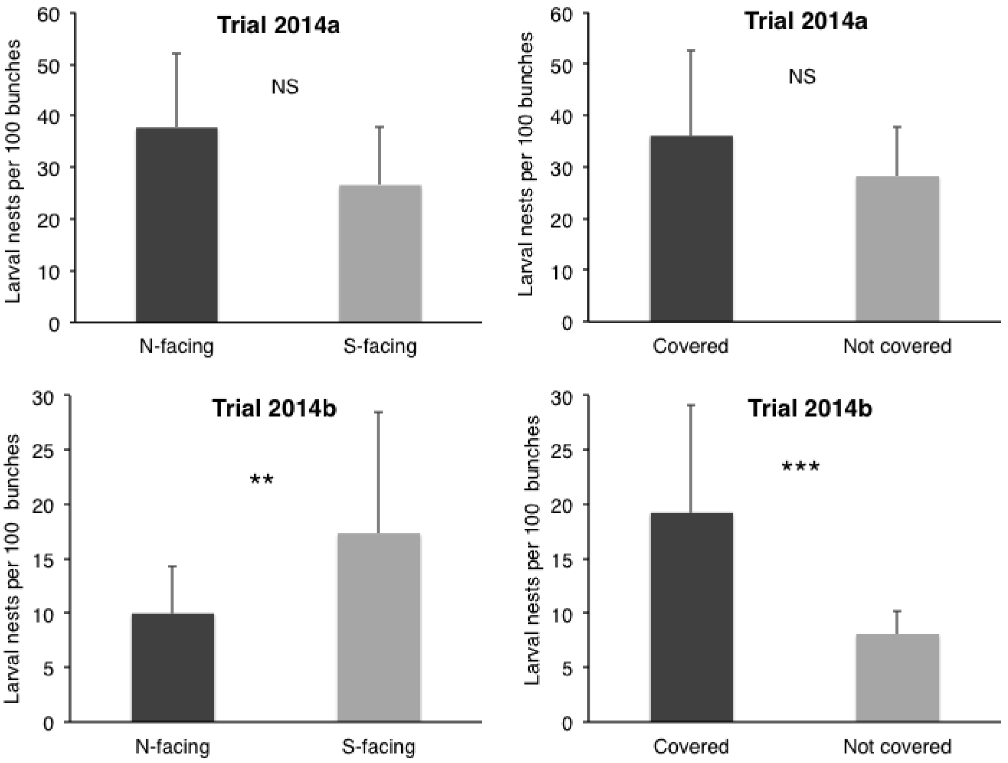

Figure 2. Second-generation infestation of *L. botrana* (mean + SD) observed in the two parallel trials conducted in 2014 on bunches located on the north- or south-facing sides of the rows and covered or not covered by leaves (i.e., exposed to sunlight by leaf removal). NS, **, *** indicate respectively not significant differences, significant differences at 0.01 level and significant differences at 0.001 at two-ways ANOVA.

the covered bunches were significantly more infested than the not-covered bunches ($F_{1,12} = 24.72$, $P = 0.0003$) (figure 2). Only in Trial 2014b the interaction between the two factors was significant ($F_{1,12} = 13.43$, $P = 0.003$) since the higher infestation recorded in the south-facing bunches was due exclusively to those covered by leaves (27.0 and 7.7 larval nests on 100 bunches in covered and not covered groups, respectively).

In Experiment 2013, *L. botrana* females laid more eggs on the sun-exposed side than on the shaded side of the south-facing bunches not covered by leaves (figure 3).

Discussion

Influence of bunch-zone leaf removal on *L. botrana* and grey mould

Bunch-zone leaf removal carried out within ten days of the beginning of the *L. botrana* second-flight, before the expected beginning of egg hatching, reduced larval infestation of the second generation by about 50%, confirming the data reported in Villani *et al.* (1997) that had previously studied the effect of leaf removal specifically on this moth generation. Our study demonstrated for the first time that the positive effect of the leaf-removal, performed to control the second generation, persisted even into the third generation, whose eggs are laid about a month after the green-pruning practice. This result is of practical relevance because the third generation causes higher yield losses (Pavan and Sbrissa, 1994) and favours more grey mould than the second generation (Pavan *et al.*, 2014a).

In Trial 2008, the control of the third generation of *L. botrana* was tendentially better guaranteed by the application with indoxacarb against the second generation than by the specific application with *B. thuringiensis*. The lack of efficacy of *B. thuringiensis* could be explained by the bunch compactness, that does not ensure thorough coverage of berries by the product sprayed, and by the very high UV radiation, that is known to inactivate *B. thuringiensis* toxin (Ignoffo and Garcia, 1978). The efficacy of indoxacarb could be explained by its high persistence that was able to partially control the third generation (Pavan *et al.*, 2014b), even though it was applied against the second generation.

During this study high grey mould levels were observed at harvest time both in Trials 2007 and 2008, but the incidence of grey mould was higher in 2007 than in 2008 in accordance with rainfall conditions recorded in the two years. Contrary to expectations, only in 2008 leaf removal significantly reduced the incidence of *B. cinerea*. It could be due to high rainfall recorded in 2007 and hence to the very high number of hours of wetness, which nullified the benefit of leaf removal. The reduction in rotten berries involved both those non-contiguous to *L. botrana* larval nests (not associated with moth activity) and those contiguous to larval nests (associated with moth activity). Leaf removal had a greater effect on non-contiguous rotten berries, but in

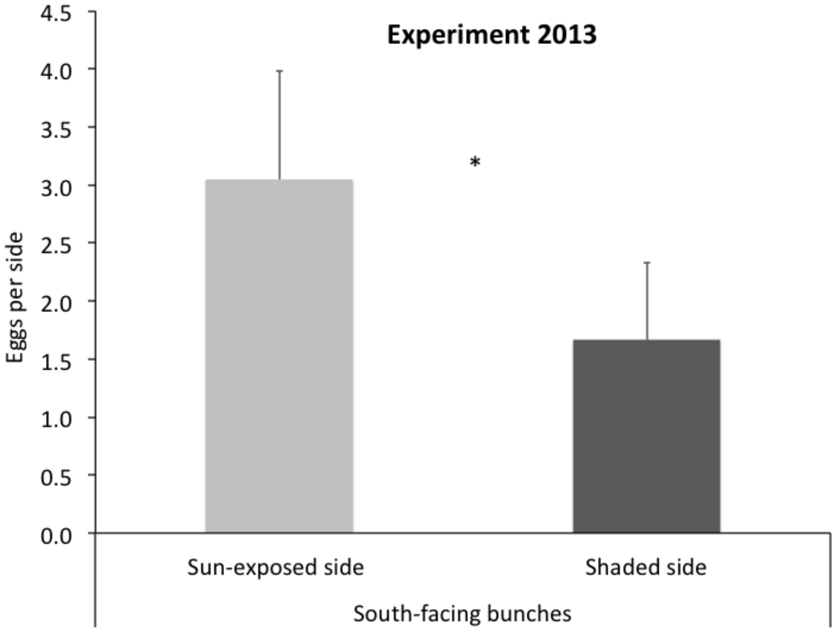

Figure 3. Partitioning of eggs (mean + SE) laid by *L. botrana* females between the two sides of south-facing bunches. Females were confined by tulle bags on 21 shoots with two bunches not covered by leaves. A significant difference was reported at the 0.05 level according to the Wilcoxon matched-pairs signed-ranks test (W = 106, No = 20, P = 0.0484).

any case the reduction in the number of contiguous rotten berries was more than proportional to the reduction in the number of larval nests.

Spatial distribution of *L. botrana* in relation to sunlight exposure

In Israel, Zahavi *et al*. (2003) showed that females of *L. botrana* prefer to lay eggs on bunches that are less exposed to sunlight and they hypothesized that this is due to different characteristics of the exposed berries. Our data showed that on south-facing bunches females preferred to lay eggs on the side of bunch that had been exposed to sunlight in the hours before. Therefore, the females that laid eggs after sunset did not avoid berries that had been previously exposed to sunlight but even showed a slight preference for them. The fact that *L. botrana* females prefer to lay on the berries of the ancestral host plant *Daphne gnidium* L., that are not naturally covered by leaves, than on grape berries, indirectly confirms that exposed fruits are not avoided for oviposition (Maher and Thiery, 2006). Higher temperatures in Israel than in northern Italy could explain differences in egg-laying preference. However, the data of Zahavi *et al*. (2003) showed that females did not completely avoid sun-exposed bunches, but that they simply laid 20-25% fewer eggs on these. In any case, these differences in oviposition were not sufficient to explain the three times lower levels of infestation observed in the field on the sun-exposed bunches. Other factors must be considered to explain such differences, and egg/larval mortality is one of these.

In the vineyard with east-west oriented rows located on the south slope of a hill, the infestation in south-facing bunches (i.e., sun-exposed row-side) was lower in bunches not covered than in those covered by leaves. These results could be explained by a lower level of egg laying or by a higher level of egg/larval mortality on sun-exposed bunches. However, considering that females did not avoid laying eggs on the sun-exposed side of bunches and that the shaded side of these bunches is not directly exposed to sunlight as well as the bunches covered by leaves, the egg/larval mortality hypothesis seems to be more plausible. In this regard, egg susceptibility to higher temperature has been demonstrated (Coscollá *et al*., 1986; Götz, 1941). In the laboratory, Coscollá *et al*. (1986) showed that the critical temperatures are above 40 °C and that the incidence of mortality increases with low relative humidity and exposure time. The same authors suggested that in the field the eggs directly exposed to sunlight could have a higher temperature than the air. In this regard, many studies have shown that berries exposed to sunlight have a higher temperature, ranging up to 10 °C or more above air temperature (Kliewer and Lider, 1968; Millar, 1972; Pieri and Fermaud, 2005; Smart and Sinclair, 1976). Therefore, berries can exceed the critical temperature (i.e., 40 °C), even when the air temperature is lower than this value. A negative effect of high constant temperatures even on larvae was shown (Rapagnani *et al*., 1988), but these results are not directly applicable to field conditions where the temperatures are variable over the day. The role of high temperatures associated with low relative humidity in egg/larval mortality could explain why in the hilly vineyard, differently from the flatland vineyard, the bunches exposed to sunlight were significantly less infested than those covered by leaves.

Conclusions

The data collected in this study allowed us to add two important knowledge on the role of bunch-zone leaf removal on *L. botrana* and grey mould control, as they showed that: (i) part of the grey-mould reduction is due to moth control; and (ii) leaf removal, carried out during the second flight of *L. botrana*, reduced not only the second- but also the third-generation of the moth.

These results suggest that leaf removal affects *L. botrana* larval infestation by increasing egg/larval mortality. Indeed, female non-avoidance of laying eggs on the sun-exposed side of bunches reject the hypothesis that leaf removal affects female fecundity as a consequence of greater difficulty in finding suitable sites for egg laying. However, more research is necessary to determine with certainty whether egg/larval mortality hypothesis is true.

Bunch-zone leaf removal is an advisable cultural practice in an Integrated Pest Management context because it also allows a better bunch coverage by insecticides and anti grey-mould applications. Because bunch-zone leaf removal influences yield and must quality, and can be associated with sunburn (e.g., Verdenal *et al*., 2013), the choice to adopt this practice must be made while taking into account all the possible positive and negative effects.

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