Agronomic Techniques to Control *Lobesia botrana*

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The grapevine moth *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) is a key pest of grapevines in Greece. As part of a broader study on integrated pest management, the effects were investigated of different cultural methods on the establishment and survival of *L. botrana*, specifically: application of different nitrogen levels (30 and 100 units of ammonium sulfate or 70 units of Agrobiosol); summer leaf and shoot pruning; application of growth regulators (Regalis, prohexadione-calcium; or Falgro, gibberellic acid). There were significant differences among the three levels of N application. The lowest *L. botrana* infestation rates were found in plots treated with 30 units of $(NH_4)_2SO_4$ and plots that received some summer pruning. Following the application of plant growth regulators, the lowest *L. botrana* infestation levels occurred in the plots treated with Regalis or Falgro at the manufacturers' recommended concentrations. On vines where growth regulators had been applied, the clusters had fewer berries than those not treated with growth regulators.
KEY WORDS: Ammonium sulfate; cluster compactness; cluster infestation; growth regulators; IPM; pruning; vine fertilization.

INTRODUCTION

Infestation levels of *Lobesia botrana* are known to be associated with a range of grapevine plant growth characteristics (2,7). The compactness of clusters is a major factor in the variable susceptibility to *L. botrana*. This appears to be due largely to the extent of berry-to-berry spread of larvae (1). A larva of *L. botrana* may penetrate not only one grape but it may subsequently attack other, adjacent grapes. Grapes penetrated by a larva will rot, and the rot may spread to adjacent grapes (19). Moreover, grapes may crack due to pressure within the cluster, providing moisture and nutrients for the growth of *Botrytis cinerea* (8). There is clearly a crucial relationship between *L. botrana* and *B. cinerea* (16,17,22).

One reason to produce vines with 'loose' bunches (fewer berries per bunch) is the fact that cluster compactness has a profound effect on disease and pest development. Fermaud (7) found that cluster looseness had an adverse effect on the survival of larvae of *L. botrana*. Other studies, carried out by Snjezana (23) and Baldacchino and Moleas (2), also revealed that grape cultivars with compact clusters were more susceptible to attack by *L. botrana*. With looser clusters, one might think that the crop yield and, thus, the economic return would be minimized. However, there is a cultural practice carried out by the growers that involves the thinning of the crop by reducing the number of clusters in late June or early

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July (unripe berry diameter: 2-4 mm). The above practice aims to increase the quality of the crop and by 'loosening' clusters the yield is not at risk.

Practical methods of 'loosening' clusters include the use of gibberellic acid (GA) sprays. Berry set or cluster compactness may be excessive in many cases and gibberellins have been found to produce loose clusters when applied at specific times (15,18,26,29,30). Prohexadione-calcium is a relatively new plant growth regulator, developed jointly by BASF (Limburgerhof, Germany) and Kumiai Chemical Industry (Tokyo). It has been registered in the USA and several European countries for the control of excessive growth on apple and pear (14). Prohexadione-calcium has been found to be an effective shoot growth inhibitor for apples (14,28). Its effect on grape fruit set and yield has recently been investigated (11,12).

Appropriate fertilization of vineyard soils is also an important cultural practice in vineyards to control plant growth. Vineyard pest management can also be influenced by the nutritional status of grapevines. The density of the leafhopper *Erythroneura variabilis* was found to be closely related to the nitrogen status of grapevines; first generation *E. variabilis* nymph densities were numerically higher within the vines treated with the elevated levels of synthetic nitrogen (13). Leaf nitrogen levels also influenced the population dynamics of the Pacific spider mite *Tetranychus pacificus* (31).

Summer pruning involves the removal of living shoots, leaves and other vegetative parts of the vine, to reduce canopy density and thus increase fruit exposure to light, improve ventilation, reduce relative humidity within the fruiting zone (6,27) and aid spray coverage (24,25). Pruning also affects the development and multiplication of pests and diseases. Leaf removal in the fruiting zone of the canopy has proved important for optimal control of Botrytis bunch rot (3,5,9,20,32), but no work related to the effect of pruning on the infestation by insects has been published.

The purpose of the present study was to evaluate the effects of different levels of nitrogen applied as $(NH_4)_2SO_4$ or Agrobiosol (an organic fertilizer), different types of pruning, and the growth regulators Regalis (active ingredient, prohexadione-calcium) and Falgro (active ingredient, gibberellic acid) on the infestation levels of *L. botrana*. In addition, the effects of nitrogen and growth regulators on cluster compactness were investigated.

MATERIALS AND METHODS

Effect of cultural practices on the infestation levels of *L. botrana* The experiments were conducted in 2004 and 2006 in two 10-year-old commercial vineyards of 'Sauvignon Blanc' (5 ha each in size, divided into 108 plots) located in Agios Pavlos Chalkidiki, Greece. The experiment was factorial in a completely random design with three fixed factors: pruning techniques (shoot and leaf removal, leaf removal, untreated control), and growth regulators (Regalis, Falgro, untreated control). Each of the 27 combinations of levels of the treatments was replicated four times in plots of approximately 126 vines each.

Using a medium type rotary hoe (1.68) (Zanon Macchine Agricole, Campodarsego, Italy), the products – $(NH_4)_2SO_4$ (supplied by Intrachem Hellas Ltd., Athens, Greece) at rates of 170 kg (30 N units) or 500 kg (100 N units) per ha and Agrobiosol (Sandoz; supplied by Lydra Ltd., Greece) at a rate of 600 kg (70 N units) per ha – were mechanically incorporated into the soil to a depth of 1–20 cm on the 5th and 7th of April (just before blooming) in 2004 and 2006, respectively, in the appropriate plots.

Following the experimental design, relevant plots were treated with the plant growth regulators Regalis (BASF; supplied by BASF Hellas, Greece) at rates of 200 mg l^{-1}, and the others with the product Falgro (Fine Agrochemicals Ltd.; supplied by Hellafarm S.A., Greece) at rates of 30 mg l^{-1} (only the grape clusters were sprayed). Spray solutions were prepared according to manufacturers' recommendations, by mixing each product with the appropriate amount of tap water. In 2004 applications were done on the 3rd and 7th of May, when the shoot of the current year was approximately 10–20 cm in length. In 2006 applications were done on the 8th and 10th of May, when the plants were at the same stage of development.

In the relevant plots, three different summer pruning techniques were applied (on the 10th and 12th of June in 2004 and 2006, respectively): shoot and leaf removal, shoot removal, or no summer pruning (control). Leaf removal involved the removal of three or four leaves around the clusters; shoot removal involved the removal of the shoots developing from those buds located at the base of the leaves on the current year's shoot, to the point above where clusters were located.

At harvest time, 100 clusters in each experimental plot were collected and observed for infestation by *L. botrana*. When a larva or larval damage was detected on the cluster, it was considered to be infested. The number of infested clusters in each plot was counted. Sampling was done using a completely randomized design. To compare infestation levels among treatments, data were analyzed using analysis of variance (ANOVA) after angular transformation because the raw data were taken in percentages. Treatment means were separated by Duncan's Multiple Range Test (P=0.05) using the SPSS v12.0. The results are presented as the original, non-transformed data.

Effect of cultural practices on cluster compactness In the plots where no pruning was carried out, the number of berries per cluster was recorded at harvest time. The experiment was factorial in a completely randomized design with two fixed factors each having three levels: fertilization (30 and 100 units of $(NH_4)_2SO_4$ or 70 units of Agrobiosol) and growth regulators (Regalis, Falgro, untreated control). There were nine combinations of levels of the treatments that were replicated four times. Thus, four replicates of 100 clusters were examined.

RESULTS

Effect of cultural practices on the infestation levels of *L. botrana* There were significant treatment effects on *L. botrana* infestation levels. The main effects of level of nitrogen (N) applied, type of pruning (Pr), application of growth regulator treatments (GR), and the interaction effect between N and Pr, were significant in 2004 and 2006 (Table 1).

Among the different N fertilization treatments, there were significant differences between the three levels of nitrogen applications in both 2004 and 2006 (Table 2). In both years the lowest infestation levels caused by *L. botrana* were found in the plots that were treated with 30 units of $(NH_4)_2SO_4$: 22.1% and 22.3% in 2004 and 2006, respectively (Table 2).

Among the pruning treatments, there was a significant difference in *L. botrana* infestation levels in both years (Table 3). The lowest infestation levels caused by *L. botrana* were found in the plots that received some pruning, whether this was shoot and leaf removal or just shoot removal. However, in both years there was no significant difference between the two types of pruning.

TABLE 1. ANOVA of the effects of nitrogen (N), pruning (Pr) and growth regulators (GR) on *Lobesia botrana* infestation (angular transformation of percent of infested bunches), in 2004 and 2006

Effect	df	F-statistic (*P*-value)	
		2004	2006
N	2	79.335 (<0.001)	69.175 (<0.001)
Pr	2	12.808 (<0.001)	15.631 (<0.001)
GR	2	4.902 (0.010)	7.236 (0.001)
N * Pr	4	3.177 (0.018)	4.520 (0.002)
N * GR	4	0.158 (0.959)	0.157 (0.959)
P * GR	4	0.271 (0.896)	1.304 (0.275)
N*Pr*GR	8	0.177 (0.993)	0.443 (0.892)

TABLE 2. Effects of nitrogen fertilization on the infestation levels caused by *Lobesia botrana* in 2004 and 2006

Nitrogen level (units ha^{-1})	% Infested clusters (mean ±S.E.)[z]	
	2004	2006
100	34.3 (0.7) c	34.2 (0.8) c
70	26.5 (0.7) b	25.3 (0.8) b
30	22.1 (0.7) a	22.3 (0.8) a

[z] Within columns, numbers followed by the same letter do not differ significantly at *P*=0.05 (DMRT).

TABLE 3. Effects of pruning on the infestation levels caused by *Lobesia botrana* in 2004 and 2006

Type of pruning	% Infested clusters (mean ±S.E.)[z]	
	2004	2006
Shoot + leaf removal	25.4 (0.7) a	24.6 (0.8) a
Shoot removal	27.0 (0.7) a	26.5 (0.8) a
Control (no summer pruning)	30.4 (0.7) b	30.6 (0.8) b

[z] Within columns, numbers followed by the same letter do not differ significantly at *P*=0.05 (DMRT).

TABLE 4. Effects of growth regulators on the infestation levels caused by *Lobesia botrana* in 2004 and 2006

Growth regulator	% Infested clusters (mean ±S.E.)[z]	
	2004	2006
Control	29.4 (0.7) b	29.4 (0.8) b
Prohexadione-calcium	26.4 (0.7) a	26.8 (0.8) a
Falgro	27.0 (0.7) a	25.5 (0.8) a

[z] Within columns, numbers followed by the same letter do not differ significantly at *P*=0.05 (DMRT).

Among the growth regulator treatments, there was a significant difference in *L. botrana* infestation levels found in the untreated control and the two growth regulator treatments, and in both years the lowest infestation levels caused by *L. botrana* were found in the plots that were treated with growth regulators (Table 4). However, there was no significant difference in either year between the two types of growth regulators.

The interaction between N fertilization and pruning treatments is shown in Tables 5 and 6. The analysis of data in both experimental years revealed that the plots that were treated

TABLE 5. Interaction between nitrogen (N) and pruning (Pr) treatments in 2004 (Data refer to percent infested bunches)

Treatment	Pr1 (shoot + leaf removal)	Pr2 (shoot removal)	Pr3 (no summer pruning)	Mean
N1 (100 U ha^{-1})	29.67 a^zB^y	35.08 bB	38.00 bC	34.3 C
N2 (30 U ha^{-1})	21.67 aA	21.00 aA	23.00 aA	22.1 A
N3 (70 U ha^{-1})	25.00 aAB	25.00 aA	29.00 aB	26.5 B
Mean	25.4 a	27.0 a	30.4 b	27.6

^{z}Within rows, numbers followed by lower-case letter indicate no statistical difference at P=0.05 (DMRT).
^{y}Within columns, numbers followed by a common capital letter indicate no statistical difference at P=0.05 (DMRT).

TABLE 6. Interaction between nitrogen (N) and pruning (Pr) treatments in 2006 (Data refer to percent infested bunches)

Treatment	Pr1 (shoot + leaf removal)	Pr2 (shoot removal)	Pr3 (no summer pruning)	Mean
N1 (100 U ha^{-1})	28.33 a^zB^y	35.75 bB	38.42 bB	34.2 C
N2 (30 U ha^{-1})	22.58 aA	20.17 aA	24.08 aA	22.3 A
N3 (70 U ha^{-1})	22.92 aA	23.58 aA	29.42 bA	25.3 B
Mean	24.6 a	26.5 a	30.6 b	27.2

^{z}Within rows, numbers followed by the same lower-case letter indicate no statistical difference at P=0.05 (DMRT).
^{y}Within columns, numbers followed by the same capital letter indicate no statistical difference at P=0.05 (DMRT).

with 100 units of $(NH_4)_2SO_4$, and shoot and leaf removal gave the lowest infestation level caused by the pest. Within the plots that were treated with 30 units of $(NH_4)_2SO_4$, there was no significant difference between the infestation levels caused by the pest, regardless of pruning types (including the control). In 2006, within the plots that were treated with 70 units of Agrobiosol there was a significant difference in the infestation levels between the two pruning types and the control (lower infestation rates were obtained in plots that received some pruning); this was not the case in 2004.

Within the plots where shoots and leaves were pruned, the vines that had been treated with 30 units of $(NH_4)_2SO_4$ gave the lower infestation levels (2004 and 2006). In those plots that had been treated with 100 units of $(NH_4)_2SO_4$ and 70 units of Agrobiosol, infestation levels did not differ significantly between them in 2004; however, in 2006 the difference was significant.

Within the plots where shoots had been removed, or received no summer pruning, the vines that had been treated with 100 units of $(NH_4)_2SO_4$ gave the higher infestation levels, which differed significantly from the vines in plots that had been treated with 70 units of Agrobiosol (2004 and 2006). Within the plots where the vines were left without any pruning, those that had been treated with 30 units of $(NH_4)_2SO_4$ gave the lower infestation rates and differed significantly from the plots that were treated with 100 units of $(NH_4)_2SO_4$ and 70 units of Agrobiosol in 2004. In 2006, the results obtained from the plots treated with 30 units of $(NH_4)_2SO_4$ differed significantly from those obtained from plots treated with 100 units of $(NH_4)_2SO_4$; however, there was no difference between those treated with 30 units of $(NH_4)_2SO_4$ and 70 units of Agrobiosol.

Effect of cultural practices on cluster compactness As far as grape cluster compactness was concerned, analysis showed that the growth regulators factor proved to be significant in 2004 and 2006, whereas the N fertilization factor was not significant, nor was the N * GR interaction (Table 7).

In both experiment years there were significant differences between grape cluster compactness on plots treated with the two growth regulators (prohexadione-calcium and Falgro), compared with the control (Table 8). On vines where growth regulators had been applied, the clusters were found to be looser (lower berry number per cluster) than those that were not treated with growth regulators (control). There was no significant difference between the two growth regulators in berry number per cluster.

TABLE 7. ANOVA of the effects of nitrogen (N) and growth regulators (GR) on cluster compactness in 2004 and 2006

Effect	df	F-statistic (*P* value in parentheses)	
		2004	2006
N	2	0.194 (0.825)	0.082 (0.921)
GR	2	19.649 (<0.001)	28.656 (<0.001)
N * GR	4	0.433 (0.784)	0.627 (0.647)

TABLE 8. Effects of growth regulators on cluster compactness in 2004 and 2006

Growth regulator	Mean (±S.E. = 2.3) berry number per cluster[z]	
	2004	2006
Control	92.3 b[z]	103.7 b[z]
Prohexadione-calcium	75.7 a	81.3 a
Falgro	73.7 a	83.0 a

[z] Within columns, numbers followed by the same letter do not differ significantly at P=0.05 (DMRT).

DISCUSSION

The different agronomic treatments applied in this study significantly affected *L. botrana* infestation levels, and some treatments affected cluster compactness. With regard to summer pruning treatments, the lowest pest infestation levels were found on vines that had received some summer pruning, whether by shoot removal, or by shoot plus leaf removal.

In 2004, in the case of 'shoot removal' or 'no summer pruning', growers could avoid applying 100 units of $(NH_4)_2SO_4$ because the infestation rates would be the highest. Instead, growers could apply 30 units of $(NH_4)_2SO_4$ or 70 units of Agrobiosol, respectively. In the case of shoot plus leaf removal, there was no significant difference in the infestation rates between 100 units of $(NH_4)_2SO_4$ and 70 units of Agrobiosol, which resulted in the highest infestation levels; therefore growers could apply 30 units of $(NH_4)_2SO_4$. In 2006, in all cases of pruning there was a significant difference between the infestation rates obtained from the plots that were treated with 100 units of $(NH_4)_2SO_4$ (highest infestation rates) and the plots that were treated with 70 units of Agrobiosol or 30 units of $(NH_4)_2SO_4$.

In the case of 100 units of $(NH_4)_2SO_4$, a lower infestation level was obtained when both shoot and leaf removal was carried out. Thus, if growers need to apply 100 units of $(NH_4)_2SO_4$ then they should also carry out shoot and leaf removal. However, if growers decide to apply 30 units of $(NH_4)_2SO_4$, then they could save labor, time and expense by not doing any type of pruning. However, long-term application of such low doses of N is not advisable as it can adversely affect vine health and grape quality (4,10).

When considering the growth regulator treatments, the lowest pest infestation levels were found on vines that had received plant growth regulators, whether prohexadione-calcium or gibberellic acid. Prohexadione-calcium is primarily used for the control of shoot growth in apple (14) and it has the potential to reduce the fruit set of specific grape varieties when it is applied pre-bloom (12). Although it is used primarily for the above purposes, it has been found that it also reduces plant susceptibility to fire blight (caused by the bacterium *Erwinia amylovora*) in pome fruits when used prophylactically (21). When prohexadione-calcium was applied pre-bloom to the grape varieties 'Cabernet Franc', 'Cabernet Sauvignon' and 'Chardonnay', it had the potential to bring about severe reductions in shoot growth (11). Application of prohexadione-calcium pre-bloom and during bloom to the above grape varieties caused a reduction in fruit set, whereas application of prohexadione-calcium one or two weeks post-bloom caused a reduction in berry weight and had less effect on fruit set. However, prohexadione-calcium did not reduce the crop yield below the economic threshold (12). In wine grape cultivation very high yields are not desirable, because they negatively affect the quality of wines.

Study of the effects of the growth regulators on cluster compactness showed that the clusters that were treated with the growth regulators were looser than those that were left untreated. Although the numerical differences among treatments were small, the difference was statistically significant. Our results are consistent with experiments carried out by others (15,18,26,29,30).

From the above, it may be concluded that the use of growth regulators reduced cluster compactness, which in turn had a positive effect in the management of infestations caused by *L. botrana*. However, the above results require further investigation. Thus, in an IPM program, plant growth regulators can be a useful tool to reduce the infestation of *L. botrana* and therefore to reduce the need for insecticides. It is important to encourage and motivate farmers to reduce insecticide use and possible environmental contamination and non-target effects, all of which can be achieved in part through the use of agronomic techniques.

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