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EFFECT OF APPLICATIONS OF ISONET L PLUS, THE CONTROLLED-RELEASE DISPENSER IN THE PROTECTION OF VINEYARDS AGAINST *LOBESIA BOTRANA* AND *EUPOECILIA AMBIGUELLA* IN THE SOUTHERN SLOVAKIA

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The grapevine moth (*Lobesia botrana*) and the grape berry moth (*Eupoecilia ambiguella*) are the main pests in southern Slovakia vineyards. While *L. botrana* represents the most common species in the vineyards, *E. ambiguella* exists in some sites only. In this study we monitored the occurrence and dynamics of the flights of the grapevine moth and the grape berry moth in the vineyard area Dvory nad Žitavou. The size of the examined area was 60 ha in 2008 and 90 ha in 2009. On these areas, we used pheromone traps type Deltastop LB for grapevine moth and Deltastop EA for grape berry moth. To control the movement of the pests, a set of three pheromone traps for each type was used in observation points which were placed at least 50 m from each other. Monitoring was carried out regularly every second to third day. The control area was located in immediate vicinity of the area. As a threshold, we consider 20 moths for 2 days at an average of three pheromone traps. In 2008, we recorded the first, second and third generation of *Lobesia botrana* and only the second generation of *Eupoecilia ambiguella*. In 2009, we saw all three generations for both types of moths. We also examined effectiveness of the protection method of grapevine based on the mating disruption using pheromone dispensers type ISONET L plus. Just before the anticipated start of swarming we distributed 420 dispensers/ha evenly over the entire vineyard; on the edges of the treated area, the basic dose of dispensers i.e. 500 pieces/ha was left. To assess the effectiveness of the product ISONET L plus in 2008 we reached more than 70% efficiency in the border plots, inside 96%. We achieved 69% efficiency in 2009. Based on the results it is be clear that the long-term use of this method of protection has a positive impact in reducing the population of moth. It is suitable for use in an integrated and biological protective system against *Lobesia botrana* and *Eupoecilia ambiguella*.

Keywords: grapevine, *Lobesia botrana*, *Eupoecilia ambiguella*, mating disruption, ISONET plus

European grape vine moth (*Lobesia botrana*) and European grape berry moth (*Eupoecilia ambiguella*) are the most important grape pests in vineyards in the southern Slovakia from economic point of view. Current world trends in viticulture mean a return to nature and the search of environmentally friendly farming systems based on the principle of sustainable viticulture. In addition, this integrated, biodynamic or organic systems totally or almost completely eliminated foreign substances from the environment, keep harmful organisms below the threshold and they are necessary to reserve the biodiversity conservation. Implementation of these basic rules in viticulture brings chance to get the products from integrated production or organic products, which are preferred by consumers. For these products with a special designation, i.e. excellent wines with mostly attributes or the character of vines – terroir, it is possible to achieve significantly higher prices when selling (Goode, 2010).

Mating disruption is an insecticide-free pest management strategy, where we use synthetically produced female pheromones that disrupt communication between the sexes and provide effective protection without the environmental impact. In the past 10 years, its use has been rapidly increasing worldwide, but in the Czech Republic, Slovakia and Hungary it has not been well established yet (Hluchý et al., 2006).

The aim of our study was to evaluate the occurrence of two tortricid species *Lobesia botrana* and *Eupoecilia ambiguella* (*Lepidoptera*: *Tortricidae*) in the southern Slovakia, and to determine the effectiveness of the protection method of mating disruption against them.

Material and methods

The study was carried out in the experimental vineyard area in Dvory nad Žitavou during two consecutive years, 2008 and 2009, over a surface of 60 ha in the first year and 90 ha in the second year. In this area we monitored the incidence and dynamics of flight activity of moths *Lobesia botrana* and *Eupoecilia ambiguella* during the first, second and third generation into pheromone traps and determined the optimal date of protection measures. Then we made protection by mating disruption that we applied by the 3rd year. The treated area was covered by several table grape varieties: Pinot Blanc, Welschriesling, Grüner Veltliner, and the variety Chardonnay on the control area. Control area was divorced from the treated area 500 m.

To monitor the flight activity of male moths, a set of pheromone traps, Deltastop LB for grape vine moth and Deltastop EA for grape berry moth (Propher, the Czech Republic) was installed. Pheromone traps were observed in both years, evenly distributed over the entire investigated

vineyard, a week before the beginning of moth first flight, i.e. between 10th April and 15th August. A point set of three pheromone traps of each species located 50 m apart was used for one observation due to the homogeneous location. Evaluation of the number of captured adults took place every second to third day (controls were practiced on Monday, Wednesday and Friday). Duration of flight and the number of captured males were continuously recorded, depending on the generation and species of pests. As a threshold of harmfulness we consider trapping 20 pieces of adults per 2 days in an average of three pheromone traps.

In our experiment we aimed to verify the effectiveness of pheromone mating disruption with the Isonet L plus dispensers (ShinEtsu Chemical Company Ltd.), which contained a total of 172 mg (E, Z)-7,9-Dodecadienyl acetate and only 18 mg (Z)-9-Dodecenyl acetate, the principal components of the pheromone blend of *Lobesia botrana* and *Eupoecilia ambiguella*, respectively. The application rate was 420 dispensers per hectare at a distance of 50 m from each other, before the expected beginning of the first flight of adults, i.e. from 5th April. On the edges of the treatment area, we kept the basic dose of dispensers. In addition, the treated area was divided into four plots; in each one we controlled 500 randomly selected shrubs, on every shrub 5 to 10 randomly selected clusters. On the edges, the first three randomly selected shrubs of 5 to 10 randomly selected clusters were evaluated. In the same way we evaluated the control area, which was located beside the treated area.

The effectiveness of the pheromone treatment was evaluated every week; equally the occurrence of the first generation of larvae in inflorescence bunches, as well as the occurrence of the second generation in clusters. The threshold of harmfulness was determined by the grape moths' infestation rate of above 5%. The biological efficacy of the Isonet L plus was evaluated by a physical inspection of harmful agents under grape in percentages and then determined according to the Abbot Scheme:

$$Efficiency\ (\%) = [(K - N) / K] \times 100$$

where:
K – the number of infected plants in the control area
N – the number of infected plants in the treated area

In 2008, the control area has not been treated, however in 2009, the control area was treated by chemical treatment with diflubenzuron (Dimilin 48 SC; Chemtura Europe Ltd.), in a dose of 0.02 %. Due to prolonged residual effect of preparation it was necessary to be treated 1–2 days before the first flight of moths, thus at the beginning of egg laying. The first application of treatment was provided on 15th May at growth stage BBCH 57, compared to the second generation at 10th July in phenological stage BBCH 77. The achieved results of protection method by mating disruption were confronted with the untreated control plot and the control plot treated by the chemical treatment.

Results and discussion

The monitoring of flight activity and the fluctuation of the number of captured adults of *Lobesia botrana* and *Eupoecilia ambiguella* in the pheromone treated vineyard was evaluated in 2008 as well as in 2009 (Table 1 and Table 2, Figure 1 and Figure 2). Our results have shown that the catches of LB in baited traps were significantly higher than the catches of EA in baited traps. The reason may be too high temperature occurred in summer, which dramatically reduced the number of captured males of European grape berry moth. Year 2008 was warmer and drier, while the year

Table 1 Overview of flight activity and the fluctuation of the number of moth adults European grape vine moth (*Lobesia botrana*) and European grape berry moth (*Eupoecilia ambiguella*) captured in pheromone traps in the vineyard area of Dvory nad Žitavou in 2008

Pests	*Lobesia botrana*		*Eupoecilia ambiguella*	
Generation	**Flight activity**	**Number of moths**	**Flight activity**	**Number of moths**
1.	25. 04. – 31. 05.	631	–	0
2.	23. 06. – 21. 07.	387	18.06. – 04.07.	97
3.	11. 08. – 08. 09.	41	–	0
Total		1059		97

Table 2 Overview of flight activity and the fluctuation of the number of moth adults European grape vine moth (*Lobesia botrana*) and European grape berry moth (*Eupoecilia ambiguella*) captured in pheromone traps in the vineyard area of Dvory nad Žitavou in 2009

Pests	*Lobesia botrana*		*Eupoecilia ambiguella*	
Generation	**Flight activity**	**Number of moths**	**Flight activity**	**Number of moths**
1.	15.04. – 03.06.	586	24.04.	2
2.	15.06. – 20.07.	457	15.06. – 22.06.	65
3.	05.08. – 04.09.	141	17.08. – 24.08.	26
Total		1184		93

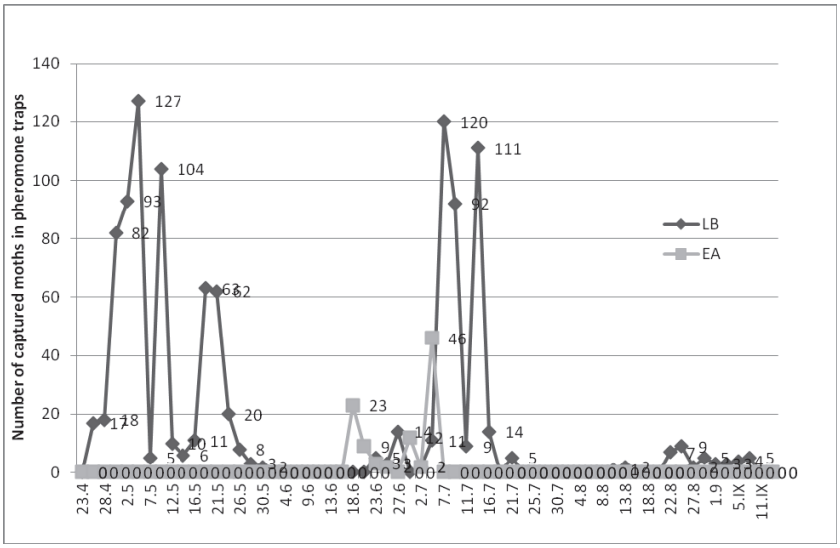

Figure 1 Flight activity of male moths European grape vine moth (LB) and European grape berry moth (EA) in the vineyard area of Dvory nad Žitavou in 2008

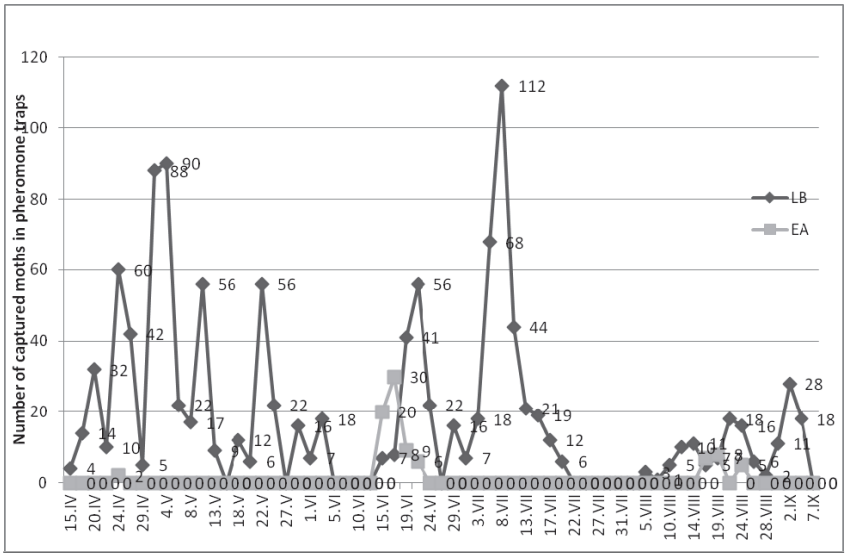

Figure 2 Flight activity of male moths European grape vine moth (LB) and European grape berry moth (EA) in the vineyard area of Dvory nad Žitavou in 2009

2009 was characterized by variable weather altering, abundant rainfalls and hot, dry periods.

In 2008, we set the infestation of grape bunches with larvae moths as follows (Figure 3):

- in the case of the first generation in phenological growth stage BBCH 75 on 24th Jun with attack of bunches 5.30% on the edges, 0.80% in the centre of pheromone treated area and 18.75% on the untreated control plot,
- during the flight of the second generation in phenological stage BBCH 85 on 22nd September with attack of bunches 2.50% on the edges, 0.50% in the middle of the pheromone treated area and 13.50% on the untreated control plot,
- without assessment of the third generation.

In 2008, evaluation of infestation on grape bunches depending on the grape variety was conducted as follows:

- the first generation: 6% of infected bunches on the upper edge of the experimental area, 6% on the lower edge, on 4% the edge, 1% in the centre for the variety Pinot Blanc and 0–1% for the varieties Welschriesling and Grüner Veltliner. The untreated area for the variety Chardonnay marked 18.75% of infested grape bunches.
- the second generation (Figure. 2): 2% of infected bunches on upper edge of the experimental area, 3% on the lower edge, 2.5% on the edge of, 1% in the middle for the variety Pinot Blanc and 0% for the varieties Welschriesling and Grüner Veltliner. However, in the untreated area for Chardonnay there was 13.50% of infested grape bunches.

In the first generation we recorded 72% of effectiveness on the edges and 96% in the middle of the vineyard. In the second generation the infestation of grapes showed 82% on the edges and 96% in the middle.

In 2009, we set the infestation of grape bunches with larvae moths as follows (Figure 4):

- in the first generation in phenological stage BBCH 65 on 3rd June with attack of bunches 1.70% in the experimental area and 5.40% in the chemically treated control plot,
- in the second generation in phenological stage BBCH 85 on 9th September with attack of bunches 0.60% in the experimental area and 2.25% in the conventionally treated control plot,
- without assessment of the third generation.

In 2009, evaluation of infestation on grape bunches depending on the grape variety was conducted as follows:

- the first generation: 0.8 % of infected bunches on the upper edge of the experimental area, 0.4% on the lower edge, 6.8% on the edge, 0.5% in the centre for the variety Pinot Blanc and 0% for the varieties Welschriesling and Grüner Veltliner. The untreated area for the variety Chardonnay marked 5.4% of infested grape bunches.
- the second generation: 0.2% of infected bunches on upper edge of the experimental area, 0.1% on the lower edge, 2.5% on the edge, 0.1% in the middle for the variety Pinot Blanc and 0% for the varieties Welschriesling and Grüner Veltliner. However, the chemical treated area for Chardonnay marked 2.25% of infested grape bunches.

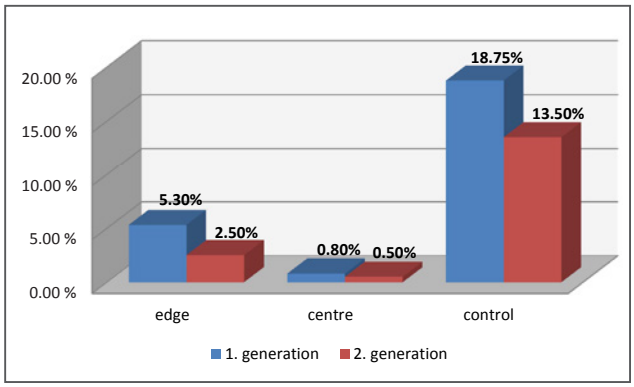

Figure 3 Infestation of grape bunches with larvae moths European grape vine moth (*Lobesia botrana*) and European grape berry moth (*Eupoecilia ambiguella*) in the vineyard area of Dvory nad Žitavou in 2008

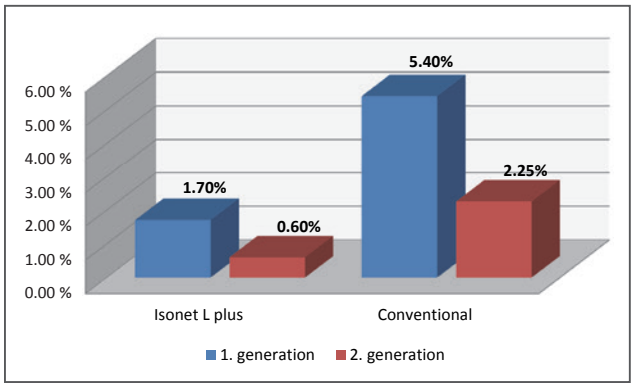

Figure 4 Infestation of grape bunches with larvae moths European grape vine moth (*Lobesia botrana*) and European grape berry moth (*Eupoecilia ambiguella*) in the vineyard area of Dvory nad Žitavou in 2009

In the first generation we recorded 69% of effectiveness and in the second generation the effectiveness of infestation of grapes showed 73%.

The results comparison of two years' trials and the technology showed excellent efficiency against moths in the vineyard where mating disruption is applied for several consecutive years. Accordingly, we achieved a very good efficiency, which was above 90%, in two of three grape varieties up to 100% on approximately 60 than 90 ha. Similar results were obtained in 2005 and 2006 by Hluchý et al. (2006), then in 2007 and 2008 by Broklová and Hluchý (2009) and subsequently by Lucchi et al. (2012) in all California countries in 2010, 2011 and 2012.

We continue our experiments with gradual dose reduction. According to Bagnoli et al. (2006) in Tuscany (Italy) and Louis and Schirra (2001) in Germany the success of our research depends on the plot size and the number of years when the method is used.

However, Kast (2001) also pointed out the disadvantages of using pheromone dispensers where he tested protection method mating disruption for 12 years in vineyards of the Wuertemberg region, Germany. According to him, the use of pheromones in viticulture seems to be a social problem rather than a technical one.

Briand et al. (2012) offer the possibility of more easily evaluation of field-deployed pheromone dispensers. They successfully tested the suitability of small insect field cages for a pre-evaluation of the impact of sex pheromones on mating using the grape moths. Study confirmed that small cages provide a fast and cheap method to compare the effectiveness of pheromone dispensers under standardised semi-field conditions.

Conclusion

In this study we monitored the incidence and dynamics of flight activity of two tortricid species *Lobesia botrana* and *Eupoecilia ambiguella* (*Lepidoptera*: *Tortricidae*) during the first, second and third generation into pheromone traps, Deltastop LB for grape vine moth and Deltastop EA for grape berry moth in the vineyard area Dvory nad Žitavou during two consecutive years, 2008 and 2009. Then we determined the effectiveness of the protection method of mating disruption with Isonet L plus dispensers against them.

Based on these observations we can state the following conclusions:

- In the southern Slovakia two types of moths, *Lobesia botrana* and *Eupoecilia ambiguella* occur most commonly, which in the observed vineyards regularly produce two or three generations.
- The proportion of spread was primarily depending on the weather conditions. The grapevine moth likes hot and dry periods, while the grape berry moth occurs mainly in colder and more humid periods.
- In 2008 we monitored the dynamics of flight activity of moths; we recorded all tree generations of *Lobesia botrana* and only second generation of *Eupoecilia ambiguella*. However, in 2009, we had all three generations of both tortricid species.
- In 2008 and 2009 in the vineyard area of Dvory nad Žitavou, the efficiency was above 90% in two of the three varieties observed up to 100%. These results were achieved after the application of the pheromone dispenser ISONET L plus.
- According to the achieved results it could be stated that the long-term use of this method of protection has a positive impact on reducing the population of male moths.
- Mating disruption in viticulture will be effective for those who will understand the importance of local environmental factors. It requires a long process of putting this method into wide practice. However, the use of environmentally friendly pest control technique requires compliance with certain principles associated with its use.
- The effectiveness of pheromone mating disruption with Isonet L plus dispensers is presented as a promising protection measure against *Lobesia botrana* and *Eupoecilia ambiguella* in Slovak vineyards; in the future, a rapid increase of its use is expected. High efficiency of this method helps to reach high levels of profitability of grape production.
- Finally, it is predictable that the usage of mating disruption in Slovakia will seriously expand in the following years due to the decreasing number of the registered insecticides, the appearance of resistance of the pests against some

group of insecticides and the increasing of the need for residue-free (and organic) products.

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