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Lobesia botrana (Lepidoptera: Tortricidae) larval population assessment by damage to grape flowers: could empty larval nests monitoring be useful?

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Abstract: A correct accurate determination of the population size is the basis of successful pest management procedures and is of primary importance in the protection against the European grapevine moth (EGVM, *Lobesia botrana*). During three consecutive years, we quantified the time course of larval damage to grape flower buds and the presence of the different larval instars of EGVM in an experimental vineyard (INRA Bordeaux Aquitaine research center). From a total of 1003 so called larval damage (glomerula), 704 living larvae were obtained. We determined the larval instars of all samples. There was a significant correlation between damage per larva and larval population densities. Intra-specific competition between larvae and avoidance of larval parasitism are the most probable causes of empty glomerulae, and of the relation we observed. We assume that grape damage could efficiently be quantified also by estimating the number of empty glomerulae as a good indicator of larval density.

Key words: grapevine; *Lobesia botrana;* damage; larval instar; behavior; parasitism

Introduction

Lobesia botrana (Den. & Schiff., Lepidotera: Tortricidae), commonly known as European grapevine moth (EGVM), is the grape moth most often found in European vineyards (Delbac *et al.*, 2010; Harari *et al*., 2011; Ioriatti *et al*., 2011). This insect, by the feeding activity of its larva on the grape clusters counts among the major pests of the vine that require constant monitoring and control (Roehrich & Boller, 1991). The damage caused by EGVM can be very important and often varies from one year to another. EGVM mainly attacks fruit parts and damage magnitude is a function of larval age (Delbac & Thiéry, 2015). Depredation in the first generation can also be spectacular but has little effect on the harvest except in low yield and expensive production vineyards (Thiéry, 2008). During the second or third generation, larvae colonize the berries and eat the pulp (Galet, 1982), and when the population density is too large, the bunch can be completely destroyed (Thiéry, 2011).

Damage made during late summer may also affect the quality of wine. An indirect harmfulness is observed before the harvest due to bites on the berry that facilitates the growth of microorganisms on the bunch. Besides the risk of gray mold (*Botrytis cinerea*) (Fermaud & Le Menn, 1989), other rots may develop, such as *Aspergillus* black rot, with significant health problems due to ochratoxins productions (Cozi *et al.*, 2006).

L. botrana performs several reproductive cycles per year depending on the region, usually two to four in France, with typically three generations in the Bordeaux region (Delbac *et al.*, 2010). Fairly regularly, insecticide treatment should be performed depending on the pest pressure, essentially against the summer generations. Traditionally, the decision to actively control or not the larval EGVM populations is based on measurements of the population of the previous generation (Delbac *et al*., 2006). This monitoring employs traps for counting adults and assessment of plant damage (Thiéry, 2011). Damage can be used as an adequate and simple tool for estimating the population dynamics of the different larval instars of EGVM in a vineyard (Delbac & Thiéry, 2015), even if the link is often debated and sometimes contested by the growers and technicians. The larval population size of *L. botrana* in the plots is however an important management indicator which has been recently reappraised (Delbac and Thiéry, 2015). Wrong evaluation of population size in spring often leads to misestimate the risk in the second generation.It is then necessary to characterize better the relationship between level of larval populations and damage generated in the plots.

The objective of our study was to determine the relationship between the *L. botrana* larval instar and the level of damage generated per larva. The present work also intended to consider the occurrence of empty larval nests – which is an easy criterion to examine – and its relation with larval population size.

Material and methods

Experimental vineyards

We used data from our survey database (1996 to 1998) [experimental vineyard [INRA Bordeaux Research Centre, Villenave d'Ornon (France)]. The surveyed plot of 1250 m², naturally infested with wild EGVM,was surrounded by vineyards under conventional management. Our experimental vineyard (described in Delbac *et al*., 2010) was planted with Merlot. No insecticide was used, but a conventional fungicide program was applied to protect bunches against downy mildew and powdery mildew (cymoxanil with dithiocarbamate, fosetyl with folpel, demethylation inhibitors, wettable sulfur).

Larval sampling

Larval activity was monitored from hatching to pupation. Damaged flowers (inflorescences) were collected during the first generation from 1996 to 1998 (Table 1); they were individually? placed in Petri dishes and immediately stored for less than one week in a cold chamber (4 °C) to stop larval development before laboratory observations.

Laboratory measurements

We checked the damage using a steromicroscope (X10 magnification) in order to confirm the presence of larvae and count the proportion of empty foci. When present, the larvae were transferred into 70% ethanol and their head capsules were measured (Figure 1) for instar determination (Delbac *et al*., 2010). Each year, we assessed the level of damage generated in spring by *L. botrana* population by counting all the bunches selected randomly from 20 winestocks. Using this sampling procedure, we obtained a mean number of clusters per winestock and could evaluate the level of damage of EGVM population in the field.

Table 1. Number of damaged flowers and larvae of different instars during the three years.

Year	1996	1997	1998
Period	May 22-June 7	May 7-June 2	May19-June 2
Larval instars			
1	14	6	52
2	80	24	56
3	111	21	61
4	162	32	47
5	25	4	9
Number of larvae	392	87	225
Number of damaged flowers (glomerulae)	635	106	262
Damage per larva	1.62	1.22	1.16

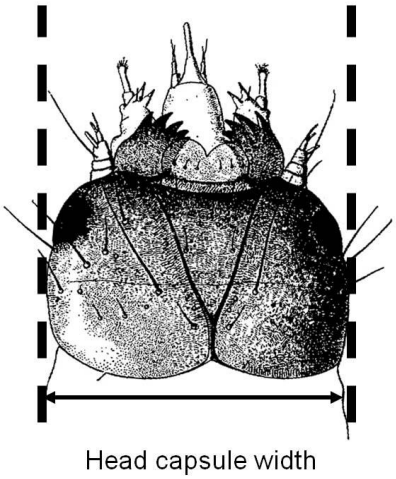

Figure 1. Principle of head-capsule measurement which considers the wider sclerified part of the head.

Statistical analysis

We used linear correlation analysis of damage per larva and damage density per hectare to assess the relationship between damage and larvae. To improve statistical analysis, we incorporated data collected in four plots from previous studies: two in 1996 (Badenhauser *et al.*, 1999) and two in 2013 (Sage *et al.*, 2014). Statistical test was performed with a type-I error rate of 0.05 under SYSTAT® 11 software.

Results

The timing of EGVM development was slightly different between years. We assessed G1 damage on several dates between mid May and early June of each year in order to obtain sufficiently large sample sizes. From 1996 to 1998, we collected 1003 G1 damages that hosted 704 living larvae (Table 1).

In 1996, most of the larval instars were L2 to L4 and we found 1.62 damage (glomerulae) per larva. In 1997, the population was significantly smaller (4 larvae per 100 bunches) than in 1996 (~ 9 larvae per 100 bunches) and few damaged flowers were collected with an average of 1.22 damage per larva. In 1998, the population size was between that of 1996 and 1997. There was an average of 1.16 damage per larva, the lowest amount of the 3 sampled years. The number of density of larvae per hectare as a function of damage per larva for G1 (Figure 2) was significantly and positively correlated (Pearson's r = 0.943, p = 0.001).

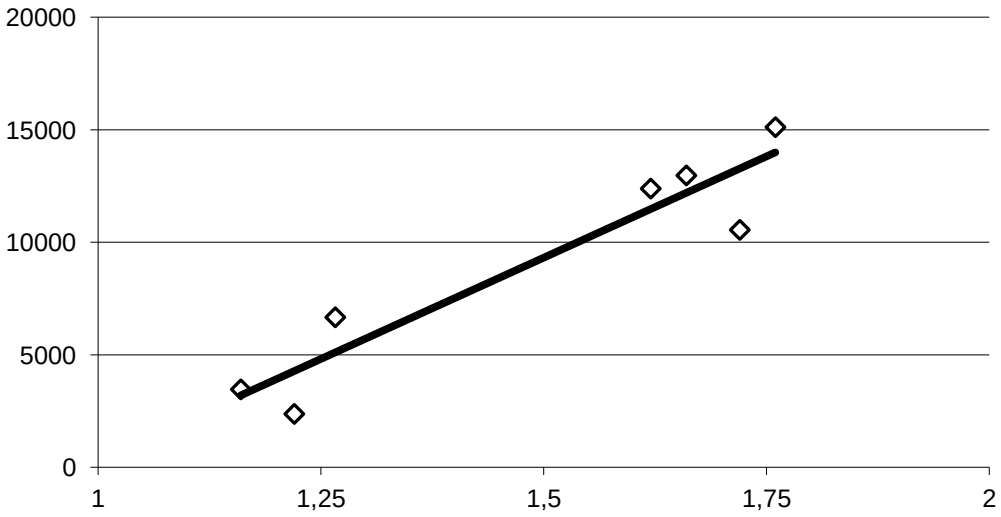

Figure 2. Linear regression of damage per larva (abscissa) and population density of larvae per hectare (ordinate) in the first generations (Pearson's r = 0.943, p = 0.001).

The larval age distribution and the inhabitation of glomerulae was determined as a function of time (Figure 3). In the year with higher infestation (1996) (Figure 3 A) the empty glomerulae increased from 25% to 72.5% of the samples while the maximum numbers of empty glomerulae was lower (26.5%) in the years with low infestation (1997 and 1998) (Figures 3 B and 3 C).

Discussion

In our study, the empty glomerulae abandoned by last larval instars were not due to pupation and possibly related to escape in response to danger or to a natural enemy luring behavior which has never been studied in this species. The Correlation analysis obtained indicated a relationship between plant damage per larva and larval population size. In literature, several other factors can affect the larval population size like climatic or geographic factors

(Coscolla, 1997), mating rates, oviposition preferences and dynamics among different cultivars (Maher & Thiéry, 2004; Thiéry *et al.*, 2014), neonate larvae installation (Gabel & Roehrich, 1995), and exposure to natural enemies (Xuéreb & Thiéry, 2006; Moreau *et al.*, 2010).

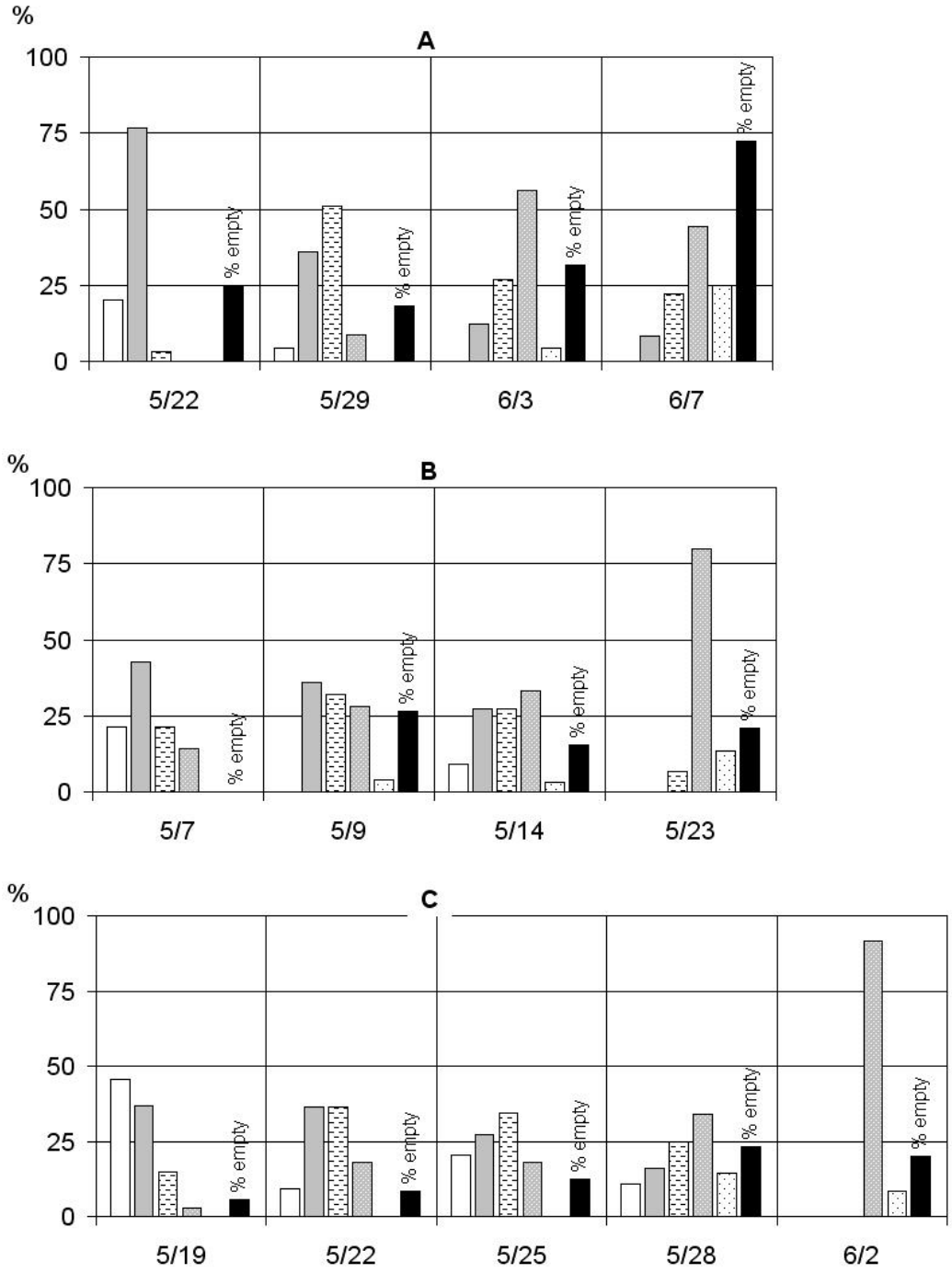

Figure 3. Percentage of different larval instars during 1996 (A), 1997 (B), and 1998 (C) in full damage and empty ones. White bars: 1st instar; Grey bars: 2nd instar; White bars with black dash: 3rd instar; Grey bars with white points: 4th instar; White bars with black points: 5th instar; Black bars: % of empty damage.

As observed in our study, increase in larval population in spring when the cluster size is reduced can lead to an increase in larval movement. In this solitary species, intra-specific competition has been documented during the egg-laying period (Gabel & Thiéry, 1992; Thiéry & Gabel, 1993) and hypothesized to occur in larvae (Gabel & Roehrich, 1995; Thiéry, 2008). The maximum number of larvae in a grape bunch at the end of development varies from 10 to 30, depending mainly on its size (Thiéry, 2008) and the cultivar. For the Merlot cultivar in Bordeaux vineyards, the maximum number is 15 larvae per bunch (Delbac *et al.*, 2012). Neonate larvae of EGVM penetrate the flower bud at the vicinity of their eggs, but never beneath the eggs, and live in the same bunch throughout their development (Thiéry, 2008) with very limited capacities of escape. Therefore, moving in the same bunch remains the only solution to avoid neighboring competition for *L. botrana*.

Empty glomerulae is a common trait in *L. botrana* spring generation and its frequency clearly varied in our study according to the larval population size. Leaving a glomerula may be the result of an adaptive behavior that allows larvae escaping from danger. Living close to feces may generate danger in many insect pests by attracting parasitoids (Weiss, 2006). This attraction often depends on the quantities of frass volatiles (Steidle & Fisher, 2000). Hence, the feces produced by *L. botrana* larvae can attract parasitoids, like *Dibrachys cavus* (Chuche *et al*., 2006). Xuéreb & Thiéry (2006) suggested that the abundance of *Campoplex capitator*, one of the most common larval parasitoids of EGVM in French vineyards (Moreau *et al*., 2010; Thiéry *et al*., 2001) could also depend on that. Therefore, when population is high, a larva could reduce the risk of being parasitized or predated by leaving its first shelter (here glomerulae). Silk is another larval by-product that can be attractive for parasitoids (Afsheen *et al*., 2008). Thus, for *L. botrana*, building several silk nests can lure parasitoids by complicating their searching behavior.

Conclusion

L. botrana larva lives in a single bunch for its complete development. If larval density per bunch is high, the larvae must then move and disperse inside the bunch to avoid intra-specific competition for food and avoid possible adverse effects of the attractiveness of its feces to natural enemies. The present study shows that damage is a function of larval age and population density. Our results suggest that taking into account empty glomerulae would improve the population size evaluation.

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