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Temporal differences in *Lobesia botrana*'s lifecycle at local scale, the example of the Saint Emilion vineyard

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Abstract: The temporal differences in *Lobesia botrana* lifecycle were studied at the local scale of the Saint Emilion vineyard during the spring generation. Comparisons between sites are based on trap catches during the emergence flight thanks to a 230 classical sticky delta trap network and on head capsule width determination of the first generation larvae. 56 temperature sensors were placed next to sticky traps to explore the link between temperature and start of the emergence flight. Results demonstrated the existence of clear temporal variation in 2013, with a time lag of 34 days between extreme values at the start of the first flight. This trend was also spatially defined; its distribution being modeled by kriging. Head capsule width measures confirm the existence of temporal differences in the phenology of *L. botrana* in this vineyard. Although defined by a temporal pattern, the flight onset did not occur at the same degree-days between sites. This result requires further investigation but highlights the difficulty to extend modeling result obtained on a precise location to a wider scale. It permits to improve winegrower practices by scheduling insecticide spraying with better precision.

Key words: grapevine moth**,** phenology**,** first flight, temperature sum

Introduction

Lobesia botrana (Den. & Schiff.) is the most common grape moth in the Bordeaux vineyards, where its lifecycle includes often three different generations and sometimes four (Delbac *et al.*, 2010). The first larval generation has generally no consequence on yields and grapes quality except for low yield varieties like Sauternes, but the second and third generations can affect grape quality both by destruction of the berries and by enhancing the development of *Botrytis cinerea* (Fermaud & Le Menn, 1992) and of various other rots. Larvae populations regularly reach the economic damage threshold in the Pomerol and Saint Emilion vineyards and need to be lowered using mating disruption or insecticide sprayings. Mating disruption is still poorly represented in this region (6% of the total area) firstly for its cost, secondly the small average surface of the Châteaux plots, and thirdly the technical difficulties. Therefore, winegrowers use to apply insecticide on the second or third generation to lower populations. *Lobesia botrana* is a typical example of an insect in which scheduling an insecticide application requires to target a precise stage of development to obtain high efficacy.

Trap monitoring and modeling are the most common tools used by advisers to perform this task, such monitoring being sometimes completed by eggs or glomerulae countings. Insecticides, either ovicidal and larvicidal have generally a persistence of action ranging from 7 to 20 days and this requires an accurate application time. Monitoring methods are thus critical for efficiency and inaccurate information at local scale often leads to efficiency loss, causing doses increase repeated applications and, often, heavy damage.

Nowadays the knowledge on *L. botrana* lifecycle temporal variability among different spatial scales is still limited. The variability in the date of the flight onset, when comparing vineyards distant 200 km and more, was noticed by different authors (Thiéry, 2005; Gallardo *et al.*, 2009) but short scale comparisons are missing. Genetic differences inducing different climate responses were supposed to be part of the explanation. According to the pioneering works of Roehrich (1969) and Roditakis (2001), photoperiod and temperature are major cues that influence the beginning of the flight, even if larval food quality (cultivars or fungi in the food) probably play an important role (Savopoulou-Soultani *et al.*, 1988; 1990; Thiéry & Moreau, 2005; Thiéry *et al.*, 2014). Recent research also showed that an over investment in the larval immune system as a result of natural enemies or larval diseases will be paid by differential larval growth characteristics from which speed (see Vogelweith *et al*., 2013a ; 2013b). All these factors affect either the induction, the end of diapause and the larval growth speed. The temperature remains an important parameter for pupal development even after the end of diapauses. In natural conditions, the adult flight can also be limited during spring by cold temperature and also rainfalls with consequences on delayed oviposition.

Filling such gaps in knowledge by studying at a very local scale (< 20 km) how variable is the phenology of *L. botrana* is thus an important task. The further objective of such a study is to use this information attempting to apply it at a wider scale.

As a tool to improve this variability, pheromone traps are known to be climatic dependent. The trapping efficiency is affected by winds, rainfalls (Gallardo *et al.*, 2009) or air temperature. Furthermore, the relation between number of catches inside a trap and the adult level of population in the field is not predictable. Important differences of *L. botrana* adult populations between the vineyards could involve time difference in trap response due to trap detection threshold (sensitivity of traps to adult populations). As another tool, the head capsule width allow to evaluate time lag difference between the vineyards by avoiding a possible population effect or climatic effect from the trap network (Delbac *et al*., 2010).

The objectives of our work presented here was (1) to study the variability of *L. botrana'*s phenology in spring in this specific region and then (2) to evaluate if the sum of temperature was a sufficient parameter to explain this variability at a local scale.

Material and methods

Study vineyards

The experiment took place in a 12 200 ha vineyard (1200 growers), localized in the Saint-Emilion and Pomerol regions (50 kilometers away South East from Bordeaux, France). The maximal distance from the east to the west, or from south to north, is above 20 kilometers, and grapevine is the main crop, covering 63% of total ground surface.

Male flight monitoring

Males flight activity was monitored using a network composed of 230 sexual traps (Figure 1). They were positioned as one trap per 50 ha of vineyard; their precise localization was obtained by GPS. Each trap consists to a yellow delta trap baited with 2 µg of the synthetic pheromone of *L. botrana* female, E_7-Z_9 DDA. Catches were checked once a week, from mid-April to the end of June, *i.e.* during the first flying period. Sticky plates and pheromones lures were replaced every two weeks.

For analysis, data were considered only if total catches per trap is at least 10 butterflies. The abundance of each trap was summed weekly and a proportional cumulated catches curve was calculated using the formulation:

$$\{\%\ catches\}_t = \sum_{i=1}^{t} \frac{[catches]_i}{total\ number\ of\ catches} * 100$$

A type 4 logarithmic curve $y = a\left(\frac{(b-a)}{\left(1+e^{\frac{(c-x)}{d}}\right)}\right)$ with a = 0 and b = 1 was fitted by least squares method then replaced the original curve in order to obtain continuous variation among time data. This method allows to infer temporal data with a daily precision. In each logarithmic curve, the start of the flight refers to the date when 10% of the total catches were obtained. An empirical variogram, exploring the relation between traps localization and the date of the beginning of the flight, was built using the equation:

$$\gamma e\ (h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z\ (t_i - t_{i\ +}\ h)]^{\ 2}$$

with ti = date of the start of the flight previously estimated and N(h) = number of pairs distant from h, then fitted with an exponential model.

Anisotropic effects were not taken in account. The model equation permitted to represent the time lag between vineyards using kriging method.

Larval instar monitoring

Six different plots (Figure 1), planted with Merlot cultivar, were selected among the total trap network for their regular high grape moth populations. On each of these plots, 50 larvae were randomly assessed at same date before the first compulsory insecticide application against "Flavescence dorée" leafhopper's vector. Collected larvae were placed in ethanol and their head capsule width (HCW) was thus measured to determine their larval instar, as described by Delbac *et al.* (2010). For studying if there was a difference between sites, an ANOVA test was done and was completed by a post-hoc Tukey test when necessary.

Temperature measures

Temperature was recorded hourly using 56 data-loggers type Tiny-Talk 2® placed on wooden posts at 1.3 meters above the ground, next to a sexual trap.

Computation of degree-days corresponding to emergence of the first fly was calculated using the Roehrich's formula (Roehrich, 1989):

$$[T°sum]t = \sum_{i=1}^{t} \left[\left(\frac{(T\ \min + Tmax)}{2}\right) i\right]$$

with Tmin and T max are respectively minimum and maximum daily temperature in °C, i =1 =1st of February, t is t the date of start of the flight on the nearest trap

The lowest threshold temperature used was 0 °C.

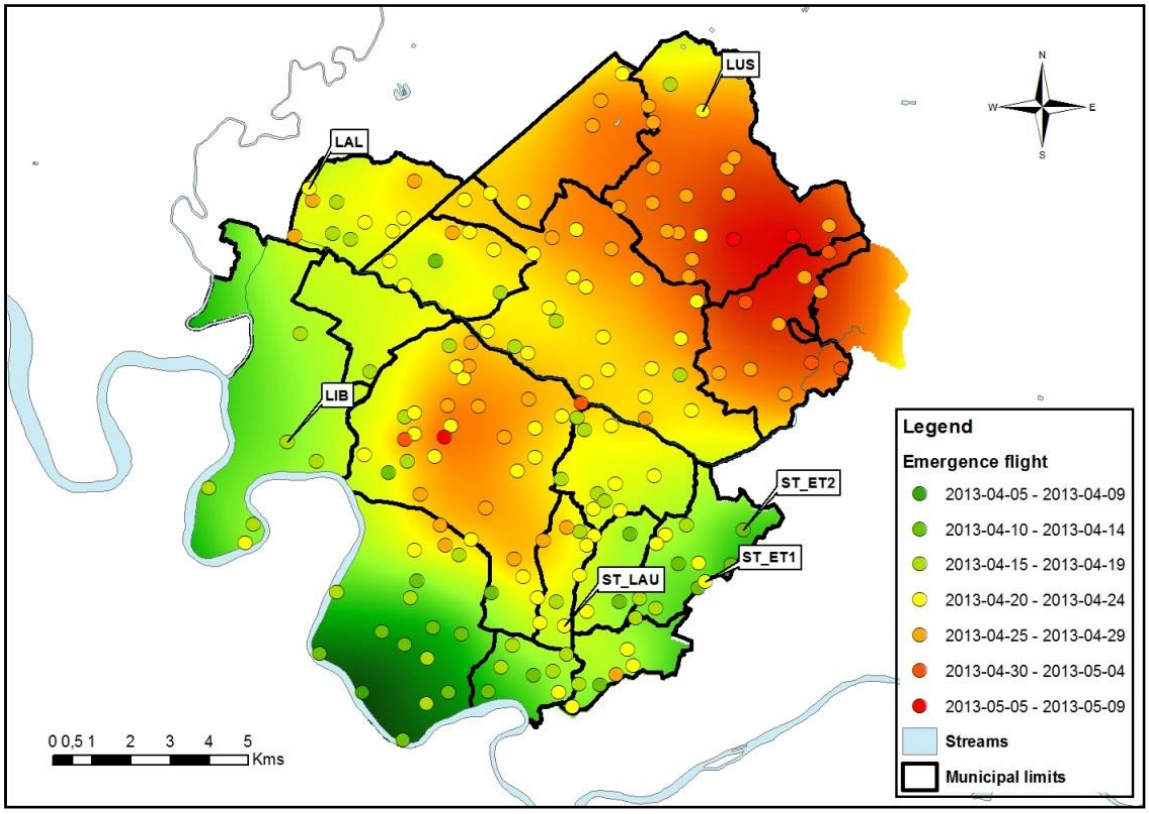

Figure 1. Localization of the trap (disk) network and larval sampling spots (labels). Time difference at the start of the emergence first flight and resulting kriging was materialized as coloring from green (earlier catches) to red (later catches).

Results

Time difference in the first flights between sites

The dates when the emergence flight starts are presented and are associated to the kriging model on Figure 1. The beginning of the emergence can vary from the 5th of April to the 9th of May, which represents a time lag of 34 days between extreme values. Results showed a clear spatial trend: the adult emergence was forward in the vineyards localized south and extreme west and was more and more delayed when moving to the north east direction. These results were confirmed by the empirical variogram function and its fitted model (Figure 2). This variogram proved the existence of a spatial-dependence pattern concerning the dates of emergence flight between all the traps.

Head capsule width measure

The larvae sampling inside the LUS plot was interrupted by the first compulsory insecticide application against “Flavescence dorée” leafhopper’s vector, leading to a smaller number of collected larvae than in other plots. LAL and LIB, in the west part of the vineyards, presented the largest HCW (Table 1). Larvae populations were composed almost only of 4th and 5th instars with a higher proportion of the last one, varying from 51 to 75% of the total sample. STLAU, STET1 and STET2, in the center and the south of the vineyards show quite similar results of HCW measures, with values smaller than LAL and LIB. The larval instar determination show a predominance of the 4th one, ranging from 65 to 82% of the sample.

Finally, the plot LUS, in the eastern part of the vineyard, showed completely different results with the smallest measures. Larval development stages were distributed differently from other plots: the proportion of larvae at 2nd or 3rd stages of development is above 50 % whereas there is no larva at last larval instar.

The significant variation in larval age pyramid distribution from a plot to another proves that the hatching egg dates varied a lot which possibly explain important time lags. Larval instar differences were in good correlation with the time lag map obtained by kriging method (Figure 1).

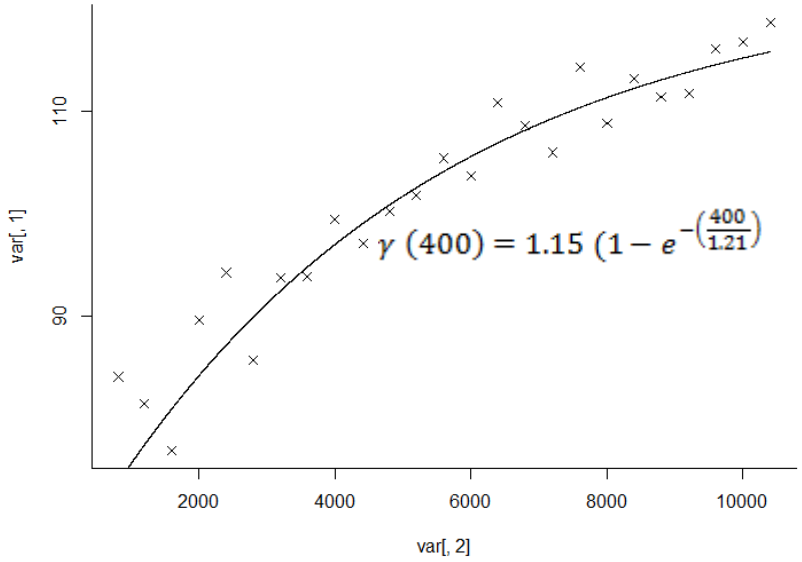

Figure 2. The horizontal axis is the distance between the traps (in km). The vertical axis is a measure of similarity between values. Quite similar dates at the start of the flight between the traps lead to low values on vertical axis. The range (negligible variation on vertical axis) means that points of observation become uncorrelated for the observed parameter. The empirical variogram is represented by crosses and the exponential fitted variogram is represented by line.

Table 1. Head capsule width measurements and larval instar determination based on head capsule width

Date	Plots	NB of larvae collected	larval instar determination results					Average HCW
			L1 (%)	L2 (%)	L3 (%)	L4 (%)	L5 (%)	
2013-06-12 to 2013-06-14	LAL	53	0	0	2	23	75	0,7133a
	LIB	53	0	0	4	45	51	0,6697a
	ST ET 1	55	0	2	4	65	29	0,594b
	STET 2	50	0	0	6	82	12	0,5574b
	ST LAU	54	0	0	15	72	13	0,5306b
	LUS	19	0	11	37	53	0	0,4306c

Relation date of emergence / temperature sum

During the first flight, the daily temperature accumulated on each micro-sensor since the 1st of February to the start of the flight ranged from 588 °C to 961 °C (mean 743 °C ± 64.69) (Figure 3). This intra-annual sum of temperature variation from a site to another cannot be explained by localized rainfalls or flight limitation due to cold evening temperature: during the period from the 12th to the 25th of April, the cumulative rainfall was near from zero and the hourly temperature during the moths' activity was generally higher than 12 °C and sometimes reached 18 °C, even in the coldest part of the vineyards. The Figure 3 highlights that at local scale the sum of temperature at the beginning of the first flight is submitted to intra-annual variations.

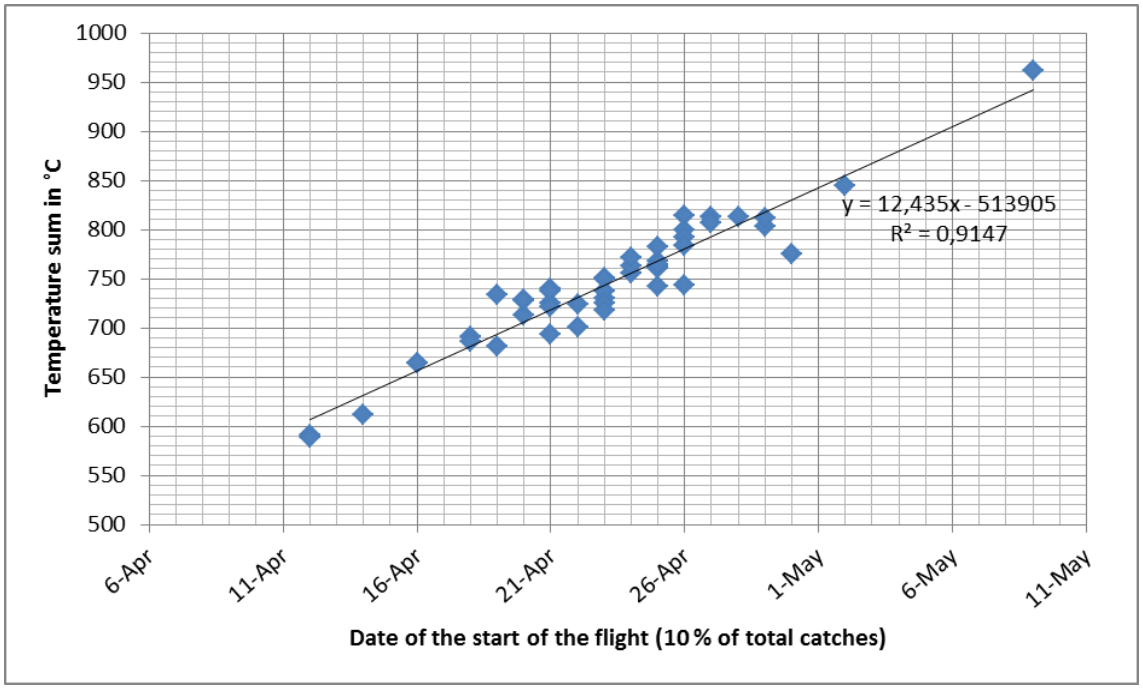

Figure 3. Relation between the start of emergence flight and the degree-days since the 1st of February (0 °C threshold).

Discussion

The temperature accumulation from the end of diapause to the start of the emergence flight was precisely investigated by Roehrich in the specific case of the Bordeaux vineyard. The minimal sum of temperature found during the study was 565 °C and was then used as a reference, but Roehrich mainly underlined the inter-annual variation concerning these data. Intra-annual variations in temperature were already known when comparing distant vineyards (Gallardo *et al*., 2009) and are newly demonstrated between proximity fields in this study.

In our case, the eventual effect of genetic differentiation could possibly be excluded considering the short distance separating the vineyards, and environmental factors listed in introduction are more likely to be the drivers. The termination of diapauses is known to be controlled by a conjugated effect of photoperiod and temperature (Roehrich *et al.*, 1969; Roditakis & Karandinos, 2001). The vineyards were localized in the same latitude which *a*

priori excludes photoperiod effect, but we have no evidence that diapauses induction occurred at the same time for individuals from the different vineyards. This time lag between vineyards was also obtained at the beginning of the following summer flights and was already demonstrated during previous years (Mary *et al.*, 2012). This time difference was enhanced if analyses were done at peak trap catches period. A time lag in diapauses induction would indirectly impact pupae by photoperiod effect. There is also no difference in cultivars since all this area is planted in huge majority with merlot. However, interestingly, Thiéry *et al.* (2014) found in a natural population occurring in another Bordeaux vineyard more than 40 days delay –varying with the sex – in 3rd generation between the first and the last emerging adults. Mechanisms explaining that are not yet fully understood, but food quality variation from stock to stock, larval density per bunch, larval exposure to entomopathogenic fungus or parasitoids could be involved.

This result could also be explained by a difference between vineyards at the end of diapause (effect of temperature). If the end of diapause was not synchronized between all sites, it would involve differences of development that are not taken in account with our calculation. Temperature data were recorded at 1.3 meters above the ground, and were supposed to be independent from ground effect. In natural conditions, the pupae were generally localized under the bark which possibly diminishes temperature variation. Temperature variation is also dependant from the grass cover and from the type of soil, and creates a micro-climate which could impact grape moth development. In that respect temperatures recorded by the sensors could differ from those to which pupae are subjected. Finally, after terminating diapauses, *L. botrana* response to temperature is proved to be greatly affected by intra-day hourly temperature variations that remain uncalculated when working with daily temperature (Picard, 2009).

Application for the winegrowers and their advisers

At local scale, the *L. botrana*'s phenological stages were spatially correlated between fields at short distance (≈ 3 km) and then varyied quickly at higher distances. A ten day lag is currently observed at the beginning of the flight between vineyards and could reduce insecticide efficiency if the date of spraying is not adapted to local context. In these vineyards, as HCW confirmed the phenology trend, a trap result can be used as a temporal high reliability index for collective advice in neighboring wineries. This result allows us to refine the monitoring framework needed to increase technical bulletin quality by defining earlier and later zone.

Forecasting models are generally uniquely based on temperature. They estimate the start of the flight by a sum calculation from a date when diapauses is supposed to be ended. In a second time, models are generally fitted on a precise location using previous year data obtained by traps and observation (Baumgartner *et al.*, 2012). The important temporal variability found in our vineyards underlines the diversity of responses at local scale which is never considered in most modelisation works. The comparison between our field data and modeling result has still has to be done.

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