

# Different emergence phenology of European grapevine moth (*Lobesia botrana*, Lepidoptera: Tortricidae) on six varieties of grapes

D. Thiéry<sup>1,2\*</sup>, K. Monceau<sup>1,2</sup> and J. Moreau<sup>3</sup>

<sup>1</sup>INRA, ISVV, UMR 1065 Santé et Agroécologie du Vignoble, F-33883 Villenave d'Ornon, France; <sup>2</sup>Université de Bordeaux, ISVV, UMR 1065 Santé et Agroécologie du Vignoble, Bordeaux Sciences Agro, CS 20032, F-33882 Villenave d'Ornon, France; <sup>3</sup>Université de Bourgogne, Equipe Ecologie Evolutive, UMR 6282 Biogéosciences, 6 Bd Gabriel, F-21000 Dijon, France

## Abstract

The phenology of insect emergence affects reproductive success and is especially critical in short-lived species. An increasing number of studies have documented the effects of thermal and other climatic variations and of unpredictable habitats on the timing of adult insect emergence within and between populations and years. Numerous interacting factors may affect the phenology of adult emergence. Host-plant quality and availability is a key factor that has been largely neglected in studies of the phenology of phytophagous insects. The purpose of this study was to determine the effect of host plant characteristics on the rate of larval growth and the pattern of emergence in a wild population of *Lobesia botrana* (European grapevine moth), a significant pest in European vineyards. The phenology of emergence differed significantly among the six tested varieties of grapes. The percentage of bunches harboring pupae was similar among the different grape varieties, and the total number of pupae collected was similar to the number of emerging adults per bunch. Among the six varieties of grapes, 0–25 pupae were produced on each bunch. Each of the grape varieties had a single wave of emergence, in which males emerged before females, but their emergence phenology differed significantly in Chardonnay, Chasselas, and Pinot grapes. Both genders had extended durations of emergence in Merlot grapes. Together, the present results show that the characteristics of the grape host plant affect the emergence phenology of *L. botrana*.

**Keywords:** phytophagous insect, host plant, emergence, protandry, grape pest

(Accepted 7 May 2013; First published online 7 June 2013)

## Introduction

The phenology of adult insect development can be an important determinant of individual and population

reproductive success (Singer, 1972; Taylor, 1981; Cushman *et al.*, 1994; Weiss & Weiss, 1998; Weiss *et al.*, 1993). Significant asynchrony in the emergence of males and females (Weiss *et al.*, 1993) or emergence when food resources are unavailable can lead to reproductive failure. For example, the oviposition of the phytophagous winter moth (*Operophtera brumata*) coincides with host plant availability (Visser & Holleman, 2001). Developmental synchrony of males and females may be more critical in short-lived insects or in environments where mate location is difficult (Calabrese & Fagan, 2004). In such

---

\*Author for correspondence

Fax: +33 5 57 12 26 21

Phone: +33 5 57 12 26 18

E-mail: denis.thiery@bordeaux.inra.fr

contexts, males often emerge shortly before females. This 'protandric emergence', which is common in the Lepidoptera (Thomas, 1989; Wiklund *et al.*, 1993; Savopoulou-Soultani *et al.*, 1994), shortens the pre-reproductive period of proovogenic females (females emerging with a fixed egg load), because most males are available for mating when they emerge (Fagerström & Wiklund, 1982), increasing the probability of mating (Carvalho *et al.*, 1998). The dynamics of the emergence of the two sexes within each generation may be a key factor in the reproductive success of the population.

The duration of larval development is also an important component of fitness, especially in the presence of competition and/or parasitism. A shorter developmental time is often favored because it reduces exposure to parasitoids and reduces the risk of death due to stochastic events. These parameters are critical for the survival of larvae in the wild (Hagstrom & Larsson, 1995; Benrey & Denno, 1997; Thiéry & Moreau, 2005). In addition, multivoltine organisms typically have shorter development times. The consequential reduction of adult size can have consequences on fitness, because insect body size is often associated with fecundity (Honek, 1993).

Numerous studies have shown that the duration of larval development and the timing of adult emergence vary significantly among populations and between years within individual populations (see Weiss *et al.*, 1993 for an example). This may be due to numerous interacting factors, such as genetic differences among individuals and populations, environmental factors (weather conditions, photoperiod, and temperature), and local habitat characteristics (Taylor, 1981; Curry & Feldman, 1987; Danks, 1994, 2002; Weiss & Weiss, 1998; Chuche & Thiéry, 2012; but see Forrest & Miller-Rushing, 2010 for a review). The effect of varying thermal regimes on larval development has been investigated (Weiss *et al.*, 1993; Ellwood *et al.*, 2012), but the effect of juvenile resource quality on larval development is often neglected. In phytophagous insects, the larval host plant may have profound effects on the growth rate of juveniles and consequently on the dynamics of emergence (Scriber, 1981; Nylin & Janz, 1993; Thiéry & Moreau, 2005). Indeed, there can be significant variation in host plant quality and in host choice by ovipositing females, and these can have important effects on the offspring. Nevertheless, the effect of the host plant on the pattern of offspring emergence has often been neglected. Tikkanen *et al.* (2000) showed that changes in the host plant can significantly disrupt the timing of male and female emergence and lead to a reduction of population density. In general, lack of information regarding the effect of host plants on insect emergence limits our understanding of the natural variation in the reproductive success of phytophagous insects.

The purpose of the present study was to determine the effect of larval host plants on the emergence phenology of a wild population of *Lobesia botrana* (Lepidoptera: Tortricidae, Denis & Schiffermueller), a significant insect pest in vineyards of Europe, North Africa, and West Asia (Roehrich & Boller, 1991; Thiéry, 2005). In particular, we measured the emergence phenology of males and females that developed on six different grape varieties during a single year in an experimental vineyard in southwestern France. *Lobesia botrana* is an ideal model organisms for such studies because larval fitness and adult reproductive success of this species are strongly related to the nature of the host plants on which the larvae have fed (Savopoulou-Soultani *et al.*, 1990; Torres-Vila *et al.*, 1999; Thiéry & Moreau, 2005; Moreau *et al.*, 2006a,b,c, 2007). In addition, adult emergence is an important determinant of

reproductive success; so it is very important to understand the effect of host characteristics on the pattern of adult insect emergence in order to implement effective pest management practices.

## Materials and methods

### *Study vineyard and grape varieties*

All experiments were performed on a wild population of the European grapevine moth (*L. botrana*) in a 30-year-old experimental vineyard of about 1 ha that was planted with different grape varieties and located in the INRA Bordeaux-Aquitaine of south-western France (GPS: N44°47'29.58" W0°34'46.50"). This study was performed from August to November. The six studied varieties were Chardonnay, Chasselas, Merlot, Pinot, Riesling, and Noah, the first five of which were used in a previous study of variation in reproductive life history traits in laboratory experiments (Moreau *et al.*, 2006b,c). The experimental plot was surrounded by vineyards of classical Bordeaux varieties (Merlot, Cabernet Sauvignon, and Sauvignon). The moth population was monitored by pheromone sticky delta traps (Thiéry, 2008).

### *Model insect and experiments*

*Lobesia botrana*, whose original food source is believed to be the Thymeleacea *Daphne gnidium*, has become a significant pest in European vineyards (Maher & Thiéry, 2006). Its larvae are polyphagous and can develop on almost all grape varieties and more than 25 other hosts (Thiéry & Moreau, 2005). In our latitude, *L. botrana* has three or four generations. First-generation larvae appear in June and damage the inflorescences, second-generation larvae appear in July, and third-generation larvae appear in August. Females oviposit single eggs at a time (Gabel & Thiéry, 1996) and several larvae can develop on one bunch of grapes, with the number limited by bunch size. All of our experiments were performed on third-generation individuals during August, because our initial pheromone trap monitoring in the experimental vineyard indicated small numbers of the first two generations (spring and early summer), but an abundant third generation. Before oviposition started, 25 grape bunches of similar size and phenology (based on visual examination) from each variety (one bunch per stock) were packaged in tube gauze bags (25 cm length, 12 cm diameter, closed at the two ends) on August 1 to prevent undated egg laying. During the peak of captures in the pheromone traps (Thiéry, 2011), the isolation bags were removed for 48 h, allowing for two consecutive nights of oviposition. Gauze bags were then put back until the harvest date (33 days after bag closure) to prevent additional egg laying. In order to prevent early adult losses, each grape bunch was harvested 1 week before the expected first date of emergence. This allowed for nearly complete larval development (five instars until pupation for the faster growers). Each collected bunch was individually packed in a 1-liter parallelepiped box that was aerated and placed in a climatic chamber. This chamber was maintained at 22 ± 1°C, 45 ± 10% relative humidity, and with a photoperiod of 15 h light: 8 h dark + 1 h of dusk. The first emergence occurred on September 10, 38–39 days after egg laying. Thus, the first emerging adults spent about 87% of their total development time in the vineyard. Our procedure ensured the complete and accurate collection of nearly all adult emergences.

After laboratory storage, all grape bunches were checked daily for collection of visible pupae. Pupae were isolated in glass tubes (70 × 9 mm diameter) that were closed with cotton plugs, labeled, and stored in the same climatic chamber (Thiéry & Moreau, 2005). Pupal weight was determined in a randomly chosen sub-sample of at least 50 pupae (range: 53–74) in each grape variety. Individual weight was recorded to within 0.01 mg. The number of pupae in each grape bunch was also recorded. Overall parasitism in this study for the six cultivar populations was less than 1%; so its effect on pupal development was disregarded.

The following variables were measured: (i) percentage of grape bunches that harbored pupae, (ii) pupal population size in each grape variety, (iii) emergence rate of all collected pupae (thereafter corresponding to pupal mortality), (iv) pupal weight, (v) total development time (egg + larvae + pupae), and (vi) adult sex ratio.

### Statistical analysis

The percentage of grape bunches that harbored pupae in the different grape varieties were compared using Fisher's exact test. Pupal population sizes were analyzed using a Negative Binomial generalized linear model (GLM) to compare the number of isolated pupae on different grape varieties and between grape bunches within a variety. The statistical significance of each term was assessed using likelihood ratio-based Chi-square test associated with a pairwise Wilcoxon multiple comparison test. A Negative Binomial GLM accounts for overdispersion bias associated with count data, and so it was preferred over a classical Poisson model (Sileshi, 2006). The percentage of emerging moths, sex ratios in different grape varieties, and sex-ratio bias within varieties were tested using Pearson's Chi-square test. Pupal weight variation among grape varieties, bunches, sexes, and within each variety was tested using ANOVA, and the statistical significance of each parameter was assessed by an *F*-statistic associated with the Tukey HSD test.

The bimodality of the emergence pattern was tested using a bimodality coefficient (*b*) defined as:

$$b = (S^2 + 1) / [K + (3 \times (n + 1)^2) / ((n - 2) \times (n - 3))],$$

where *S* represents the skewness (asymmetry) of the distribution, *K* represents the kurtosis (peakedness) of the distribution, and *n* represents the sample size. A *b*-value greater than 0.55 indicates a bimodal distribution (Vega & Grundy, 1993) and *S* and *K* values of 0 indicate a normal distribution. Deviation from a normal distribution was tested by comparing skewness (*S* < 0: left-tailed, *S* > 0: right-tailed) and kurtosis (*K* < 0: platykurtic, *K* > 0: leptokurtic) to the theoretical value from Student's *t*-test for infinite degrees of freedom with an *a* risk of 5% ( $t_{0.05, \infty} = 1.96$ ) as:

$$ts = S/s_S \quad \text{and} \quad tk = K/s_K,$$

where  $s_S = \sqrt{[6n(n-1)/(n-2)(n+1)(n+3)]}$  and  $s_K = \sqrt{[24n(n-1)^2/(n-3)(n-2)(n+3)(n+5)]}$ .

The emergence patterns of the two genders and in the different grape varieties were compared using a Cox proportional hazards regression model and the statistical significance of each parameter was assessed with likelihood ratio-based Chi-square test. The relationship between mean emergence delay (emergence delay = adult birth date – oviposition date) and mean pupal mass for males and females

was assessed separately using Pearson's product-moment correlation test.

All statistical analysis was performed with R software (v. 2.10.1 R Development Core Team, 2008) implemented with the *e1071* package for skewness and kurtosis computation and the *survival* package for the Cox proportional hazards regression model.

## Results

### Population characteristics of moths from different grape varieties

The percentage of grape bunches that harbored pupae was similar in all six grape varieties (Chardonnay: 95%, Chasselas: 80%, Merlot: 90%, Noah: 90%, Pinot: 100%, and Riesling: 100%; Fisher's exact test:  $P = 0.15$ ), but pairwise comparisons indicated significant differences in the total number of pupae in the different grape varieties (GLM:  $\chi^2_5 = 62.27$ ,  $P < 0.0001$ , fig. 1). Moreover, the distribution was not homogenous in grape bunches within a variety (range: 0–25 pupae per bunch,  $\chi^2_{14} = 442.92$ ,  $P < 0.0001$ ). Wilcoxon pairwise multiple comparison tests indicated significantly more pupae on Merlot and Chardonnay than on Riesling and Chasselas (fig. 1). The percentage of emerging moths was significantly different in the six grape varieties (Chardonnay: 71%, Chasselas: 91%, Merlot: 85%, Noah: 89%, Pinot: 88% and Riesling: 92%; Pearson's Chi-square test:  $\chi^2_5 = 35.84$ ,  $P < 0.0001$ ), and this difference was mainly due to the low percentage on Chardonnay. The adult sex ratio was similar in the six grape varieties (Pearson's Chi-square test:  $\chi^2_5 = 2.39$ ,  $P = 0.79$ ) and there was an approximately equal ratio of males and females on each variety (Pearson's Chi-square test: all *P*-values > 0.05).

### Pupal weight of moths from different grape varieties

The weight of pupae differed significantly in the six grape varieties (ANOVA, variety effect:  $F_{5, 281} = 2.53$ ,  $P = 0.03$ , fig. 2). This difference can be explained by the low weight of pupae on Merlot and the high weight of pupae on Pinot (Tukey HSD test:  $P = 0.01$ ). Pairwise tests indicated that none of the other comparisons of weight were significant ( $P > 0.26$ ). Overall, female pupae were heavier than male pupae (sex effect:  $F_{1, 281} = 534.75$ ,  $P < 0.0001$ ) in all grape varieties (interaction sex × variety:  $F_{5, 281} = 0.77$ ,  $P = 0.57$ ) and there was no significant difference in bunches within each grape variety (nested effect of bunches within varieties:  $F_{94, 281} = 1.15$ ,  $P = 0.19$ ). The pupal population size was not correlated to the pupal weight ( $r$  Pearson =  $-0.32$ ,  $P = 0.53$ ).

### Timing of emergence of moths from different grape varieties

Among the six grape varieties, the emergence of adults lasted up to 45 days (Table 1, fig. 3), and faster growers needed 39 days to complete their development. Nevertheless, for both genders within each grape variety, the bimodality coefficient was less than 0.55 (range indicating bimodal distribution: 0.30–0.52), indicating that each emergence occurred as a single wave. The distribution of female emergence deviated from normality in Chardonnay, Merlot, and Pinot grapes and the distribution of male emergence deviated from normality in Chasselas and Merlot grapes. In all cases, deviations were due to early massive emergence ( $S > 0$ ) in a short time ( $K > 0$ ), except for females on Chardonnay (no excess of kurtosis).

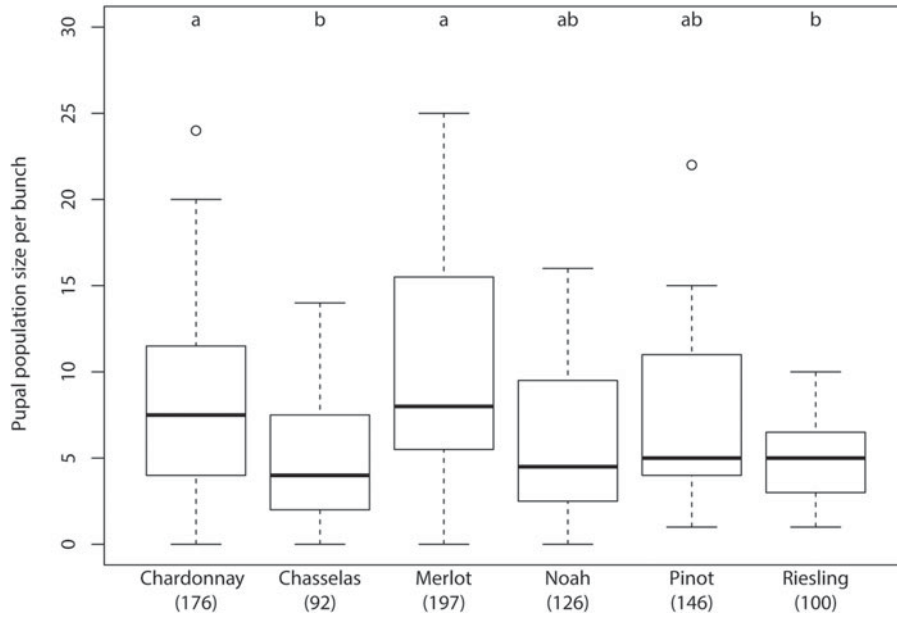


Fig. 1. Number of *L. botrana* pupae per bunch in different grape varieties. Bold line, median; box: middle two quartiles; dashed lines, 1.5 × interquartile range; open circle, extreme value. The numbers inside parentheses indicate number of pupae per 20 bunches. Columns with the same letter are not significantly different ( $P > 0.05$ ) based on a nonparametric pairwise Wilcoxon multiple comparison test.

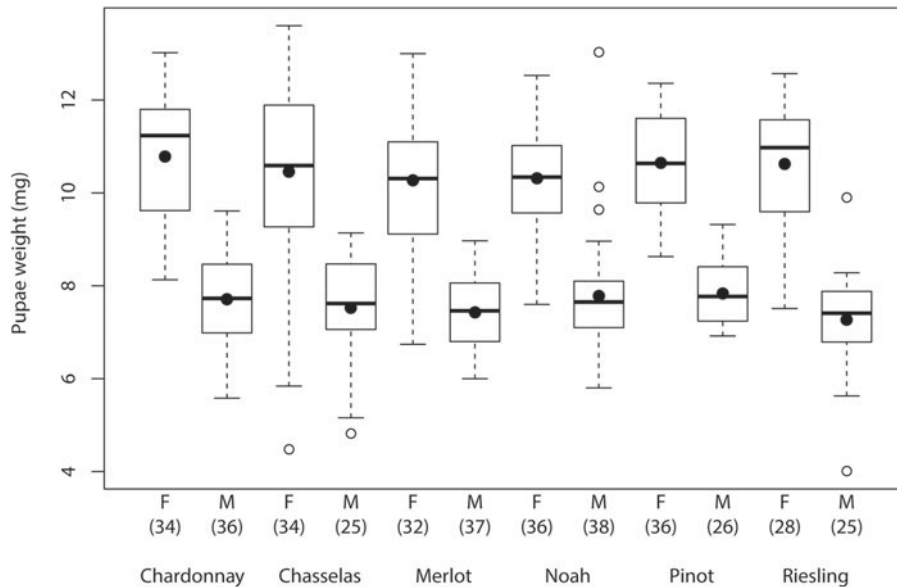


Fig. 2. Weight of male and female *L. botrana* pupae in different grape varieties. Bold line, median; box, middle two quartiles; solid circle, mean; dashed lines, 1.5 × interquartile range; open circle, extreme value. The numbers in parentheses indicate number of pupae that were weighed.

Analysis of emergence delay indicated that gender and grape variety had significant effects on emergence time, but that there was no interaction of these variables (Cox proportional hazards regression model, sex effect:  $\chi^2_1 = 11.15$ ,  $P < 0.001$ ; variety effect:  $\chi^2_5 = 96.34$ ,  $P < 0.0001$ ; interaction sex × variety:  $\chi^2_5 = 7.13$ ,  $P = 0.21$ , *fig. 4*). Males emerged an average of two days before females (emergence delay after the first emergence [mean ± SD males:  $15.94 \pm 6.25$  days, females

$17.77 \pm 6.06$  days, *fig. 4*). Emergence occurred first on Pinot grapes and last on Merlot grapes (*fig. 3*).

#### *Relationship of emergence delay and pupal mass*

Finally, our results indicate that there was no significant relationship between emergence delay and pupal weight in

Table 1. Analysis of the variation in emergence phenology of male and female *L. botrana* on six different host grape plants.

Variety	Gender	<i>N</i>	<i>W</i>	<i>P<sub>w</sub></i>	<i>S</i>	<i>P<sub>s</sub></i>	<i>K</i>	<i>P<sub>k</sub></i>	<i>b</i>
Chardonnay	F	66	0.94	<0.01	0.83	<0.01	0.32	0.29	0.49
	M	59	0.98	0.43	-0.32	0.15	0.08	0.45	0.34
Chasselas	F	47	0.97	0.26	0.39	0.13	0.24	0.36	0.33
	M	37	0.82	<0.0001	1.83	<0.0001	5.71	<0.0001	0.48
Merlot	F	85	0.91	<0.0001	1.19	<0.0001	1.51	<0.01	0.52
	M	82	0.89	<0.0001	1.26	<0.0001	3.08	<0.0001	0.42
Noah	F	51	0.97	0.14	-0.47	0.08	0.51	0.22	0.33
	M	61	0.98	0.39	-0.24	0.22	-0.05	0.46	0.34
Pinot	F	65	0.92	<0.001	1.19	<0.0001	4.64	<0.0001	0.31
	M	64	0.98	0.25	0.18	0.27	-0.07	0.45	0.34
Riesling	F	48	0.98	0.71	0.02	0.47	-0.56	0.20	0.38
	M	44	0.98	0.68	-0.04	0.46	0.10	0.44	0.30

*N*, sample size; *W*, Shapiro–Wilk test; *P<sub>w</sub>*, *P*-value associated with Shapiro–Wilk test; *S*, skewness; *P<sub>s</sub>*, *P*-value associated with *t*-test for skewness bias; *K*, kurtosis; *P<sub>k</sub>*, *P*-value associated with *t*-test for kurtosis bias; *b*, bimodality coefficient (see the text for details).

females (Pearson's product–moment correlation,  $r = -0.78$ ,  $P = 0.07$ , fig. 5a) or in males ( $r = -0.54$ ,  $P = 0.26$ , fig. 5b).

## Discussion

The present study of the emergence phenology of *L. botrana* on six varieties of grape hosts had four major findings: (i) more adult moths develop on certain grape varieties; (ii) the host plant for the larvae affects adult emergence phenology; (iii) male moths emerged before female moths in all grape varieties; and (iv) the grape variety that hosted larvae affected the phenology of emergence, and had different effects on males and females. It is well-established that abiotic and biotic factors affect the timing of many phenological characteristics (Forrest & Miller-Rushing, 2010). Our results clearly show that the characteristics of the larval host plant influence the timing of emergence of *L. botrana* adults.

Two of our most important results are: (i) the number of pupae varied significantly between grape bunches and (ii) some grape varieties harbored more pupae than others. This second point is consistent with the findings of a previous study (Sharon *et al.*, 2009). The difference in pupal population size among grape varieties could be due to differences in egg laying and/or differences in larval mortality (Maher *et al.*, 2001; Moreau *et al.*, 2007, 2008). The differential acceptability of a grape variety is controlled by stimulants and deterrents perceived by ovipositing females (Thiéry *et al.*, 1992; Maher *et al.*, 2001, 2006; Maher & Thiéry, 2004). Our results indicate that females: probably oviposited more on Merlot and Chardonnay, suggesting a preference for these varieties. Previous research documented different larval mortalities earlier in the season (before or at bunch closure) among different grape varieties (Gabel & Roehrich, 1995). These authors attributed this to host plant resistance, but a part of their observed differential mortality may have been due to larvae dropping from the bunches. However, recent laboratory studies, in which ripened dried berries were offered to *L. botrana*, did not confirm the presence of different mortality in different grape varieties (Moreau *et al.*, 2006a,b). Thus, the variation in the number of adults in the different grape varieties that we observed most likely results from differences in oviposition preference.

Female pupae were heavier than male pupae in each of the six grape varieties. This occurs in many Lepidoptera because the females store nutrients for egg production (Raven, 1961; Slansky & Scriber, 1985). Females may become larger by feeding for a longer time, resulting in slower development (Thiéry & Moreau, 2005; Moreau *et al.*, 2006a,b,c). Consequently, males tend to emerge before females. This protandry is common in fruit tortricids and several other Lepidoptera (Rodríguez-del-Bosque *et al.*, 1989; Roehrich & Boller, 1991; Wiklund *et al.*, 1993). Protandry appears to provide at least two advantages: (i) maximization of copulation opportunities for males (reviewed in Wiklund & Fagerström, 1977; Bulmer, 1983), and (ii) minimization of the pre-reproductive period of females, so that males are available upon females' emergence (Fagerström & Wiklund, 1982). Consistent with our results, Torres-Vila *et al.* (1995) showed that 3-day-old *L. botrana* males were the most efficient at mating.

We also found that grape variety affected the duration of larval development and pupal weight. Size and rate of development are typically inversely related (Danks, 1994), but our results seem to contradict this pattern. Although the correlation that we observed was only marginally significant (possibly due to the small number of tested varieties), our results indicated that larvae developed faster and were heavier on Pinot grapes, and developed slower and were lighter on Merlot grapes. It should be noted that the presence of high quality or quantity of food can disable the trade-off of size and rate of development (Reznick *et al.*, 2000; Wissinger *et al.*, 2004). Prolongation of development is potentially an important component of fitness, because it can affect larval exposure to predators and parasites (Benrey & Denno, 1997).

One of our most interesting results is that the emergence phenology of *L. botrana* was different on different grape varieties. Many abiotic factors, such as temperature and humidity, influence adult emergence (Curry & Feldman, 1987; Weiss *et al.*, 1993; Chuche & Thiéry, 2012). Nevertheless, the different grape varieties in our study were close together, grew on similar soil, and were exposed to similar climate; hence we believe that environmental differences had only a minor role in causing differences in emergence. Genotypic variation among different populations that are locally adapted to their hosts may also cause variability in adult emergence

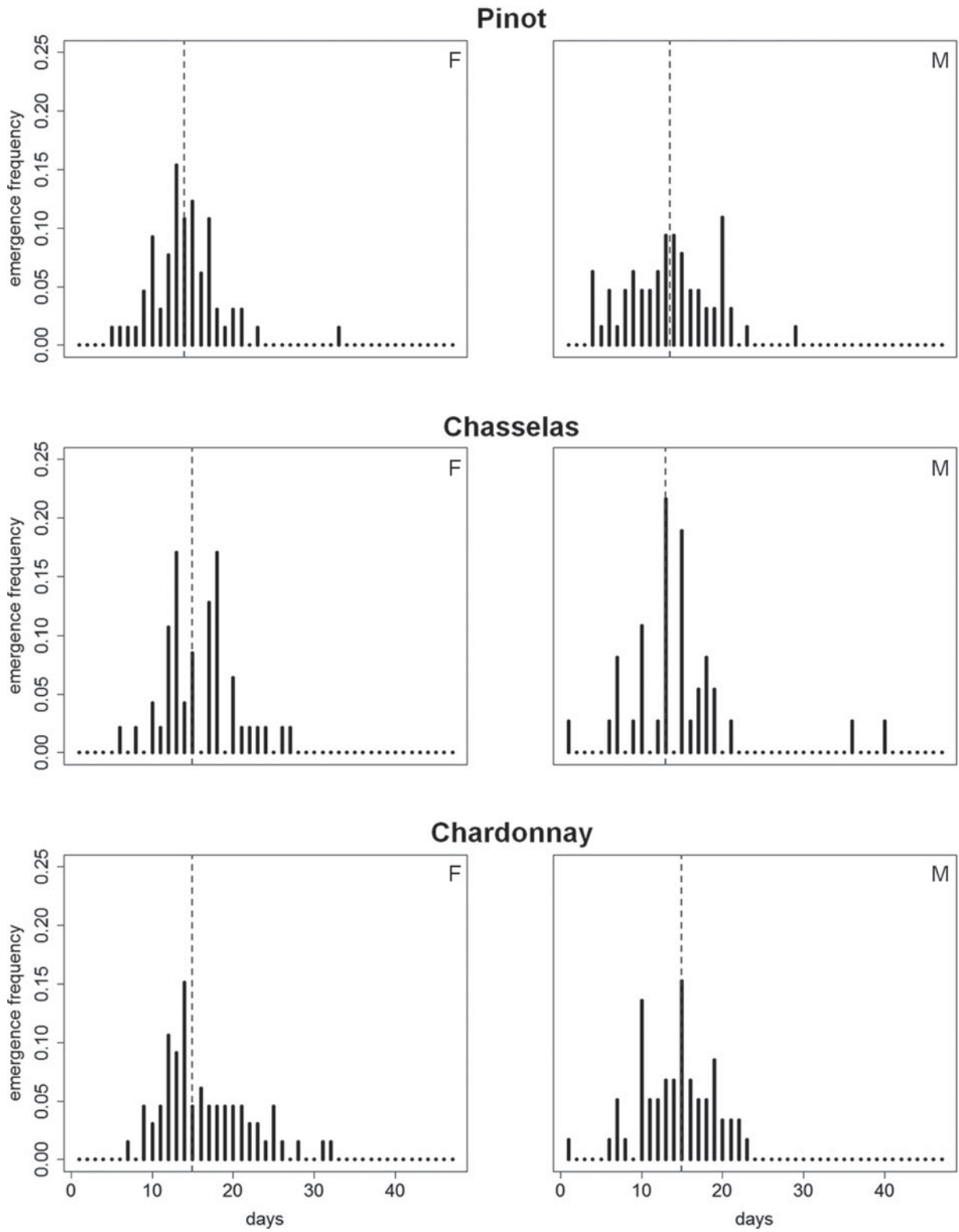


Fig. 3. See the following page for legend.

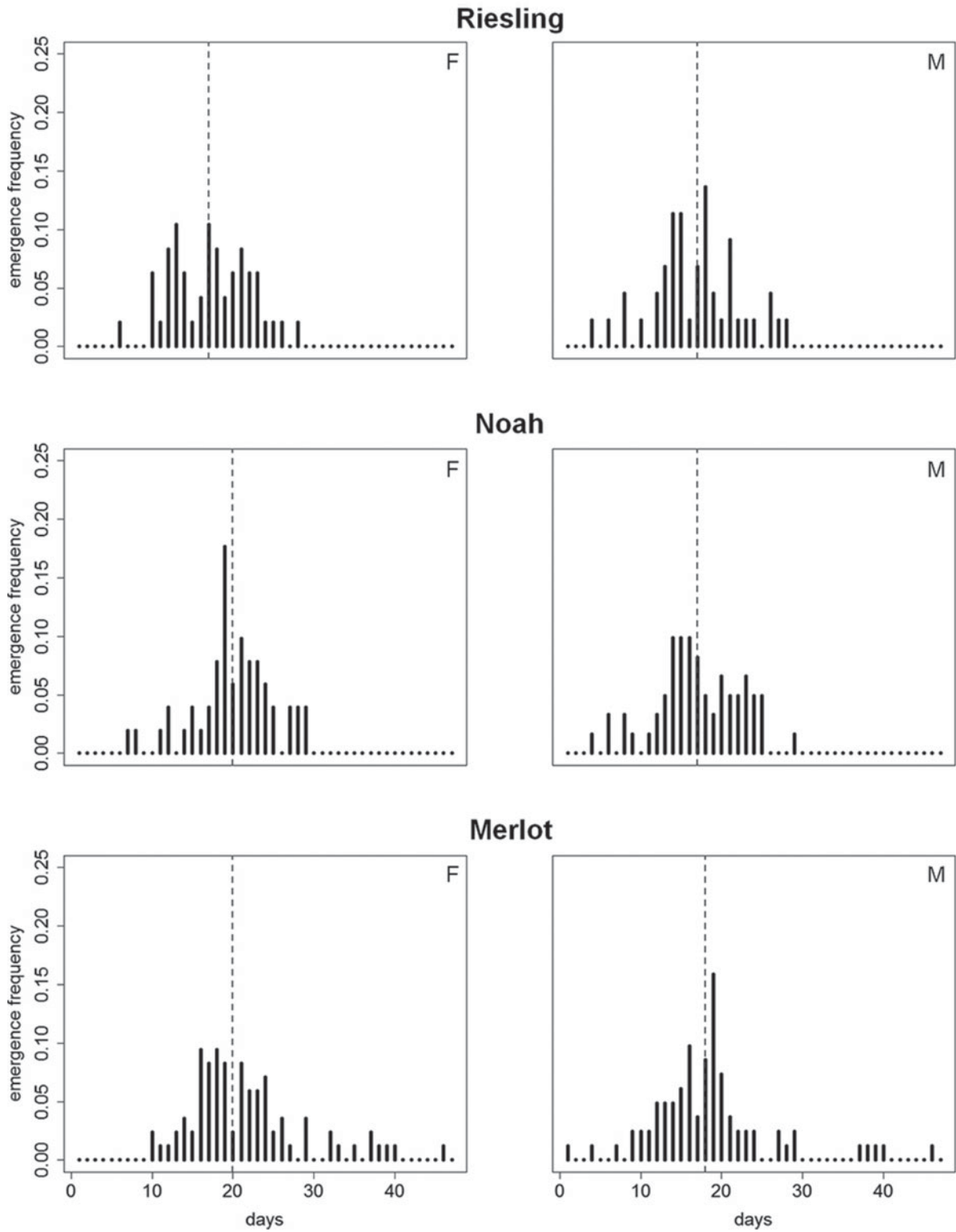


Fig. 3. Emergence of *L. botrana* females (F) and males (M) in different grape varieties. Grape varieties are listed in the order of pupal emergence, from earliest to latest. Vertical dashed line indicates mean emergence time.

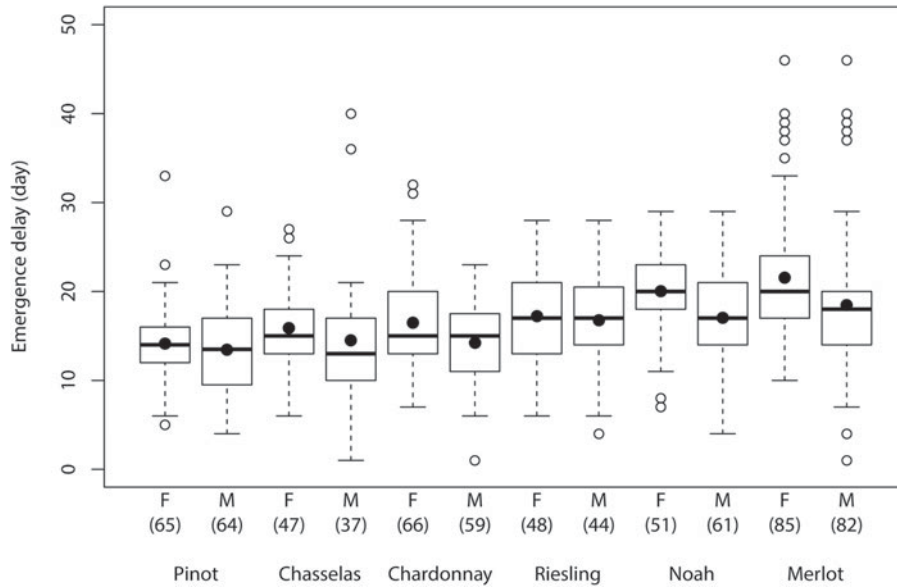


Fig. 4. Variations in emergence delay according to the varieties and sexes, from the earliest (Pinot) to the latest (Merlot). Bold line, median; box, middle two quartiles; solid circle, mean; dashed lines,  $1.5 \times$  interquartile range; open circle, extreme value. The numbers in parentheses indicate the total number of emerging adults.

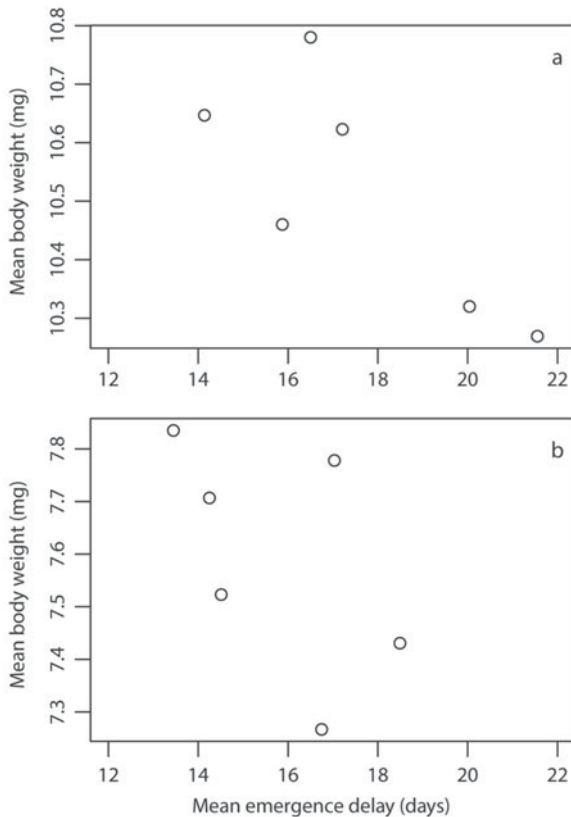


Fig. 5. Relationship between mean emergence delay and pupal mass of (a) female moths and (b) male moths on each of the six grape varieties.

(Wood *et al.*, 1990; Feder *et al.*, 1993; Feder, 1995; Groman & Pellmyr, 2000; Thomas *et al.*, 2003). However, the moths in our small experimental vineyard were from a single population, so genotypic variation is also an unlikely cause of the variation in emergence that we observed. Thus, grape variety seemed to play the major role in determining the phenology of adult emergence of *L. botrana* in our study. Interestingly, for each grape variety, emergence occurred as a single wave. Furthermore, in Chardonnay, Chasselas and Pinot, the emergence phenology was different for males and females. This suggests that males and females have different food requirements and/or assimilation abilities. Male and female moths both had an extended period of emergence on Merlot. Merlot was also associated with prolonged larval development and lighter pupae. A complete understanding of factors that affect emergence phenology is not yet available, but nutrients or secondary compounds present in grapes, which can vary significantly in different grapes (Zhu *et al.*, 2012), undoubtedly play a role. The rootstock and soil characteristics could also affect emergence of moths. Merlot contains high levels of flavan-3-ols (flavanols), including dihydroquercetin and stilbenes (Zhu *et al.*, 2012), and such molecules could potentially affect emergence phenology. We suggest further study of this issue.

The variation in adult emergence phenology that we observed agrees with previous studies which reported that quantitative plant defenses should limit the annual number of insect generations by extending the larval development time (see Cizek *et al.*, 2006 for an example). It is possible that the size and shape of the grape bunches in the different varieties, as well as other characteristics of the larval micro-habitat, may influence development time. For instance, different larval micro-distributions of the Bay Checkerspot butterfly (*Euphydryas editha bayensis*) vary in development time by up to



11 days (Weiss *et al.*, 1993; Weiss & Weiss, 1998). Humidity and solar radiation are the most important micro-climatic parameters for larval development, because development time depends on temperature due to the small size of larvae (Willmer, 1982; Weiss *et al.*, 1993). In the case of grapes, the compactness of clusters differs among varieties, leading to differences in sunlight exposure and temperature (Fermaud, 1998; Pieri & Fermaud, 2005), but we cannot exclude the possibility that there is a genetic basis for increased variation of emergence, a 'bet-hedging strategy' of *L. botrana* (Forrest & Miller-Rushing, 2010 and references therein). Further experiments that employ fine temperature measurements inside grape bunches are needed to establish the contribution of such factors.

The mechanism(s) underlying the variation of emergence of *L. botrana* is unknown. Nonetheless, the greater variation in emergence of larvae that feed on Merlot could be advantageous for individuals of temperate regions, in which there is greater variation of environmental factors such as temperature and precipitation. In the last *L. botrana* generation (at the end of summer), the emergence of all adults within a narrow window of time could be risky, because inclement weather at this time could affect flight and oviposition (Goulson, 1993). Thus, it might be expected that increased variation in emergence would improve overall survival of the final generation. In other words, an extended period of adult emergence could be a 'bet-hedging strategy' (Danforth, 1999; Hopper, 1999), even if such a strategy depends on population genetic diversity. Increased variation of adult emergence might allow some individuals to escape detrimental conditions and contribute to long-term persistence of populations in a vineyard.

Large-scale field experiments are clearly needed to evaluate the fitness of early- and late-emerging adults under controlled conditions. In addition, a field survey analyzing the reproductive success of subsequent generations of moths that have the most extreme phenologies (those that emerged on Merlot and Chasselas) should provide important information regarding the contribution of the reproductive capacity of individuals to the foundation of the following year's population. Optimally, such a survey should also consider the dispersive capacities of adults that have rapid and slow larval development, and the effect of parasitoids on the rate of development (Xuéreb & Thiéry, 2006). This last point should receive attention because it is important to have population-level data on this significant vineyard pest. The data provided here on adult emergence phenology of *L. botrana* could also be useful for the development of age-structured mathematical models of vineyard infestation (Ainseba *et al.*, 2011). Such knowledge should improve the assessment of pest outbreaks in subsequent generations.

## References

- Ainseba, B., Picart, D. & Thiéry, D. (2011) An innovative multi-stage, physiologically structured, population model to understand the European grapevine moth dynamics. *Journal of Mathematical Analysis and Applications* **382**, 34–46.
- Benrey, B. & Denno, R.F. (1997) The slow growth-high mortality hypothesis: a test using the cabbage butterfly. *Ecology* **78**, 987–999.
- Bulmer, M.G. (1983) Models for the evolution of protandry in insects. *Journal of Theoretical Biology* **35**, 195–206.
- Calabrese, J.M. & Fagan, W.F. (2004) Lost in time, lonely, and single: reproductive asynchrony and the Allee effect. *American Naturalist* **164**, 25–37.
- Carvalho, M.C., Queiroz, P.C.D. & Ruzsczyk, A. (1998) Protandry and female size-fecundity variation in the tropical butterfly *Brassolis sophorae*. *Oecologia* **116**, 98–102.
- Chuche, J. & Thiéry, D. (2012) Egg incubation temperature differently affects female and male hatching dynamics and larval fitness in a leafhopper. *Ecology and Evolution* **2**, 732–739.
- Cizek, L., Fric, Z. & Konvicka, M. (2006) Host plant defences and voltinism in European butterflies. *Ecological Entomology* **31**, 337–344.
- Curry, G.L. & Feldman, R.M. (1987) *Mathematical Foundations of Population Dynamics*. College Station Texas, Texas A and M University Press.
- Cushman, J.H., Boggs, C., Weiss, S.B., Harvey, A., Murphy, D.D. & Ehrlich, P.R. (1994) Estimating female reproductive success in a threatened butterfly: influence of emergence time and host plant phenology. *Oecologia* **99**, 194–200.
- Danforth, B.N. (1999) Emergence dynamics and bet hedging in a desert bee, *Perdita portalis*. *Proceedings of the Royal Society of London B* **266**, 1985–1994.
- Danks, H.V. (ed.) (1994) *Insect Life-cycle Polymorphism: Theory, Evolution and Ecological Consequences for Seasonality and Diapause Control*. Dordrecht, Kluwer Academic.
- Danks, H.V. (2002) The range of insect dormancy responses. *European Journal of Entomology* **99**, 127–142.
- Ellwood, E.R., Diez, J.M., Ibanez, I., Primack, R., Kobori, H., Higushi, H. & Silnader, J.A. (2012) Disentangling the paradox of insect phenology: are temporal trends reflecting the response to warming? *Oecologia* **168**, 1161–1171.
- Fagerström, T. & Wiklund, C. (1982) Why do males emerge before females? Protandry as a mating strategy in male and female butterflies. *Oecologia* **52**, 164–166.
- Feder, J.L. (1995) The effects of parasitoids on sympatric host races of *Rhagoletis pomonella* (Diptera: Tephritidae). *Ecology* **76**, 801–813.
- Feder, J.L., Hunt, T.A. & Bush, L. (1993) The effects of climate, host plant phenology and host fidelity on the genetics of apple and hawthorn infesting races of *Rhagoletis pomonella*. *Entomologia Experimentalis et Applicata* **69**, 117–135.
- Fermaud, M. (1998) Cultivar susceptibility of grape berry clusters to larvae of *Lobesia botrana* (Lepidoptera: Tortricidae). *Journal of Economic Entomology* **91**, 974–980.
- Forrest, J. & Miller-Rushing, A.J. (2010) Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society of London B* **365**, 3101–3122.
- Gabel, B. & Roehrich, R. (1995) Sensitivity of grapevine phenological stages to larvae of European grapevine moth *Lobesia botrana* Den. & Schiff. *Journal of Applied Entomology* **119**, 127–130.
- Gabel, B. & Thiéry, D. (1996) Oviposition response of *Lobesia botrana* females to long chain free fatty acids and esters from its eggs. *Journal of Chemical Ecology* **22**, 161–171.
- Goulson, D. (1993) The evolutionary significance of bimodal emergence in the butterfly, *Maniola jurtina* (Lepidoptera: Satyriinae) (L.). *Biological Journal of the Linnean Society* **49**, 127–139.
- Groman, J.D. & Pellmyr, O. (2000) Rapid evolution and specialization following host colonization in a yucca moth. *Journal of Evolutionary Biology* **13**, 223–236.

- Haggstrom, H. & Larsson, S. (1995) Slow larval growth on a suboptimal willow results in high predation mortality in the leaf beetle *Galerucella lineola*. *Oecologia* **104**, 308–315.
- Honek, A. (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* **66**, 483–492.
- Hopper, K.R. (1999) Risk-spreading and bet-hedging in insect population biology. *Annual Review of Entomology* **44**, 535–560.
- Maher, N. & Thiéry, D. (2004) A bioassay to evaluate the activity of chemical stimuli from grape berries on the oviposition of *Lobesia botrana* (Lepidoptera: Tortricidae). *Bulletin of Entomological Research* **94**, 27–33.
- Maher, N. & Thiéry, D. (2006) *Daphne gnidium*, the possible native host plant of the European grapevine moth *Lobesia botrana*, stimulates its oviposition. Is a host shift relevant? *Chemoecology* **16**, 135–144.
- Maher, N., Jolivet, M. & Thiéry, D. (2001) Préférence de ponte de l'eudémis, *Lobesia botrana* (Lépidoptère, Tortricidae) entre différents cépages de vigne: influence de l'information chimique à la surface des baies. *IOBC/WPRS Bulletin* **24**, 103–108.
- Maher, N., Thiéry, D. & Städler, E. (2006) Oviposition by *Lobesia botrana* is stimulated by sugars detected by contact chemoreceptors. *Physiological Entomology* **31**, 14–22.
- Moreau, J., Arruego, X., Benrey, B. & Thiéry, D. (2006a) Parts of *Vitis vinifera* berries cv. Cabernet Sauvignon modifies larval and female fitness in the European grapevine moth. *Entomologia Experimentalis et Applicata* **119**, 93–99.
- Moreau, J., Benrey, B. & Thiéry, D. (2006b) Assessing larval food quality for phytophagous insects: are facts as simple as it appears? *Functional Ecology* **20**, 592–600.
- Moreau, J., Benrey, B. & Thiéry, D. (2006c) Grape variety affects larval performance and also female reproductive performance of the European grapevine moth (*Lobesia botrana*). *Bulletin of Entomological Research* **96**, 205–212.
- Moreau, J., Thiéry, D., Troussard, J.P. & Benrey, B. (2007) Grape variety affects female but also male reproductive success in wild European grapevine moths. *Ecological Entomology* **32**, 747–753.
- Moreau, J., Rahme, J., Benrey, B. & Thiéry, D. (2008) Larval host plant origin modifies the adult oviposition preference of the female European grapevine moth *Lobesia botrana*. *Naturwissenschaften* **95**, 317–324.
- Nylin, S. & Janz, N. (1993) Oviposition preference and larval performance in *Polygonia c-album* (Lepidoptera: Nymphalidae): the choice between bad and worse. *Ecological Entomology* **18**, 394–398.
- Pieri, P. & Fermaud, M. (2005) Effects of defoliation on temperature and wetness of grapevine berries. *7th International Symposium on Grapevine Physiology and Biotechnology*, University of California, Davis, CA; *Acta Horticultura* **689**, 109–116.
- Raven, C.P. (1961) *Oogenesis: The Storage of Developmental Information*. Oxford, Pergamon Press.
- R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, available online at <http://www.R-project.org> (accessed 19 May 2012).
- Reznick, D., Nunney, L. & Tessier, A. (2000) Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology and Evolution* **15**, 421–425.
- Rodriguez-del-Bosque, L.A., Smith, J.W. & Browning, H.W. (1989) Development and life-fertility tables for *Diatraea lineolata* (Lepidoptera: Pyralidae) at constant temperatures. *Annals of the Entomological Society of America* **82**, 459–469.
- Roehrich, R. & Boller, E. (1991) Tortricids in vineyards. pp. 507–514 in Van der Gesst, L.P.S. & Evenhuis, H.H. (Eds) *Tortricid Pests, Their Biology Natural Enemies and Control*. Amsterdam, Elsevier.
- Savopoulou-Soultani, M., Stavridis, D.G. & Tzanakakis, M.E. (1990) Development and reproduction of *Lobesia botrana* on vine and olive inflorescences. *Entomologia Hellenica* **8**, 29–35.
- Savopoulou-Soultani, M., Stavridis, D.G., Vassiliou, A., Stafilidis, J.E. & Iraklidis, I. (1994) Response of *Lobesia botrana* (Lepidoptera: Tortricidae) to levels of sugar and protein in artificial diets. *Journal of Economic Entomology* **87**, 84–90.
- Scriber, J.M. (1981) Sequential diets, metabolic costs and growth of *Spodoptera eridania* feeding on dill, lima bean, and cabbage. *Oecologia* **51**, 375–380.
- Sharon, R., Zahavi, T., Soroker, V. & Harari, A.R. (2009) The effect of grape vine cultivars on *Lobesia botrana* (Lepidoptera: Tortricidae) population levels. *Journal of Pest Science* **82**, 187–193.
- Sileshi, G. (2006) Selecting the right statistical model for analysis of insect count data by using information theoretic measures. *Bulletin of Entomological Research* **96**, 479–488.
- Singer, M.C. (1972) Complex components of habitat suitability within a butterfly colony. *Science* **176**, 75–77.
- Slansky, F. & Scriber, J.M. (1985) Food consumption and utilization. pp. 87–163 in Kerkut, G.A. & Gilbert, L.I. (Eds) *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Oxford, Pergamon Press.
- Taylor, F. (1981) Ecology and evolution of physiological time in insects. *American Naturalist* **117**, 1–23.
- Thiéry, D. (2005) *Vers de la grappe, les connaître pour s'en protéger*. Bordeaux, France, Vigne et Vins Publishers.
- Thiéry, D. (2008). Les tordeuses nuisibles au vignoble. In *Les ravageurs de la Vigne*. France, Férét Publisher.
- Thiéry, D. (2011) Gaps in knowledge for modern integrated protection in viticulture: lessons from controlling grape berry moths. *IOBC/WPRS Bulletin* **67**, 305–311.
- Thiéry, D. & Moreau, J. (2005) Relative performance of European grapevine moth (*Lobesia botrana*) on grapes and other hosts. *Oecologia* **143**, 548–557.
- Thiéry, D., Gabel, B., Farkas, P. & Pronier, V. (1992) Identification of an oviposition regulating pheromone in the European Grapevine Moth, *Lobesia botrana* (Lepidoptera: tortricidae). *Cellular and Molecular Life Sciences (Experientia)* **48**, 697–699.
- Thomas, A.W. (1989) Food consumption and utilization by 6th-instar larvae of spruce budworm, *Choristoneura fumiferana*: a comparison on three Picea (spruce) species. *Entomologia Experimentalis et Applicata* **52**, 205–214.
- Thomas, Y., Bethenod, M.T., Pelozuelo, L., Frérot, B. & Bourguet, D. (2003) Genetic isolation between two sympatric host-plant races of the European corn borer, *Ostrinia nubilalis* Hubner. I. Sex pheromone, moth emergence timing and parasitism. *Evolution* **57**, 261–273.
- Tikkanen, O.P., Niemela, P. & Keranen, J. (2000) Growth and development of a generalist insect herbivore, *Operophtera brumata*, on original and alternative host plants. *Oecologia* **122**, 539–536.
- Torres-Vila, L.M., Stockel, J. & Roehrich, R. (1995) Le potentiel reproducteur et ses variables biotiques associées chez le mâle de l'Eudémis de la vigne *Lobesia botrana*. *Entomologia Experimentalis et Applicata* **77**, 105–119.
- Torres-Vila, L.M., Rodriguez-Molina, M.C., Roehrich, R. & Stockel, J. (1999) Vine phenological stage during larval feeding affects male and female reproductive output of

- Lobesia botrana* (Lepidoptera: Tortricidae). *Bulletin of Entomological Research* **89**, 549–556.
- Vega, G.L. & Grundy, S.M.** (1993) Two patterns of LDL metabolism in normotriglyceridemic patients with hypoalphalipoproteinemia. *Arteriosclerosis and Thrombosis* **13**, 579–589.
- Visser, M.E. & Holleman, L.J.M.** (2001) Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proceeding of the Royal Society of London B* **268**, 289–294.
- Weiss, S.B. & Weiss, A.D.** (1998) Landscape-level phenology of a threatened butterfly: a GIS-based modelling approach. *Ecosystems* **1**, 299–309.
- Weiss, S.B., Murphy, D.D., Ehrlich, P.R. & Metzler, C.F.** (1993) Adult emergence phenology in checkerspot butterflies: the effect of macroclimate, topoclimate, and population history. *Oecologia* **96**, 261–270.
- Wiklund, C. & Fagerström, T.** (1977) Why do males emerge before females? *Oecologia* **31**, 153–158.
- Wiklund, C., Kaitala, A., Lindfors, V. & Abenius, J.** (1993) Polyandry and its effect on female reproduction in the green-veined white butterfly (*Pieris napi* L.). *Behavioral Ecology and Sociobiology* **33**, 25–33.
- Willmer, P.G.** (1982) Microclimate and the environmental physiology of insects. *Advances in Insect Physiology* **16**, 1–57.
- Wissinger, S., Steinmetz, J., Alexander, S. & Brown, W.** (2004) Larval cannibalism, time constraints, and adult fitness in caddisflies that inhabit temporary wetlands. *Oecologia* **138**, 39–47.
- Wood, T.K., Olmstead, K.L. & Guttman, S.I.** (1990) Insect phenology mediated by host-plant water relations. *Evolution* **44**, 629–636.
- Xuéreb, A. & Thiéry, D.** (2006) Does natural larval parasitism of *Lobesia botrana* (Lepidoptera: Tortricidae) vary between years, generation, density of the host and vine cultivar? *Bulletin of Entomological Research* **96**, 105–110.
- Zhu, L., Zhang, Y., Deng, J., Li, H. & Lu, J.** (2012) Phenolic concentrations and antioxidant properties of wines made from north American grapes grown in China. *Molecules* **17**, 3304–3323.