

Male flight phenology of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae) in different wine-growing regions in Spain

V. Ortega-López^{1*}, M. Amo-Salas², A. Ortiz-Barredo³
and A.M. Díez-Navajas³

¹Civil Engineering Department, University of Burgos, Calle Villadiego s/n, E-09001 Burgos, Spain: ²Mathematics Department, University of Castilla-La Mancha, Camino de Moledores s/n, E-13071 Ciudad Real, Spain:

³Plant Production and Protection Department, NEIKER-Tecnalia, E-01080 Vitoria-Gasteiz, Spain

Abstract

Lobesia botrana is the most significant pest of grape berries in Spain. Further knowledge of its phenology would enable wine growers to decide on an optimal treatment schedule. The aim of this study is, therefore, to predict the flight peaks of *L. botrana* in seven wine-growing regions of Spain. The main goal is to provide a prediction model based on meteorological data records. A logistic function model, based on temperature and humidity records, together with an exhaustive statistical analysis, were used to compare the wine-growing regions in which the male flight phenology of *L. botrana* displays similar patterns and to sort them into groups. By doing so, a joint study of the dynamics of the moth is possible in the regions within each group. A comparison of the prediction errors before and after applying the Touzeau model confirmed that the fit of the latter model is not sufficiently accurate for the regions under study. Moth flight predictions with the logistic function model are good, but accuracy may still be improved by evaluating other non-biotic and biotic factors.

Key words: climatic areas, pheromone traps, adult population dynamics, logistic function, phenological models, flight peak

(Accepted 1 April 2014; First published online 9 May 2014)

Introduction

Lobesia botrana (Denis & Schiffermüller) (Lepidoptera: Tortricidae) is the most important grape moth in European vineyards, attacking the berry and berry-like fruits of more than 20 host families in the Palearctic region

(Savopoulou-Soultani *et al.*, 1990; Thiery & Moreau, 2005), and more recently identified in vineyards in Chile, Argentina and California (Ioratti *et al.*, 2011; Gutierrez *et al.*, 2012).

This insect completes between two and four generations annually in European vineyards (Thiery & Moreau, 2005), attacking grapes by oviposition in berry clusters. Larvae of the first generation damage the flowers and the second and third generations damage the green, ripening and ripe berries. First generation larval infestations are noticeable from a silken web produced by the larvae linking together flowers or fruit clusters, and the second and third larval generations bite the berries for feeding purposes, providing access to fungal

*Author for correspondence
Phone: +34 947259078
Fax: +34 947259478
E-mail: vortega@ubu.es

pathogens, such as *Botrytis cinerea* and *Aspergillus* spp. (Fermaud & Le Menn, 1989; Cozzi *et al.*, 2006; Delbac *et al.*, 2010).

Pheromone-baited traps are employed to detect adult males of this pest and to monitor them, while mating disruption techniques have been extensively used for population control (Witzgall *et al.*, 2005; Vacas *et al.*, 2011); capture of the males figuring among the best means of monitoring adult phenology throughout the season (Anshelevich *et al.*, 1994; Witzgall *et al.*, 2000; Anfora *et al.*, 2005). Control of eggs and young larvae by chemical treatments or insecticides containing *Bacillus thuringiensis* toxins (Ruiz de Escudero *et al.*, 2007), or regulation by natural enemies (Xuéréb & Thiéry, 2006) have also been described. Oviposition dynamics are quite difficult to forecast with sexual pheromone traps alone and should be complemented with egg counting on grape bunches. Pest damage in the crop may be estimated, if peak flight times and egg-laying density are known. The timing and the density of larvae populations are the main concerns for grape moth management in the vineyard, due not only to the direct damage that they cause, but also because this stage is sensitive to plant protection chemicals. Therefore, the existence of models, which determine the moment of maximum flight of *L. botrana*, combined with knowledge on the timing of oviposition, egg hatching, and larval development, will greatly facilitate the optimization of spray frequency and dosage, which is the aim of any advisory system.

Larval population levels appear to be influenced by different grapevine aspects. Berry cluster compactness influences their exposure to sunlight and internal temperatures (Fermaud, 1998). Grape variety plays an important role in larval and female fitness (Moreau *et al.*, 2006a; Thiéry & Moreau, 2006), in female and male reproductive success (Moreau *et al.*, 2007) and in the phenology of insect emergence (Thiéry *et al.*, 2013). The phenological development of the vine and diet quality during larval feeding significantly affect adult sexual behaviour (Savopoulou-Soultani *et al.*, 1999; Torres-Vila *et al.*, 1999, 2005; Moreau *et al.*, 2006b, c). Besides, grapevine moth population dynamics vary in accordance with each geographical area and the particular climatic variables of each season. The number of generations in a season depends on weather conditions in late summer (Coscollá, 1997).

Several models have been proposed to describe/predict grapevine moth phenology. Many of them are based on the computation of temperature accumulations, in order to obtain the correlation with the pheromone trap catches of the adult males (Touzeau, 1981; Gabel & Mocko, 1984; Cravedi & Mazzoni, 1994; Milonas *et al.*, 2001). All of them propose degree-day accumulations, to make predictions about the development of *L. botrana*. Del Tío *et al.* (2001) demonstrated the statistical significance of a positive linear relationship between temperature accumulation and the pheromone trap catches of male moths. Other authors have attempted to include the relative influence of other specific climatic factors on the dynamics of this insect in their models, such as relative humidity, rainfall, and wind speed, although these are without importance for the duration of the larval stages. The only environmental factor involved in the prediction of population size throughout the breeding season was temperature (Schmidt *et al.*, 2003). However, relative humidity combined with temperature affected the duration of early developmental stages. Furthermore, drought conditions shorten the lifespan of eggs and larvae and increase egg

mortality in very hot wine-growing areas (Torres-Vila *et al.*, 1996, 1999).

Other authors have proposed models with more complex mathematical tools. Thus, Sáenz-De-Cabezón *et al.* (2011) used simplicial sets, Shape theory or Morse theory to predict functions for *L. botrana* capture data sets with spatial distribution. Aïnseba *et al.* (2011) took into account a stage-structured population model, based on partial differential equations, describing the period and the length of population dynamics for egg, larval and male and female adult stages. Moravie *et al.* (2006) used a Bayesian approach and hierarchical modelling to overcome the usual limits of models based on field data. Amo-Salas *et al.* (2011) proposed an empirical and biologically plausible model based on logistic transformations of daily temperatures, proving a better fit and permitting simple inclusion of additional variables such as relative humidity. By doing so, they redefined the parameters for the Touzeau model in the Ribera del Duero region in Northwestern Spain.

The main goal of our study is to provide a model that predicts the flight peaks of *L. botrana* in different wine-growing regions of Spain, characterized by flight periods that are similar to those of the tortricid. For this purpose, the model proposed by Amo-Salas *et al.* (2011) was used for comparing and grouping the flight activity of the moth from the different growing areas. Thus, the data records from different regions may be joined, to obtain more robust models. These models would be of use to an agricultural pest control advisor, to help predict the peak flight in each generation, based on meteorological variables.

Materials and methods

Data from pheromone traps

Cardboard delta traps with sticky inserts baited with synthetic sex pheromone (ECONEX. (E,Z)-7,9-dodecadienyl acetate 1 mg) were used to monitor the flight activity of male *L. botrana*. Traps were hung at a height of 1–1.5 m above the ground level. In all vineyards, traps were hung before the first flight (March–April), corresponding with the phenological state of budburst, and were maintained until the end of the season (usually October). Traps were checked once a week (every 7 days) or twice a week (every 3 or 4 days, always the same weekday). Sticky inserts were changed as needed and the pheromone lures were refreshed once every 4–6 weeks, following the manufacturer's recommendations. The data from trap catches over the years provided information on the duration of each generation under field conditions.

Pheromone trap catch data were collected over different periods, throughout seven regions in Spain. The time frame of the data used in this study ranged from 2004 to 2011. The regions under study (and the number of vineyards in each one) were: Bierzo (26), Ribera del Duero (21), Arlanza (4), Bizkaiko Txakolina (7), Rioja Alavesa (21), Navarra (22) and Yecla (10) (fig. 1). Rioja Alavesa is located in the northern area of the Rioja Designation of Origin, on the left bank of the Ebro River (<http://uk.riojawine.com/en/6-production-areas.html>).

Two or three traps were placed in each vineyard, consequently, the information corresponded to the catches in each trap, the average of the traps and the average catch per trap per day (CTD); in other words, the ratio between the average number of the traps and the number of days between two inspections.

Flight curves were plotted with this information, which express the captures per trap and day throughout the year.

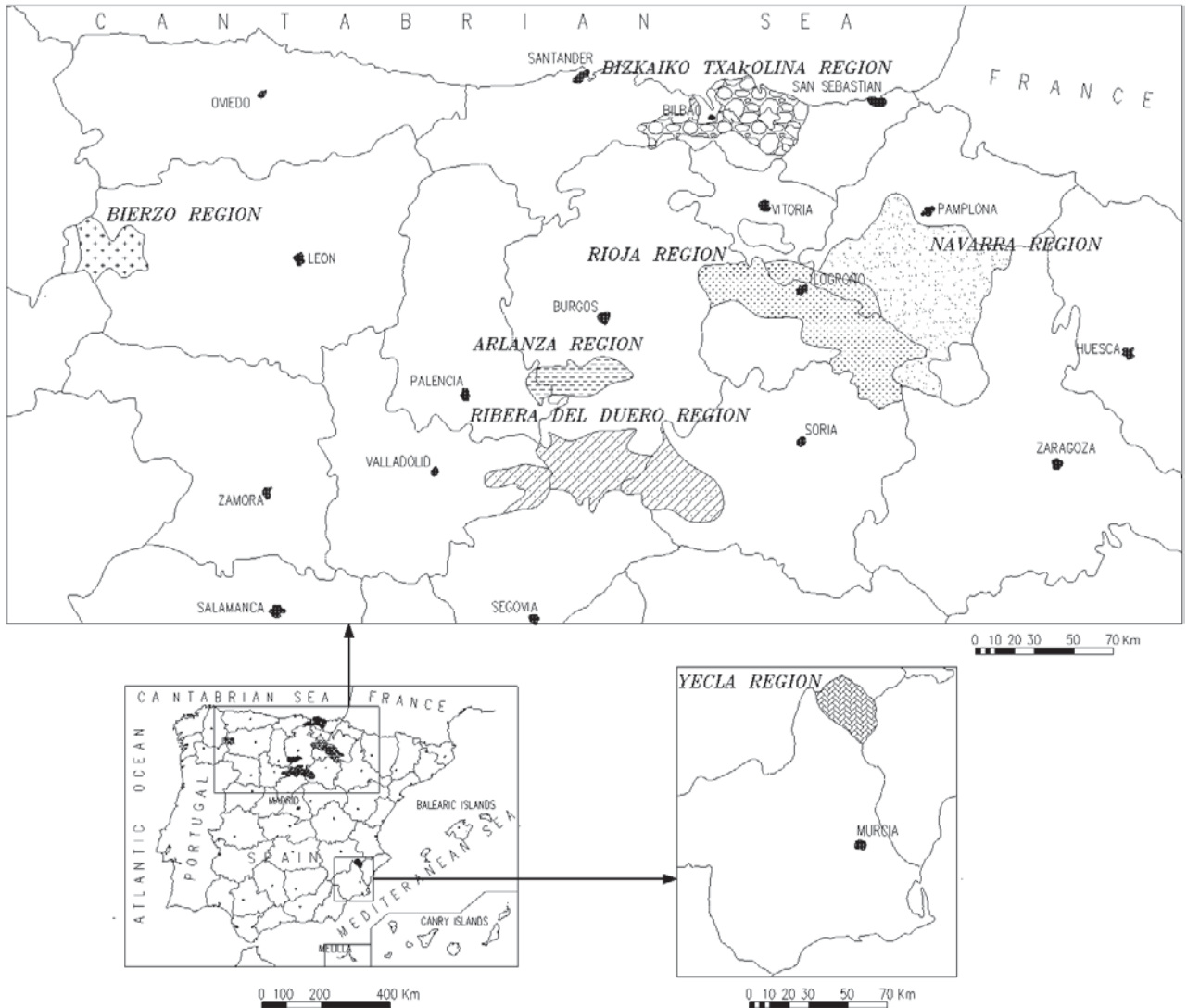


Fig. 1. Location of the wine-growing regions in the study.

This methodology enabled the estimation of the actual time of maximum flight in each generation, once these maximum flights had taken place; a method was therefore needed to predict these times.

Weather data

Data on weather patterns were collected, over the years of the study, from weather stations distributed throughout the different regions. These stations provide daily maximum, minimum and average temperatures and readings of these and relative humidity, solar radiation, precipitation and wind speed, at 30 or 60 min intervals.

Each vineyard in the study was assigned to a weather station on the basis of closeness criterion. The largest and smallest distances between a studied plot and its corresponding weather station were 25 and 0.1 km, respectively. Table 1 lists the locations, coordinates and altitudes of these weather stations.

Touzeau model

In 1980, J. Touzeau (1981) developed a degree-day prediction model, a detailed description of which may be found in Amo-Salas *et al.* (2011). The Touzeau function is mathematically expressed by

$$f_{\text{Tou}}(T) = \max\{0, (T - 10)\} \tag{1}$$

where, T is the real variable standing for the average temperature. The cumulative value of the degree of development on day k of the year is

$$S_k(\tau) = \sum_{d=1}^k f_{\text{Tou}}(T_d) \tag{2}$$

where, $\tau=(T_1, T_2, \dots, T_k)$ is the sequence of average daily temperatures recorded by the corresponding weather station from January 1st and T_d is the average temperature on day d of the year.

Table 1. Locations, coordinates and altitudes of the weather stations (WE).

Region	Municipality (WE)	Coordinates UTM (ED 1950)			Altitude (m)
		Zone	X	Y	
Bierzo	Arganza	29T	689,905	4,723,776	622
	Cacabelos	29T	685,250	4,719,155	504
	Priaranza del Bierzo	29T	692,386	4,711,997	525
	San Lorenzo	29T	697,334	4,711,997	587
	Villafranca	29T	679,696	4,719,150	532
	Carracedelo	29T	686,486	4,715,064	475
Ribera del Duero	Aranda de Duero	30T	439,525	4,611,525	830
	La Horra	30T	427,814	4,623,318	762
	Nava de Roa	30T	421,072	4,608,749	706
	Peñafiel	30T	408,006	4,605,558	742
	Sotillo de la Ribera	30T	430,797	4,622,423	761
	Villanueva de Gumiel	30T	448,875	4,620,895	848
Arlanza	Covarrubias	30T	455,018	4,656,124	920
	Lerma	30T	445,779	4,652,812	844
	Quintana del Puente	30T	403,216	4,659,341	800
Rioja Alavesa	Durana	30T	546,878	4,711,153	558
	Las Muñecas	30T	542,314	4,705,914	433
	Matacaballos	30T	533,153	4,711,225	538
	Villabuena	30T	527,347	4,710,810	502
	Carabriñas	30T	513,873	4,716,011	464
	La Llana	30T	513,732	4,713,151	441
	Espirbel	30T	515,225	4,714,658	484
	Montebuena	30T	517,515	4,714,864	508
	La Hueta	30T	516,696	4,717,330	586
	Salinillas	30T	513,232	4,720,145	483
	Párganos	30T	533,035	4,712,180	575
Rodezno	30T	513,360	4,707,845	577	
Bizkaiko Txakolina	Bakio	30T	514,637	4,807,711	46
	Iurreta	30T	528,101	4,781,335	111
	Zalla	30T	488,294	4,783,886	97
Yecla	Yecla Norte	30S	657,918	4,280,624	658
	Yecla-Pinillos	30S	664,558	4,270,147	567
Navarra	Ablitas	30T	612,462	4,650,683	336
	Cascante	30T	605,832	4,654,795	333
	Olite	30T	611,257	4,699,457	374

Table 2 shows the thresholds, established by Touzeau, which display the timing of maximum moth flight per generation (flight peak), expressed in degree-days ($DD > 10^{\circ}\text{C}$). These times can be compared with those observed in the field with monitoring methods (actual times of maximum flight) to test the precision of this model.

The logistic function model

Amo-Salas *et al.* (2011) developed a model based on the accumulation of the meteorological variables temperature and relative humidity for the Ribera del Duero region (Spain). This is a logistic function model, in which the sum of daily developments started on 1st January. The three parameters of the model and the thresholds for each generation were estimated, so that the timing of maximum moth flight per generation (peak flight) could be predicted with the same equation.

In this model, ‘suitable prediction’ was defined as a precise fit between predicted and actual days of maximum flight. Therefore, the error was the addition of the absolute values of the intervals between the predictions of maximum and the actual days of maximum flight

$$Err = \sum_{r,y,p,g} |k_{r,y,p,g} - \tilde{k}_{r,y,p,g}| \quad (3)$$

Table 2. Thresholds of the Touzeau model.

Step	Temperature sum ($^{\circ}\text{C}$) DD > 10°C	Accumulated temperature sum ($^{\circ}\text{C}$) DD > 10°C
1st flight	125	125
2nd flight	375	500
3rd flight	450	950

where, $k_{r,y,p,g}$ are the actual (observed) dates of maximum flight in the region r , of the year y , on the plot p , of the generation g and $\tilde{k}_{r,y,p,g}$ are the corresponding predictions.

The basic building block of the mathematical model considered was the logistic function given by the following formula:

$$f_1(T, H | \alpha, \beta, \gamma) = \frac{1}{1 + e^{-(\alpha + \beta T + \gamma H)}} \quad (4)$$

where, T and H are real arguments and α , β and γ are real parameters. In the function, the value $f_1(T, H | \alpha, \beta, \gamma)$ quantifies in intervals of [0,1] the development of *L. botrana* throughout 1 day with an average temperature of T and an average relative humidity of H . Daily data were accumulated as in equation (2),

the cumulative value in the new equation on day k being defined by

$$S_k(\tau|\alpha, \beta, \gamma) = \sum_{d=1}^k f_1(T_d, H_d|\alpha, \beta, \gamma)$$

An estimate of the day of maximum flight of generation g (g is 1, 2 or 3) was determined by the threshold θ_g applied on the cumulative values S_k (see details in Amo-Salas *et al.*, 2011).

The estimates of the dates of maximum flight strongly depend on the values of parameters $\alpha, \beta, \gamma, \theta_1, \theta_2$ and θ_3 . Hence, the model was calibrated, which required finding the values of the parameters to minimize the prediction error. The error criterion of the form in the logistic function model was

$$Err(\alpha, \beta, \gamma, \theta_1, \theta_2, \theta_3) = \sum_{r,y,p,g} |k_{r,y,p,g} - \hat{k}_{r,y,p,g}(\tau_{p,y}|\alpha, \beta, \gamma, \theta_g)| \quad (5)$$

where, $k_{r,y,p,g}$ are real (observed) days of the maximum flight in the region r , of the year y , on the plot p , of the generation g , and $\hat{k}_{r,y,p,g}(\tau_{p,y}|\alpha, \beta, \gamma, \theta_g)$ are the corresponding predictions yielded by the model with parameters $\alpha, \beta, \gamma, \theta_g$ under the temperature record $\tau_{p,y}$ on plot p throughout year y . The sum was obtained by adding the errors pertaining to all the recorded combinations of regions, plots, years and generations in the data.

Statistical analysis

The data from 2004 to 2010 were used to compute the parameters of the logistic function model and those of 2011 were used to check it. Likewise, those same data were used to estimate the error committed in the predictions of the maximum flight with this model and with the Touzeau model.

Data were checked for the assumption of independency, normality and homoscedasticity by the Wald–Wolfowitz runs test, the Kolmogorov–Smirnov test and Levene’s test, respectively. One-way analysis of variance (ANOVA) (Wolfram Mathematica version 8.0 for Windows) was used to assess whether there was any significant effect of region on the average error of the logistic function model. The planned hypothesis test was:

H_0 : The behavior of *L. botrana* is similar in all regions

H_1 : No H_0

The regions were compared by the average committed error for the computed model. Thus, the hypothesis test could be written as:

$$H_0 : \overline{Err}_1 = \overline{Err}_2 = \dots = \overline{Err}_k$$

$$H_1 : \text{No } H_0$$

where, \overline{Err}_i is the average error in region i .

Comparisons between regions were evaluated with Tukey’s *post hoc* test. Differences between mean values were considered statistically significant at $P < 0.05$. The one-way ANOVA and the *post hoc* test were applied to the total data set, including the data on 2011. The regions were grouped according to their similarity based on ANOVA results and analysed as different cases; the parameters of the logistic function model were estimated and validated for each case. The mean prediction error was also defined in each region for these parameters. Finally, the Akaike Information Criterion (AIC) was used to compare the Touzaeu model with the

Table 3. Estimation of the parametric values and thresholds of the logistic function model.

Model	α	β	γ	θ_1	θ_2	θ_3
1st model	−5	0.38	−0.001	27	79	122
2nd model	−5.64	0.42	−0.0032	23.2	73.2	117.6
3rd model	−4.7	0.42	−0.015	27	76	121
4th model	−6	0.24	0.035	26	73	112

other models through the relative likelihood of the Touzeau model with respect to the logistic function model ($\exp(0.5*(AIC_{logistic} - AIC_{Touzeau}))$).

Results

Data from the seven studied regions were taken into account to compute the parameters of the logistic function model and to evaluate their committed error and the committed error of the Touzeau model. The data from 2004 to 2010 were used to estimate the parameters of the model and to compute the committed error in the predictions of the flight peaks of *L. botrana*, and the data from 2011 were used to validate this model.

The maximum flight predictions of the Touzeau model were made using equations (1) and (2) and the thresholds of table 2. The cumulative committed error of the predictions generated by the Touzeau model for the data up to 2010, using equation (3), was 14,862 days. Note that this error is cumulative in the sense that it depends on the total amount of data considered. The total amount of data considered above was 791; the relative error was 18.79 days per region, year, plot and generation.

The predictions of the logistic function model were based on the temperature and humidity records of the weather stations, meaning that the predictions for the plots assigned to the same weather station were necessarily the same. However, the actual days of maximum flight differed even for plots with the same weather station, which implies that there is a lower limit on the achievable error. This error limit was computed by taking the median of the set of days of maximum flight for each region, year, weather station and generation as the optimum prediction. Thus, the minimum error was 2608 days, where the predictions in this case are the median of the set of days of maximum flight for each region, year, weather station and generation. The median was chosen as a robust measure against outliers. In this case, the relative error was 3.30 days per region, year, plot and generation. Note the large interval between the minimum error and the error of the Touzeau model.

The error function defined by equation (5) was minimized for the values of the parameters shown in the first row of table 3, in order to estimate the parameters of the logistic function model and the thresholds. The achieved error value was 7437.35 days, which amounted to 9.40 days per region, year, plot and generation. Using AIC, the relative likelihood of the Touzeau model with respect to the logistic function model was less than 0.001, showing that the Touzeau model is <0.1% as likely to minimize the information loss as the logistic function model. The validation of both the logistic function model and the Touzeau model was with data from 2011. In this first case, the error of the Touzeau model was 23.23 days per region, year, plot and generation and the error of our

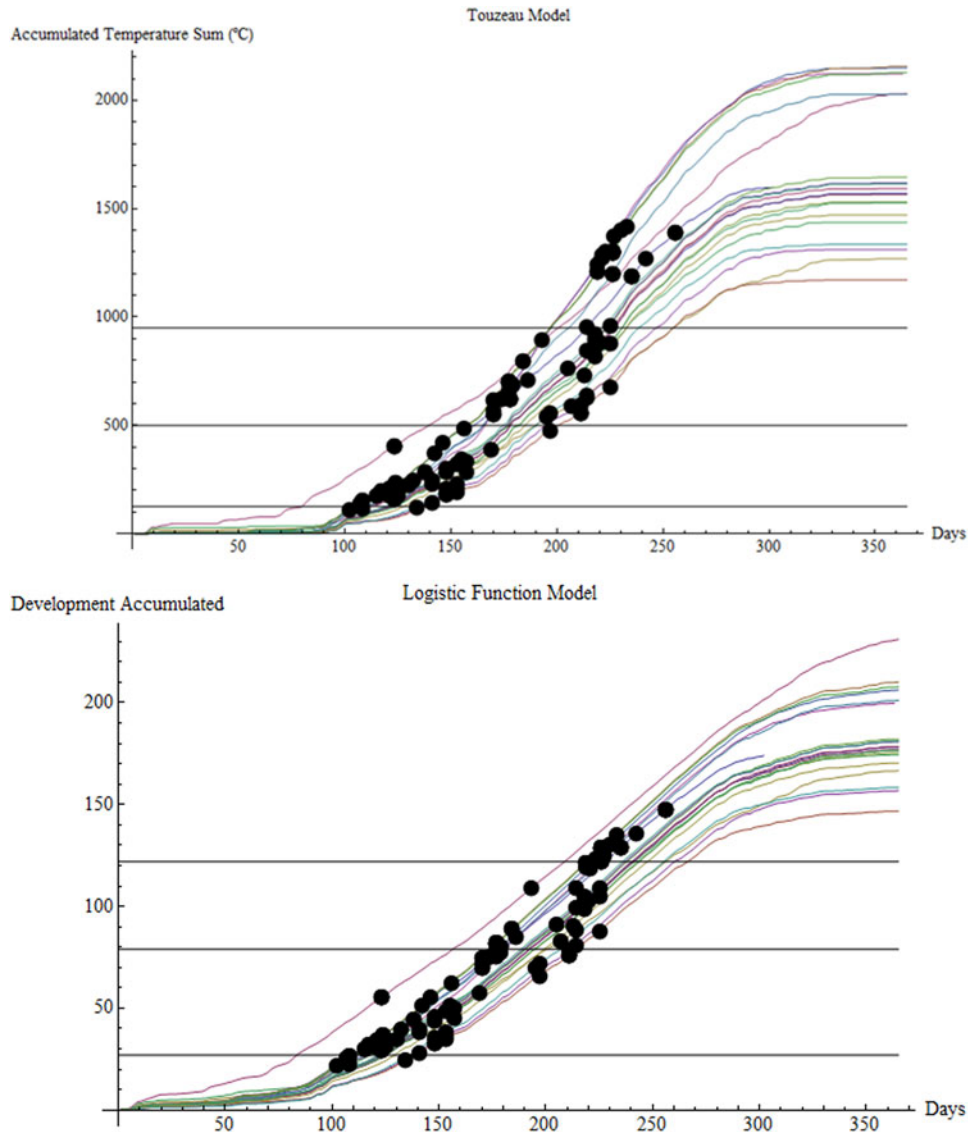


Fig. 2. Plot of the accumulation of temperatures using the Touzeau model (first plot) and accumulated development using the Logistic Function model with variable temperature and humidity (second plot) throughout 2011. Black dots stand for the actual days of maximum flight and horizontal lines stand for the thresholds of maximum flight according to the corresponding model.

model with the new parameters was reduced by almost 12 days, with an achieved value of 11.79 days per region, year, plot and generation; an error margin that could still be considered too large.

Fig. 2 plots the fit of the data collected in 2011 with both the Touzeau model and the logistic function model. The predictions of the first flight with the Touzeau model were acceptable; however, the differences between the actual peak flights and the predictions were not of sufficient accuracy for the second and the third flights. The logistic function model offered a better fit than the Touzeau model, although its fit could still be improved.

The above model assumes that the phenology of *L. botrana* is similar in the different regions under consideration. The total amount of data differs between the regions, although this would be of no importance, if the previous assumption is true.

Otherwise, the model's predictions achieve a better fit with the data from the region with a higher total amount of information. To verify this assumption, one-way ANOVA was used over the total data set (2011 included). If the phenology of *L. botrana* is similar in all the regions under study, then the average committed error will be similar in all the regions computed by the model. Thus, the hypothesis test was based on the average committed error of the computed model. Table 4 shows the average error of the model computed in each region for different data sets (including the data for 2011).

In this first situation, the average error for the Bizkaiko Txakolina region was excessively large in comparison with the other regions. The one-way analysis showed significant differences between the average errors ($F=28.96$, $d.f.=6935$, $P<0.001$). The *post hoc* test indicated that the data from the Bizkaiko Txakolina region was significantly different from the

Table 4. Average error of the logistic function model, expressed in days per region, year, plot and generation.

Region	Average error $\pm \sigma$ (days)			
	Case 1st	Case 2nd	Case 3rd	Case 4th
Bierzo	12.39 \pm 7.94 ^a	12.51 \pm 8.43 ^a	–	9.32 \pm 6.52 ^a
Ribera del Duero	6.99 \pm 5.88 ^b	6.79 \pm 5.51 ^b	6.55 \pm 5.54 ^a	–
Arlanza	10.56 \pm 6.55 ^{ab}	9.77 \pm 5.99 ^{ab}	8.87 \pm 5.12 ^a	13.50 \pm 11.46 ^{ab}
Rioja Alavesa	12.72 \pm 9.59 ^a	12.62 \pm 9.12 ^a	–	17.69 \pm 13.09 ^b
Bizkaiko Txakolina	16.99 \pm 11.81 ^c	–	–	–
Yecla	8.40 \pm 8.96 ^{ab}	8.39 \pm 8.58 ^b	7.90 \pm 7.99 ^a	–
Navarra	5.82 \pm 5.91 ^b	5.80 \pm 5.78 ^b	5.59 \pm 5.59 ^a	–
Global mean in each case	9.78 \pm 8.28	9.26 \pm 7.76	6.52 \pm 5.75	11.98 \pm 9.94

^{a, b, c} Superscripts indicate the groups associated with Tukey's *post hoc* test.

data from other regions. Moreover, significant differences were found between Bierzo and Ribera del Duero, Bierzo and Navarra, Rioja Alavesa and Navarra, and Rioja Alavesa and Ribera del Duero. Different situations were studied by removing data from the regions with significant differences from the original data set. These situations are referred to as *cases*, the first of which refers to all the regions.

In the second case, the data on Bizkaiko Txakolina were removed from the data set due to the large difference between the average error for that region and the average error for the other regions. The estimation of the parameters of the model after removing these data is shown in the second row of [table 3](#). The average error of the model with the above parameters was 8.99 days per region, year, plot and generation and its validation for 2011 achieved 10.71 days, representing around one day of reduction with respect to the first case. The average error of the Touzeau model for this data set was 19.54 days per region, year, plot and generation, and the average error for the data collected in 2011 was 18.76 days per region, year, plot and generation, which implies a difference of over 8 days with regard to the error of the logistic function model. The relative likelihood, based on AIC, of the Touzeau model with regard to the logistic function model was also less than 0.001.

For the second data set, significant differences were detected among the average errors ($F=28.99$, $d.f.=5881$, $P<0.001$). The *post hoc* test showed two groups: the first comprised Ribera del Duero, Arlanza, Yecla and Navarra, which was the third case in the study; and the second group was composed of Bierzo, Arlanza and Rioja Alavesa, which corresponded to the fourth case under study. Note that the Arlanza region was initially included in both groups.

In the third case, the average error of the Touzeau model for the data set was 17.47 days per region, year, plot and generation. The parameters of the logistic function model in this case are shown in the third row of [table 3](#). The relative error of these parameters was 6.58 days per region, year, plot and generation. There was an important improvement in the prediction of the flight peaks. Moreover, the minimum achievable relative error for this data was 3.24 days per region, year, plot and generation. The relative error of the Touzeau model for the validation data set was 21.77 days per region, year, plot and generation; however, with the new estimated parametric values of the model, the relative error changed to 6.19 days per region, year, plot and generation. As in the previous cases, the relative likelihood of the Touzeau model with regard to the logistic function model was less than 0.001. The one-way ANOVA was not significant ($F=2.52$, $d.f.=3502$, $P>0.06$),

therefore the phenology of *L. botrana* could be considered similar in these regions.

Finally, for the fourth case, the relative error of the Touzeau model was 21.29 days per region, year, plot and generation. The parametric estimations of the logistic function model for this data set are shown in the fourth row of [table 3](#). The relative error for the logistic function model was 10.17 days per region, year, plot and generation. The relative error of the Touzeau model for the validation data set was 23.88 days per region, year, plot and generation, whereas the relative error of the logistic function model for the same data set was 14.48 days per region, year, plot and generation. The relative likelihood of the Touzeau model with respect to the logistic function model was less than 0.001 despite the relative error of the logistic function model being greater than in the previous cases. There were significant differences between regions ($F=33.77$, $d.f.=2394$, $P<0.001$), where Rioja Alavesa differed from Bierzo. Even though Rioja Alavesa and Bierzo belong to the same group in this analysis, *L. botrana* does not necessarily have a similar phenology in both regions. Thus, in this case where both regions were jointly studied without the confusing effect of other regions, it was observed that the model did not provide good predictions for Rioja Alavesa region, even though it achieved a better fit for the data from Bierzo. Moreover, the relative error for the Arlanza region, in this case, was too large. Therefore, the predictions for this region should be based on the parameters of the previous group.

Discussion

Efficient application of control methods, such as mating disruption with pheromones and chemical treatments, rely on knowledge of the differential susceptibility of each pest stage to the control strategy. Thus, predicting the development of the grapevine moth *L. botrana* could be of enormous assistance in the determination of an optimal treatment schedule (Moravie *et al.*, 2006). Considering the increased interest in biorational insecticides, where precise timing of treatments is crucial, daily temperature and relative humidity models could be a useful tool in improving their efficiency. The empirically-based model presented in this work was intended to predict the timing of maximum moth flights in different wine grape producing regions in Spain, by counting the males trapped in pheromone traps and by considering average daily temperature and relative humidity.

The proposed model is based on the logistic function, which is biologically more realistic than the Touzeau model and is more suited to the incorporation of additional

quantitative variables, in situations where the development of the moth only increases linearly with the temperature, without a higher developmental threshold. Implementation of the logistic function model is also relatively simple and the model can be employed regardless of the number of peak flights. Based only on the empirical data, the model predicts that the development of the moth is negligible under 10°C and that any temperature increase over the limit of approximately 22°C has no further positive effect. These results agree with other models based on the accumulation of growing degree-days, which adopt a lower threshold temperature of 8°C and upper threshold of 28°C (Tzanakakis *et al.*, 1988; Cossu *et al.*, 1999).

Considering the data set for all the regions, the committed error in the predictions, at around 10 days per region, year, plot and generation, was too large. Nevertheless, this error was half that of the Touzeau model. This paper shows that the fit of the Touzeau model in the regions under study was not sufficiently accurate. This lack of fit might be the result of the differences between the region where the Touzeau model was developed (Midi-Pyrénées, South France) and the different Spanish Designations of Origin in Castilla y León where it is applied in this study and where the climatic conditions are quite unlike those of the South of France (Armendáriz *et al.*, 2010).

Even though the prediction error of the logistic function model, expressed in days, in several cases, remains higher than the residual effect of biological insecticide sprays, the model that has been developed, coupled with knowledge on ovoposition found in the literature (Touzeau, 1981; Amo-Salas *et al.*, 2011) and monitoring of egg hatching over the peak flight moment, could significantly improve the control of *L. botrana* with insecticide sprays that target eggs and especially larvae, which is the most susceptible stage of treatment.

Statistical analysis showed vineyard regions where *L. botrana* had similar male flight phenology that could be grouped. Thus, parameters of the model and flight peak thresholds may be adjusted for each of those different groups with the aim of simplifying the method.

The study revealed that the Bizkaiko Txakolina region is different from the other regions analysed in this study, in that its oceanic climatic conditions are characterized by smooth temperatures, small annual thermal oscillation and abundant rainfalls, well distributed throughout the year. It would therefore be interesting to extend the study by gathering information from other Cantabrian regions with the same climatic conditions.

Using the same parameters of the logistic model, the relative error of the group comprising the regions of Ribera del Duero, Arlanza, Yecla and Navarra, was around 6.5 days per region, year, plot and generation. This value is lower than the 7 days between two trap checking phases, so it could be considered a very good prediction. It is important to note the good fit of these parameters in the Navarra region, with even lower prediction errors than those achieved in the Ribera del Duero region where the original model was developed. The parameters of the logistic function revealed that the effect of humidity is small and negative in this group. These regions, despite the distance between them, are both characterized by a dry Continental climate, with moderate–low rainfalls, concentrated in spring and autumn. There is a large thermal oscillation between climatic seasons, with hard and long winters, frequent frosts, and warm and dry summers.

The last step verified that Bierzo and Rioja Alavesa differ between each other and from the others regions. Note that, in this case, the sign of the estimated parameter γ is positive and that relative humidity therefore has an inverse effect in relation to the previous cases. The location, a valley in the Bierzo region, has a Mediterranean microclimate, with smooth temperatures, little frost and moderate and constant moisture throughout the year.

In contrast, the Rioja Alavesa region is a transitional area between the rainy Basque Country and the semi-arid area of the Central Ebro depression, such that this (Northwestern) area of the Rioja Designation of Origin is more exposed to moisture from the Atlantic Ocean and to its thermoregulatory effect (oceanic climate).

The special weather conditions in the Rioja region, composed by Rioja Alavesa, Rioja Alta and Rioja Baja, mean that an independent study of the phenology of *L. botrana* that has a further amount of data is needed.

Moreover, other factors affecting the European grapevine moth phenology, not evaluated in the present paper, should be considered in future studies. Some of the most important regulating biotic factors of *L. botrana* populations are: grape variety, morphology and the phenological stage of the reproductive organs, affecting the installation and larval mortality (Gabel & Roehrich, 1995), the phenology of adult emergence (Thiéry *et al.*, 2013) and reproductive success (Moreau *et al.*, 2006b, 2007; Thiéry & Moreau, 2006). Different food quality, ingested by the larvae through the three generations, also affects the larvae and adult fitness. Female and male reproductive performance appear to increase with advancing plant-growth stages (inflorescences < unripe berries < ripe berries). High-quality food ingested by larvae (generally promoting higher adult body weight) enhances male and female reproductive output. These results suggest that different intervention thresholds, such as those based on pheromone catches or/and mathematical models (as in our study), may be needed for each of the three moth generations, to compensate for the increased fecundity and potential of later generations to inflict more extensive damage on crops (Torres-Vila *et al.*, 1999; Moreau *et al.*, 2006c).

Another factor to take into account for improving the model could be the effect of climatic thermal intra-day oscillations (especially during a diapause phase). The current growth threshold has been statistically estimated based on day-degrees, but it is likely that some information on moth development has been missed, especially in wine-growing regions with few warm hours per day or with a high daily difference between maximum and minimum temperatures. The study of the daily development of *L. botrana* based on different temperature ranges within the same day (warm hours and cold hours) could improve moth flight predictions, but this would add further complexity to the development of the model. It would also require access to this kind of information, which it is not always easy. It appears less interesting to do the same thing with relative humidity, as its contribution to the model is lower.

Finally, this study identified regions where the male flight phenology of *L. botrana* is similar (Ribera del Duero, Navarra, Yecla and Arlanza), in order to use their weather data records more efficiently and to predict flight peaks in different wine-growing regions with similar climatic conditions. Moreover, fewer models would be needed, which could be adapted to new regions by redefining their parameters. Such regions as Bizkaia, Rioja Alavesa and Bierzo

would need independent studies due to their specific climatic conditions.

The evaluation of other non-biotic and biotic factors might also contribute to decreased error in the model and to greater gains in accuracy, when predicting the peak flight moment and improving the assessment of pest outbreaks in each generation.

Acknowledgements

The authors acknowledge the help of the following organizations and their technicians for supplying information on meteorological conditions and data on the capture of *Lobesia botrana*: Junta de Castilla & León, Servicio de Sanidad Vegetal de the Consejería de Agricultura y Agua from the region of Murcia, Servicio Agrícola of the Diputación Foral de Bizkaia and the viticulture area in DRMAyAL of the Navarra Government. The authors also thank Dr R. M. Gleiser for advice and proofreading.

References

- Ainseba, B., Picart, D. & Thiéry, D. (2011) An innovative multistage, physiologically structured, population model to understand the European grapevine moth dynamics. *Journal of Mathematical Analysis and Applications* **382**, 34–46.
- Amo-Salas, M., Ortega-López, V., Harman, R. & Alonso-González, A. (2011) A new model for predicting the flight activity of *Lobesia botrana* (Lepidoptera: Tortricidae). *Crop Protection* **30**, 1586–1593.
- Anfora, G., Tasin, M., Backman, A.C., de Cristofaro, A., Witzgall, P. & Ioriatti, C. (2005) Attractiveness of year-old polyethylene isonet sex pheromone dispensers for *Lobesia botrana*. *Entomologia Experimentalis et Applicata* **117**, 201–207.
- Anshelevich, L., Kehat, M., Dunkelblum, E. & Greenberg, S. (1994) Sex-pheromone traps for monitoring the European vine moth, *Lobesia botrana*: effect of dispenser type, pheromone dose, field aging of dispenser, and type of trap on male captures. *Phytoparasitica* **22**, 281–290.
- Armendáriz, I., Pérez-Sanz, A. & Miranda, L. (2010) Predicción de la polilla del racimo de la vid (*Lobesia botrana*) en seis Denominaciones de Origen de Castilla y León. *Boletín de Sanidad Vegetal Plagas* **36**, 11–22.
- Coscollá, R. (1997) *La polilla del racimo de la vid (Lobesia botrana Den. & Schiff.). Generalitat Valenciana*. Conselleria de Agricultura, Pesca y Alimentación. Valencia, Spain.
- Cossu, Q.A., Delrio, G., Di Cola, G. & Gilioli, G. (1999) *Modelli matematici nella protezione integrata delle colture in Sardegna*. Collana di Agrometeorologia per la Sardegna, NotaTecnica 3. Italy.
- Cozzi, G., Pascale, A., Perrone, G., Visconti, A. & Logrieco, A. (2006) Effect of *Lobesia botrana* damages on black aspergilli rot and ochratoxin A content in grapes. *International Journal of Food Microbiology* **111**, S88–S92.
- Cravedi, P. & Mazzoni, E. (1994) Verification of the relation between degree-days and pheromone trap catches of *Lobesia botrana* (Den. & Schiff.) (Lepidoptera Tortricidae). *Redia* **77**, 109–122.
- Delbac, L., Lecharpentier, P. & Thiery, D. (2010) Larval instars determination for the European Grapevine Moth (Lepidoptera: Tortricidae) based on the frequency distribution of head-capsule widths. *Crop Protection* **29**, 623–630.
- Del Tío, R., Martínez, J.L., Ocete, R. & Ocete, M.E. (2001) Study of the relationship between sex pheromone trap catches of *Lobesia botrana* (Den. & Schiff.) (Lep., Tortricidae) and the accumulation of degree-days in sherry vineyards (SW of Spain). *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie* **125**, 9–14.
- Fermaud, M. (1998) Cultivar susceptibility of grape berry clusters to larvae of *Lobesia botrana* (Lepidoptera: Tortricidae). *Journal of Economic Entomology* **91**, 974–980.
- Fermaud, M. & Le Menn, R. (1989) Association of *Botrytis cinerea* with grape berry moth larvae. *Phytopathology* **79**, 651–656.
- Gabel, B. & Mocko, V. (1984) Forecasting the cyclical timing of the grape vine moth, *Lobesia botrana* (Lepidoptera: Tortricidae). *Acta Entomologica Bohemoslovaca* **81**, 1–14.
- Gabel, B. & Roehrich, R. (1995) Sensitivity of grapevine phenological stages to larvae of European Grapevine Moth, *Lobesia botrana* Den. and Schiff. (Lep. Tortricidae). *Journal of Applied Entomology – Zeitschrift Fur Angewandte Entomologie* **119**, 127–130.
- Gutierrez, A.P., Ponti, L., Cooper, M.L., Gilioli, G., Baumgärtner, J. & Duso, C. (2012) Prospective analysis of the invasive potential of the European grapevine moth *Lobesia botrana* (Den. & Schiff.) in California. *Agricultural and Forest Entomology* **14**, 225–238.
- Ioratti, C., Anfora, G., Tasin, M., De Cristofaro, A., Witzgall, P. & Lucchi, A. (2011) Chemical ecology and management of *Lobesia botrana* (Lepidoptera: Tortricidae). *Journal of Economic Entomology* **104**, 1125–1137.
- Milonas, P.G., Savopoulou-Soultani, M. & Stavridis, D.G. (2001) Day-degree models for predicting the generation time and flight activity of local populations of *Lobesia botrana* (Den. & Schiff.) (Lep., Tortricidae) in Greece. *Journal of Applied Entomology – Zeitschrift Fur Angewandte Entomologie* **125**, 515–518.
- Moravie, M.A., Davison, A.C., Pasquier, D. & Charmillot, P.J. (2006) Bayesian forecasting of grape moth emergence. *Ecological Modelling* **197**, 478–489.
- Moreau, J., Benrey, B. & Thiéry, D. (2006a) Grape variety effects larval performance and also female reproductive performance of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae). *Bulletin of Entomological Research* **96**, 205–212.
- Moreau, J., Benrey, B. & Thiéry, D. (2006b) Assessing larval food quality for phytophagous insects: are the facts as simple as they appear? *Functional Ecology* **20**, 592–600.
- Moreau, J., Arruego, X., Benrey, B. & Thiéry, D. (2006c) Differences in nutritional quality of parts of *Vitis vinifera* berries affect fitness of the European grapevine moth. *Entomologia Experimentalis et Applicata* **119**, 93–99.
- Moreau, J., Thiéry, D., Troussard, P.J. & Benrey, B. (2007) Grape variety affects female but also male reproductive success in wild European grapevine moths. *Ecological Entomology* **32**, 747–753.
- Ruiz de Escudero, I., Baltasar Escriche, A.E. & Caballero, P. (2007) Potential of the *Bacillus thuringiensis* toxin reservoir for the control of *Lobesia botrana* (Lepidoptera: Tortricidae), a major pest of grape plants. *Applied and environmental microbiology* **73**, 337–340.
- Sáenz-De-Cabezón, E., Hernández, L.J., Rivas, M.T., García-Ruiz, E., Marco, V., Pérez-Moreno, I. & Sáenz-De-Cabezón, F.J. (2011) A computer implementation of the partition of the unity procedure and its application to arthropod population dynamics. A case study on the European grape berry moth. *Mathematics and Computers in Simulation* **82**, 2–14.

- 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., Nikolaou, N. & Milonas, P.G. (1999) Influence of maturity stage of grape berries on the development of *Lobesia botrana* (Lepidoptera: Tortricidae) larvae. *Journal of Economic Entomology* **92**, 551–556.
- Schmidt, K., Hoppmann, D., Holst, H. & Berkelmann-Löhnertz, B. (2003) Identifying weather-related covariates controlling grape berry moth dynamics. *OEPP/EPPPO Bulletin* **33**, 517–524.
- Thiery, D. & Moreau, J. (2005) Relative performance of European grapevine moth (*Lobesia botrana*) on grapes and other hosts. *Oecologia* **143**, 548–557.
- Thiéry, D. & Moreau, J. (2006) Grape cultivar affects larval and female fitness of European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae). *Integrated Protection in Viticulture* **29**, 131–138.
- Thiéry, D., Monceau, K. & Moreau, J. (2013) Different emergence phenology of European grapevine moth (*Lobesia botrana*, Lepidoptera: Tortricidae) on six varieties of grapes. *Bulletin of Entomological Research*. Available on CJO2013. DOI: <http://dx.doi.org/10.1017/S000748531300031X>.
- Torres-Vila, L.M., Stockel, J. & Rodríguez-Molina, M.C. (1996) Efecto de la indisponibilidad de agua sobre el potencial biótico de la polilla del racimo *Lobesia botrana* Den. y Schiff. (Lepidoptera: Tortricidae). *Boletín de Sanidad Vegetal Plagas* **22**, 443–449.
- Torres-Vila, L.M., Rodríguez-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.
- Torres-Vila, L.M., Rodríguez-Molina, M.C., McMinn, M. & Rodríguez-Molina, A. (2005) Larval food source promotes cyclic seasonal variation in polyandry in the moth *Lobesia botrana*. *Behavioral Ecology* **16**, 114–122.
- Touzeau, J. (1981) Modélisation de l'évolution de l'Eudémis de la Vigne par la région Midi Pyrénées. *Bollettino di Zoologia Agraria e di Bachicoltura, ser. II* **16**, 26–28.
- Tzanakakis, M.E., Savopoulou-Soultani, M., Oustapassidis, C. S., Verras, S.C. & Hatziemmanuel, H. (1988) Induction of dormancy in *Lobesia botrana* by long day and high temperature conditions. *Entomologica Hellenica* **6**, 7–10.
- Vacas, S., Alfaro, C., Zarzo, M., Navarro-Llopis, V. & Primo, J. (2011) Effect of sex pheromone emission on the attraction of *Lobesia botrana*. *Entomologia Experimentalis et Applicata* **139**, 250–257.
- Witzgall, P., Bengtsson, M. & Trimble, R.M. (2000) Sex pheromone of grape berry moth (Lepidoptera: Tortricidae). *Environmental Entomology* **29**, 433–436.
- Witzgall, P., Tasin, M., Buser, H.R., Wegner-Kiss, G., Mancebon, V.S.M., Ioriatti, C., Backman, A.C., Bengtsson, M., Lehmann, L. & Francke, W. (2005) New pheromone components of the grapevine moth *Lobesia botrana*. *Journal of Chemical Ecology* **31**, 2923–2932.
- 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.