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Research Article

Three years analysis of *Lobesia botrana* (Lepidoptera: Tortricidae) flight activity in a quarantined area

Guillermo Heit[1, 2*], Walter Sione[3] and Pablo Cortese[1, 2]

1. Department of Plant Protection, Faculty of Agronomy, University of Buenos Aires, Buenos Aires, Argentina.
2. Bureau of Surveillance and Monitoring, National Animal Health and Agri-food Quality Service, Av. Paseo Colón 315, Ciudad Autónoma de Buenos Aires, Argentina.
3. Autonomous University of Entre Ríos, Regional Center for Geomatics, Matteri y España s/n, Diamante, Entre Ríos, Argentina.

Abstract: *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), is an important vineyard-pest in the European and Mediterranean areas and it was recently described in Argentina and Chile. Since knowledge on the *L. botrana* phenology on Argentina is still limited, the objective of this study was to develop a phenological model to predict voltinism of *L. botrana* in Argentina through a regional approach.Voltinism of *L. botrana* males was simulated based on occurrence of four non-overlapping flights. Nonlinear regression models were constructed using the weekly average trap catches from the agricultural seasons 2011-2012 to 2013-2014 and amount of degree-days accumulation. Weibull equation showed, on average for the four annual flights, the best estimate of the observed variability in the percentage of adult catches in relation to degree-day accumulation. It can be expected that 50% of male adult emergence for the first flight occurs at 443.9 DD; in the second flight at 1211.7 DD; while in the third and the fourth flights, the accumulation of degree days reaches values of 2077.8 DD and 2905 DD, respectively. The regional approach adopted in this work could explain the variation found in field data and has a reasonable predictive and explicative capability as a component in the ongoing prospective analysis of the activity of *L. botrana* in Argentina.

Keywords: *Lobesia botrana*, surveillance system, voltinism

Introduction

The European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae), an endemic pest in the Palearctic Region, widespread in all wine-growing areas, is one of the most noxious vineyard-pests in the European and Mediterranean areas (Delbac *et al.*, 2010).

L. botrana is described as polyphagous species and its presence in grapes is relatively recent, its importance as a pest in vineyards has been reported at the beginning of the twentieth century (Thiéry and Moreau, 2005). This species was considered a quarantine pest absent in South America until 2008, when it was found in Chile and subsequently, in 2010, in Mendoza Province, Argentina (González, 2010).

This pest recently introduced into Argentina, is under official control through the National Program for Prevention and Eradication of *L. botrana*. One of the

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[*] **Corresponding author**, e-mail: gheit@agro.uba.ar
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objectives of the program is to develop predictive models of the population dynamics of *L. botrana*, as phytosanitary warning tool at regional level, in order to estimate the behavior of the species in other wine regions of the country at risk of being invaded.

Potential damage of *L. botrana* to grapevines varies during the grape growing season; the later generations are the most harmful, they can seriously affect the mature grape berry harvest directly through larval feeding and indirectly by predisposing the crop to fungal infection by *Botrytis cinerea* (Armendáriz *et al.*, 2009; DallaMonta *et al.*, 2007). The number of generations per year of *L. botrana* on V*itis vinifera* differs geographically and this variability is determined by several factors including photoperiod, temperature, relative humidity, latitude and host phenology (Sciarretta *et al.*, 2008).For example, in the Palearctic region, *L. botrana* voltinism ranges from one to five flights (Pavan *et al.*, 2006). In Mediterranean areas it is usually trivoltine although in the warmest years a fourth partial generation has been reported (Ioriatti *et al.*, 2011).

In applied entomology, various empirical approaches have been used to estimate the population dynamics of insects, mainly based on the study of patterns of temporal distribution of different insect developmental stages, for example, the distribution of emergence periods of one or more developmental stages (Moravie *et al.*, 2006). Due to the great influence that temperature exerts on insect phenology, most of the models that describe insect development are temperature-driven (Damos and Savopoulou-Soultani, 2012).

Several authors have used field observations to estimate the phenology of insect populations in order to use these estimations in integrated pest management or even in pest risk analysis under climate change scenarios (Satake *et al.*, 2006; Martin-Vertedor *et al.*, 2010; Gutierrez *et al.*, 2012). Many researchers have used nonlinear regression models to describe temperature dependent processes (Damos and Savopoulou-Soultani, 2012). Milonas *et al.* (2001) used nonlinear regression to estimate the voltinism of *L. botrana* in Greece. Tobin *et al.* (2003), have used Logistic and Gompertz functions to estimate adult emergence of *Endopiza viteana* (Lepidoptera: Tortricidae) based on cumulative day degree. Milonas and Savapolous (2006) have used Logistic and Weibull functions to estimate the proportion of catches of *Adoxophyes orana* (Lepidoptera: Tortricidae) on pheromone traps.

The aim of this study was to develop a simple species-specific phenological model to predict voltinism of *L. botrana* in Mendoza (Argentina) through a regional approach.

Materials and Methods

Study area and monitoring system

The study area included the north, central and eastern Oasis of Mendoza province, Argentina (Fig. 1). The official phytosanitary surveillance system in this area included the installation of pheromone traps for monitoring the temporal and spatial variation of *L. botrana* adult population, in order to identify quarantine areas for pest eradication.

Flight activity of *L. botrana* was monitored using Delta traps with (E, Z)-7,9-dodecadienyl acetate as the major component a of the synthetic pheromone. Among thousands of traps installed by the official surveillance program, only those installed before the emergence of the first adult (late August) and that remained till the end of the season (April), were considered in this study. Traps were installed on vineyard at 1.3 and 1.5 m above the ground and were checked once a week. Sticky floors were changed frequently and pheromone dispensers were renewed at least once a month.

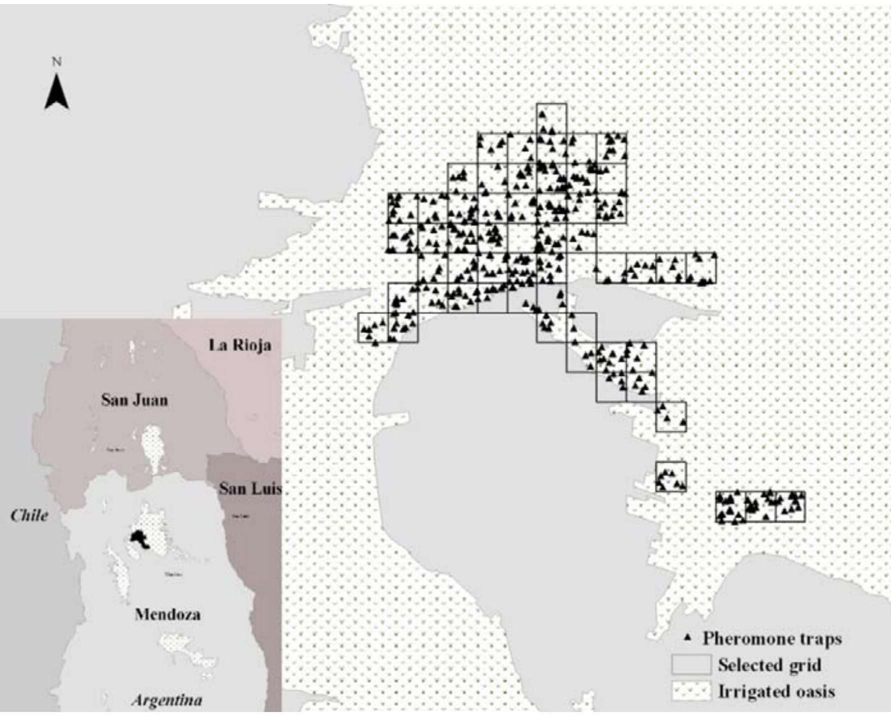

Figure 1 Distribution map of pheromone traps and selected grids in the study area.

Weather database

In order to incorporate the spatial variation of air temperature along the quarantine area on phenology model, a countrywide raster database of daily temperature was generated. Daily records of maximum and minimum air temperature (°C) provided by 124 weather stations of the National Weather Service (SMN) and the National Institute of Agricultural Technology (INTA) were interpolated at spatial resolution of 2km, according to the methodology proposed by Blanco *et al.* (2010). Digital terrain model of the Shuttle Radar Topography Mission (SRTM) was used as external drift variable for Kriging algorithm (Aalto *et al.*, 2013, Stahl *et al.*, 2006, Dodson and Marks, 1997). A total of 822 raster layers were generated, one by each day from 1 July to 30 March for each agricultural seasons included in this work, 2011-2012 to 2013-2014. These raster layers were validated by generalized cross validation (Haylock *et al.*, 2008). R software (gstat, gdal and automap libraries) and QGIS 1.8 were used (R Core Team, 2012; Quantum GIS Development Team, 2013).

GIS analysis and model selection

Regional approach for *L. botrana* flight activity was analysed by means of a homogeneous polygon grid of two km. It was used to make weekly statistics of monitoring traps installed in the study area and to get temperature values from raster layers.

Following Moravie *et al.* (2006) methodology, traps with data from a single year or less than 10 catches by agricultural season were not included in posterior analysis (n = 506 pheromone traps).

Only grids with recurrent trap catches were used as input for nonlinear regression models (n = 40 grid) and for these, weekly average catches of *L. botrana* males and degree-day accumulation were independently calculated for each of the three agricultural seasons evaluated, 2011-2012 to 2013-2014.

Voltinism of *L. botrana* males was simulated based of occurrence of a maximum of four non-overlapping flights between early September and late March. According with the approach followed by Damos and Savopoulou-Soultani (2010) and

Kumral *et al.* (2005), the start of the first annual flight was determined by the steady increase of moth capture in early spring after a period of little or no capture of adults. The start of the subsequent flights were assumed to be when trap catches began to rise consistently after a period of no catch or a significant drop in moth captures. This analysis was performed independently for each of the selected grid and agricultural season.

Degree days accumulation (DD) for each selected grid from 1 July to 30 March, were calculated according to the average method developed by Baskerville and Emin (1969), by subtracting the base temperature from the average daily temperature. In this study minimum temperature threshold for development of 7 °C was considered for any of the developmental stages of *L. botrana* (Del Tío *et al.*, 2001; Gallardo *et al.*, 2009).

Nonlinear regression models were constructed using the percentage of accumulated trap catches as the dependent variable (as values between 0 and 1) and day-degrees accumulated above the minimum temperature threshold for development as the independent variable, for each flight period. The following nonlinear regression models were used:

$$\text{Logistic model } Y = {}^{C1}/_{(1 + C2\, e^{-C3\,DD})}$$

$$\text{Weibull model } Y = 1 + e^{\left(-\frac{DD}{C2}\right)^{C3}}$$

where Y is the cumulative percentage of captured moths, DD is the sum of degree days reached at the date of trap checking. Parameters C1, C2, C3 were calculated by the nonlinear regression models using Info stat Estudiantil software (Di Rienzo *et al.*, 2013).

Model performance comparison and validation

Model performance comparisons were based on the adjusted coefficient of determination (R^2), the mean square error (MSE) and the number of iterations to achieve the lowest MSE estimated by the model. It was also taking into account that the estimated coefficients were not highly correlated. Furthermore were taken into account the Akaike information criteria (AIC) and Schewatrz or Bayesian information criterion (BIC) (Quinn and Keough, 2002; Ranjbar Aghdam *et al.*, 2011). Lack to fit test was performed to compare models of two and three parameters. Model with lower MSE, on the average for the four flights analysed, was considered as reference (Mc Meekin *et al.*, 1993; Zwietering *et al.*, 1990).

Validation of the reference model was performed using data from 15 additional randomly selected grids that were not used for making the original model (validation grid). For each estimated flight the cross-validated correlation coefficient (R^{2*}), between the validation data and the estimates of the dependent variable of the model obtained with the original data was calculated (Dos Santos and Porta Nova, 2007). Residual values calculated for both data sets were compared by means of the Kolmogorov Smirnov test.

The intrinsic variability of raster of mean daily maximum and minimum temperature, was evaluated through the estimation of the root mean square error (RMSE) of each pixel in the study area, from 1 July to 31March (Ali and Abustan, 2014, Degaetano and Belcher, 2006).

On the basis of the reference model, estimated voltinism of *L. botrana* for the last 24 growing seasons was simulated. Point based temperature statistic of weather stations in the study area, since 1990 to 2014, were considered. Subsequently the percentiles of 5% and 50% adult emergence date were calculated.

Results

Figure 2 shows the weekly evolution of trap catches of *L. botrana* and degree days accumulation in the study area, for the growing season 2011-2012, 2012-2013 and 2013-2014.

Pooled nonlinear regression equation for accumulated trap catches by grid versus day-degrees accumulation for each flight is presented in Table 1. Two models showed a very high prediction capability, as i s indicated by the mean square error values and the coefficient of determination (R^2). R^2 is above 91% for all flights and regression models considered. Therefore, it can be deduced that a high proportion of the variability observed in the cumulative percentage of male catches of *L. botrana* can be explained by the accumulation of degree days from 1 July for the four flight periods analyzed.An increased variation with succeeding generations could be observed and this deviation could be due to population sizes and overlapping generations that varied considerably among some of the data series.

Statistics for model performance comparison are presented in Table 2. In this study, Weibull equation shows, on average for the different analyzed flights, the lowest MSE, AIC and BIC values. Although compared with the Logistic model, Weibull required a greater number of iterations to achieve the best fitting. Because of this, we consider the Weibull equation achieved the best estimate of the observed variability in the percentage of adult catches in the quarantine area of Mendoza (Argentina), in relation to degree-day accumulation.

Test the lack of fit (*F*) showed significant differences with Logistic regression model in the third and fourth flight ($p < 0.05$). Differences in the AIC and BIC between the reference model and Logistic models were very strong for the 2nd, 3rd and 4th flight periods (Jan Wagenmakers and Farrell, 2004).

Estimation of the intrinsic variability of input temperature raster data, applied to calculation of the cumulative degree-days in the quarantine area, showed a Root-mean-square error (RMSE) that averaged 1.82 °C for daily maximum temperature and 2.05 °C for daily minimum temperature.

There was a good fit between the values obtained experimentally in the validation grids and the predicted equation for *L. botrana* moth phenology for all flights. Cross-validated correlation coefficient (R2*) obtained by 1st flight were of 0.871; by 2ndflight: 0.809 and by the 3rd and 4th flight of 0.795 and 0.773, respectively. Residual values calculated with both sets of data using the Kolmogorov-Smirnov test showed no statistically significant differences for any of the four flights analyzed ($p > 0.05$).

According to these results it can be expected that 50% of male adult emergence for the first flight occurs at 443.9 ± 2.3 DD; in the second flight at 1211.7 ± 4.5 DD; while in the third and the fourth flight, when the accumulation of degree days reach values of 2077.8 ± 4.7 DD and 2905 ± 3 DD, respectively (Fig. 3).Table 3 shows the percentiles of the predicted dates for 5% and 50% cumulative male catches, according to Weibull voltinism simulation, for the last 24 growing seasons.

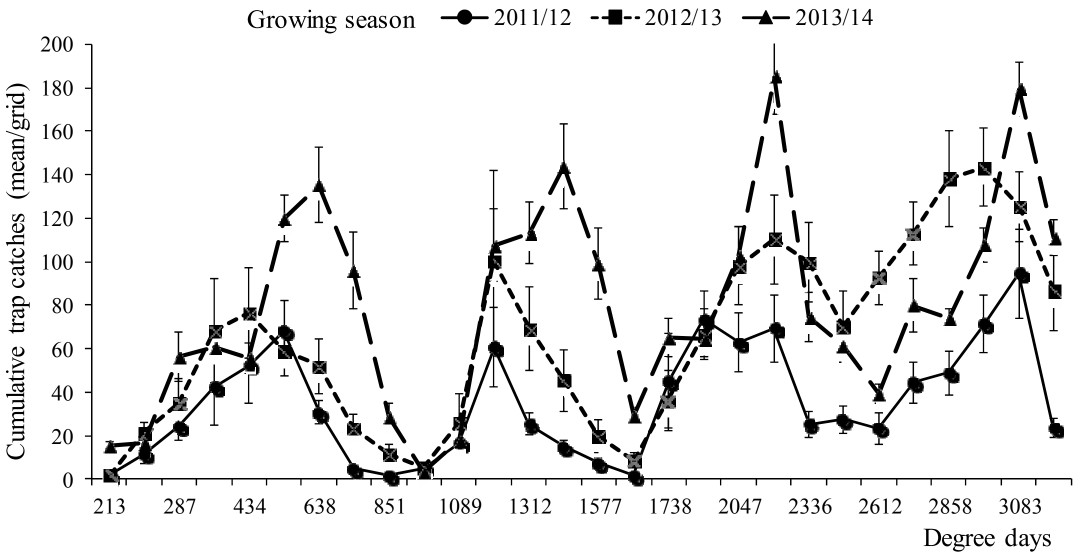

Figure 2 Evolution of *L. botrana* trap catches for each growing season. Average male catches by grid/week and standard error (n = 40).

Table 1 Parameters of the nonlinear regression models for describing the relationship between degree-days and the cumulative proportion of adult males of *Lobesia botrana*, by grid.

Voltinism	Model	Equation parameters			MSE	R^2
		C_1	C_2	C_3		
1° flight	Logistic	1.01 ± 0.01	128.0 ± 10.92	$0.01 \pm 2.2E^{-4}$	0.0029	0.97
	Weibull		496.9 ± 1.69	3.25 ± 0.05	0.0026	0.97
2° flight	Logistic	0.94 ± 0.01	$2.4E^{+7} \pm 1.4E^{+7}$	$0.02 \pm 5.5E^{-4}$	0.0135	0.93
	Weibull		1262.7 ± 3.25	8.89 ± 0.26	0.0104	0.95
3° flight	Logistic	0.97 ± 0.01	$3.4E^{+9} \pm 1.7E^{+7}$	$0.01 \pm 3.9E^{-5}$	0.0101	0.93
	Weibull		2155.5 ± 3.31	9.99 ± 0.21	0.0043	0.96
4° flight	Logistic	1.00 ± 0.01	$9E^{+9} \pm 9.7E^{+7}$	$0.01 \pm 1.5E^{-5}$	0.0114	0.91
	Weibull		2960.1 ± 2.1	19.80 ± 0.36	0.0031	0.97

Abbreviations: MSE = Mean squared error, C1, C2, C3= Parameters calculated by nonlinear regression models and standard error.

Table 2 Nonlinear regression models performance comparison.

Voltinism	Model	*df*	AIC	BIC	Δ BIC	Δ AIC	*F*	*p*
1° flight	Logistic	1077	-1373	-1356	44	48	114.35	0.074
	Weibull	1078	-1416	-1404				
2° flight	Logistic	717	-575	-561	65	68	176.72	0.059
	Weibull	718	-640	-629				
3° flight	Logistic	837	-706	-690	220	224	692.05	0.030
	Weibull	838	-926	-914				
4° flight	Logistic	717	-839	-844	60	43	686.63	0.031
	Weibull	718	-898	-887				

Abbreviations: AIC: Akaike information criterion, BIC: Bayesian information criterion.
Δi (AIC) = AICi-minAIC; Δi (BIC) = BICi-minBIC.

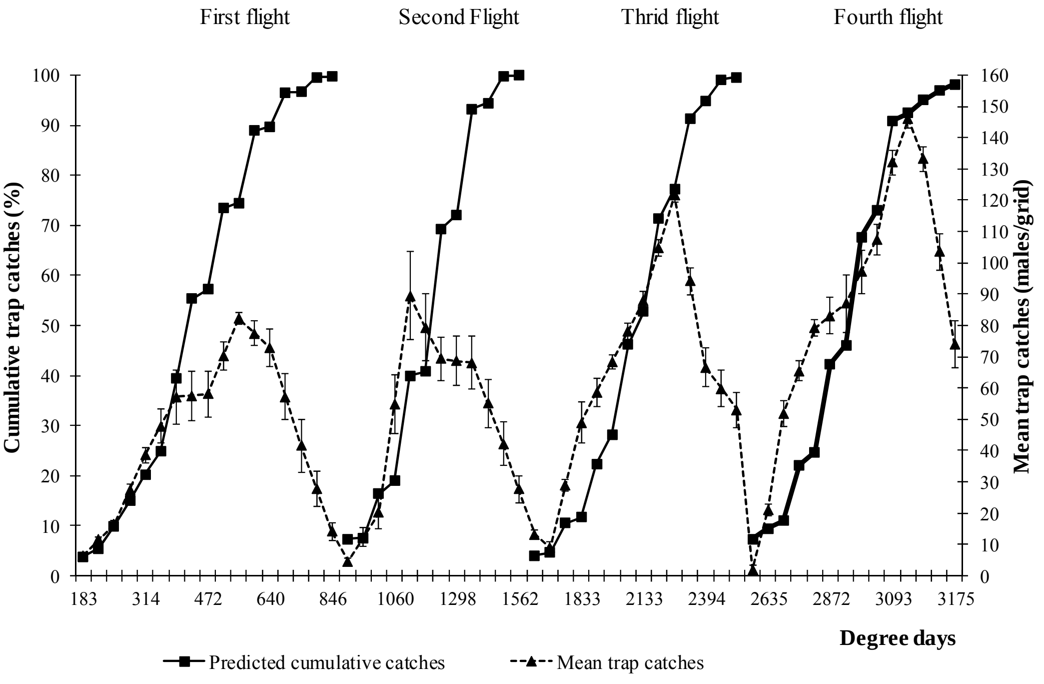

Figure 3 Observed and predicted data for adult emergence of *L. botrana* in Mendoza.

Table 3 Percentile distributions of predicted dates for cumulative male catches, according to Weibull nonlinear regression, for the last 24 growing seasons in Mendoza (Argentina).

Predicted flight activity	Percentil 10	Percentil 50	Percentil 90
5% cumulative catches			
1° flight	53 (Aug-22)	68 (Sep-06)	84 (Sep-22)
2° flight	127 (Nov-04)	134 (Nov-11)	143 (Nov-20)
3° flight	170 (Dec-17)	175 (Dec-22)	183 (Dec-30)
4° flight	220 (Feb-05)	225 (Feb-10)	239 (Feb-24)
50% cumulative catches			
1° flight	88 (Sep-26)	97 (Oct-05)	107 (Oct-15)
2° flight	146 (Nov-23)	153 (Nov-30)	162 (Dec-09)
3° flight	195 (Jan-11)	199 (Jan-15)	209 (Jan-25)
4° flight	242 (Feb-27)	247 (Mar-04)	259 (Mar-16)

Days from July 1. Estimated calendar day in Argentina in brackets.
n = 24 growing seasons (1990 to 2014).

Discussion

The present study agreed with the results from the preliminary analysis for the first two years of the monitoring program of *L. botrana* in the quarantine area in Mendoza, Argentina (Heit *et al.*, 2014). Although, *L. botrana* is trivoltine in Mediterranean latitudes (Stefanos *et al.*, 2005; Martin-Vertedor *et al.*, 2010), observational evidences suggest that *L. botrana* displays four annual flights in this newly invaded area.

Voltinism studies of *L. botrana* in Europe have only described predictive equations for two or three annual generations, using different biofix and lower developmental threshold (Del Tío *et al.*, 2001; Milonas *et al.*, 2001; Armendáriz *et al.*, 2007, 2009; Gallardo *et al.*, 2009).For this reason, it is difficult to make a comparison of the thermal constant found in this work with prior studies of *L. Botrana* voltinism in its endemic area.

For example, Amo-Salas *et al.* (2011), predicted maximum flight of the first, second and third generations of *L. botrana*, in Ribera del Duero region (Spain),at 144 DD, 666 DD and 1216 DD above a minimum threshold of 10ºC,from January 1st. Using the same biofix and base temperature, in Italy, Caffarelli and Vita (1988) estimated the occurrence of the first generation flight peakat236 DD, the second 782 DD and the third when at least 1462 heat units were accumulated.

Other authors have chosen 1 March as the date from which to start computing the degree-days. In two regions of Greece, Milonas *et al.* (2001) estimated degree days required for the first generation of *L. botrana* from 276 to 334 DD, the second from 752 to 834 DD and the third generation from 899 to 1197 DD (with a baseline of 6.45 °C). Gallardo *et al.* (2009), estimated degree-day accumulations corresponding to 50% of captures for the second generation to be 902 DD, above a minimum threshold of 7 ºC.

The high variability in the patterns of adult emergence of *L. botrana* reported under conditions of field studies, is not only limited to differences between study areas, but also occurs between different generations of the same year or between different agricultural seasons (Briere and Pacros, 1998; Del Tío *et al.*, 2001; Milonas *et al.*, 2001). However, it is not a specific attribute of *L. botrana*, since the existence of variation in adult eclosion time has been reported in other tortricid species (Rock *et al.*, 1993; Milonas and Savapolous, 2006).

Nutritional quality of the host, photoperiod, microclimatic conditions, increasing overlap between generations as grapevine phenology progresses or even the reduction of trapping efficiency of pheromone traps usually observed over time, can function as sources of variability and thus reduce the predictive power of the phenology model (Milonas *et al.*, 2001; Rakefet *et al.*, 2009; Pavan *et al.*, 2010).However from a practical point of view, the applications of temperature driven models to the study of temporal flight patterns to an invasive species could help in assembling effective forecasting systems for application in eradication programs.

Quality of the temperature data is further limited by the variable distances between the weather stations and vineyards being monitored even after the altitude correction. Limited number of official weather stations on the Cuyo Region must be assumed as a priori structural characteristic of the system itself; however, the input temperatures used in this work showed an acceptable RMSE value (Maurer and Hidalgo, 2008; Degaetano and Belcher, 2006).

Since knowledge on the *L. botrana* phenology in Argentina is still limited, this work presents an analysis of field monitoring data from three successive years and proposes a series of equations that describe the flight patterns of adults of *L. botrana* in the quarantine area of Mendoza, where this species is under official control.

The regional approach adopted in this work could explain a large proportion of the variation found in field data and has reasonable predictive and explicative capabilities as a component in the ongoing prospective analysis of the invasive potential of *L. botrana* in Argentina.

Acknowledgments

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تجزیه و تحلیل سه سال فعالیت پرواز کرم خوشه‌خوار انگور *Lobesia botrana* (Lepidoptera: Tortricidae) در یک منطقه قرنطینه

گولرمو هیت[1،2*]، والتر سیون[3] و پابلو کورتز[1،2]

۱- گروه گیاهپزشکی، دانشکده زراعت، دانشگاه بوئنس آیرس، بوئنس آیرس، آرژانتین.
۲- اداره مراقبت و پیش‌آگاهی، مرکز ملی بهداشت دامی و کیفیت محصولات غذایی، بوئنس آیرس، آرژانتین.
۳- دانشگاه خودمختار انتر ریوس، مرکز منطقه‌ای ژئوماتیکس، دیامونت، انتر ریوس، آرژانتین.
* پست الکترونیکی نویسنده مسئول مکاتبه: gheit@agro.uba.ar

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چکیده: کرم خوشه‌خوار انگور *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), یکی از آفات مهم انگور در اروپا و نواحی مدیترانه می‌باشد که اخیراً از آرژانتین و شیلی گزارش شده است. ازآنجاکه اطلاعات کمی در ارتباط با فنولوژی کرم خوشه‌خوار انگور در آرژانتین وجود دارد این مطالعه به‌منظور تهیه یک مدل فنولوژیکی برای تخمین تعداد نسل کرم خوشه‌خوار انگور در آرژانتین انجام شده است. تعداد نسل کرم خوشه‌خوار انگور براساس حشرات نر وقوع چهار پیک پرواز را نشان می‌دهد. مدل رگرسیون غیرخطی براساس متوسط شکار هفتگی و میزان روز درجه‌های تجمعی طی فصول زراعی ۲۰۱۱-۲۰۱۲ تا ۲۰۱۳-۲۰۱۴ تهیه شد. معادله ویبول نشان داد که این حشره به‌طور متوسط چهار پرواز در سال دارد که بهترین تخمین براساس درصد حشرات شکار شده و میزان روز درجه تجمعی است. می‌توان انتظار داشت که ظهور ۵۰٪ حشرات نر برای اولین پرواز در ۴۴۳/۹ روز درجه، دومین پرواز در ۱۲۱۱/۷ روز درجه اتفاق می‌افتد این درحالی است که پروازهای سوم و چهارم به‌ترتیب در روز درجه‌های ۲۰۷۷/۸ و ۲۰۹۵ مشاهده می‌شود. روش اتخاذ شده در این پژوهش می‌تواند یک پیش‌بینی منطقی از فعالیت کرم خوشه‌خوار انگور در آرژانتین را بیان نماید.

واژگان کلیدی: کرم خوشه‌خوار انگور، سیستم بازرسی، تعداد نسل