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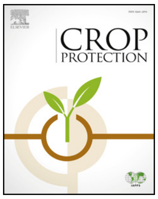

Is a biofix necessary for predicting the flight phenology of *Lobesia botrana* in Douro Demarcated Region vineyards?

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ABSTRACT

The European grapevine moth, *Lobesia botrana* (Denis and Schiffermüller), is among the most economically important vineyard pests in European and Mediterranean areas. Predicting the insect's flight phenology during the growing season is critical to improve Integrated Pest Management (IPM) tactics through better timing of sampling or control operations. The aim of this study was to characterize the flight phenology of *L. botrana* in Douro Demarcated Region (DDR) in Portugal, as well as to develop degree-day (DD) models for predicting main pest flights, based on data of male catches in sex pheromone traps and temperature data recorded over a 20-year period. Nonlinear models based on Boltzmann regression equations were developed using the cumulative percentage of male catches and accumulated DD, considering two starting points for this accumulation, a biofix (first male catch) and a calendar date (January 1st), both using 7.3°C and 33°C as lower and upper thresholds, respectively. The results obtained suggest that the cumulative percentage of *L. botrana* catches and accumulated DD are highly related, using both events as starting points for DD accumulation, although, in the case of the second and third flights, the best correlations were obtained using the model developed from January 1st. Although the use of a biofix seems to improve model's accuracy, from the practical point of view and considering large scale application for an IPM strategy, the use of a fixed calendar date (January 1st) should be preferred. These results could be useful in timing *L. botrana* control measures, especially biorational pesticides applications that require accurate information on insect phenology to be effective.

1. Introduction

The European grapevine moth, *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae), is an important vineyard pest in the European and Mediterranean areas (Ioriatti et al., 2011) and was recently found in Chile, California and Argentina (Gonzales, 2010; Varela et al., 2010). This is a multivoltine species which, according to Martín-Vertedor et al. (2010), develops four annual generations under Iberian Peninsula conditions. The larvae of first generation feed on bud clusters while the next generations feed on ripening and ripe berries. Fungi, especially grey mold, *Botrytis cinerea*, develop rapidly on the damaged grapes, causing entire clusters to rot (Fermaud and Giboulot, 1992).

The Douro Demarcated Region (DDR) is an important winegrowing area (43,670 ha) located in the Northeast of Portugal, where "Port wine" D.O.P ("Denominação de Origem Protegida") is produced. In this region, damages caused by the pest are highly variable among years, ranging from 0 to 90% of infested clusters at harvest (Carlos et al., 2014).

Traditionally, the control of *L. botrana* in DDR relies primarily on the use of insect growth regulators (IGRs), once or twice a year, against the second and/or the third generation (Carlos et al., 2010). This third generation is particularly difficult to control, since larvae quickly penetrate ripening fruit. As economic damage to grapes occurs when neonates feed on grape clusters, control measures which are applied mainly against the third generation, should primarily target *L. botrana* eggs. Therefore, precise timing of sprays with ovicides (e.g. IGRs), before the eggs hatch, is particularly important to avoid damage. Thus, the need to ensure effective and sound strategies to control this important pest requires the development of tools to support sampling programs and/or timing of insecticide sprays, to increase their efficacy,

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and so reducing their number, as well as their environmental impact. Several researchers have proposed predictive models for the development of *L. botrana,* both in the laboratory and in the field, based on the relationship between temperature and developmental rate of the insect (Baumgärtner and Baronio, 1988; Brière and Pracros, 1998; Cravedi and Mazzoni, 1994; Del Tío et al., 2001; Gabel and Mocko, 1984; Gallardo et al., 2009; Heit et al., 2015; Milonas et al., 2001; Savopoulou-Soultani et al., 1996). Other authors have studied the relationship between *L. botrana* pheromone trap catches and degree-day (DD) accumulations, using phenological models to determine the best time for spraying (Del Tío et al., 2001; Gallardo et al., 2009; Heit et al., 2015; Milonas et al., 2001; Ortega-Lopez et al., 2014). This last approach typically comprises one to several regression models. In the simpler case, cumulative counts or proportions of seasonal counts, are related to cumulative DD and predicted distribution is compared with the observed one, to measure the accuracy of the model (Hardman, 2012). Physiological models have also been developed (Amo-Salas et al., 2011; Gilioli et al., 2016; Gutierrez et al., 2012; Moravie et al., 2006; Ortega-Lopez et al., 2014; Schmidt et al., 2003), including both abiotic variables (e.g. relative humidity, photoperiod), and biotic variables (e.g. overwintering population density, mortality, fecundity, larval diet) that can impact the development of *L. botrana*. However, despite their realistic approach and their usefulness, especially when included with decision support systems, from the practical point of view and because of a lack of physiological data, the DD models have the advantages of being simpler to develop and easier to use by growers, after being validated locally. This is because timing of the emergence of *L. botrana* adults varies according to location, climate and year (Gallardo et al., 2009).

The starting point for DD accumulation is one of the main cause of uncertainty in DD models. In the previous developed models, DD accumulations started on a calendar date (e.g. January 1st used by Del Tío et al. (2001) and Lozzia and Vita (1987), March 1st used by Gallardo et al. (2009) and Milonas et al. (2001) and March 5th used by Gabel and Mocko (1984)). However, as the rate of insect development depends on temperature, forecasts should be improved by starting DD accumulations from a biological event or biofix. Accordingly, Riedl et al. (1976) and Jones et al. (2013) found that, for codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), the model accuracy could be improved by using the first moth catch to synchronize the model to field populations. Thus, Blomefield and Giliomee (2014), Jones et al. (2013) and Joshi et al. (2016) used this biological event to start computing DD for this pest. To our knowledge, no studies have investigated the possibility of using a biofix to start DD accumulation for *L. botrana.*

The present study aimed to describe the flight phenology of *L. botrana* in DDR, in relation to accumulated DD, as well as to develop DD models, as key tools to improve monitoring and forecasting of the pest in the studied region, using two different starting points for DD accumulation: a calendar date (January 1st) and a biofix (first male catches on traps). Specifically, through the analysis of pheromone trap catch data collected over a 20-year period in relation to accumulated DD, our study aims to (1) describe the beginning and the occurrence of 50% of catches of each flight of *L. botrana*; (2) develop nonlinear models considering two different starting points for DD accumulation; 3) and validate such models in DDR conditions. Such information may be important when optimizing *L. botrana* control measures within an integrated pest management (IPM) approach.

2. Material and methods

2.1. Study area

The DDR consists of three sub-regions: Baixo Corgo (BC), Cima Corgo (CC), and Douro Superior (DS) (Fig. 1 A). The western most part of the region is approximately 70 km from the coast and the eastern most areas border with Spain (Fig. 1 B).

The climate of DDR is characterized by a strong inter-annual consistency of total insolation, temperature, and potential evapotranspiration and significant inter-annual variation in precipitation. From the analysis of the WorldClim database for the 1950-2000-time period (Hijmans et al., 2005) during the active growth stages of grapevines (April to September), the average rainfall varied between 189 and 326 mm, while it was only 50–85 mm during the ripening stage (from July to September). The low precipitation along with significant temperature and radiation availability give rise to situations of intense summer water stress, particularly in the Cima Corgo and Douro Superior sub-regions. For the period 1950–2000, growing season temperatures (April–October) averaged 17.8°C, over the entire region, but ranged from 12.1°C, in the upper elevations in the Baixo Corgo, to 19.7°C, in the warmest areas in the Douro Superior. Overall, the region is 65% intermediate climate, 24% warm climate and nearly 10% 'hot climate (Jones and Alves, 2012). For the 1931–1960 time period, the variation range, in average maximum temperatures during the growing season was nearly 8°C, from 22.4 to 30.3°C. During August, which was the warmest month of summer, maximum values reached 37.0°C. For these reasons, viticulture in the DDR is carried out over a considerable area of the land under moderate to very severe water stress conditions (Jones, 2012).

The present study was conducted between 1989 and 2016 (20-years of data) in nine wine farms located in Baixo Corgo and Cima Corgo sub-regions (Fig. 1 A, Table 1). The studied vineyards were grown either on terraces or on vertical rows at varying altitude (92–222 m a.s.l) (Table 1). Vineyards were grown under IPM guidelines, with 1 or 2 applications of insecticide (mainly IGRs) against the second and/or third generation of *L. botrana*, using an economic threshold level of 1–10% damaged clusters. The soil was maintained with natural vegetation between rows and use of herbicide within rows.

2.2. Data collection

The flight activity of *L. botrana* males was monitored, in each vineyard, using a pheromone Delta trap (AgriSense BCS Ltd.) with sticky base, baited with 1 mg synthetic sex pheromone (E7,Z9-12:Ac). Traps were hung 1.0–1.3 m above ground and checked weekly.Sticky base and pheromone lures were both replaced monthly. Traps were installed in early March and maintained until late September/mid-October. Temperature data were obtained from several meteorological stations located either in farms or at its proximity ($<$ 5 km).

Males emerging from overwintering pupae constituted the flight of the overwintering generation, here designated as first flight. The beginning of the first flight was that of the first catch in early spring. According to Magalhães (2006), the peak of *L. botrana* oviposition in DDR region occurs at about the same time as the peak of male catches in pheromone traps, which in turn coincides with the period when 50% of the individuals of the flight are captured.

2.3. Development of models

Degree-days (DD) were computed using the UC IPM Web degree-day calculator developed by the University of California (http://www.ipm.ucdavis.edu/WEATHER/ddretrieve.html), using a single sine wave function (Allen, 1976). An upper intermediate cutoff was selected to slow down DD accumulation and avoid overestimation of heat units, when temperatures rise above the upper threshold.

The lower and upper development thresholds were defined as 7.3°C and 33°C, respectively (Savopoulou-Soultani et al., 1996; Brière and Pracros, 1998). DD were calculated, for each flight and each sampling station, using two events as starting point for accumulation, a calendar date (January 1st) and a biological event (first male catches).

Based on data collected over a 20-years period, flight of *L. botrana* in DDR was described in terms of DD required for the occurrence of its main flight events (beginning and occurrence of 50% of catches), with these data hereafter designated as “observed data”. Observed data are