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A degree-day phenological model for *Cydia pomonella* and its validation in a Mediterranean climate

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Abstract

The codling moth *Cydia pomonella* (L.) (Lepidoptera Tortricidae) is a pest of worldwide importance that exhibits seasonal phenology that is mainly affected by temperature. We developed and validated a simple degree-day (DD) phenology model with reference to a temperate - Mediterranean climate. Sixteen data sets were collected from apple orchards in Greece from 2011 to 2014. Using the data from 2011 and 2012, a 3-parameter non-linear regression model was constructed to fit cumulative moth captures as a function of accumulated degree days (Biofix 1st January, T_L = 10.1 °C). The model described moth phenology of all three male flights with high accuracy ($R^2 > 0.9$ in all cases). The first males of the first overwintering generation across all observation years were caught between 250-300 DD, and the population peaked at 468 accumulated degree days. The first males of the second flight were caught between 850-900 DD, and the population peaked at 1130 DD. Moreover, the first males of the third flight were caught after 1500 DD, and the population peaked at 1815 DD. Subsequently, using the moth capture data from 2013 and 2014, the predictions of the model were validated. The overall model performance was higher for the first flights compared to the second and third as reported by the root mean square error (RMSE) and enhanced counter plots. The phenological model developed in this study is expected to be useful for field applications in integrated pest management (IPM) systems, for example, to forecast optimal spray times for available insecticides and application of other control measures.

Key words: codling moth, insect phenology, voltinism, IPM, temperature.

Introduction

A key component of developing sound integrated pest management (IPM) strategies is the study and comprehension of emergence patterns and phenology of pests in the field (Welch *et al.*, 1978; Prues, 1983; Cravedi and Jörge, 1995; Cross and Berrie, 2001). Since many pest control methods must be timed precisely, managers often attempt to predict populations based on pest monitoring in order to manage outbreaks (Pajac *et al.*, 2012). Furthermore, conventional insecticides are increasingly being excluded from the European Union (EU) authorization lists, and more environmentally friendly products are becoming more widely adopted. Insect growth regulators and biological pesticides in particular can give promising results, but rely heavily on timely application to target an insect's vulnerable stages.

The codling moth *Cydia pomonella* (L.) (Lepidoptera Tortricidae) is regarded as the most serious apple pest worldwide (Riedl *et al.*, 1976; Pickel *et al.*, 1986; Barnes, 1991; Damos *et al.*, 2015) and is increasingly becoming a problem in walnuts, pears, and a few varieties of plums. For many years, the most prevalent control tool for this pest has been the application of pesticides.

The use of traditional non-selective insecticides has been associated with a variety of problems, including detrimental environmental effects, insecticide resistance, negative impacts on natural enemies, and safety issues for pesticide applicators and the food supply (Damalas and Eleftherohorinos, 2011). Moreover, although many sustainable methods have been developed over the past years and are currently used in many European and North American countries (i.e. mating disruption, attract and kill, granulosis virus and so on), the codling moth control still depends on the use of pesticides and especially stage specific compounds (Damos *et al.*, 2015).

Reasonable and effective control of the codling moth is based on male moth population monitoring with pheromone traps and appropriate treatment interventions during the optimal stage of pest development (Knight, 2000; 2010; Knight and Light, 2005). However, trap captures are affected by a range of factors (i.e. initial pest status, rain, wind) and may be not the best option for forecasting the species phenology and establishing IPM programs (Knight and Light, 2005). Therefore, based solely on moth captures, it is quite difficult to establish sound management decisions.

In applied entomology and pest management, various empirical approaches have been used to estimate the population dynamics of insects to provide forecasts (Kocourek and Stará, 2005; Kumral *et al.*, 2008). Nevertheless, due to the large influence that temperature exerts on insect phenology, most of the models that describe insect development are driven by temperature (Damos and Savopoulou-Soultani, 2012a; 2012b), and the majority of phenology modelling efforts have been established on the basis of accumulated degree days as an expression of thermal time. This is mostly due to the simplicity of the calculation of heat summations and because degree-days are easy to be estimated by plant protection advisors. Nevertheless, even so, degree-day models require that the statistical relationship between insect phenology and temperature are reliably determined to be used as practical tools for decision making in IPM (Damos and Savopoulou-Soultani, 2010).

Phenological models have been considered by many researchers as important tools to improve the management of the codling moth in many apple-producing areas worldwide (Riedl *et al*., 1976; Brunner *et al*., 1982; Tomkins *et al*., 1987; Blago, 1992; Ahmad *et al*.; 1995, Samietz *et al*., 2007; Knight, 2007; Jones *et al*., 2008; 2013, Barros-Parada *et al*., 2015). It is recognized that models that have been developed and validated in California have been used on a routine basis for that region (Welch *et al*., 1978; Pitcairn *et al*., 1992; Knight, 2007). This is also the case in Italy and Portugal, where regional agricultural extension services uses routinely forecasting models for crop protection (Bugiani *et al*., 1996; Rosa, 2000). Yet, though the number of predictive models is steadily growing, there are always concerns regarding their applicability under different scenarios (Kuhrt *et al*., 2006; Blomefield and Giliomee, 2011; Chappel *et al*., 2015), and there are cases in which a model developed for a region is inaccurate when applied under different conditions (Damos *et al*., 2014). For example, cumulative flight curves for the first generation of a *C. pomonella* model developed for one location were statistical different compared to another (Knight, 2007; Joshi *et al*., 2017). The potential impact of such change in model development and the characterization of codling moths phenology on the effectiveness of insecticide programs is obvious.

Furthermore, in most cases, the first adult capture in a pheromone trap is used as a biofix, and degree-days are accumulated thereafter. Unfortunately, a biological biofix is often difficult to be established especially in cases were population pressure is low, such as under conditions of mating disruption (MD), pesticide application, abrupt weather conditions, etc. Actually, it is of interest to establish a calendar biofix, rather a biological one, because it simplifies management and eliminates mistakes associated with poor trap catch, particularly in low-pressure situations where MD reduces trap efficiency (Jones *et al*., 2008).

It is also expected that the thermal constants that are used as thresholds for heat summations may differ among stages and/or populations of the same species due to diverse aspects of temperature acclimatisation and adaptation of each population to its environment as well as levels of tolerance. Other factors such as photoperiod, humidity, and nutrition may also affect the bionomics and related phenology of *C. pomonella* (Peterson and Hamner, 1968; Wenniger and Landolt, 2011). For instance, in Mediterranean temperate climates (e.g. Greece), the species may break and terminate diapause earlier due to higher temperatures and may complete one additional flight. Thus, a good performance for a respective model developed under particular circumstances will have no utility, if its predictions in other locations have ambiguous biological meaning.

Actually, most of the available models have been developed in areas characterized as Mediterranean like climate but normally are associated with other climates, such as much of the Pacific Northwest, much of southern Chile, part of west-central Argentina, and parts of New Zealand (Belda *et al*., 2014). Few exceptions in the Mediterranean include mostly region specific models developed in Italy (i.e. Emilia-Romagna; Butturini *et al*., 1993; Tiso and Butturini, 1999). In the Pacific Northwest, for example, the major fruit growing regions are in central Washington State and are characterized as Cwa climates according to Köppen (1923) and only few apple growing areas in Oregon State are clarified as Csa and most of them are Csb.

The climate of the Mediterranean basin and northern Greece in particular, is classified as Csa Mediterranean climatic type (Köppen, 1923). This climatic system is characterized by hot dry-summer climate in contrast to oceanic climates, which are often referred as Mediterranean, but are classified as Csb climatic type and are characterized by cool dry-summer conditions (i.e. the warmest month is below 22 °C). Furthermore, as in every climatologic domain, region specific high continental locations of the Mediterranean domain can present cooler temperatures during the winter which can sometimes prohibit the diapause termination affecting pest emergence and population phenology (Damos and Savopoulou-Soultani, 2009).

Since climate may have an impact on a species phenology and its thermal adaptation, the development of suitable regional-specific phenology models is a prerequisite for successful design, development, and application of expert system technology in modern fruit production and a major challenge for solving practical decision-making problems in region specific IPM systems. The above reasons as well as the species- and stage-specific plasticity affected by local temperature conditions give important reasons to develop and validate a region-specific phenological model of *C. pomonella* for Greece and with reference to other Mediterranean locations.

The primary objective of the current study was to develop and validate a degree-day phenological model that would reliably predict male moth emergence and population dynamics of the *C. pomonella* during the growth season in Mediterranean climates. Predicting pest phenology, such as the first flight of codling moth, is helpful to predict and detect events that are important, but not easily sampled, such as peak moth flight and the related period of oviposition activity.

Materials and methods

Study area and monitoring system

Field experiments and observations were conducted in Veria (northern Greece) to study the flight and bionomics of *C. pomonella* during 2011-2015. The experimental apple orchards consisted of four plots (≈0.8 ha each) planted with Granny Smith cultivars belonging to the fruit cooperative Messi Coop$^{®}$ (40.30089°N 22.15239°E) and were situated in the same district in a distance 3-5 km. The tree height was ≈2.5-3.5 m, and the trees were ≥ 10 years old. Three cardboard Delta traps (Pherocon, pheromone traps; Trécé Inc., Salinas, CA, USA), were used in each experimental orchard with sticky inserts baited with synthetic sex pheromone [mixture of 3.0 mg of (*E*, *E*)-8, 10-dodecadien-1-ol and 3.0 mg of ethyl (2*E*, 4*Z*)-2, 4 decadienoate]. Traps were placed on the perimeter of each orchard and having a distance > 70 m between them in a tree branch at 1.5-2 m above the ground in early April and were inspected for moth captures twice weekly from April till the end of September. No pesticide treatments were applied on the tree rows with the traps or on the adjacent ones during the observation period. Moths were removed after counting, lures were replaced every month, and sticky inserts were changed if necessary.

Males of *C. pomonella* that emerged from overwintering larvae constituted the first flight. The start of this flight was determined by the first moth catches in early spring. The second flight correspond to individuals of the first annual generation and the third flight to individuals of the second generation. The start of these subsequent flights was assumed to be the time when moth catches began to rise consistently following a period of few or no catches. Hence, the period between the start of each flight and the start of the subsequent flight was considered as the generation time (Milonas and Savopoulou-Soultani, 2006; Damos and Savopoulou-Soultani, 2010). In some cases in which the moth flight patterns were characterized by a bimodality activity during very short time intervals, they were considered belonging to the same generation.

Moth capture data from 2011 and 2012 were used to estimate the parameters for the phenological model, which was used to simulate the adult flights during the growth season, while data from 2013 and 2014 were used to validate the model predictions for the adult population dynamics.

Weather recordings and accumulated degree days

Daily minimum and maximum air temperature data were obtained by a HOBO data logger (Onset Computer Corporation, USA) placed in the experimental region and compared, for confirmative reasons, with data of the national observatory of Athens through a meteorological station, which is situated in the same area and level of the experimental orchards.

Degree days were calculated using the triangular method (Zalom *et al.*, 1983):

$$\begin{cases} T_U - T_L, & if\ T_{max}, T_{min} > T_U \\ 0, & if\ T_{max}, T_{min} < T_L \\ \dfrac{6(T_{max} + T_{min} - 2T_L)}{12}, & if\ T_{max} < T_U\ and\ T_{min} < T_L \\ \dfrac{6(T_{max} - T_L)^2}{T_{max} - T_{min}}\left(\dfrac{1}{12}\right), & if\ T_{max} < T_U\ and\ T_{min} > T_L \\ \dfrac{6(T_{max} + T_{min} - 2T_L)}{12} - \left[\dfrac{6(T_{max} - T_U)^2}{T_{max} - T_{min}}\dfrac{1}{12}\right], & if\ T_{max} > T_U\ and\ T_{min} < T_L \\ \left[\dfrac{6(T_{max} - T_L)^2}{T_{max} - T_{min}} - \dfrac{6(T_{max} - T_U)^2}{T_{max} - T_{min}}\right]\dfrac{1}{12}, & if\ T_{max} > T_U\ and\ T_{min} > T_L \end{cases}$$

We used temperature thresholds, where T_u = 34.4 °C is the upper temperature threshold, and T_L = 10.1 °C is the lower temperature threshold (Pitcairn *et al.*, 1992; Jones and Wiman, 2008; Ranjbar-Aghdam *et al.*, 2011). T_{min} and T_{max} are the minimum and maximum daily temperatures, respectively, and degree days were summed after January 1st.

Phenological model

The following three-parameter non-linear regression model was fitted to the data (Damos and Savopoulou-Soultani, 2010):

$$F(x;\alpha,\beta,\gamma) = \frac{a}{1+e^{-\left(\frac{x-\gamma}{\beta}\right)}} + \varepsilon_i \qquad (1)$$

In equation (1), F is the cumulative percentage of moth catches, x is the number of accumulated degree days, and ε_i is the standard error term that is assumed to have a normal distribution and zero variance. The behaviour of this curve is affected by three key constant variables: α, β, and γ. The model is quite flexible since the curve can be twisted around to fit most conceivable variations of its basic shape depending on the parameter values; α and β designate the upper and lower asymptotes, respectively, and set the vertical limits of the curve. The parameter γ is the gradient that sets the length of time of the curve, which represents the time (DD) of 50% moth emergence. Extreme outliers were removed, cumulative data were grouped across years (Knight, 2007) and the model was applied to each of the three successive *C. pomonella* flights using the moth capture data sets from 2011 and 2012. In all cases, parameter estimation was based on an iterative ordinary least square method (OLS) using the Levenberg-Marquard algorithm, in which the cumulative moth catches were treated as the independent variable.

Model performance statistics

Analysis of variance (ANOVA) was performed to detect the overall significance of the regression model separately for each generation flight, while the t statistic was applied to test the null hypothesis that the coefficients of the independent variable are zero (Sokal and Rohlf, 1995). The variability of the data was estimated using the residual mean square MS_{reg}:

$$MS_{reg} = SS_{reg}/df \qquad (2)$$

$$MS_{res} = SS_{reg}/df \qquad (3)$$

where SS_{reg} denotes the sum of squares, and *df* are the degrees of freedom, which represent the number of observations and variables in the regression equation.

Additionally, ANOVA was performed for the regression, and the corresponding F ratio was estimated. The F test statistics gauge the contribution of the degree-day heat summation in predicting the moth emergence and can be written as the following ratio:

$$F = MS_{reg}/MS_{res} \qquad (4)$$

A Shapiro-Wilks (W-statistic) was applied to test the null hypothesis that the data are sampled from a normal distribution and a Spearman rank correlation constant variance test was performed to detect whether or not the data have constant variance (i.e. homogeneity of variance).

Given a set of observations x_1, x_2, …, x_n, the W-statistic is defined by:

$$W = \left(\textstyle\sum_{k=1}^{n} a_k\, x_k\right)^2 / \left(\textstyle\sum_{k=1}^{n} x_k - \bar{x}\right)^2 \qquad (5)$$

where $\bar{x} = 1/n \sum_{k=1}^{n} x_k$ is the sample mean, and $a_k, k = 1,2,\dots,n$ are the weights that only depend on the sample size n. All statistical tests were performed at p = 0.05 significance level (R Development Core Team, 2008).

Model validation statistics

The forecasting performance of the phenological model was validated separately for the data sets from 2013 and 2014. Two criteria were used to validate the model: the distribution of the absolute prediction error and the root mean square error (RMSE). The absolute prediction error is (Shcherbakov *et al*., 2013):

$$\varepsilon_i = (y_i - f_i^m) \qquad (6)$$

where y_i is the measure values at observation i, f_i^m is the predicted value of observation i, which is obtained from the use of the forecasting model. The distribution of ε_i is shown using a boxplot for the predicted and observed values and is pooled over the data from 2013 and 2014.

The RMSE was used as an absolute performance statistic (Pitcairn *et al*., 1992). The RMSE is based on the difference between the predicted value $\hat{y}_i$ and the actual value y_i for the observation i, and it was estimated by the package HydroGOF in R software (R Developmental Core Team, 2008):

$$RMSE = \sqrt{1/n \sum_{k=1}^{n} (\hat{y}_i - y_i)^2} \qquad (7)$$

Lower values of RMSE indicate a better fit. The RMSE was estimated for each flight generation and year separately (R Development Core Team, 2008).

Results

Accumulated degree-days

In most cases, after the end of February to 10-20 March, the average temperatures were higher than the species lower threshold of development (figure 1). As a result, the heat summation patterns were quite similar during the four growing seasons. A slight deviation was observed in 2012, when higher temperatures occurred earlier. Hence, in northern Greece, which consist of a typical Mediterranean region, temperatures above the lower threshold of codling moth development begin after March which is quite early in the growth season.

Flight patterns of *C. pomonella*

The number of trapped males was higher in 2011 compared to 2012, 2013, and 2014 (figure 2). Moreover, in 2014, a considerably lower number of moths were captured compared to 2013. In most cases, three discrete flights were observed, although in a few cases, bimodal patterns were observed (figure 2).

During 2011 and 2012, the first captures were recorded in the end of April and early May. The peak of the moth captures occurred in May and early June and decreased after mid-June. The first moth captures of the second flight were observed in mid and late June in 2011 and 2012, respectively. Moreover, the occurrence of the highest moth captures for the second flight appeared in mid and late July during 2011 and 2012, respectively. The population of the third flight during 2011 peaked in late August, while in 2012, population dynamics showed a more constant level of captures during August. The latest captures were observed in early September.

In 2013, the overwintering generation lasted from the end of April to late May. The second flight lasted from June to mid-July, and the third lasted from late-July to late August. In 2014, the first flight patterns of *C. pomonella* were quite analogous to that of 2013. However, overall the moth captures were lower in 2013 compared to 2014 and the third flight seems to develop later in early spring and late summer.

Phenological model

The cumulative proportion of male *C. pomonella* moths captured in 2011 and 2012 was used within nonlinear regression models for each flight, and the generated models are presented in figure 3. The estimated regression parameters of the nonlinear models are listed

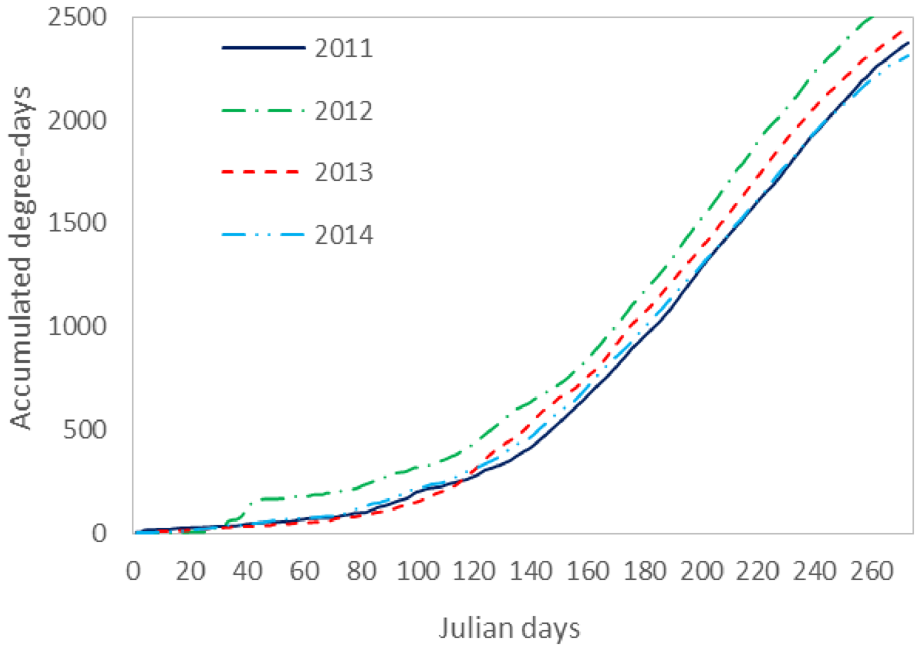

Figure 1. Effective field temperature summations (>10 °C) accumulated after January 1st in the area of Veria in northern Greece from 2011 to 2014.

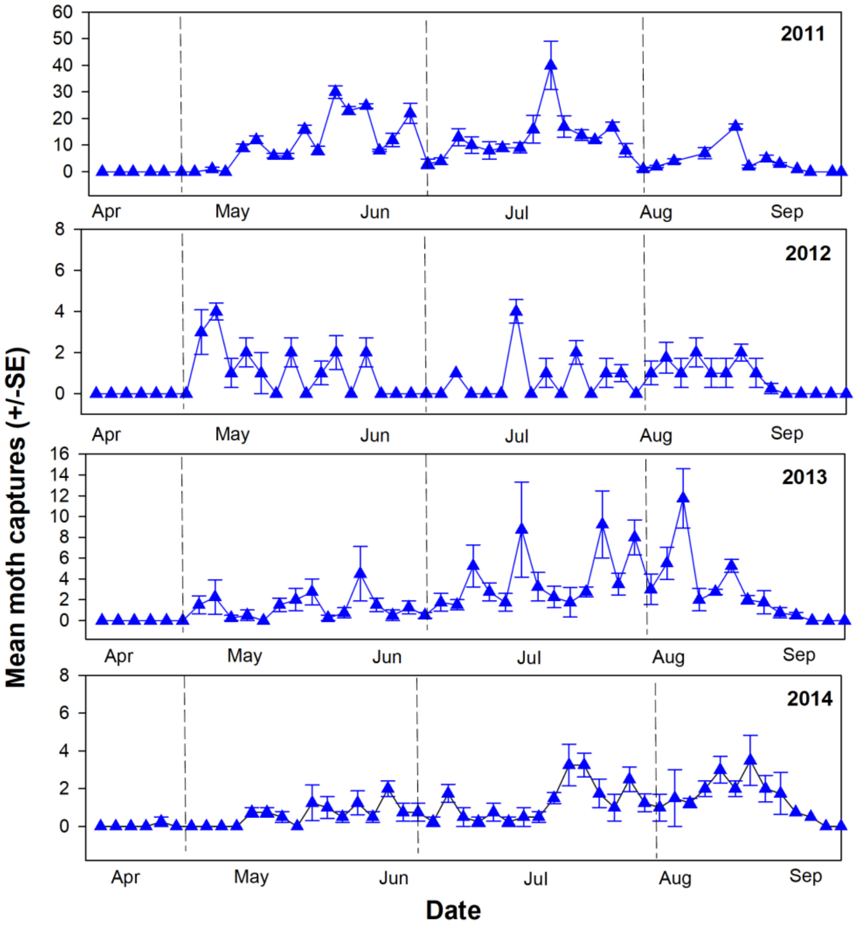

Figure 2. Flight patterns of *C. pomonella* male moths during four successive growth seasons (2011, 2012, 2013, and 2014) in northern Greece (average moths caught and standard error of mean; vertical dotted lines represent different flight periods).

Table 1. Parameter estimations of the phenological model and related nonlinear regression statistics with respect to each male moth flight.

Flight	Parameter	Model performance statistics				
		Coefficient	Std. Error	t	p	R^2
	α	98.7583	3.4820	28.3628	<0.0001	
1^{st}	β	69.5118	6.9099	10.0597	<0.0001	0.9690
	γ	468.8300	8.6795	54.0155	<0.0001	
	α	98.7505	3.4145	28.9209	<0.0001	
2^{nd}	β	99.9595	9.4227	10.6083	<0.0001	0.9704
	γ	1130.9937	12.2312	92.4682	<0.0001	
	α	106.1449	4.8139	22.0497	<0.0001	
3^{rd}	β	110.2595	12.2365	9.0107	<0.0001	0.9767
	γ	1815.8485	16.5084	109.9953	<0.0001	

in table 1. The cumulative emergence of all three flights was described well by the nonlinear regression model. The regression coefficients (R^2) were 0.9690, 0.9704 and 0.9767 for the first, second, and third flight, respectively. Furthermore, the nonlinear regressions were significant ($p < 0.05$) in all cases. Hence, a high proportion of the variability observed in the cumulative percentage of male catches of *C. pomonella* in relation to the accumulation of degree days was explained by the nonlinear regression model (table 2).

Additionally, the parameter γ, which represents the 50% of cumulative moth emergence, provided a robust estimation of the population peak for each flight. The moths of the first flight across all observation years were captured after 250-300 DD, and the population peaked at 468 accumulated degree days ($t = 54.015$, $df = 31$, $p < 0.0001$). The first males of the second flight were caught at 850-900 DD, and the population peaked at 1130 DD ($t = 92.468$, $df = 31$, $p < 0.0001$). Moreover, the first males of the third flight were caught after 1500 DD, and the population peaked at 1815 DD ($t = 109.995$, $df = 31$, $p < 0.0001$).

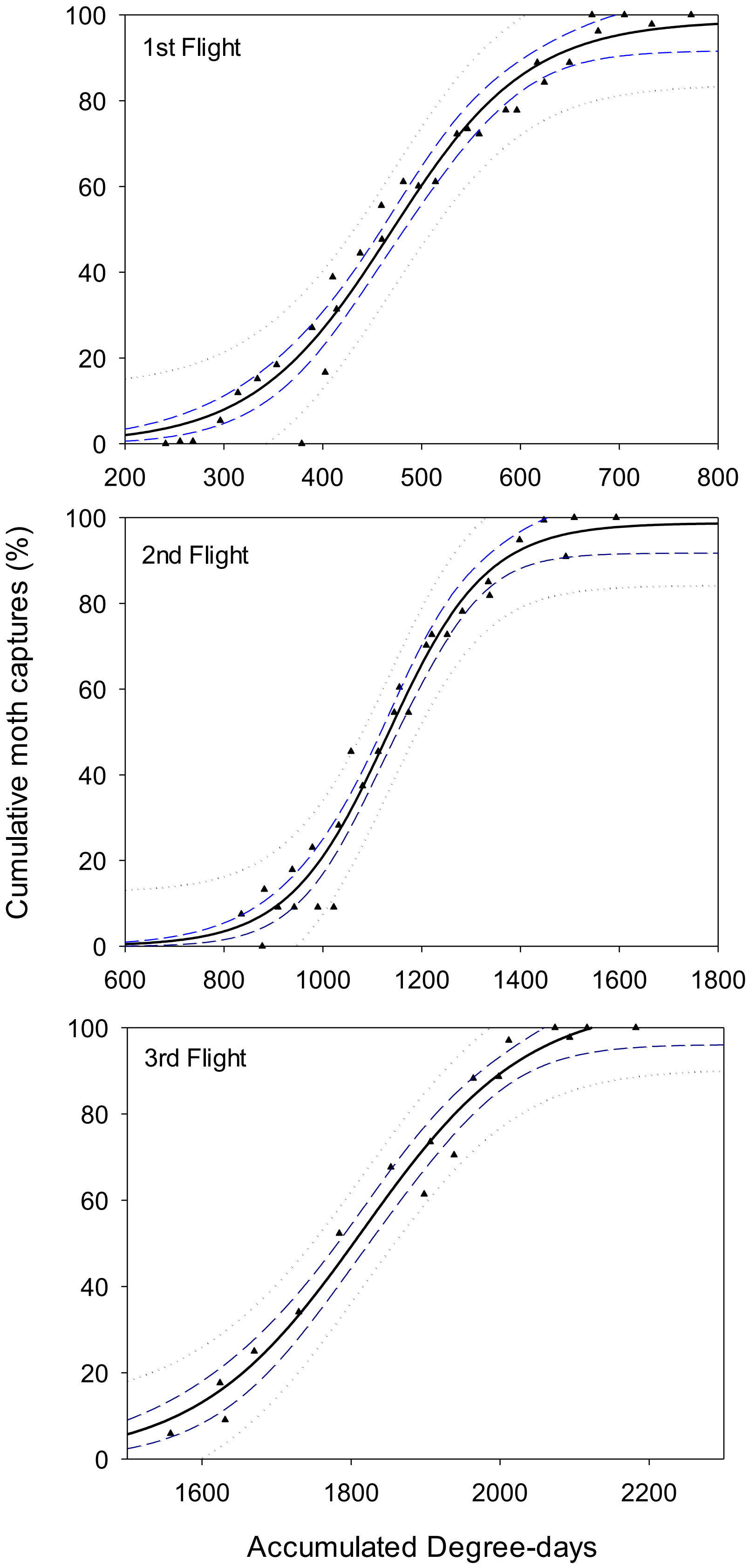

Figure 3. Cumulative *C. pomonella* captures in pheromone traps in relation to accumulated degree days and the respective nonlinear regression model (black dots: pooled data 2011 and 2012; black line: model fit; long dashes: 95% confidence bands; short dashes: 95% prediction bands). R^2 is above 98% for all flights and regression models considered.

Table2. Analysis of variance and related statistics for the nonlinear regression model of the adult male moth phenology of *C. pomonella*.

Flight				ANOVA		
		DF	Sum of Squares	Mean Squares	F	p
1st	Regression	2	35941.1070	17970.5535	437.4932	<0.0001
	Residual	28	1150.1332	41.0762		
	Total	30	37091.2402	1236.3747		
2nd	Regression	2	29620.5231	14810.2616	393.3327	<0.0001
	Residual	24	903.6784	37.6533		
	Total	26	30524.2015	1174.0078		
3rd	Regression	2	18127.4874	9063.7437	293.5472	<0.0001
	Residual	14	432.2726	30.8766		
	Total	16	18559.7601	1159.9850		

Model validation

The seasonal variation of cumulative moth captures in 2013 and 2014 followed the population emergence patterns predicted by the phenological model (figure 4). In all cases, the model prediction fitted well to the observed values, although ideally, all points should be close to the scatter chart diagonal (figure 5). The overestimations in fit performance were lower for the first and second flights compared to the third flight.

The overall patterns of the observed and predicted values were well distributed (figure 5). In all cases, the box plots showed a normal degree of dispersion, although a slight negative skewness was detected in the observed values of the first and second flights. Nevertheless, in all cases, the model performance statistics passed the W-normality and constant variance tests (table 3). These trends were to some degree expected considering that the start and end of the flights are characterized by few or very low moth captures. In addition, the bimodality of the moth flight patterns, which was especially apparent during the second and third flights of 2013, was a source of variability that the affected prediction efficacy.

The prediction phenology model for the first flights shows remarkably good fits and had the lowest RMSE compared to the second and third flights, respectively

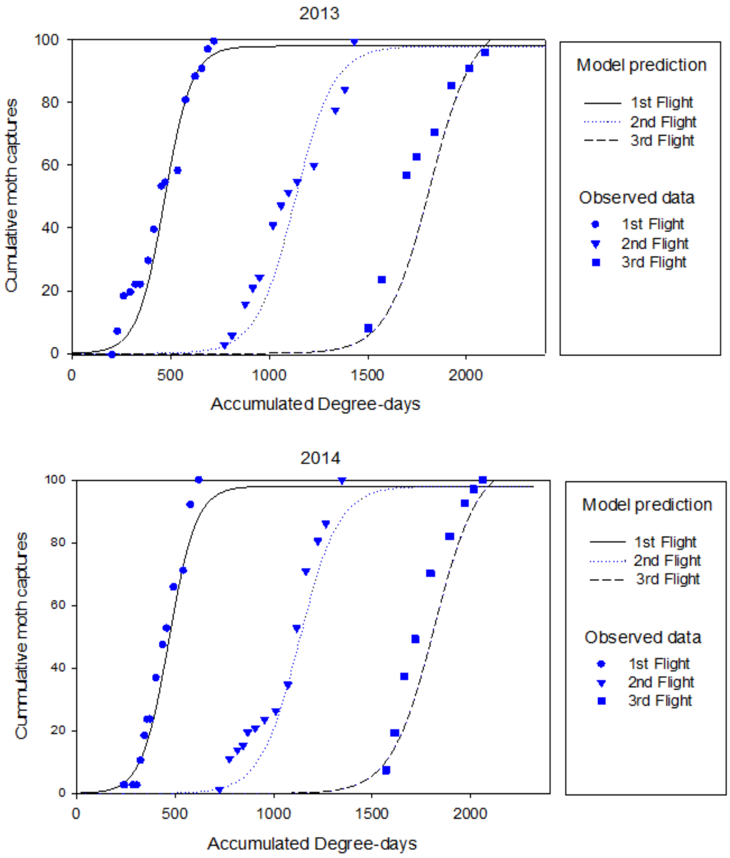

Figure 4. Predictions of cumulative moth captures as a function of degree days accumulated according to the developed phenological model and observed captures for 2013 and 2014 (Biofix: 1st January, T_L: 10.1 °C and T_u: 34.4 °C).

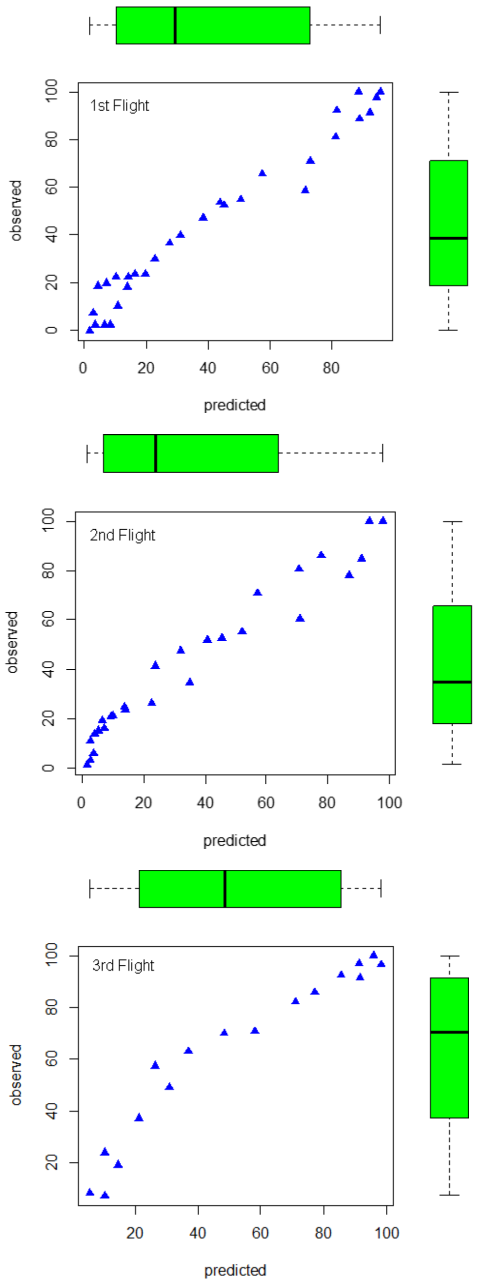

Figure 5. Enhanced scatter plots of predicted versus observed DD values of moth captures and related box plots for each case. The scatter plots are generated for the first, second, and third flight using pooled validation data from 2013 and 2014.

(figure 6). The model predictions were more accurate for the first flight of 2013 compared to 2014 (figure 6). However, the model predictions for the second and third flights were slight better for the 2014 data set compared to 2013. Furthermore, the absolute model prediction for the second flight generation was lower compared to the third. These differences may be due to the higher variability that was generally observed in the moth captures for the third flight generation.

Discussion

The codling moth *C. pomonella* is a key pest threat for pome fruit production worldwide. In Greece, in particular, its management is still based in many cases on preventive and intensive sprays with conventional insecticides. However, during the last few years pesticides registration has rapidly changed in the EU, and most of the conventional pesticides with long residual activity have been completely banned. However, the use of stage-specific biorational pesticides is increasing worldwide (Damos and Savopoulou-Soultani 2012a; Damos *et al.*, 2015). Phenological models are an essential prerequisite for successful management of *C. pomonella* with such stage selective pesticides (Knight, 2007; Barros-Parada *et al.*, 2015). Although farmers in Greece are encouraged by institutes and fruit cooperatives to move towards rational treatment and/or an IPM of *C. pomonella*, this is often not feasible due to the lack of region-specific decision tools, such as phenological models and related action thresholds.

In this work, a nonlinear degree-day phenological model has been developed for *C. pomonella* and was validated to predict the phenology of the codling moth flights in Greece. Seasonal flight patterns of *C. pomonella* in Greece could be in most cases accurately predicted using the current phenological model. Based on the regression statistics and the residual analysis, the overall model prediction performance can be judged as fairly acceptable and especially in predicting the first flight. In general, model prediction performance was higher for the first flight compared to the second and third. These deviations between predicted and actual moth activity, for the second and third flights, may be linked to the low flight activity that was observed in some cases. One additional source of variability for

Table 3. Constant variance and normality test ($p > 0.05$) for the nonlinear regression model of the adult male moth phenology of *C. pomonella*.

	Model assumptions			
	Constant variance test*	W-Statistic**	Significance level	Normality test
1st	0.5012	0.9231	= <0.0001	0.0285
2nd	0.2676	0.9557	= <0.0001	0.2940
3rd	0.2146	0.9296	= <0.0001	0.2580

* based on Spearman rank correlation between the absolute values of the residuals and the observed value of the depended variable.

**based on Shapiro-Wilk statistic that test the null hypothesis that the data are sampled from a normal distribution.

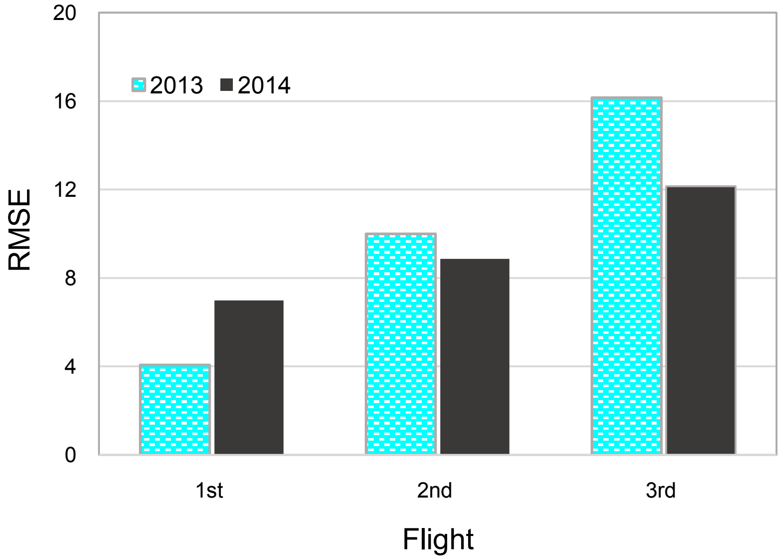

Figure 6. Root mean square error (RMSE) of the predicted versus the observed values for the moth capture data set from 2013 and 2014 and for the first (1st), second (2nd), and third (3rd) flight.

model development and validation was the occurrence of bimodal flight patterns and in some cases the overlapping of generations (i.e. the second and third).

The moth emergence of the first flight and the succeeding flight patterns during 2013 were in most cases consistent and in relatively high levels which facilitated the calibration of the model. All the same, there were instances, especially during 2014, where the catches were quite low and, thus, making hard the distinction of the last flight. Therefore, a critical question is whether the current phenological prediction will be accurate to a high degree on other locations and years as well. Apple management practices, such as MD for example, may also affect the development and phenology of *C. pomonella* and provide means to adjust phenology models (Joshi *et al*., 2017). As a result, *C. pomonella* models should be considered dynamic, by the sense that they may need to be adjusted accordingly over time and place to address new needs (Knight, 2007; Joshi *et al*., 2017).

Nevertheless, the phenology model showed a remarkably good fit, and the absolute model accuracy, which was reported as the RMSE, was very low for the first flight and within reasonable limits in most other cases (i.e. having a low deviation for the second and third flight compared to the first one). Additionally, the absolute residual error was generally very low and close to 30 DD across all generations. This deviation is within reasonable limits considering that 15-30 DD may be 1-3 days at the temperatures that occur during any given period. In fact, such slight deviations in model predictions cannot be avoided since they are related to the actual temperature oscillations around the base temperature, especially during summer, and most of the available heat summation methods do not capture these deviations. However, it should be also noted that a wider applicability of the model should take in to account also empirical validations performed by growers on other locations and years.

C. pomonella complete three flights per year in northern Greece as in other locations with warm and Mediterranean climate conditions (Mohammadi *et al*., 2016; Ranjbar-Aghdam, 2015). Spring moth emergence (overwintering generation) occurs in early May, and the succeeding second flight starts in late June and peaks in mid-July. The third flight begins in early August, peaks in mid-August, and may last (in some cases) until early September.

In general, the estimated thermal requirements for each flight duration were close to those observed in earlier studies (Bethell, 1978; Brunner *et al*., 1982; Pickel *et al*., 1986; Pitcairn *et al*., 1992). For example, the mean generation time of *C. pomonella*, according to the model of Pitcairn (T_L : 10.1 °C) and the University of California Statewide IPM program (UCIPM, 2014), is 619 DD, which is close the mean generation time observed in the current study (Pitcairn *et al*., 1992). Moreover, the duration of the mean generation time observed in Greece is in accordance with very recent DD *C. pomonella* studies performed in Pensylvania (Joshi *et al*., 2017).

Yet a direct comparison in terms of degree-day flight durations cannot be performed due the differences in methodology, the experimental set up (e.g. orchard conditions, lures) and population origin (i.e. acclimatisation, thermal plasticity, resistance). Slight differences in model predictions may be also related to the way in which the heat summations were estimated (for example, we used the triangular method instead of a sinusoidal method) as well as the established Biofix. Although there were no considerable differences in heat summations between the triangle and the sine method (data not shown), we chose the first one because it can be taken more easily by growers and advisors which calculate heat summations manually. This was to some degree expected considering that different degree day estimation methods (i.e. average, triangulation and sine waves) are more alike to one another during the warm months of the year (Rolsth *et al.*, 1999). Actually, the least deviation from hourly summation estimates is consistently associated with the triangulation method (Roltsch *et al.*, 1999).

Concerning the established Biofix, most temperature-driven models are synchronized to field populations of the first capture of overwintering moths (Jones *et al.*, 2013; Barros-Parada, 2015). Unfortunately, trap captures are affected by different factors, including the initial pest status (e.g. high or low), abrupt environmental conditions (e.g. wind, rain, chill), as well as by pest management actions (e.g. insecticides, mating disruption), which often created difficulties in detecting the emergence of moths of the first flight (Damos *et al.*, 2014).

Moreover, the Mediterranean climate system and conditions (e.g. temperature, photoperiod and dry conditions) may affect voltinism and finally the number of generations completed under field conditions (Juszczak *et al.*, 2013; Vavrovič *et al.*, 2014). It has been recorded that *C. pomonella* has four flights in Morocco (El Iraqui and Hmimina, 2016), three in Iran (Ranjbar-Aghdam, 2015), two full and a partial third in Washington State (Knight, 2007) and Chile (Barros-Parada, 2015), and one in Slovakia (Vavrovič *et al.*, 2014).

Therefore, in our study, due to the field temperatures that occur in Greece, January 1st was preferred as the Biofix instead of the time of the first captures in the pheromone traps. Moreover, increased temperatures in early spring (i.e. March) may accelerate diapause termination and moth emergence and therefore extend the number of generations, increasing the species voltinism (El Iraqui and Hmimina, 2016).

Moreover, temperatures during July to early August are high in Greece and allow for fast development before photoperiod triggers diapause induction. As a result more larvae escape diapause prolonging the last flights; this was a potential cause of variation when developing the model.

Finally, regional selection pressure imposed by insecticide use may evolve differences in the phenology of *C. pomonella* (Knight, 2007) and thus may complicate the adoption of any available model. For example, other studies found an increase in the development time of *C. pomonella* and a subsequent phenological delay of insecticide resistant individuals, which has been suggested as a pleiotropic cost of insecticide resistance (Boivin *et al.*, 2005). Thus, the current study of the *C. pomonella* phenology in Greece, which aimed to develop and validate a prediction model with a broader applicability in Mediterranean climates, is of special importance.

In conclusion, a part of few works (Bugiani *et al.*, 1996; Rosa, 2000), the codling moth phenology has not been extensively studied in the Mediterranean basin (Pajač *et al.*, 2011), and in Greece particularly the management actions are mostly based on empirical instructions from fruit cooperatives and plant protection advisors.

The phenological model developed in this study is expected to be useful in IPM systems to anticipate optimal spraying times of available insecticides as well as applications of other control measures. Additionally, the model can be incorporated into existing real-time decision-support systems (Damos, 2015) or can be used by national plant protection authorities and institutes as a means to provide temperature-driven predictions of *C. pomonella*. Finally, the model can be improved by incorporating the developmental events of more stages, and long-term field evaluations should be conducted to ensure the model is reliable to remain applicable.

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