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Application of accumulated heat units in the control of the olive moth, *Prays Oleae* (Bern.) (Lep., Praydidae), in olive groves in Southern Spain

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Abstract: Olive moth, *Prays oleae*, is one of the most important phytophagous of olive cultivation in the Mediterranean basin. Its control is based on the application of chemical insecticides, sometimes combined with the release of natural enemies. The need for application is established according to population thresholds. However, the wide range of environmental conditions requires the adoption of complementary elements that allow the adequate time of applications to the phenological stages responsible for the damage. During the years 2013, 2014 and 2015, weekly observations of the insect phenology have been performed in 10 plots of olive trees at different altitudes (253 m to 1017 m) in the province of Jaén (southern Spain), recording daily temperature variations at 4-hour intervals. This has allowed determining the thermal integrals for the three insect generations and to specify the influence of the altitude on its development periods, more especially in those corresponding to the philophagous and carpophagous generations. This knowledge has allowed the development of a model-chronogram for the adjustment of treatment dates based on crop altitude.

Index terms: Degree-days, thermal integral, accumulated heat units, altitude, *Prays oleae*.

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Aplicação de unidades de calor acumulado no controle da traça da oliveira, *Prays Oleae* (Bern.) (Lep., Praydidae), em oliveiras do sul da Espanha

Resumo: A traça da oliveira, *Prays oleae*, é um dos mais importantes fitófagos da olivicultura da bacia mediterrânica. Seu controle é baseado na aplicação de inseticidas químicos, às vezes combinados com a liberação de inimigos naturais. A necessidade de aplicação é estabelecida de acordo com limites populacionais. No entanto, a ampla gama de condições ambientais exige a adoção de elementos complementares que possibilitem o momento adequado das aplicações aos estádios fenológicos responsáveis pelo dano. Durante os anos de 2013, 2014 e 2015, foram feitas observações semanais da fenologia do inseto em 10 parcelas de oliveiras, em diferentes altitudes (253 m a 1.017 m), na província de Jaén (sul da Espanha), registrando variações diárias de temperatura em intervalos de 4 horas. Isso permitiu determinar as integrais térmicas para as três gerações do fitófago e especificar a influência da variável altitude em seus períodos de desenvolvimento, mais especialmente naqueles correspondentes às gerações fitófago e carpófago. Este conhecimento permitiu a realização de um modelo-cronograma para o ajuste das datas de tratamento com base na altitude das culturas.

Termos para indexação: Graus-dias, integral térmica, unidades de calor acumulado, altitude, *Prays oleae*.

Introduction

Integrated Pest Management (IPM) represents a substantial improvement over Conventional management (EHI-EROMOSELE et al., 2013). Among the main contributions, its main objective is the optimization of the entomophagous activity of natural enemies through the stimulation of crop biodiversity (CALABRESE et al., 2012; GÓMEZ et al., 2018). This requires better understanding of the agroecosystem based on better knowledge of the biology, behavior and ecology of pests and their natural enemies. The determination and knowledge of factors of influence in the regulation of its development is, in this sense, of essential interest to optimize the effectiveness of any control strategy against this pest.

Among the factors that determine the development of insects, temperature plays a primordial role (ALDANA, 1983) in determining the speed of enzymatic reactions that regulate the metabolic activity of organisms. The influence of temperature on biochemical processes (especially in poikilothermic organisms) has led many researchers to adopt prediction tools to estimate the duration of insect development phases based on the knowledge of their relationship with ambient temperature (WAGNER et al., 1984; PITZALIS et al., 1985; BELCARI et al., 1989; FAN et al., 1992; COUTINHO et al., 1997; BRÉVAULT; QUILICI, 2000; ASPLANATO; FARCÍA-MARÍ, 2001; GALLARDO et al., 2009; GONÇALVES; TORRES, 2011; HAAVIK et al., 2013; PETACCHI et al., 2015). Insect development takes place exclusively within a relatively narrow temperature range and is determined by two temperature threshold values:

The lower threshold, or baseline temperature, is the critical temperature value from which the metabolic processes are activated and insect development begins. From this temperature value, there is increase in the speed of enzymatic reactions until reaching maximum development rate. Once the second temperature value corresponding to the upper threshold is exceeded, the development rate progressively slows down, until reaching temperature value or thermal maximum, from which development stops and determines the end of the development and entry into larvae estivation, reported by Civantos, (1998) and Kumral et al. (2005), and the death of the organism may even occur. Crovetti et al. (1982) in their study on the olive fly *Bactrocera oleae* (Rossi, 1790) (Dip., Tephritidae), determined the lower and upper temperature thresholds in laboratory, estimating them at values of 8.99°C and 30°C, respectively. Gallardo et al. (2009) in their study on *Lobesia botrana* (Denis & Schiffermüller, 1775) (Lep., Tortricidae) indicated values of 7°C and 30ºC, very similar to those reported by Gabel and Mocko (1986) and Tío Moreno (1996).

Since the conditions suitable for insect development, or *effective temperature*, are determined by a defined interval between lower and upper thresholds, Chiang (1985) defines the *thermal constant*, expressed in degree-days (DD), as "the amount of heat that each species requires to complete its cycle or part of it, regardless of temperature to which it is exposed". Pitzalis et al. (1985) indicate that degree-day models are based on a close linear temperature/development relationship. For its calculation, they consider the number of degrees that exceed the lower threshold in a period of 24 hours, proceeding, once the development is finished, to the calculation of the *thermal integral*, as a result of the sum of the degree-days accumulated during the entire period.

The determination of the thermal integral allows developing a method for the estimation of the date of appearance of the adult stage, in its case, of the different phenological stages. This method represents a very useful tool for precise estimations and allows forecasting the emergence of the different phenological stages in advance, making it possible to focus our attention on those of special interest, such as those responsible for crop damage.

The olive moth, *Prays oleae* (Bernard, 1788) (Lep., Praydidae), is one of the most economically important phytophagous of this crop, in the Mediterranean basin, the Black Sea, the Middle East and the Canary Islands (TZANAKAKIS, 2003). The damage caused by this pest, according to available studies, causes estimated production losses between 49% and 63% (RAMOS et al., 1998; PATANITA; MEXIA, 2002). During the annual cycle, this insect develops three generations: the philophagous generation, whose larvae attack leaves (autumn-winter-spring), the anthophagous generation, whose larvae feed on flowers (spring), and the carpophagous generation, whose larvae feed on the fruit mesocarp (summer-autumn).

This implies that, in its annual cycle, the species is affected by climatologically very different environmental conditions, which directly affect its development rate and ability to survive. It has been indicated that temperatures above 30ºC very negatively affect the larvae mobility, hindering the process of penetration into fruits (CIVANTOS, 1998), although up to now, the thermal maximum value has not been specified, from which mortality of any of the insect stages would occur. Regarding minimum values, it has been indicated that temperatures below 10º C slow down the activity of adults, which can die when they drop below 7ºC (CIVANTOS, 1998; DE ANDRÉS CANTERO, 2001). On the other hand, a close relationship between the mortality of larvae and pupae has been associated with the number of days with minimum temperatures below 0ºC (RAMOS et al., 1978).

The control of *P. oleae* is mainly based on strategies focused on the application of synthetic or natural chemical insecticides, in combination with biological control strategies, either through the use of *Bacillus thuringiensis* subsp. *kurstaki*, or through the release of natural enemies, particularly lacewings (Neu., Chrysopidae). Until now, the monitoring of their populations is carried out by means of traps baited with synthetic pheromones and the decision for the application of control means is established based on population thresholds using samples of vegetative organs. However, the diversity of environmental conditions and the difficulty of sampling large areas over a short period of time prevent the application of control means with the desired precision.

In general, the result of a pattern or general calendar of uniform application over a wide territory, without considering the microclimatic particularities, implies loss of effectiveness of control measures, and the consequent waste of human and economic resources. In the province of Jaén (southern Spain), where 20% of the world's olive oil is produced (MUÑOZ PÉREZ, 2020), crops are located both in rural areas and in low and medium mountain areas, with altitudes between 250 m and 1100 m (a.s.l.).

Based on the above, the adoption of complementary tools that allow control strategies to be adapted to the microclimatic characteristics of the different areas is needed, which would represent a substantial advance in the effectiveness of any measure used to control this pest. Consequently, given the notable thermal differences among cultivation areas, especially considering the maximum temperatures of spring and summer months, it is suggested to perform in-depth studies on the influence of temperature on the development duration of this insect, especially with regard to anthophagous and carpophagous generations, which are the object of plant health treatments.

Improving the knowledge of the thermal influence would allow adapting treatment dates to the phenology of this insect, which is the aim of this work.

Material and Methods

The study was carried out during the years 2013, 2014 and 2015 in the province of Jaén (southern Spain), where 10 olive producing areas were selected. In these areas, all crops belong to the 'Picual' variety, aged 20-80 years, planted in a 10 x 10 m scheme and cultivated under irrigation. Plots are heterogeneous in terms of slope, inclination and orientation. During the study period, treatment against pests has been carried out according to the Integrated Pest Management, and if necessary, treatment can be based on the application of organophosphate insecticides, mostly Dimethoate® 40% © (400 g / l) (BASF), or by applying *B. thuringiensis*. With the selection of these 10 areas, whose altitudinal characteristics are shown in Table 1, a complete representation of the topographic conditions of olive cultivation in the province of Jaén has been sought, where altitudes vary between 253 m and 1017 m (a.s.l.).

Table 1. Geographical and altitudinal characteristics of olive groves selected for the study in the different areas.

Areas	Code	Altitude	Latitude	Longitude
Andújar	#1	253m	37° 59' 41.33" N	04° 01' 24.37" O
Fuerte del Rey	#2	289m	37° 55' 10.19" N	03° 56' 18.25" O
Puente del Obispo	#3	327m	37° 57' 27.63" N	03° 31' 50" O
Garcíez (pedanía)	#4	484m	37° 49' 00" N	03° 52' 55.88" O
La Cerradura	#5	504m	37° 42' 55.10" N	03° 40' 05.68" O
Torre del Campo	#6	705m	37° 46' 35.58" N	03° 52' 32.76" O
Baeza	#7	710m	37° 59' 21.07" N	03° 27' 39.74" O
Cambil	#8	927m	37° 41' 43.49" N	03° 31' 42.70" O
Cabra Santo Cristo	#9	960m	37° 42' 39.72" N	03° 17' 26.06" O
Noalejo	#10	1017m	37° 32' 18.17" N	03° 38' 17.93" O

Sampling

During the three experimental years, periodic sampling was carried out at weekly intervals (between January 1 and December 31), in each of the 10 study areas. Its purpose was to detect the presence of the different phenological stages of *P. oleae*, and where appropriate, to determine the development degree. On each date and area, 3 olive trees have been randomly selected, considering in each of them, the number of replicates indicated below:

- Sampling on the *philophagous generation*: The first sampling stage took place at weekly intervals between maturation (phenological stage I of the olive tree), which occurred at the end of September until the detection of the first eggs, corresponding to the philophagous generation. In the first stage, the sampling consisted of the random selection of 100 leaves from each of the three randomly selected olive trees, which were examined in laboratory under magnifying lens, with the established criterion being the detection of at least 1 egg per 100 leaves. With this sampling, assessment of the population density is not intended, but rather the establishment of the oviposition start date. The sample size (100 leaves) is due to the fact that the female of this insect lays eggs widely scattered on olive tree leaves, and very rarely more than one egg per leaf is observed. The second stage of weekly sampling began on January 1, ending with the beginning of flowering of olive trees (stage F1). This corresponded, for the year 2013, with the first week of May and mid-June for the low and high areas, respectively; with the first week of May and first of June for the year 2014, and the end of April and the first week of June for the year 2015. The aim of the second sampling stage was to determine the phenological evolution of the different development stages during the philophagous generation. In each sampling, 20 leaves affected by *P. oleae* larvae were selected from each olive tree. These were later examined under magnifying lens, which allowed determining the earliest date of appearance of the different larval stages, as well as the pupal stage. For the philophagous generation, this study has two replicates, corresponding to the 2013/14 and 2014/15 seasons.
- Samplings on the *anthophagous generation*: Carried out weekly between the beginning of flowering (stage F1 of olive trees) until fruiting (stage G of olive trees). The start of fruiting corresponded in 2013 to the beginning and end of June for low

and high areas, respectively; in mid-May and end of June in 2014; and mid-May and June in 2015. In each of the three randomly selected olive trees, 20 inflorescences affected by larvae were selected (nt = 60 inflorescences/area/week). Inflorescences were subsequently examined in laboratory under magnifying lens, confirming the presence of eggs/larvae/pupae, which allowed determining the dates of the first appearance of each of these development stages, and, in the case of larvae, the corresponding age (L1 to L5).

- Sampling on the *carpophagous generation*: The first stage of weekly sampling took place between the start of fruiting (stage G), whose start dates have been previously indicated, and the end of the oviposition period of *P. oleae* on fruits. In these samplings, 100 fruits were randomly selected from each of the three selected olive trees, observed under magnifying lens in laboratory, which allowed differentiating between live eggs, recently laid eggs, hatched eggs and predated eggs. Fruits that presented hatched eggs were dissected, verifying the presence of the larva in the fruit mesocarp.

The second period of fruit sampling took place between the end of stage H (stone hardening), which took place at the end of August in low areas and mid-September in high areas, and the beginning of maturation (stage I of olive trees), whose dates have been previously indicated for the beginning of samplings of the philophagous generation. Fruits corresponding to the second period were dissected, observing the presence/absence of 5th age larvae (L5), which allowed determining their date of emergence, prior to the pupal stage, with approximation of one week. The detection of the first larval emergences determined the date of start of samplings of the philophagous generation. Given the interval in which the study was carried out (January 1, 2013 to December 31, 2015), there were three replicates for anthophagous and carpophagous generations (2013, 2014 and 2015).

For the monitoring of adults, 3 delta-type traps were installed in each of the areas in 3 randomly selected trees, placing them in the outer area of olive trees and at height of 150-170 cm from the ground. Traps were provided with a synthetic pheromone capsule: 1% z-7-tetradecenal (1 mg/capsule) and were weekly examined from the beginning of the flight of the anthophagous generation. The appearance of the first captures in traps indicated the date of start of the sampling of floral organs of olive trees, which took place between the beginning of corolla formation (stage DI of olive trees) and the end of the full flowering stage (stage FII of olive trees). In each sampling, the number of individuals captured per trap was counted. Synthetic pheromone capsules were renewed every two months.

Record of temperature data and calculation of thermal integrals

In each of study areas, Tinytag Transit 2 TG-4080© datalogger temperature recording device was installed. These devices were programmed to record values every 4 hours, so the number of records was 6/day. These remained in the selected olive grove areas throughout the sampling period. Data were downloaded every six months using inductive ACS-3030 pad to facilitate the process.

To calculate degree-days (DD), and to avoid, as much as possible, overestimation of heat units when the temperature exceeds the upper threshold, the *sine wave with vertical shift* method was used (HERMS, 2004; MURRAY, 2020).

The temperature value considered as the lowest threshold was 10.85ºC (KUMRAL et al., 2005). For the upper threshold temperature, Kumral et al. (2005) and Civantos (1998) point out a slowdown in development and even mortality of neonate larvae above 30°C.

The calculation of degree days for each of the generations has been established as the sum of degree days in the period between the appearance of the first egg of two consecutive generations. Specifically, the calculation of the thermal integrals of each of *P. oleae* generations has been defined by:

- *Philophagous generation*: Period elapsed between the appearance of the first egg of the philophagous generation until the first egg of the anthophagous generation.
- *Anthophagous generation*: Period elapsed between the appearance of the first egg of the anthophagous generation until the first egg of the carpophagous generation.
- *Carpophagous generation*: Period elapsed between the appearance of the first egg of the carpophagous generation until the first egg of the philophagous generation.

For the practical determination of the optimal dates for the application of control treatments, January 1 has been established as the starting point for the accumulation of degree-days, as indicated by Touzeau (1981), Castro et al. (2008), Armendariz, et al. (2010), Gonçalves and Torres (2011) and Canales et al. (2014).

Statistical analysis

For the statistical analysis, the Statgraphics Centurion XVII (2016) and STATISTICA (13.0) packages were used. The normality of distributions was verified using the Shapiro-Wilk normality test. To determine the interrelation between variables, simple regression analysis was performed, with altitude (m) being the independent variable and degree days the dependent variable.

To determine significant differences ($p<0.05$) between data sets, one-way analysis of variance, ANOVA, was applied. The test of least significant differences, Fisher's LSD was used to determine differences between groups, with each of these being data resulting from the degree days of the three years corresponding to each altitude and, on the other hand, to determine differences between the number of days for each generation in each area. To analyze the influence of altitude on degree days (DD) and development duration (number of days, D), altitude ranges have been established, so that the different areas under study have been grouped into the following categories: high areas (>900 m), low areas (<400 m), and intermediate areas (between 400 and 900 m).

Thus, to determine the existence of significant differences in the DD/D ratio as a function of altitude, grouping areas into categories has allowed achieving a sufficient number of replicates for each altitude category. However, to establish a linear relationship between DD and altitude (carpophagous generation) through the application of linear regression, the specific altitudes of the different areas have been taken into account, and therefore altitude is considered as a continuous variable.

Results and Discussion

Based on periods considered to calculate the sum of degree days of the different *P. oleae* generations, the results are presented in Tables 2, 3, and 4.

Philophagous generation

The global average value (2013/14 and 2014/15 seasons) was 638.44 DD (Table 2), with the annual values being 551.88 DD and 725.00 DD (respectively for 2013/14 and 2014/ 15 seasons).The annual values calculated for the 10 cultivation areas represent an average variation of 13.56% with respect to the global average value. There are no significant differences in the number of degree-days according to the crop altitude category (d.f.=9; F=0.18; p>0.05).

In view of data presented in Table 2, a significant increase in the development duration can be observed for this generation at altitudes above 900 m, unlike what occurred with degree days, in which there is no great variation. Below 900 m, the calculated period ranges from 189 to 224 days ($\bar{x}$ =220.15 days), with the first eggs appearing on inflorescences (corresponding to the anthophagous generation) in the first fortnight of May (Table 2).

On the other hand, above 900 m, this period increases significantly (d.f.=2; F=39.83; p<0.001) up to 238-252 days ($\bar{x}$ =242.67 days), observing eggs on inflorescences on much later dates, in the first half of June. This increase in the development period in higher cultivation areas is related to the lower temperature and the metabolic activity rate of the insect.

Table 2. Total number of degree-days (DD) and days (D) counted for the complete development of the philophagous generation of *P. oleae* in the study areas (years 2013/14 and 2014/). The dates of appearance of the first eggs are indicated, corresponding to consecutive philophagous-anthophagous generations (egg phi/egg ant). ($\bar{x}$: mean; ERR: standard error).

	Code area (h)	**egg phi / egg ant**	***(D)***	**DD**
2013/14	**#1 (253 m)**	oct 10/may 08	*(210)*	636.44
	#2 (289 m)	oct 24/may 08	*(189)*	539.85
	#3 (327 m)	oct 28/may 05	*(189)*	506.39
	#4 (484 m)	oct 25/may 16	*(203)*	515.81
	#5 (504 m)	oct 22/may 13	*(203)*	550.04
	#6 (705 m)	oct 18/may 09	*(203)*	558.90
	#7 (710 m)	oct 21/may 12	*(203)*	553.03
	#8 (927 m)	oct 09/jun 04	*(238)*	578.84
	#9 (960 m)	oct 16/jun 11	*(238)*	522.99
	#10 (1017 m)	oct 15/jun 10	*(238)*	556.51
	$\bar{X}$ + ERR (2013/14)		*(212.10+5.90)*	551.88+11.70
	Code area (h)	**egg phi / egg ant**	***(D)***	**DD**
2014/15	**#1 (253 m)**	oct 02/may 07	*(217)*	808.45
	#2 (289 m)	oct 09/may 14	*(217)*	743.95
	#3 (327 m)	oct 06/may 11	*(217)*	745.09
	#4 (484 m)	oct 03/may 15	*(224)*	713.53
	#5 (504 m)	sept 30/may 12	*(224)*	767.07
	#6 (705 m)	oct 03/may 15	*(224)*	745.55
	#7 (710 m)	oct 06/may 11	*(217)*	685.14
	#8 (927 m)	sept 24/jun 03	*(252)*	745.16
	#9 (960 m)	oct 01/jun 03	*(245)*	653.68
	#10 (1017 m)	oct 07/jun 09	*(245)*	642.37
	$\bar{X}$ + ERR (2014/15)		*(228,20+4,33)*	725,00+16,32
	$\bar{X}$ + ERR		***(220.15 + 4.01)***	**638.44 + 22.14**

Anthophagous generation

The calculation of degree-days corresponding to the anthophagous generation (Table 3) presented general mean value of 173.50 DD. Regarding this value, the integrals calculated for the different areas represent average deviations of 9.98%, 12.30% and 8.50% (years 2013, 2014 and 2015, respectively), with global average value of 10.26%. No significant differences were observed regarding the altitude variable (d.f.=9; F=2.04; p>0.05).

Table 3. Days (D) and total number of degree-days (DD) counted for the complete development of the anthophagous generation of *P. oleae* in the ten areas under study (years 2013, 2014 and 2015). For each year, the dates of appearance of the first eggs are indicated, corresponding to consecutive anthophagous-carpophagous generations (egg ant/egg car). (x̄: mean; ERR: standard error).

	Code area (h)	egg ant / egg car	(D)	DD
2013	#1 (253 m)	may 16/jun 13	(28)	186.95
	#2 (289 m)	may 30/jun 20	(21)	173.10
	#3 (327 m)	may 20/jun 17	(28)	209.12
	#4 (484 m)	may 31/jun 28	(28)	185.21
	#5 (504 m)	may 28/jun 18	(21)	152.65
	#6 (705 m)	may 24/jun 21	(28)	207.60
	#7 (710 m)	may 20/jun 17	(28)	188.50
	#8 (927 m)	jun 19/jul10	(21)	182.75
	#9 (960 m)	jun 26/jul 17	(21)	194.48
	#10 (1017 m)	jun 25/jul 16	(21)	161.75
	X̄+ERR (2013)		(24.50+1.16)	184.21+5.70
	Code area (h)	egg ant / egg car	(D)	DD
2014	#1 (253 m)	may 08/may 29	(21)	151.35
	#2 (289 m)	may 08/may 29	(21)	167.31
	#3 (327 m)	may 05/may 26	(21)	157.73
	#4 (484 m)	may 16/jun 16	(21)	144.53
	#5 (504 m)	may 13/jun 03	(21)	155.87
	#6 (705 m)	may 09/jun 06	(28)	223.73
	#7 (710 m)	may 12/jun 09	(28)	213.95
	#8 (927 m)	jun 04/jun 25	(21)	184.54
	#9 (960 m)	jun 11/jul 02	(21)	162.88
	#10 (1017 m)	jun 10/jul 01	(21)	163.07
	X̄+ERR (2014)		(22.40+0.93)	172.50+8.45
	Code area (h)	egg ant / egg car	(D)	DD
2015	#1 (253 m)	may 07/may 28	(21)	149.1
	#2 (289 m)	may 14/jun 04	(21)	186.68
	#3 (327 m)	may 11/jun 01	(21)	161.94
	#4 (484 m)	may 15/jun 05	(21)	145.22
	#5 (504 m)	may 12/jun 02	(21)	175.14
	#6 (705 m)	may 15/jun 05	(21)	183.53
	#7 (710 m)	may 11/jun 01	(21)	166.01
	#8 (927 m)	jun 03/jun 24	(21)	173.83
	#9 (960 m)	jun 03/jun 24	(21)	164.75
	#10 (1017 m)	jun 09/jun 30	(21)	131.76
	X̄+ERR (2015)		(21,00+0)	163,80+5,52
	X̄+ERR		(22.63+0.55)	173.50+4.04

The absence of an altitudinal gradient in the development of the anthophagous generation can most likely be attributed to the relatively moderate temperatures between late spring and early summer, predominantly located within the optimal temperature range for insect development. This also determines that the development period has been shorter for this generation, ranging from 21 to 28 days, with average value of 22.63 days.

However, the delay observed in the date of oviposition of adults of the anthophagous generation in olive groves at higher altitudes (>900 m) would be a consequence, as previously indicated, of the fact that these development rates of the philophagous generation are delayed even during an average period of 20 days with respect to low levels. It is important to point out that this delay in the phenology of the insect is parallel to the later flowering of olive trees in these higher areas (stage F), in which this stage begins a month later than in lower areas.

Carpophagous generation

Regarding the carpophagous generation, the sum of accumulated degree-days was, by far, the highest (Table 4), being between 662.21 and 1208.41 DD, with mean values of 918.70 DD; 953.70 DD and 1023.14 DD (years 2013, 2014 and 2015) and with general average value of 965.18 DD. Regarding this value, the average difference of deviations obtained for each area is 13.51%. As indicated in Figure 1, values corresponding to crops at altitudes below 700 m do not vary significantly as a function of their altitude (d.f.=5; F=0.07; p>0.05), and in these, the mean thermal integral is 1038.59 DD. However, once this level is exceeded, statistically significant decrease in the number of degree-days is observed (d.f.=4; F=91.62; p<0.01), with mean value of 891.77 DD. This decrease in DD with increasing altitude from 700 m is satisfactorily adjusted using a linear regression model, according to the following function: DD =1912.89 -1.18 h (r^2 = 0.9575).

The development duration of this generation ranged from 91 days to 147 days, with annual mean value of 114.10 days; 114.80 days and 133.70 days, respectively (years 2013, 2014 and 2015), with global mean value of 120.87 days. As in the case of degree-days, statistically significant decrease in the development duration is observed in mountain olive groves (h > 900 m) (Table 4), where duration presented minimum values, with mean of 100.33 days (d.f.=1; F=44.74; p<0.001).

Unlike what was observed in philophagous and anthophagous generations, in the case of the carpophagous generation, the increase in temperature in the spring-summer months is the limiting factor that seems to exert the greatest influence. As shown in Figure 2, during this generation, the mean temperatures exceeded the development threshold (30ºC) between the beginning of July and end of August in low areas (<400 m). It has been indicated that once this upper threshold is exceeded, a slowdown in development is observed, given that it involves biological processes, this slowdown in activity, although had begun gradually since the optimum temperature range was exceeded, reached the development upper temperature threshold value before. It is therefore obvious to consider that, during the summer months, the daily increase in temperature above the upper threshold is a significant limitation for larval activity. This is because, on these days, once the upper temperature threshold is exceeded, larvae would decrease or even completely stop their activity, reactivating when the temperature drops below the upper threshold. It is therefore evident that the generation time is considerably longer than that recorded for the anthophagous generation, whose development takes place during the end of spring, when temperatures do not reach extreme values, and are generally found in the medium area of the optimum threshold for the development of this species.

Table 4. Days (D) and total number of degree-days counted for the complete development of the carpophagous generation of *P. oleae* in areas under study (years 2013, 2014 and 2015). For each year, the dates of appearance of the first eggs are indicated, corresponding to consecutive carpophagous-phyllophagous generations (egg car/egg phy). (x̄: mean; ERR: standard error).

	Code area (h)	egg car / egg phy	(D)	GD
	#1 (253 m)	jun 13/oct 10	(119)	915.79
	#2 (289 m)	jun 20/oct 24	(126)	1069,64
	#3 (327 m)	jun 17/oct 28	(133)	1039,91
	#4 (484 m)	jun 28/oct 25	(119)	886.34
	#5 (504 m)	jun 18/oct 22	(126)	1011,96
2013	**#6 (705 m)**	jun 21/oct 18	(119)	1062,18
	#7 (710 m)	jun 17/oct 21	(126)	1021,87
	#8 (927 m)	jul 10/oct 09	(91)	749.55
	#9 (960 m)	jul 17/oct 16	(91)	767.58
	#10 (1017 m)	jul 16/oct 15	(91)	662.21
	x̄ + ERR		**(114.10+5.22)**	**918.70+46.66**
	Code area (h)	**egg car / egg phy**	**(D)**	**GD**
	#1 (253 m)	may 29/oct 02	(126)	1030,44
	#2 (289 m)	may 29/oct 09	(133)	1208,41
	#3 (327 m)	may 26/oct 06	(133)	1103,80
	#4 (484 m)	may 06/oct 03	(119)	910.59
	#5 (504 m)	may 03/sep 30	(119)	994.05
2014	#6 (705 m)	may 06/oct 03	(119)	1091,13
	#7 (710 m)	may 09/oct 06	(119)	1019,34
	#8 (927 m)	jun 25/sep 24	(91)	740.37
	#9 (960 m)	jul 02/oct 01	(91)	767.89
	#10 (1017 m)	jul 01/oct 07	(98)	671.00
	x̄ + ERR		**(114.80+5.02)**	**953.70+55.81**
	Code area (h)	**egg car / egg phy**	**(D)**	**GD**
	#1 (253 m)	may 28/oct 22	(147)	1101,23
	#2 (289 m)	jun 04/oct 22	(140)	1151,27
	#3 (327 m)	jun 01/oct 26	(147)	1096,25
	#4 (484 m)	jun 05/oct 23	(140)	1029,36
	#5 (504 m)	jun 02/oct 13	(133)	1029,83
2015	**#6 (705 m)**	jun 05/oct 16	(133)	1117,79
	#7 (710 m)	jun 01/oct 26	(147)	1151,12
	#8 (927 m)	jun 24/oct 14	(112)	854.5
	#9 (960 m)	jun 24/oct 28	(126)	941.81
	#10 (1017 m)	jun 30/oct 20	(112)	758.27
	x̄ + ERR		**(133,70+4,23)**	**1023,14+41.99**
	x̄ + ERR		**(120.87+3.18)**	**965.18+28.21**

The daily fluctuation towards values close to the upper threshold can probably represent a significant drawback for the calculation of degree days. This is due to the fact that the algorithm used in this study to calculate the degree-days takes into account the number of degrees that are daily counted above the lower threshold, although considering only the average daily values. The source of error is also due to the fact that full days are considered for its calculation, provided that the average values do not exceed 30ºC, not allowing to correct the error derived from the development slowdown when values approach the upper threshold.

The accumulated distortion would explain the deviation of the number of degree days calculated with respect to the anthophagous generation, in which this error would not affect, as the most stable temperatures are found at the end of spring, without generally approaching the lower and upper thresholds. This may explain the deviation with respect to calculations for philophagous and anthophagous generations, and differences regarding the calculation of the degree days for this generations with respect to olive groves in northern Spain, characterized by notably more moderate summers (ARMENDÁRIZ et al., 2009).

In this sense, several authors such as Knight et al. (1991); Rock et al. (1993); Tzanakakis (2003), consider high temperatures as the main source of error in the calculation of the thermal integral for the carpophagous generation, resulting in excessively overestimated values. Consequently, the inverse relationship between altitude and maximum temperatures during the summer, and the effect of the increase in temperature, explain why thermal integrals present maximum values at altitudes below 400 m, minimum values at altitudes above 900 m, and intermediate values at altitudes between 400 m and 900 m (Table 4).

On the other hand, the slowdown and subsequent stoppage of the larva development (more frequent during the summer the lower the altitude of the olive grove), and data recorded for the dates of appearance of adults of the carpophagous generation (Figure 2) indicate maximum periods (egg/adult) in olive groves at lower altitudes (<400 m, $\bar{x}$ =133.77 days), minimum periods in olive groves at higher altitudes (>900 m, $\bar{x}$ = 100.33 days), and intermediate periods in olive groves located between 400 m and 900 m ($\bar{x}$ =126.58 days).

This is due to the fact that the estivation period has significantly shorter duration in elevated areas (d.f.=2; F=24.56) compared to lower (400 m) ($p<0.001$) and intermediate elevations (400-900 m) ($p<0.001$).

The effect of the daily oscillation in maximum and minimum temperatures towards the upper and lower threshold temperature values, and the slowdown in the larva development, represents the main source of error for the calculation of thermal integrals, which implies that the extrapolation of results to other distant areas would be more reliable the more similar the geographical and topographical conditions (photoperiod, latitude and altitude) of areas being compared.

However, from the practical point of view, this anomaly does not seem to represent a major inconvenience, since it occurs on dates when chemical control measures against the pest are not applied.

Temperature does not only influence the insect phenology, but also the phenological changes of olive trees. In this aspect, fruiting presents large gaps between high and low areas. As indicated above, fruiting in low areas occurs one month earlier than in high areas.

Although referring to another insect associated with olive trees, Petacchi et al. (2015) in their study on *Bactrocera oleae* (Rossi, 1790) (Diptera: Tephritidae) also highlight the influence of altitude as a determining factor in the emergence of adults. Similarly, Kumral et al. (2005) and Armendariz et al. (2009) found great variability in the number of degree-days between the different generations of the olive moth in olive groves in northern Spain and in Turkey, respectively. Armendariz et al. (2009) found values very similar to those presented here for the anthophagous and carpophagous generations (162.59 DD and 974.09 DD, respectively). However, the philophagous generation obtained value significantly different (327.39 DD) from that obtained in this study, a deviation that, on the other hand, is not surprising, in view of the great climatological differences between the northern and southern regions of the Iberian Peninsula.

Environmental variations between different seasons of the year are reported as the cause of the intergenerational variability of the DD of other polyvoltine moths such as *Cydia pomonella* (PITCAIRN et al., 1992), *Platynota idaeusalis* (Walker, 1859) (Lep., Tortricidae) and *Argyrotaenia velutinana* (Walker, 1859) (Lep., Tortricidae), (ROCK et al., 1993) and *L. botrana* (MILONAS et al., 2001).

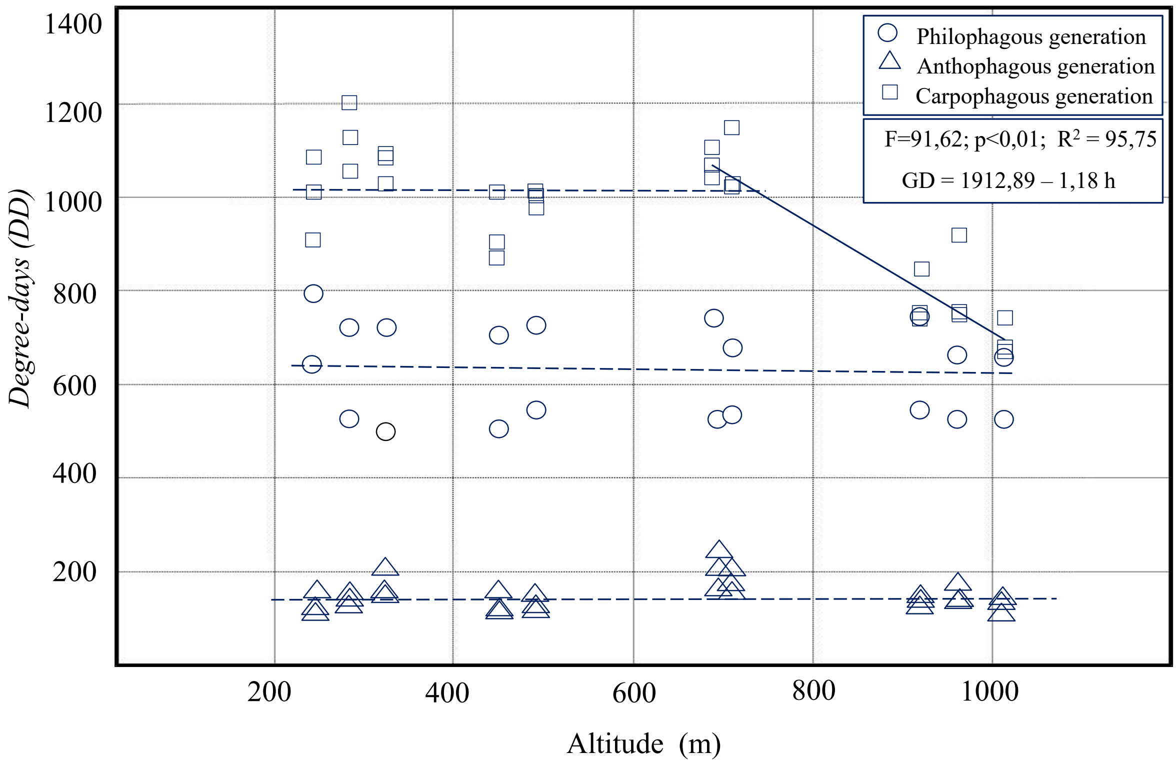

Figure 1. Distribution of values of thermal integrals corresponding to the 10 areas selected for the three *P. oleae* generations as a function of altitude. The regression line for the carpophagous generation is represented in areas with altitude greater than 700 m.

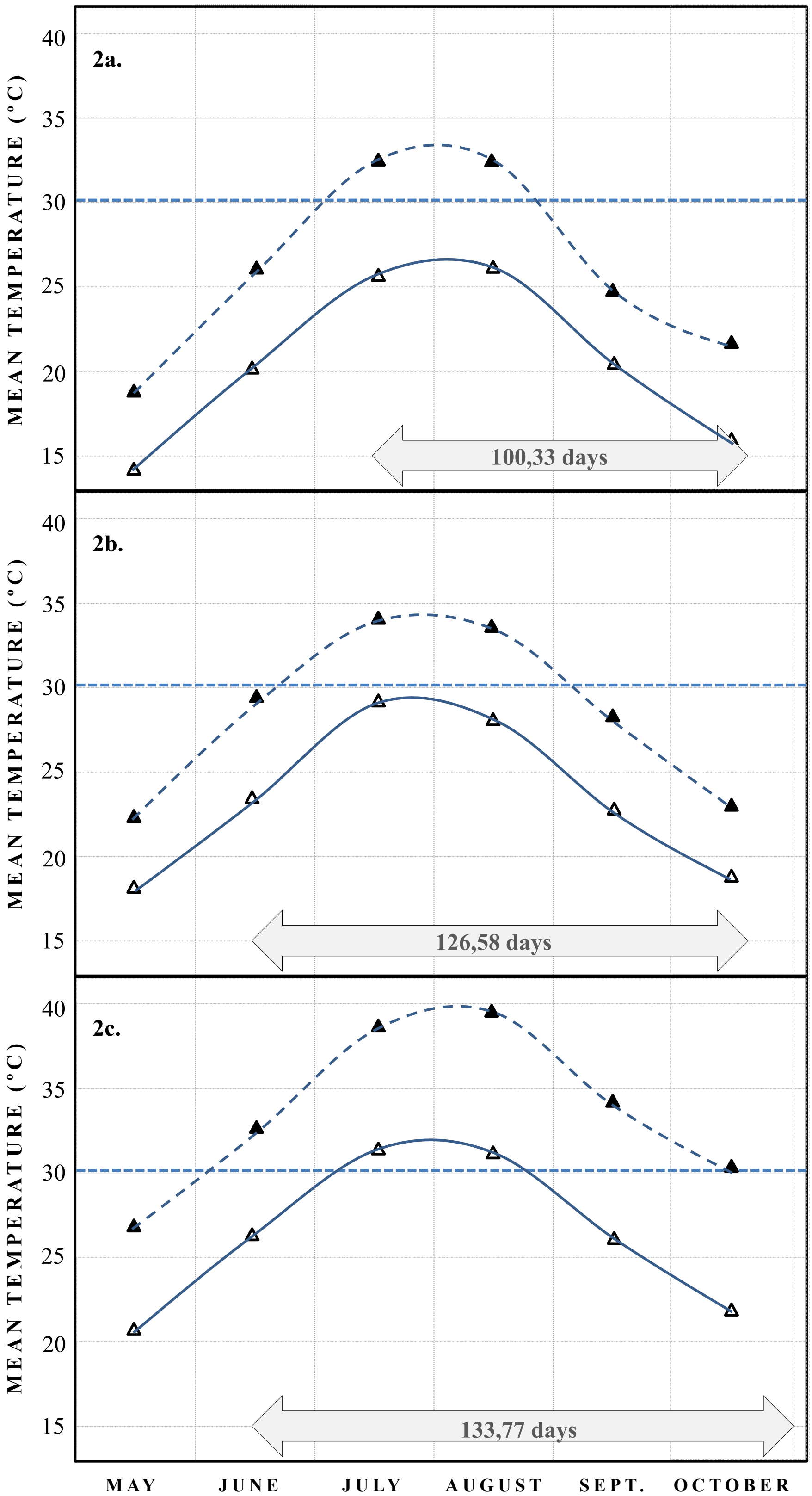

Figure 2. Graphical representation of the mean monthly temperatures (triangles on white background) and the mean maximum temperatures (black triangles) during the development period of *P. oleae* carpophagous generation in the selected olive groves, grouped according to their altitude: h >900m (2a.); h 400-900m (2b.); h < 400 m (2c.). Arrows indicate the mean development duration in each case. The horizontal dashed line represents the upper threshold temperature value (30°C).

Practical application of thermal integrals in the control of *P. oleae*.

As previously indicated, the precise calculation of dates on which the phenological events that determine the application of control measures take place, and which corresponds to the appearance of the first eggs of anthophagous and carpophagous generations, has been performed according to the sum of degree-days counted from January 1, in accordance with Touzeau (1981); Castro et al., (2008); Armendariz et al., (2010); Gonçalves and Torres, (2011); Canales et al., (2014).

The resulting thermal integrals at the beginning of oviposition are specified in Tables 5 and 6 for the different areas, with general mean values for both generations being 412.13 DD and 585.72 DD, respectively.

Table 5. Number of accumulated degree-days (DD) and days (D) elapsed since January 1 (years 2013, 2014 and 2015) and the appearance of the first *P. oleae* eggs in the flower buds of olive trees in the different areas under study (x̄: mean; ERR: standard error).

Code area (h)	2013		2014		2015		x̄ DD	*(x̄ D)*	ERR DD	*(ERR D)*
	DD	*(D)*	DD	*(D)*	DD	*(D)*				
#1 (253 m)	395.35	*(136)*	401.41	*(128)*	438.41	*(127)*	411.72	*(130)*	13.46	*(2.85)*
#2 (289 m)	437.26	*(150)*	409.35	*(128)*	451.60	*(134)*	432.73	*(137)*	12.41	*(6.57)*
#3 (327 m)	410.21	*(140)*	389.97	*(125)*	429.28	*(131)*	409.82	*(132)*	11.35	*(4.36)*
#4 (484 m)	418.03	*(151)*	410.08	*(136)*	401.38	*(135)*	409.83	*(141)*	4.81	*(5.17)*
#5 (504 m)	410.01	*(148)*	409.29	*(133)*	414.20	*(132)*	411.16	*(138)*	1.53	*(5.17)*
#6 (705 m)	371.04	*(144)*	383.04	*(129)*	433.08	*(135)*	395.72	*(136)*	18.00	*(4.36)*
#7 (710 m)	381.08	*(140)*	420.51	*(132)*	397.97	*(131)*	399.85	*(134)*	11.43	*(2.85)*
#8 (927 m)	384.70	*(170)*	416.32	*(154)*	453.00	*(154)*	418.01	*(159)*	19.74	*(5.33)*
#9 (960 m)	392.66	*(177)*	398.51	*(161)*	410.21	*(154)*	400.46	*(164)*	5.16	*(6.81)*
#10 (1017 m)	393.17	*(176)*	428.91	*(162)*	473.89	*(160)*	431.99	*(166)*	23.35	*(5.03)*
x̄ DD (x̄ D)	**399.35**	***(153)***	**406.74**	***(139)***	**430.30**	***(139)***	**412.13**	***(144)***		
ERR DD (ERR D)	**6.21**	***(4.88)***	**4.39**	***(4.56)***	**7.81**	***(3.75)***	**4.28**	***(2.76)***		

Table 6. Number of accumulated degree days (DD) and days (D) elapsed from January 1 (years 2013, 2014 and 2015) until the appearance of the first *P. oleae* eggs in the fruits of olive trees of the different areas under study (x̄: mean; ERR: standard error).

Code area (h)	2013		2014		2015		x̄ DD	*(x̄ D)*	ERR DD	*(ERR D)*
	DD	*(D)*	DD	*(D)*	DD	*(D)*				
#1 (253 m)	582.30	*(164)*	555.76	*(149)*	587.51	*(148)*	575.19	*(154)*	9.83	*(5.17)*
#2 (289 m)	610.36	*(171)*	576.66	*(149)*	638.28	*(156)*	608.43	*(159)*	17.81	*(6.49)*
#3 (327 m)	619.33	*(168)*	547.70	*(146)*	591.22	*(152)*	586.08	*(155)*	20.83	*(6.57)*
#4 (484 m)	603.24	*(179)*	554.61	*(157)*	546.60	*(156)*	568.15	*(164)*	17.70	*(7.51)*
#5 (504 m)	562.66	*(169)*	565.16	*(154)*	589.24	*(153)*	572.35	*(159)*	8.47	*(5.17)*
#6 (705 m)	578.64	*(172)*	606.77	*(157)*	616.51	*(156)*	600.64	*(162)*	11.35	*(5.17)*
#7 (710 m)	569.58	*(168)*	634.46	*(160)*	563.98	*(152)*	589.34	*(160)*	22.62	*(4.62)*
#8 (927 m)	567.45	*(175)*	600.86	*(176)*	626.83	*(175)*	598.38	*(175)*	17.19	*(5.17)*
#9 (960 m)	587.14	*(198)*	561.39	*(183)*	574.96	*(175)*	574.50	*(185)*	7.44	*(6.74)*
#10 (1017 m)	554.92	*(197)*	591.98	*(182)*	605.65	*(181)*	584.18	*(187)*	15.15	*(5.17)*
x̄ DD (x̄ D)	583.56	*(176)*	579.53	*(161)*	594.07	*(160)*	585.72	*(166)*		
ERR DD (ERR D)	**6.78**	***(4.07)***	**8.89**	***(4.40)***	**8.98**	***(3.73)***	**2.46**	***(2.31)***		

The average accumulated temperature values for the two generations (anthophagous and carpophagous) are reached after an average period of 144 and 166 days, respectively, which corresponds to May 25 and June 15, respectively. However, the selection of these two dates for the application of control measures would not allow the insect to be controlled except in only one third of olive groves under study, so adequate control requires considering the microclimatic characteristics of the different areas: in the case of the anthophagous generation (Figure 3) in crops located at altitudes of up to 710 m, applications should preferably be made between May 10 and 20, while at higher altitudes (>900 m), in which flowering very frequently occurs in the month of June (the later the higher the altitude), applications should be preferably performed between June 10 and 20.

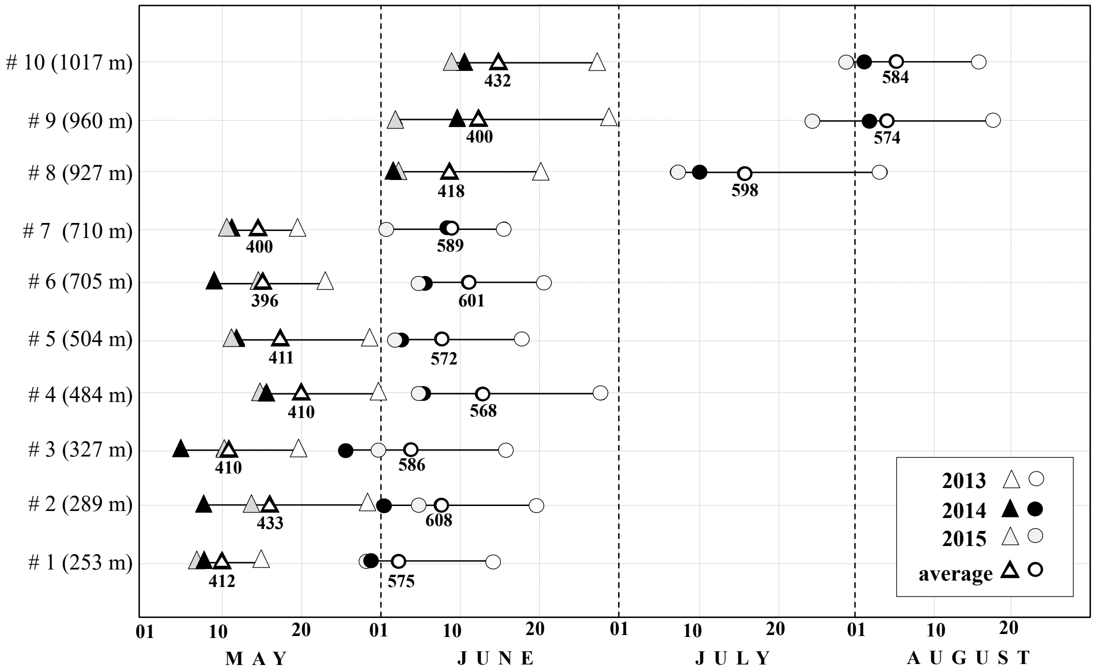

Figure 3. Representation of dates of appearance of the first eggs corresponding to the anthophagous (triangles) and carpophagous (circles) generations in the years 2013, 2014 and 2015, as well as the average dates for each of the 10 olive cultivation areas, according to the altitude. Figures indicate the average number of degree-days for each area, in both generations.

Likewise, with regard to the carpophagous generation, the application of synthetic insecticides is a frequent practice, although before adopting this decision, previously evaluating the predatory potential of lacewing larvae (Neu., Chrysopidae) is recommended in the area. In this sense, Ramos and Ramos (1990), in a 20-year study, reported mean predation values of 71% in olive groves in the province of Granada, while González-Ruiz et al. (2008) found predation values above 90%. Equally high are data reported in olive groves in the province of Jaén, with predation rates greater than 80% (CIVANTOS et al., 2017). It is obvious that, as the Integrated Pest Management has been adopted, the use of chemical insecticides on this generation would not only be unnecessary, but also counterproductive for the interests of the farmer, due to its detrimental effect during the depredation process of lacewings. Despite this, the application of any of the authorized products against carpophagous generation (Acetamiprid, Betaciflutrin, Deltametrin, Fosmet, Lambda Cihalotrin, or Spinetoram) is very common. According to data obtained, they allow adapting dates of intervention on this generation according to the altitude:

- In olive groves located at altitudes of up to 710 m, the accumulated temperature values determine the average date of the beginning of oviposition in fruits during the first half of June.
- In olive groves at altitudes above 900 m, the onset of oviposition takes place during the second fortnight of July, with the most delayed areas chronologically corresponding to olive groves at higher altitudes (between 960 m and 1017 m), where the average date of onset of oviposition is observed in the first days of August, which represents an average difference of almost 2 months compared to lower areas.

Conclusions

The mean value observed for thermal integrals of *P. oleae* was 638.44 DD, 173.50 DD and 965.18 DD for philophagous, anthophagous and carpophagous generations, respectively. The crop altitude does not represent an influencing factor in the thermal integrals of philophagous and anthophagous generations. However, in the carpophagous generation, a decrease in the number of degree-days is detected in areas above 700 m.

The average development duration has been 220 and 121 days for philophagous and carpophagous generations, respectively. In relation to these, the considerably shorter average duration corresponds to the anthophagous generation, of only 23 days, which takes place during spring, when average temperatures are within the optimum temperature range for this insect. On the contrary, the maximum value, corresponding to the philophagous generation, of approximately 7 months (between the beginning of autumn and beginning of spring), occurs when the average temperatures are frequently below the lower threshold.

The intermediate duration, of just over 3 months, corresponds to the carpophagous generation, which takes place between the end of spring and beginning of the following autumn. In this case, its longer duration is attributable to the high maximum temperatures that exceeded the upper threshold during the summer, and that determined the entry of larvae in the diapause drought.

A close influence between altitude of the cultivation area and the duration of philophagous and carpophagous generations has been confirmed. In the first, the duration increases significantly, although only in olive groves located at altitudes above 900 m. The greatest influence of altitude on generation time affects the carpophagous generation, whose maximum and minimum values correspond to olive groves located at the lowest levels (h<400 m), and mountain olive groves (h>900 m), respectively.

The estimates of the average number of degree-days counted from January 1 to the appearance of the first eggs, corresponding to the anthophagous and carpophagous generations, were 412.13 DD and 585.72 DD, respectively. The influence of altitude on the development duration implies that these values are reached in a relatively wide time interval, which implies mean differences of 1 month for the anthophagous generation, and 2 months for the carpophagous generation, depending on the altitude. This fact constitutes a factor of great importance from the practical point of view, for which data of this study have allowed the development of a model-chronogram where temporary intervals are represented for an optimal effectiveness of plant health treatments according to crop altitude.

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