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Host–Pest Interactions: Investigating *Grapholita molesta* (Busck) Larval Development and Survival in Apple Cultivars under Laboratory and Field Conditions

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Abstract: Phenology models are widely used in Decision Support Systems to predict the phenology of pests. *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), a polyphagous pest, has a high prevalence in apple trees (*Malus domestica* Borkh.) (Rosales: Rosaceae) in certain areas, despite the fact that apple trees are considered secondary hosts. Its natural behavior of feeding inside plant tissue at the larval stage makes monitoring and planning methods of control critical for Integrated Pest Management. The development times and survival rates of two field populations of *G. molesta* larvae on apple cultivars ‘Gala’, ‘Golden’, and ‘Fuji’ were determined under different temperature (constant versus field fluctuation) and feeding conditions (diet versus apples attached to the tree and detached from the tree). The results showed that *G. molesta* fitness in apples is affected by fluctuating temperature regimes in the field, the condition of the fruit, whether or not the fruit is attached to the tree, and the cultivar. The temperature was the main cause of the differences in the studied parameters. Larvae development time was delayed due to fluctuating field temperatures. Larvae had a shorter development time in fruits detached from the tree compared to attached fruits, and apple cultivars influenced development time in both laboratory and field conditions, with the ‘Golden’ cultivar having better fitness and a higher rate of larvae survival in the fruits attached to the tree. These factors need to be considered to properly adjust the phenology models and improve the Integrated Pest Management system of apples.

Keywords: fluctuating temperatures; attached fruits; fitness; apple cultivars; phenology model; Tortricidae



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1. Introduction

Phenology forecast models are useful tools that predict pest population dynamics and can be used in IPM (Integrated Pest Management) programs to determine the optimal moment for interventions to control the target pest [1,2]. Phenology models can predict the stage of a given species, and for this reason, they have been widely used in Decision Support Systems to predict the phenology of pests in commercial orchards [3–6]. The incidence of a pest in a host depends on its survival, development, and offspring production, and all of these parameters are influenced by the suitability of the host [7] and abiotic factors [8]. In poikilothermic organisms, such as insects, temperature plays a major role in their development [6,9–11]. Many insect phenology models use development data from constant temperatures [6,9,10] collected under controlled environmental conditions with

fruits detached from trees [7,12–16] to model the relationship between temperature and development. Even though information from studies at constant temperatures is crucial for modeling the relationship between development rate and temperature [6], these conditions differ from those in the field.

Grapholita molesta (Busck) (Lepidoptera: Tortricidae) is a pest found worldwide. Peach is generally considered its primary host, although it can infest many fruit trees in the Rosaceae family [17]. Its larvae feed inside fruits [17], causing direct damage to the fruit; they can also feed inside green shoots [18,19] and apple burr knots [20]. *Grapholita molesta* spends most of its larval stage inside host tissues [17]. During larval development, the options to manage the pest are greatly reduced. Therefore, precisely monitoring this pest is especially important to target the most accessible stages [21]. *Grapholita molesta* can have three to six generations depending on the region [1,2,15,22,23]. During the adult stage, *G. molesta* can colonize other areas in search of better conditions; it can move from a harvested orchard to another where fruits are still present, or simply to a host that is in a more optimal phenology stage for their offspring [24,25]. Therefore, the time required for *G. molesta* to complete its development can change during the season due to fluctuating temperatures and varying host and host quality food conditions (e.g., maturity) [19], creating difficulties in predicting its phenology.

In Catalonia, a region in northeastern Spain, *G. molesta* is present in the two major fruit production areas: Girona in the northeast, close to the Mediterranean Sea, and Lleida in the interior. Notably, it shows different behavior in these areas. In the Girona province, apple is the most common host for *G. molesta*, and its presence in alternative hosts is scarce. In this province, *G. molesta* has been causing serious losses in apples over the last decade [26]. On the contrary, in the province of Lleida, where there is a mixture of host species, its main hosts (peaches and nectarines) are present in the areas where apple and pear are dominant. However, no significant losses in apples caused by *G. molesta* have been reported so far, even though significant numbers of *G. molesta* adults are also detected in apple orchards [27]. Further differences can be found in the number of generations; *Grapholita molesta* has four generations in Girona and five in Lleida, even though both provinces have similar temperature and climatic conditions [27]. The fitness of *G. molesta* is influenced by many factors; in general, it is higher in peach than in other hosts such as plum or apple [12–15,19]. However, the level of fruit maturation affects the development and survival of *G. molesta* [15,16], and the fitness of *G. molesta* is reversed in immature fruits, with higher fitness in apples than in peaches [15]. In addition, differences in the development time of *G. molesta* larvae reared on different cultivars of apples and peaches have also been reported [7,14,19]. So, in an area with a predominant crop, as in the Girona province, the incidence of the main apple cultivars may be important in the modeling of the pest. The most common apple varieties cultivated in Catalonia are ‘Golden’, followed by ‘Gala’, and ‘Granny Smith’ or ‘Fuji’, covering a maturation period from August to October.

This study aims to investigate the development time and survival rate of two Spanish populations of *G. molesta* (one feeding on apples and the other on peaches) on three apple cultivars (‘Gala’, ‘Golden’, and ‘Fuji’) under different temperature conditions: laboratory (constant temperatures) versus field (fluctuating temperatures), and various food conditions (diet, apples still attached to the tree, and detached apples). The incidence of these factors in pest modeling is discussed.

2. Materials and Methods

2.1. Insects

Two populations of *G. molesta* from Northeast Spain were captured, one from orchards around the village of Ventalló in the province of Girona (hereafter referred to as the Girona population), and the other from Menàrguens village in the province of Lleida (hereafter referred to as the Lleida population). Adults and larvae were captured in Girona in 2018 and in Lleida during 2017–2018. Adult insects were captured using Ajar-like traps baited with terpinyl acetate, while larvae were collected from infested fruits and shoots. The

Girona insects were collected in apple orchards, while the Lleida insects were collected in peach orchards. Adult insects were kept in an environmental chamber at 25 ± 1 °C under a 16:8 h light/dark photoperiod, and larvae were reared at the same temperature and photoperiod in a semi-artificial diet modified from that of Ivaldi-Sender [28]. The main diet components were agar, maize flour, and beer yeast. Fresh eggs (0–24 h), collected from wax paper that was constantly available for the females to lay eggs, were used to infest the fruits.

2.2. Plant Material

The orchards involved in the study were located at the IRTA Mas Badia research center (42.054271 N, 3.062348 E, La Tallada d'Empordà, Girona, Spain). The maximum distance between the apples used in the experiment was 250 m in a continuous apple area. Three different apple cultivars were used in the experiment: 'Gala Schniga', 'Golden Crielaard', and 'Fuji Toshiro', all of them on M9 rootstock. Planting spaces were 3.75×1 m, 3.8×1 m, and 3.75×1.2 m for 'Gala', 'Golden', and 'Fuji'. The 'Gala' orchard was planted in 2004, and 'Golden' and 'Fuji' were planted in 2006. The experiments were conducted on trees managed with a special Integrated Pest Management (IPM) system called "Fruit.net", which aims to obtain fruits without residues at harvest [29,30]. Mating disruption was used for codling moth (*Cydia pomonella* (L.), Lepidoptera: Tortricidae), mass trapping for medfly (*Ceratitis capitata* Wied., Diptera: Tephritidae), and Phytoseiidae for controlling European red mite (*Panonychus ulmi* Koch, Trombidiformes: Tetranychidae), and punctual sprays were applied when population levels reach the recommended threshold. During the years of the experiments, no insecticide treatments were used following the flowering of the apple trees. Three apple cultivars, 'Gala', 'Golden', and 'Fuji', were used to test the development of *G. molesta*, which are usually harvested in August, September, and October, respectively, in Girona province. To avoid the effect of the fruit maturation stage on the development of *G. molesta* [15], experiments on each cultivar started when the fruits reached 75% maturation for the corresponding cultivar. The period considered in this study as fruit maturation started at "full bloom" (phenology stage BBCH 65) and ended on the expected harvest day. The dates at which these stages of apple tree phenology were reached were provided by the personnel responsible for the experimental orchards, who are specialists in apple tree physiology.

2.3. Development Time and Survival of Two Populations of *G. molesta* under Laboratory Conditions

Four different environmental chambers were used to test five temperatures (16, 18, 22, 26, and 30 °C) in the laboratory. The chamber temperature was monitored throughout the experimental periods using data loggers (HOBO ext temp/RH pro v2 or HOBO pendant, Onset Computer Corporation, Bourne, MA, USA) (Table S1).

At 75% fruit maturation for each cultivar, apples were harvested for laboratory experiments and stored at 4 °C in fruit conservation chambers for up to 4 months. One day before the start of each temperature experiment, the apples were placed at the corresponding temperature. To infest the apples with *G. molesta* eggs, a piece of wax paper containing one egg less than 24 h old was placed in the calyx of an apple. The calyx was then covered with parafilm. The infested apples were kept in individual plastic containers with corrugated cardboard inside to allow larval pupation and to facilitate the detection of mature larvae. The containers were closed with a mesh and an elastic band to prevent the larvae from escaping. One hundred eggs of each population were placed on fruits of each cultivar ('Gala', 'Golden', and 'Fuji'). Each temperature was tested in two different chambers at different dates (50 eggs each time) to reduce any possible effect of the chamber and any maturation that happened during storage in the conservation chambers.

Egg hatching and mature larval emergence from the fruit were recorded daily to calculate the larval development time. The egg was considered to have hatched when it showed iridescence following the black head stage, and larval maturation was established

when the larva exited the apple and began to form the pupal cocoon. The phenology model described by Croft et al. [24] was used to determine the end of the experiment at each temperature. This model is currently available on the Ruralcat website [31], which offers services to advisers and producers in the study area regarding pest phenology during fruit growth. The hourly temperature recorded by the data loggers was used to calculate the number of degree-days (DD) at each temperature. DD was calculated using the thresholds described in Croft et al. [22] (7.2 and 32.2 °C) with a horizontal cut-off. When the DD accumulated for each temperature exceeded the development time for egg and larval development described in the phenology model (~300 DD) plus 150 DD, the apples in which the larva had not emerged were checked to detect the presence of living larvae that had not finished their development.

2.4. Development Time and Survival of Two Populations of *G. molesta* under Field Conditions

In the field experiments, apples were infested with eggs following the same methodology used in the laboratory experiments unless specified otherwise. Infestation occurred when each cultivar reached 75% maturation. The time at which larvae emerged from fruits was recorded daily. This time corresponds to the egg plus larval development time as the time of egg hatching was not recorded in field conditions. As the possible effect of fruit detachment needs to be considered in the assays [19,32], three experiments were conducted in the field: (i) fruits attached to the trees, (ii) fruits detached from the trees but kept in the same field, and (iii) diet control for the experimental period of each apple variety. Throughout the experimental period, air temperature in the experimental fields was monitored using two data loggers (HOBO ext temp/RH pro v2, Onset Computer Corporation, Bourne, MA, USA) (see Table S1 for details).

The procedure for each experiment was as follows:

(i) Fruits attached to the trees: Apples infested with *G. molesta* eggs were left naturally attached to the trees. This treatment was carried out for three consecutive years. In 2018 and 2019, 300 eggs from each population were placed on the same number of apples over two consecutive days (150 each day) at 75% maturation of each of the three apple cultivars. In 2020, 150 eggs from each insect population origin were placed on the 'Gala' cultivar and 100 on the 'Golden' and 'Fuji' cultivars on the corresponding dates. Just before each experiment began, branches containing 4–10 apples were covered with mesh sleeves to prevent predators and parasitoids from entering and *G. molesta* larvae from escaping. The selected branches were evenly distributed over the entire tree canopy. During the entire experiment period, two data loggers were installed at the top and middle of the canopy. The phenology model described in Croft et al. [22] and the temperatures recorded in situ with the data loggers were used to predict the end of larval development. When the accumulated DD was close to the end of larval development, the emergence of larvae from apples was checked daily. After the first mature larva was detected in each repetition, the inspection was maintained until a further ~175 DD were accumulated to ensure enough larvae were recovered to allow for statistical analysis. When the larvae were near the end of their development, corrugated cardboard was placed on the top end of the sleeves to provide a desirable place for larval pupation and facilitate the detection of mature larvae. The whole content of the mesh was examined to locate the mature larvae, and notably, most of the larvae (>70%) were detected in the cardboard. When the specified DD were reached, all infested apples were collected and examined. The presence of living larvae inside the fruit or any indication of larval feeding was recorded. Since egg hatching was challenging to record under field conditions, detecting larval feeding in infested apples was considered evidence of egg hatching, and the number of apples with larval feeding was treated as the number of hatched eggs.

(ii) Fruits detached from trees: In 2020, one day after the field experiment with attached fruits started, apples from the same trees were detached from the trees and infested with *G. molesta* eggs following the same procedure described earlier. The process was repeated for each cultivar at 75% maturation. A total of 150 eggs of each *G. molesta* population

were individually placed in detached ‘Gala’ cultivar fruits, and 100 of each population were placed in apples of ‘Golden’ and ‘Fuji’ cultivars. Infested apples were placed in two mesh-lined field cages beneath the same trees used in the attached fruit experiment. The procedure was repeated for each cultivar. The phenology model [22] and the temperatures recorded with data loggers placed inside the cages were used to determine when the larvae were nearing the end of their development. Larval emergence from apples was checked daily until 175 DD after the first larva emergence was detected. When the experiment ended, all apples were examined to detect living larvae or indications of larval feeding, as in the attached fruit experiment. Larval feeding was also used as an indicator of egg hatching.

(iii) Diet control: In 2019, *G. molesta* development in the standard semi-artificial larval diet was monitored during field experiments in the corresponding period of each cultivar. On the same day that the experiment with attached apples was initiated in each cultivar, 300 eggs from each population were placed together inside vented cages on a semi-artificial diet (the same used for larval growth in insect breeding). To guarantee that the eggs developed unimpeded, 200 g of diet was placed in each cage, four times the quantity used in regular rearing for the same number of eggs. The ventilated cages were then placed in field cages protected from direct sunlight in the same research station near the experimental orchards. A data logger was placed inside the cage to record the temperature. The proportion of hatched eggs was registered, and the emergence of mature larvae was checked daily until no more mature larvae were detected.

2.5. Statistical Analysis

Different statistical methodologies can be used to analyze development and mortality data in entomology [7,12–16,19]. “Survival” analysis is common for mortality data (e.g., [33–35]). However, it can also be used for many other “time-to-event” data, such as development times [36]. Survival analysis has some characteristics that make it useful for studying insect development. For example, it can incorporate censored (incomplete) data into the analysis (e.g., insects that have not finished their development in the period studied) [36,37]. These individuals are usually omitted or considered to have finished their development in most development studies. However, including them as censored data in survival analysis can lead to more accurate conclusions [37]. Additionally, survival analysis compares the whole follow-up period of the data [38], not just the mean, as in most usual methods (e.g., ANOVA).

All data analyses were performed using R 4.1.1 [39]. Larval survival under laboratory conditions was calculated as the proportion of mature larvae that emerged plus the remaining living larvae inside the fruit with respect to the number of hatched eggs. In field conditions, egg hatching was not recorded; therefore, larval survival was calculated as the proportion of mature larvae that emerged plus living larvae with respect to the number of apples with evidence of larval feeding. Survival data were analyzed using a generalized linear model with a binomial function for the error distribution. Model selection started from the simplest model containing no main effects. Then, each main factor was sequentially added, and finally, interactions between significant factors were tested. For model comparison, the likelihood ratio test (LRT) and the Akaike information criterion (AIC) were used, and models with lower AIC values and significantly different LRT were selected. Means of survival for each significant factor were compared using Tukey’s test for multiple pairwise comparisons. For laboratory conditions, the effects of temperature, population, and cultivar were analyzed. For the field-attached fruit experiment, population, cultivar, and year were analyzed. For the field detached fruit experiment, population and cultivar were analyzed. Data from the different experiments (laboratory conditions, attached fruits, detached fruits, and field controls) were analyzed independently.

Development time analyses were conducted using the Kaplan–Meier method [40]. Larvae still inside the apple at the end of the experiments were added as right-censored

individuals (i.e., the event did not occur at the end of the experiment). The log-rank test was used to evaluate the effects of individual factors on the cumulative proportion of larvae that completed development as a function of development time expressed in DD. Multiple pairwise comparisons were made to compare the levels of significant factors using a log-rank test and a Benjamini–Hochberg correction to avoid false positives. In laboratory experiments, larval development time was analyzed. In field experiments, egg plus larval development was analyzed, as well as comparisons between field and laboratory conditions.

3. Results

3.1. Survival

The statistical analysis of laboratory experiments conducted at constant temperatures revealed that “temperature” was the most significant factor in explaining larval survival (Deviance [Dev.] = 628.29, $df = 2$, $p < 0.001$). The highest survival rate was observed at 22 °C and 26 °C (87% and 83%, respectively), while the lowest survival rate was recorded at 14 °C (23%) (Figure 1). Additionally, the insect population origin had a significant effect on survival (Dev. = 10.97, $df = 1$, $p < 0.001$), with the survival rate in the Girona population being 7% higher than that of the Lleida population (72% and 66%, respectively). No significant differences were detected among cultivars (Dev. = 4.83, $df = 2$, $p = 0.09$), and the interaction between temperature and insect population origin was not significant (Dev. = 8.03, $df = 4$, $p = 0.09$).

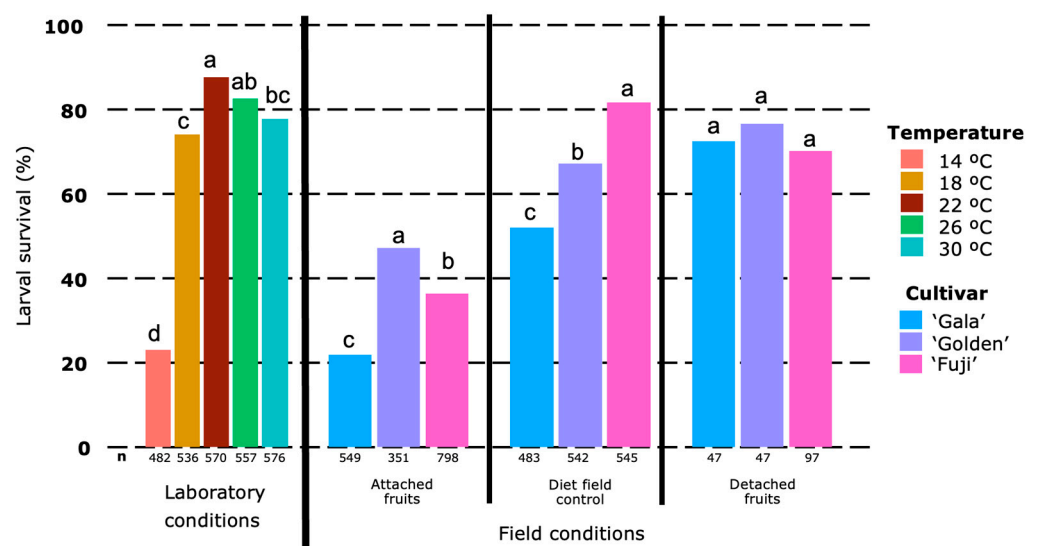


Figure 1. Effect of temperature on *Grapholita molesta* larvae survival under laboratory conditions and effect of cultivar on larval survival under field conditions. Values within the same experimental conditions (within vertical lines) with different letters indicate significant differences (Tuckey test: $p < 0.05$). The initial number of larvae for each treatment is located under the corresponding column. Note: in diet field control, “cultivar” corresponds to the period of the year in which the field experiments were conducted for each cultivar; insect population origin and year of field experiment have been combined in this plot to facilitate representation.

Experiments conducted under field conditions revealed that: (i) in fruits attached to the tree, larval survival was primarily influenced by the factor “cultivar” (Dev. = 88.23, $df = 2$, $p < 0.001$) and “year” (Dev. = 62.01, $df = 1$, $p < 0.001$), with no interaction between the two factors detected (Dev. = 0.46, $df = 2$, $p = 0.79$). The insect population origin did not affect larval survival in attached fruits under field conditions (Dev. = 0.02, $df = 1$, $p = 0.88$). The ‘Golden’ cultivar had the highest survival rate, while ‘Gala’ had the lowest (Figure 1). Larval survival was 1.6 times higher in 2018 (43%) than in 2019 (26%). In 2020, due to high mortality in eggs (84%), the final number of emerged larvae was very low (1–8 depending on the cultivar and population), so data from attached fruits in 2020 were not

used for statistical analysis. (ii) In detached fruits (in 2020), larval survival was not affected by either cultivar (Dev. = 0.68, df = 2, $p = 0.71$, Figure 1) or population (Dev. = 0.09, df = 1, $p = 0.76$). (iii) In the diet field control (in 2019, with an artificial diet), differences were found in larval survival between the cultivar periods (Dev. = 103.45, df = 2, $p < 0.001$, Figure 1) but not between population origins (Dev. = 0.15, df = 1, $p = 0.69$).

3.2. Development Time

The median development time for larvae reared under laboratory conditions was 248.07 DD, which was 33.07 DD higher than the 215 DD estimated in the phenology model used to calculate the DD [22]. Considering egg plus larval development (294.4 DD) [22], the recorded development time was also higher in all the conditions analyzed, with 319.59, 431.50, and 501.39 DD being the median development times for laboratory detached fruits, field-detached fruits, and field-attached fruits, respectively.

Under laboratory conditions, the factors “cultivar” ($\chi^2 = 97.77$, df = 2, $p < 0.0001$, Figure 2A) and “temperature” ($\chi^2 = 268.15$, df = 4, $p < 0.0001$, Figure 2B) had a significant effect on larval development time, with no significant differences observed between populations ($\chi^2 = 0.17$, df = 1, $p = 0.59$). The development time of ‘Gala’ was significantly shorter than that of the other two cultivars (‘Golden’ and ‘Fuji’), and ‘Golden’ was significantly shorter than that of ‘Fuji’ (Figure 2A). The shortest development time was registered at 26 °C and the longest at 14 °C. The development time increased significantly from 26 °C to 22 °C, 18 °C/30 °C, and 14 °C, respectively (Figure 2B). At 14 °C, larval development times were equal in all cultivars; at 18 °C and 26 °C, *G. molesta* larvae had significantly shorter development times in ‘Gala’ and ‘Golden’ cultivars compared to ‘Fuji’; and at 22 °C and 30 °C, differences in larval development time were found among all three cultivars studied (Figure S1). The effect of temperature on development time was similar across all cultivars (Figure S2).

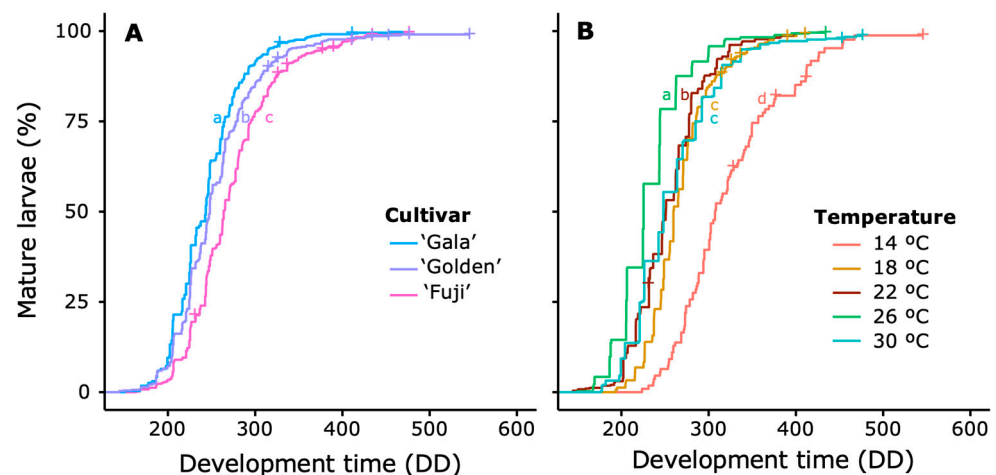


Figure 2. Cultivar (A) and temperature (B) effect on cumulative proportion of *Grapholita molesta* larvae that completed development as a function of development time since egg hatching (larval development time expressed in degree-days, DD) under laboratory conditions. Different letters indicate significant differences between curves (Log-rank test $p < 0.05$). The number of larvae alive at the end of the assay was 619, 624, and 647 for ‘Gala’, ‘Golden’, and ‘Fuji’ cultivars (A), and 109, 395, 467, 449, and 440 for 14, 18, 22, 26, and 30 °C (B). Crosses mark times when censored data occurred.

Under field conditions:

(i) In the attached fruits during 2018, the irrigation system in the ‘Golden’ cultivar orchard failed, resulting in a water deficit for the plants. The size of the affected fruits was clearly reduced, and probably the performance of the larvae was also altered. In these attached apples, the median development time (50% of mature larvae) was approximately 100 DD shorter than that recorded in the same orchard in 2019 (Table S2). The larvae

of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) also preferred feeding on drought-stressed apple leaves [41], suggesting a benefit from feeding on stressed plants. For this reason, data from ‘Golden’ 2018 were excluded from the statistical analysis. In general (excluding ‘Golden’ 2018), the cultivar had a significant effect on egg plus larval development time in attached fruits ($\chi^2 = 53.13$, $df = 2$, $p < 0.0001$) (Figure 3A). There were no significant differences between ‘Gala’ and ‘Fuji’ in either 2018 or 2019. Egg plus larval development time in the ‘Golden’ cultivar was significantly shorter in 2019 than in the ‘Fuji’ cultivar. Significant differences in development time between the years 2018 and 2019 were also found ($\chi^2 = 74.29$, $df = 1$, $p < 0.0001$). The development time in 2019 at 50% of larval emergence was around 40 DD shorter than that in 2018; however, the temperatures recorded during each cultivar period were very similar (Table S1). Data from 2020 was not used due to the low number of mature larvae recovered. Insect population origin did not affect development time in fruits attached to the tree ($\chi^2 = 0.25$, $df = 1$, $p = 0.61$).

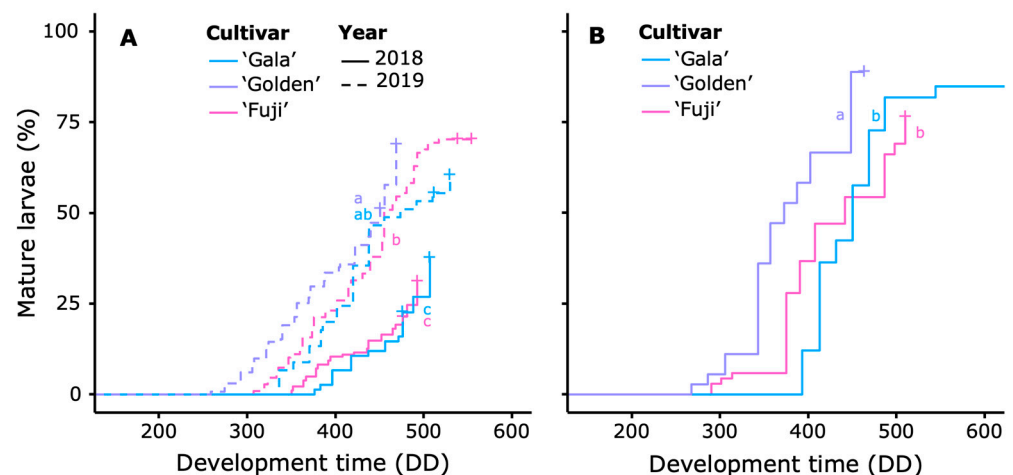


Figure 3. Cumulative proportion of *Grapholita molesta* larvae that completed development as a function of development time since egg laying (egg plus larval development time expressed in degree-days, DD) in field conditions, (A) fruit attached to the trees, (B) fruit detached from the trees (2020). Crosses mark times when censored data occurred. Different letters indicate significant differences between curves (Log-rank test $p < 0.05$). The number of larvae alive at the end of the assay was 75 and 182 for ‘Gala’ and ‘Fuji’ cultivars in year 2018, and 45, 131, and 108 for ‘Gala’, ‘Golden’ and ‘Fuji’ cultivars in year 2019 (A); and 33, 36, and 68 for ‘Gala’, ‘Golden’ and ‘Fuji’ cultivars in year 2020 (B). Crosses mark times when censored data occurred.

(ii) In detached fruits, egg plus larval development time was significantly affected by the cultivar ($\chi^2 = 23.28$, $df = 2$, $p < 0.0001$), with the ‘Golden’ cultivar resulting in a shorter development time (Figure 3B). This experiment found no significant difference in population origin ($\chi^2 = 1.51$, $df = 1$, $p = 0.22$).

(iii) The diet field control in 2019 showed differences in egg plus larval development time between the periods in which the attached fruit experiment was conducted for each cultivar ($\chi^2 = 346.43$, $df = 2$, $p < 0.0001$). The development time was shorter for the ‘Gala’ period, followed by the ‘Golden’ period, while the ‘Fuji’ period showed the longest development time (Table 1).

Table 1. *Grapholita molesta* egg plus larval development time (expressed in degree-days, DD) for 25, 50, and 75% of the total larvae recovered in the field control experiment with semi-artificial diet. Different letters indicate significant differences between curves (Log-rank test $p < 0.05$), and n indicates the number of larvae alive at the end of the assay.

Cultivar Period	T (°C)	n	Egg Plus Larval Development Time (DD)			
			25%	50%	75%	
‘Gala’	25.01	249	237.04	255.91	255.91	a
‘Golden’	24.56	362	249.55	264.37	280.45	b
‘Fuji’	22.15	440	269.44	282.64	294.88	c

3.2.1. Laboratory vs. Field Comparisons

Differences were observed in egg plus larval development time within constant temperatures in the laboratory (14, 18, 22, 26, and 30 °C) and between them and field-attached fruits and detached fruits ($\chi^2 = 1817.79$, $df = 12$, $p < 0.0001$). Constant temperatures had a similar effect on egg plus larval development as on larval development alone; development time was shortest at 26 °C and increased at 22, 18, 30, and 14 °C (Table 2). Development in all temperatures tested in the laboratory was shorter than in all field conditions, except at 14 °C, which had no significant differences from the development in the detached ‘Golden’ cultivar (Table 2).

Table 2. *Grapholita molesta* egg plus larval development time (expressed in degree-days, DD) for 25, 50, and 75% of the total larvae recovered. Different letters indicate significant differences between curves (Log-rank test $p < 0.05$), and n indicates the number of larvae alive at the end of the assays.

Fruit Condition	Year	T (°C)	Cultivar	n	Egg + Larval Development Time			
					25%	50%	75%	
Detached Lab.		26		470	279.62	300.96	319.59	a
Detached Lab.		22		498	290.30	315.17	339.69	b
Detached Lab.		30		444	296.10	317.97	362.03	c
Detached Lab.		18		395	329.36	341.83	372.22	d
Detached Lab.		14		114	373.90	406.06	454.00	e
Detached Field	2020	25.20	‘Golden’	36	343.27	372.79	448.51	e
Detached Field	2020	22.80	‘Fuji’	68	375.17	441.63	509.89	fg
Detached Field	2020	28.42	‘Gala’	33	412.92	450.26	486.76	fg
Attached Field	2019	24.17	‘Golden’	131	356.29	450.70	NA	f
Attached Field	2019	25.03	‘Gala’	45	419.76	473.35	NA	fg
Attached Field	2019	21.11	‘Fuji’	108	401.87	455.26	NA	g
Attached Field	2018	25.64	‘Gala’	75	487.89	NA	NA	h
Attached Field	2018	21.72	‘Fuji’	182	492.74	NA	NA	h

Grapholita molesta larvae in detached apples develop faster at constant laboratory temperatures than at field temperatures (‘Fuji’ detached—average field T: 22.08 °C vs. lab detached—average T: 22 °C; ‘Golden’ detached—average field T: 25.2 °C vs. lab detached—T: 26 °C; ‘Gala’ detached—average T: 28.4 °C vs. lab detached—T: 26 °C or T: 30 °C).

3.2.2. Field Attached vs. Field Detached Comparisons

In general, larvae in detached fruits had a shorter development time than in attached fruits (Table S3). Also, in experiments of the year 2020, when the comparison between attached and detached is more direct since it was performed simultaneously, the development time was shorter in the attached fruits than in the detached ones (Table S2). Temperatures under detached field conditions were between 3.8 °C and 2.1 °C higher than in attached field conditions (Table S1). However, these results must be treated with caution since only a small number of mature larvae were recovered, and no statistical analysis could be performed. If we consider the development of each cultivar and year independently

(Table S2), there were no differences in the cultivars ‘Gala’ and ‘Fuji’ between the detached fruits and attached fruits of the year 2019. It should be noted that the temperature difference (Table S1) found between ‘Gala’ detached and ‘Gala’ attached 2019 (3.39 °C) was much higher than that of the same ‘Golden’ treatments (1.03 °C), where significant differences were found.

4. Discussion

When the variable related to feeding was uniform, such as in the laboratory conditions assays with detached apples of the different cultivars, or in the field conditions, using an artificial diet, the temperature was the main factor explaining the significant differences in larval survival. In the diet field control, survival was highest during the period of ‘Fuji’ ripening, followed by ‘Golden’ and ‘Gala’, with significant differences between the two. The mean temperatures at these moments were 22.15 °C, 24.56 °C, and 25.01 °C, respectively, with a very similar thermal amplitude ($T_{\max}-T_{\min}$) ranging between 18.3 °C and 20.9 °C. These mean temperatures coincide with the range of the most favorable conditions in the laboratory (22 °C and 26 °C) [42], 22 °C being the best and the closest to the average in the ‘Fuji’ variety period, which shows the highest survival within the diet control.

No differences were found in survival between cultivars in the detached fruits assays, neither in the laboratory conditions nor in the field, where the percentage of survival larvae was even higher than the one obtained in the field diet assay, with more than 70% in all cultivars. A similar larval survival rate was found by Sharker et al. [15] in detached apples of the ‘Fuji’ cultivar infested with *G. molesta*. However, the survival rate of *G. molesta* [7] or *C. pomonella* [43] larvae was affected by the cultivars in other assays with detached apples. Nevertheless, in the attached fruit assay there were significant differences in survival among the cultivars.

The fruit penetration rate of *G. molesta* first instar is strongly influenced by the firmness of the fruits [7,15], but it can be also influenced by other surface properties such as wax, smoothness, and different secretions of the surface [44–47]. These characteristics change when the fruits are detached from the tree [48,49], which may have contributed to the observed differences between cultivars. In apples, different maturation rates have been reported between attached and detached fruits [48–50]. This change in maturation has been shown to affect the survival rate of *Cryptophlebia illepidia* (Butler) (Lepidoptera: Tortricidae) larvae [32] and could influence the results of other similar studies, as also suggested by Myers et al. [19]. Moreover, the differences in the maturation process in attached and detached apples can vary among cultivars. In cultivars ‘Gala’ and ‘Golden’, this process is greatly intensified after fruits are harvested [49,50], while this effect in ‘Fuji’ is much lower [49]. Therefore, interaction between cultivar and fruit conditions (attached vs. detached) is to be expected.

In the attached fruits, larval survival was higher in the ‘Golden’ cultivar than in ‘Fuji’. However, in the diet control assay, where differences in larval survival were due to the temperatures, survival was higher in the ‘Fuji’ season time. The temperatures recorded during the experimental periods of the ‘Golden’ and ‘Fuji’ varieties were very similar in the attached fruit trial and in the diet control trial. Therefore, the cultivar seems to have an effect on the survival of larvae when the fruits are attached to the tree. Additionally, direct and indirect plant defense responses triggered by herbivore feeding [51,52] could have interfered with larval survival. The concentration of secondary metabolites can affect herbivore performance [53] and could have been responsible for variations in the survival rate. For instance, growth rate and survival of the leaf beetle (*Chrysomela falsa* (Brown)) (Coleoptera: Chrysomelidae) decreased linearly with condensed tannins from birch (*Betula resinifera* (Sarg.)) (Fagales: Betulaceae) [54]; *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) was found to increase the presence of a secondary metabolite (maltol) when feeding on its plant hosts [55]; and the presence of maltol in plant tissue has been associated with a decrease in the survival of *C. fumiferana* and an increase in its

development time [56]. In apples, a flavonoid (phloridzin) has been related to induced defense against *S. littoralis* [57]. Therefore, the activation of plant defense mechanisms could have reduced the fitness of *G. molesta* larvae in attached apples, as detected in this study.

Insects can evolve to overcome plant defense systems. In some tortricid moths, specific host races have been identified [58,59]. Host races perform better in certain plant species within the total species host's range. In *G. molesta*, adaptation to different host cultivars has been previously reported [60]. *Grapholita molesta* exhibits worldwide genetic variation [61] and also varies among close populations [62,63]. Some of the genetic differences may be due to specific host races. However, it does not appear that the *G. molesta* population in the Girona province has undergone significant adaptation to feed on apples. No differences in development and survival rates were detected between Lleida and Girona populations under field conditions, and only a small but significant increase in survival was detected in the Girona population when developing in apple fruits under laboratory conditions. The reduced difference in larval fitness among populations with different main host species indicates the pest's ability to easily adapt to different food sources and its potential capacity to attack multiple hosts in mixed crop areas.

For practical reasons, research on the effect of temperature on *G. molesta* (and similar species) is typically conducted under controlled environmental conditions with fruits detached from trees [7,12–16]. In this study, field conditions were also investigated to validate the results obtained in a controlled environment; thus, a phenology model was used to compare the development time of egg plus larvae at different temperature regimes.

Although phenology models take into consideration the effect of temperature on development time, differences among temperatures were detected in this study. There are two main explanations for this discrepancy. First, the phenology model used [22] assumes a linear relationship between temperature and development [3]. However, the poikilotherm development rate is a nonlinear function of temperature. The relationship between temperature and development is almost linear near the optimum temperature, but at temperatures near the development thresholds, this linear relation is lost [6,11,64]. In the laboratory assays, at constant temperatures, the shortest development time (in DD) recorded was at 26 °C, which is closest to the optimum temperature (25 °C) found in another study [42]. The development time increased as temperatures deviated from the optimum until it reached the longest development time at 14 °C. The second consideration is described by the Kaufmann effect [53], which recognized the Jensen inequality of non-linear functions as a source of error in degree-day models of insect development and plant phenology.

In general, the larval development time recorded under laboratory conditions was longer than that predicted by the model (215 DD, [22]). Nevertheless, this delay in development (~43 DD) is consistent with other studies on *G. molesta* development in apples [12–14,19]. Under field conditions (both attached and detached fruits), with fluctuating temperatures, the results showed that *G. molesta* had a longer development time than under laboratory conditions at temperatures closest to the mean temperatures recorded during field experiments. Chen et al. [42] found that fluctuating temperatures reduced the development time of *G. molesta* compared to similar constant temperatures. However, in this paper, 20 eggs were inoculated in each fruit, but the mean number of larvae present in each apple was not reported. The effect of the competence for feeding among the larvae and the possible consequences to the development were not discussed. In other species, such as *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), development was found to be shorter under fluctuating temperatures below 25 °C compared to constant temperatures with the same means [64]. Fluctuating temperatures are more energy-demanding than constant temperatures in a highly fluctuating environment, mainly in the warming part of a daily cycle [65,66], as were the field conditions in our assay (detached assay 2020: 11.7 °C–47.5 °C, 'Fuji' period; attached assay 2019: 14.9 °C–44.3 °C, 'Gala' period; attached assay 2018: 15.7 °C–39.2 °C, 'Gala' period), suggesting that high temperatures did impair

performance to some degree. The exposition to harmful temperatures under a fluctuating temperature condition delayed development compared to constant temperatures as in other assays [67,68]. These delays are likely a consequence of direct cold or heat injuries and the costs of subsequent physiological and biochemical repair [69,70].

The effect of excising fruits on larval fitness was also observed in the development time. Under field conditions, the development time was significantly shorter for the ‘Golden’ cultivar in detached fruits than in attached fruits in 2019 and was also shorter in all detached fruit cultivars than in attached fruit ‘Gala’ and ‘Fuji’ cultivars in 2018. The mean temperature in detached ‘Golden’ was 1 °C higher and had a wider range of temperatures than in ‘Golden’ attached fruits. There were significant differences in the development time between the 2019 and the 2018 attached fruit assays. Nevertheless, there were no remarkable differences between the temperature conditions in the attached ‘Gala’ and ‘Fuji’ in 2018 and 2019. Some of the observed variations could be attributed to the phenology model’s difficulties in predicting insect growth in variable temperature conditions. In fact, in the diet field control with the same food source, the DD required to complete egg and larval development were lower in the period of the ‘Gala’ cultivar than in the period of the ‘Fuji’ cultivar, further confirming the inaccuracy of the model. However, other factors previously discussed (e.g., fruit maturation, and plant defenses) may have also interfered.

The ‘Golden’ cultivar had, in general, a better fitness for *G. molesta* larvae development in the field conditions (attached and detached fruits) under fluctuating temperatures. In the laboratory, in detached fruits at constant temperatures (26 °C, 18 °C, and 14 °C), ‘Fuji’ was the cultivar with lower fitness. As in survival, the properties of different cultivars and the effect of detachment over fruit maturation [49,50,71] have influenced *G. molesta* larvae development, as has been reported in other species [32]. Hence, the likely influence of fruit detachment must be considered when using laboratory data to model insect development. Additionally, some fruits attacked by *G. molesta* in natural conditions fall prematurely from the tree [72]. Finally, the information obtained from detached fruits could also be used to include larvae that developed in fallen fruits into a more complex phenology model.

5. Conclusions

There were no appreciable differences in the development and survival of the *G. molesta* populations from Girona and Lleida, despite the fact that the Lleida population does not usually feed on apples. Temperature had the greatest impact on larval survival and larval development time in *G. molesta*, both at constant and fluctuating temperatures. The effect of highly fluctuating temperatures registered in the field, particularly those near to or surpassing the pest’s temperature upper limit of growth, was to considerably slow their development compared to that at constant temperatures. This delay may explain the inaccurate prediction made by the linear model used in the area to forecast pest development. Using more complex models that can deal with temperatures approaching the thresholds will most likely improve field forecasts, especially as temperatures rise due to climate change. Cultivar should also be considered, as it has been proved in the experiments reported here that *G. molesta* larvae had different fitness in different cultivars, being the best in the ‘Golden’ cultivar. At the same time, it was proved that larvae develop faster in detached fruits than in attached fruits. All the results highlight the need to improve the currently used linear phenology model to deal with the natural conditions encountered in the field. All this new knowledge will facilitate an improvement not only in the forecast of pest development but also in the implementation of better IPM systems for apples in the areas studied, and it may also help in other similar regions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae10101016/s1>, Figure S1: Effect of cultivar in the development time within temperatures, Figure S2: Effect of temperature in the development time within cultivars, Table S1: Summary of experimental temperatures, Table S2: Detailed field development times, Table S3: Field vs. laboratory development times.

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