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*Research Article*

# Effects of Environmental Temperature on *Capnodis tenebrionis* Adult Phenology

**Carmelo Peter Bonsignore**

*Dipartimento Patrimonio Architettonico ed Urbanistico (PAU) e Laboratorio di Entomologia ed Ecologia Applicata, Università Mediterranea di Reggio Calabria, Salita Melissari, Feo de Vito, 89100 Reggio Calabria, Italy*

Correspondence should be addressed to Carmelo Peter Bonsignore, cbonsignore@unirc.it

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The phenology of *Capnodis tenebrionis* adults was presented with reference to two different climate conditions. In a temperate moderate-warm climate, adult density showed two separate peaks during the year: one in early summer of the overwintering generation and one with beetles emerging in the late summer. In a warmer semiarid climate, the overwintering adults and the new generation overlapped during summer with a continuous increase of adult density. The difference in the average annual temperature between areas during the study period was almost 3°C, and, in the warmer area, the new generation of *C. tenebrionis* emerged at least one month earlier. To make a prediction of adult presence, a model utilizing degree-days was developed from data collected over a five-year period. Models obtained from equations (Logistic 4-parameter, $y(x) = yo + a/(1 + (x/xo)^b)$) of each year were developed to describe the relationship between degree-day accumulation (with a minimal threshold activity temperature of 14.21°C calculated in the laboratory) and the cumulative percentage of adult presence. According to the overall model, the 50% of overwintering beetles occurred at 726 degree-days (Biofix: 1st March) and the emerging beetles occurred at 801 degree-days (Biofix: 1st July). The results show that a change in temperature is an important aspect that highlights the adaptability of this species.

## 1. Introduction

Abiotic factors including climate may limit the abundance of poikilothermic species and affect their distribution. In particular, variations in the ambient temperature have a dramatic impact on a range of fundamental biological processes including reproduction [1, 2]. Similarly, the relationship between biological events and temperature may provide useful information for predicting the same events, to define the most appropriate time for pest control using presence simulation and seasonal dynamics in regressive models [3].

The buprestid beetle *Capnodis tenebrionis* L. seriously damages *Prunus* spp. L., especially apricot, cherry, and plum [4–6], and it is capable of constraining the organic cultivation of these tree species [7]. Presence of the beetle has serious effects in orchards, and trees can be rapidly killed by the destructive action of its endophytic larvae. The species is widespread in the Mediterranean region, even in areas where their presence has been considered sporadic [8]. Outbreaks in areas previously not affected by the insect have allowed for the presence of the host, possibly as a consequence of global warming [9]. The adults of this species can live longer than one year and some hibernate twice (C. P. Bonsignore, unpubl. data). The females of *C. tenebrionis* are larger and heavier than males, with a sex ratio of 1 : 1 in the population [10]. During the reproductive phase of their lifecycle, the adults of beetle make male-biased aggregations and mated females lay their eggs at the base of host plants, after which, the larvae penetrate the roots of the tree. When the summer months are cold and wet, the number of eggs laid by one female drastically decreases [11]. The species overwinters in the adult stage or with different larval instars that can be simultaneously present on trees (range: 1–7 cm). The adult is active during day and flies during warm days [12], seriously damaging the plant by feeding on the young bark of shoots, buds, and at the base of the petiole of leaves, which drop off.

The presence of overwintering adults in fields starts in spring and is characterized by a gradual emergence when the temperature rises [7]. The presence of the new generation of adults of the beetle takes place gradually over the summer, with variation in different areas relating to climatic conditions. The timing of adult occurrence is not always predictable, which can make pest control difficult, as measures are generally targeted at the adult stage, eggs, and emerging larvae [13, 14].

Developing models of population phenology can enhance decision-making processes around pest control and provide greater opportunity to control the pest within integrated pest management programs [3, 15]. There are not many references concerning the phenology of *C. tenebrionis*, perhaps due to the cryptic habits of the juveniles and the long life cycle that characterizes the species.

The relationship between temperature and adult beetle activity has been partially explored [12]. In the present study, the movement of the insect has been considered a fundamental feature of the initial activity and presence of the adults in orchards. The objective of this study was to verify the influence of temperature on adult phenology and to describe a development and phenology model able to predict the presence of *C. tenebrionis* adults.

## 2. Materials and Methods

*2.1. Study Site and Experimental Design.* Studies on *C. tenebrionis* were conducted on apricot plantations in two different regions of southern Italy. One was in Sicily, in the hilly area of Serradifalco (CL) (37°25′52′′N, 13°52′37′′E 500 m above sea level), and the second was in the coastal area of Gioiosa Ionica (RC) in Calabria (38°11′16′′N, 13°11′56′′E 50 m above sea level).

The climate of the Sicilian orchard, according to the climatic index of De Martonne [16], is defined as "moderate warm," described here as temperate, with an annual mean temperature of 15-16°C [17]. The second site, in Gioiosa Ionica (RC), according to the climatic index of De Martonne, falls into the category of "subhumid" with a tendency towards semiarid, with an annual mean temperature of 17-18°C [18]. Moreover, this is one of Calabria's driest areas, owing to its orographic characteristics. The minimum precipitation is near the coastline as a consequence of the shielding effect of the mountains, because Mediterranean storms usually impact on Calabria from west to east [19, 20].

In the study areas, tree crops are predominantly grown, such as peach, grape, and apricot in Sicily and citrus and olive groves in Calabria. Over the past few decades, *C. tenebrionis* has been abundant in these areas. Observations were conducted in 2005, 2006, and 2007 in Sicily and in 2008 and 2009 in Calabria. The apricot orchards grafted onto Mirabolano (*Prunus cerasifera* Ehrh.) were, respectively, 11 and 9 years old, and the former had been organically managed since planting. No phytoiatric interventions against insects and mites were made in the orchards during the study years. The trees were arranged in a 4 × 4 m layout and grown in the form of a vase 2.5–3.0 m high.

*2.2. Sampling of C. tenebrionis.* Each year, observations were made weekly, or at least every 10 days. Observations of overwintering adults started in early spring and continued until their disappearance. The new adult generation of *C. tenebrionis* was assumed to start when the adult beetles began to emerge during summer. All insects detected on the sampled trees were manually collected, counted and identified to sex and generation then released back into the tree.

To identify which generation adults belonged to, the mandibles were examined. They were sharp and pointed in the specimens of the newly emerged generation and more blunt in the overwintered generation [6]. To evaluate possible difference between the populations of the two areas, body size measurements were taken for ∼60 male and 60 female specimens. At least 24 plants were sampled in the orchard in Sicily and 48 plants in the orchard in Calabria. The observations concerned the number, sex, and generation of adults on each plant.

*2.3. Laboratory Experiment.* Laboratory trials were carried out in a climatic chamber to investigate the relationship between adult beetle movement and temperature to find the threshold temperature (t0) at which the movement rate was zero. At this thermal threshold, the insect would not be expected to carry out activities and therefore would not be detected in the field. Experimental adults were sourced from the field population by collecting overwintering adults a week before the start of trials. Captured individuals were separated by sex and then provided with *ad libitum* apricot tree shoots. Twelve hours before the observations began, each adult was placed in a cage measuring 25 × 25 × 35 cm. An apricot shoot with at least 12 leaves was placed in the middle of the cage. The base of each shoot was placed in a plastic tube containing water. The cages were kept at 15, 20, 25, 30, 35, 40, or 45 ± 1°C, relative humidity of 50 ± 5% and a photoperiod of 13L : 11D. Seven observations of 60 min each, with an interval of one hour, were carried out at each temperature. Each temperature was replicated eight times. During each observation period, the number of 5-min sequences in which the insect was stationary was recorded.

The rate of movement activity of the insect was calculated using the formula $1/(n+1)$, with $n =$ the number of sequences with no movement for five minutes. This transformation made it possible to use periods of inactivity of any duration with a maximum value of 1, which corresponds with continual movement and also stabilized the variance of the data [21]. The regression method was used to find the threshold temperature (t0) at which the movement rate was zero, estimated by the $x$-intercept based on linear regression models [22]. The threshold temperature (t0) at which the movement rate was zero was used to calculate degree-day accumulation.

*2.4. Data Analysis.* The average number of adults per plant was calculated for each monitoring date, and the date of new generation adults emerging was noted for each experimental area and for each year of observation.

Paired $t$-tests were used to compare the body size of each sex between the two locations. The mean daily temperature was obtained from data loggers (Hobo, Onset Computer Corporation) and was calculated from bihourly data. The daily degree-day was calculated with summation of the difference in mean daily temperature and the minimum temperature threshold of beetle movement. In each year, the biofix starts on the 1st of March for overwintering beetles and 1st of July for new emerging adults. To compare the difference between areas, the annual mean temperatures were calculated.

A Logistic equation (4-parameter) commonly used for phenology modeling [23–25] was applied to the cumulative percentage of overwintering and emerging adult beetles and was fitted for each year of the data:

$$y(x) = yo + \frac{a}{1 + (x/xo)^{b}}, \tag{1}$$

in which $y$ is the cumulative percentage of adults, $x$ is the accumulated degree-days, and $a$, $b$, $xo$, and $yo$ are the constants. From these, the parameter $xo$ has biological meaning and represents the degree-days of 50% of beetle presence. Moreover, two Logistic overall equations were constructed to find 50% of the cumulative percentage of overwintering and emerging adults considering all years and the two areas. The predicted values of each year and area obtained from overall equations were linearly regressed against the observed cumulative percentages of the adult to verify the fit between observed and predicted data [26].

The values of degree-days at 50% of cumulative percentage obtained with the nonlinear regression were also compared with values obtained from the ordinary least square (OLS) method, where the cumulative percentage of the observed beetle was used as independent variable versus degree-days.

For each year, the time between the appearing and subsequent increase of adults in each generation was calculated using degree-days. This was defined as time between the onset of generations (TBG). For each area, this was calculated by the average of TBG. All analyses were performed with SigmaPlot and SPSS software.

## 3. Results

*3.1. Laboratory Results.* Using data from the movement activity of the beetle, a minimum threshold temperature for movement was calculated. The increase in temperature in relation to the rate of insect movement followed a sigmoidal function (Figure 1). Given the average daily temperature trend in the experimental areas is long periods under 25°C, we considered the first three temperatures used (up to 25°C). The movement rate was almost linear, and the linear regression method ($y = a + bx$) calculated the temperature threshold to be 14.21°C. From this temperature, the value of the accumulated degree-days was calculated for overwintering and emerging adults.

The different thermal conditions of the two areas are shown in Figure 2. In the warmer area (Calabria), the temperature is higher in the first and last months of the year. The average annual temperature estimates were 15.81 ± 0.34°C for the temperate area and 18.79 ± 0.33°C for the semiarid area. These temperatures are consistent with the reported data available for the two areas [17, 18]. Usually, females were larger than males, but paired $t$-test found no difference in the size of each sex between the two experimental areas: male $t = 1.221$, $n = 61$, $P = 0.227$ or female $t = 1.111$, $n = 64$, $P = 0.271$.

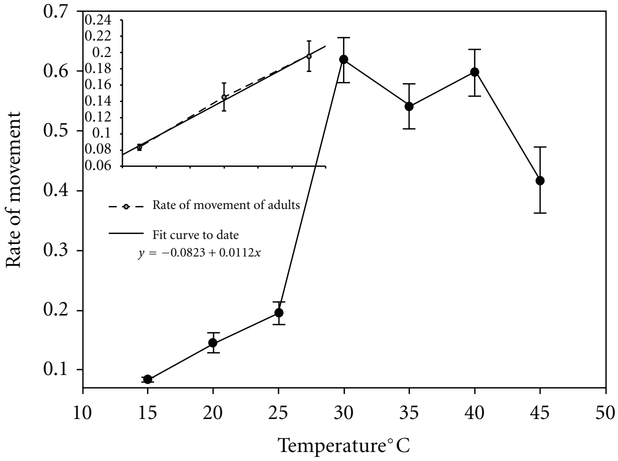


FIGURE 1: Rate of movement of *Capnodis tenebrionis* adults at different temperatures. Linear rate of movement at the first three temperatures can be observed ($R^2 = 0.996$; $F = 302.37$; df = 1, 2; $P = 0.037$).

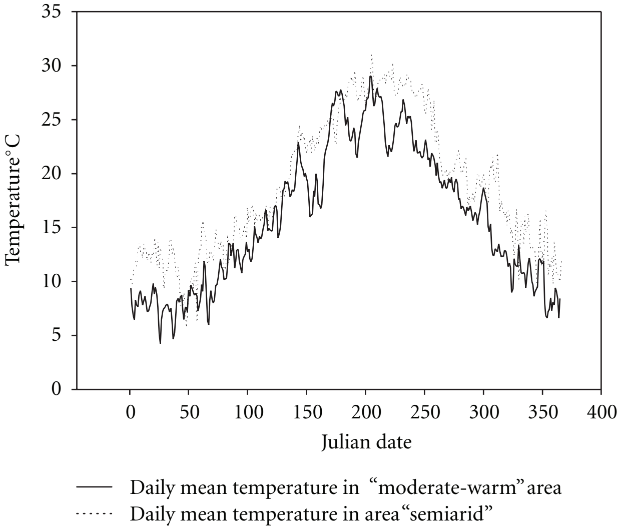
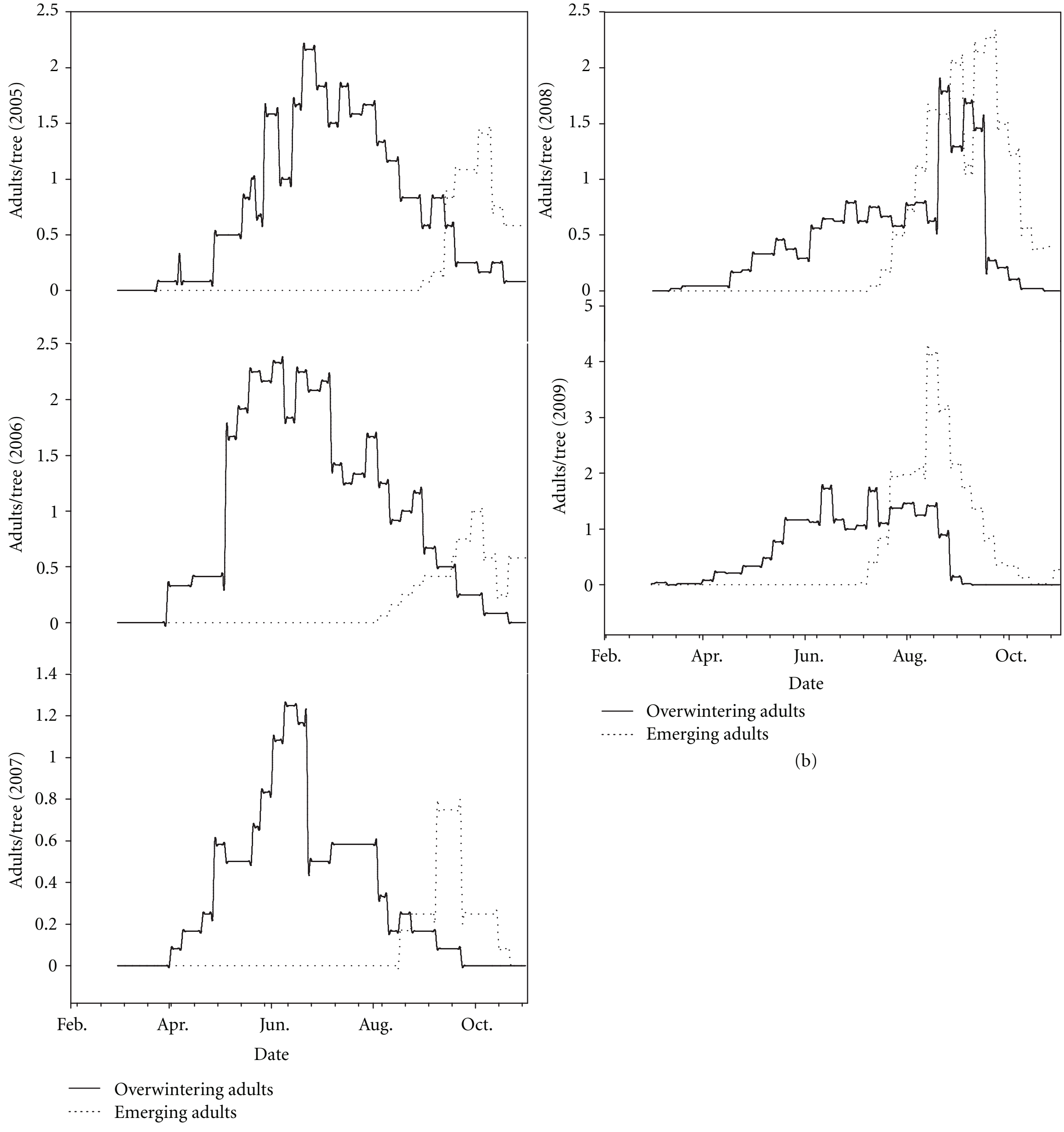


FIGURE 2: Daily mean temperature of temperate climate (Sicily), years 2005, 2006, and 2007; daily mean temperature of semiarid climate (Calabria), years 2008 and 2009.

*3.2. Beetle Phenology.* The presence of *C. tenebrionis* adults, although it has shown some variation in density over the years, has been widely documented in the two areas of investigation.

In the temperate area, the maximum density of adults in the field is reached with the overwintering generation,



(a)

(b)

Figure 3: Adult presence of *Capnodis tenebrionis* in a temperate area (*a*) and in a semiarid area (*b*). The early onset and the overlapping of generations of adults are evident in the warmer area.

and the lowest density of adults was found in 2007 (1.25 adults per tree), which was connected with the disappearance of plant resources due to *C. tenebrionis* attacks. The first adult maximum density in the temperate climate was reached in the middle of June in 2005 and in the first 10 days in June in 2006 and 2007. In the semiarid area of investigation in 2008 and 2009, the adult maximum density was reached in the middle of August (Figure 3), at which time the overwintering generation overlapped with the newly emerged adults. Research in the Calabrian orchard was suspended in 2010 due to the disappearance of the host resource and, subsequently, the disappearance of beetles. The overwintering generation appeared in open fields from late March and gradually spread as temperatures rose.

The appearance of adult beetles in 2005, 2006, and 2007 varied slightly in the temperate area, with the new adults emerging in August. In 2008 and 2009, the new generation emerged in the first 20 days of July, in accordance with the more elevated temperatures of this area. The emergence of adults in the two areas differed by around one month (Figure 4).

The analysis of the parameters obtained with logistic equations for overwintering gave the value of the parameter *xo* (time of 50% adult emergence), which ranged from 320

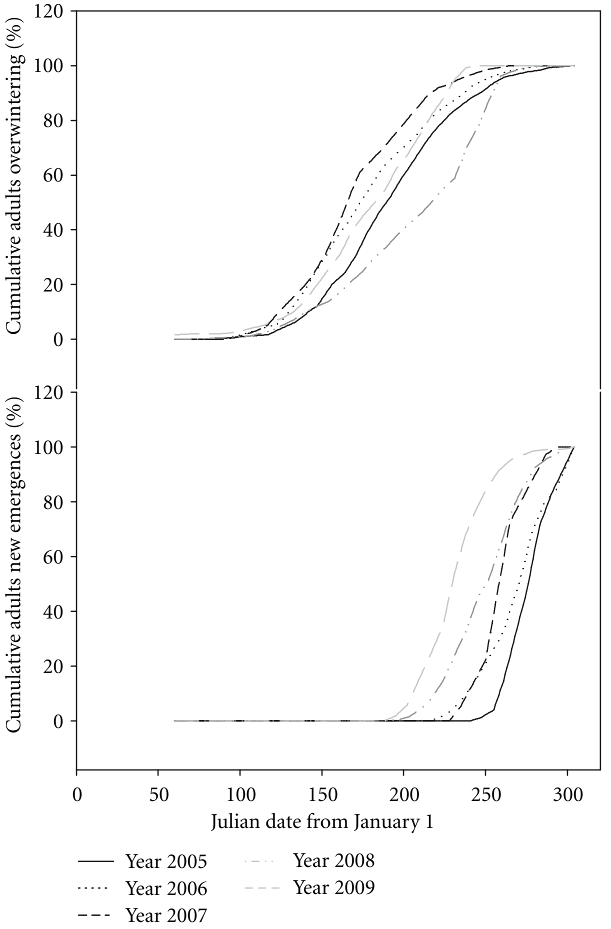


Figure 4: Cumulative percentage of *Capnodis tenebrionis* (overwintering and emerging) adults. The grey line refers to semiarid climate, and the black line refers to temperate area.

degree-days (DD) (2007) to 930 DD (2005) for the temperate area. In the semiarid climate, *xo* ranged from 1163 DD (2008) to 768 DD (2009) (Table 1). The overall equation for the analysis of data allowed us to obtain a value of 726 DD for 50% of cumulative adults. It should be noted that, for 2008, the logistic equation of the data was not adjusted to the programmed iterations of the software, so the Boltzmann equation was utilized.

For the emerging generation of *C. tenebrionis* whose onset is in midsummer, the values obtained were a minimum of 812 DD (2005) and a maximum of 1199 DD (2006). In the semiarid climate, the values were 1385 (2008) and 722 DD (2009). The overall analysis of data allowed us to obtain the figure of 801 DD for 50% accumulation of adults emerging (Table 1).

The comparison with the linear regression for each year of the observed cumulative percentage (overwintering and emerging adults) versus the predicted values with the overall logistic equation showed a close fit between the model and observed data (Table 1). The comparison of the DDs at 50% of cumulative adults obtained with the nonlinear regression with values from the OLS method has been highlighted in Table 1. In this case, only the overall values of DDs are similar, and, in general, the value for this linear method is lower, with differences reduced between years in the OLS method.

The average time between the appearance of each generation (TBG) and calculated degree-days showed in the temperate area that for the three years (2005–2007), the TBG value was greater (DD = 858 ± 51 SE, $n = 3$), while in the semiarid area, the value remained the lowest (DD = 631 ± 46 SE, $n = 2$).

## 4. Discussion

This study of *C. tenebrionis* showed the crucial role of temperature in the emergence of adult beetles. The thermal differences between the two different locations affected the development of species and caused the early onset of the new generation in the summer in the semiarid area. In contrast, overwintering adults emerged in spring in both areas, though the timing appeared to be more uniform in the temperate area. Interestingly, initial emergence of *C. tenebrionis* at both sites coincided with the opening of the earliest flowers at each site; however, the increase of adults at each location was slow, and the maximum density occurred when plants were fully vegetative. Thus, the emergence of beetles in spring appears not to be bound by the phenology of the plant, perhaps because its feeding is independent of the flowering plant.

The temperature seems to be responsible for spring adult appearance, and this strong dependence was expected, because the average daily temperatures for the first four months often remain below the threshold calculated (see Figure 2). It is necessary to obtain more years of monitoring dates of appearance to verify whether climatic fluctuations are primarily responsible for the interannual variability in spring appearance phenology. An early onset brought about by increasing temperature is seen in other species of insects, such as *Apis mellifera* (L.) and *Pieris rapae* (L.) [27]. However, other factors may influence the phenology of *C. tenebrionis* and their subsequent appearance in orchards. For these, we can refer to the microhabitats of orchards, which can influence the activity of adults. For example, in some orchards, tillage of the soil is not carried out, leaving a ground layer of grassy vegetation. In this case, the heating process of the basal part of the plant is reduced, which may result in retardation of the emergence of overwintering adults and formation of new adults. The effects of the grass, as discussed by Snyder et al. [28], could explain the delayed appearance of the overwintering adults in the semiarid orchard compared with the temperate orchard. Although during winter in the semiarid area specimens were rarely present at the base of the plants, they would be unable to feed due to the absence of plant resources.

The difference of recorded temperature in the two study areas was nearly 3°C, and this made it possible to identify the effects of temperature on the phenology of the species in the field, but a manipulation of temperature in the laboratory is desirable for further investigation. *Capnodis tenebrionis* can be reared in the laboratory, although certain technical issues make it a difficult species to culture [29].

Table 1: The parameter estimate of a nonlinear model describing *Capnodis tenebrionis* adult phenology. The comparison with the linear regression for each year of the observed cumulative percentage versus the predicted values with the global logistic equation. Degree-day values at 50% of cumulative adults calculated with ordinary least squares regression for *Capnodis tenebrionis* adults (overwintering and emerging adults of the year) for two different Mediterranean areas (S: Sicily; C: Calabria).

| Parameter estimates (SE) with logistic equations and regression statistics | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Overwintering adult | | | | | | Emerging adult | | | | |
| | Log. equat. parameter | | Log. regression values versus observed cumulative | | OLS regression | | Log. equat. parameter | | Log. regression values versus observed cumulative | | OLS regression |
| **2005 (S)** | $a$ | 167.99 (1.7) | $y = 0.91x + 2.22$ | $R^2 = 0.997$ | | **2005 (S)** | $a$ | 109.70 (1.21) | $y = 0.831x + 6.33$ | $R^2 = 0.922$ | |
| | $b$ | −1.22 (0.01) | | $(N = 245)$ | | | $b$ | −24.75 (0.48) | | $(N = 123)$ | |
| | $x0$ | **930.95** (15.22) | | | **560.73** | | $x0$ | **832.96** (0.98) | | | **727.41** |
| | $y0$ | 0.49 (0.07) | | | $(r^2 = 0.9829)$ | | $y0$ | 0.06 (0.1) | | | $(r^2 = 0.5147)$ |
| | $R^2$ | 0.9998 | | | | | $R^2$ | 0.9984 | | | |
| **2006 (S)** | $a$ | 161.56 (4.04) | $y = 0.93x - 2.49$ | $R^2 = 0.991$ | | **2006 (S)** | $a$ | 571.93 (180.13) | $y = 0.942x + 0.31$ | $R^2 = 0.978$ | |
| | $b$ | −0.92 (0.02) | | $(N = 245)$ | | | $b$ | −6.21 (0.23) | | $(N = 123)$ | |
| | $x0$ | **753.58** (41.16) | | | **517.23** | | $x0$ | **1198.71** (83.18) | | | **725.70** |
| | $y0$ | −0.83 (0.27) | | | $(r^2 = 0.9360)$ | | $y0$ | 0.11 (0.14) | | | $(r^2 = 0.7166)$ |
| | $R^2$ | 0.9982 | | | | | $R^2$ | 0.9970 | | | |
| **2007 (S)** | $a$ | 117.52 (1.35) | $y = 1.01x - 4.00$ | $R^2 = 0.973$ | | **2007 (S)** | $a$ | 108.88 (1.13) | $y = 0.876x + 1.52$ | $R^2 = 0.997$ | |
| | $b$ | −1.22 (0.02) | | $(N = 245)$ | | | $b$ | −13.22 (0.31) | | $(N = 123)$ | |
| | $x0$ | **319.92** (7.12) | | | **488.05** | | $x0$ | **812.03** (1.93) | | | **690.76** |
| | $y0$ | 0.511 (0.29) | | | $(r^2 = 0.8845)$ | | $y0$ | 0.13 (0.14) | | | $(r^2 = 0.7416)$ |
| | $R^2$ | 0.9974 | | | | | $R^2$ | 0.9979 | | | |
| **2008 (C)** | $a$ | 120.79 (2.67) | $y = 1.007x+13.90$ | $R^2 = 0.904$ | | **2008 (C)** | $a$ | 228.14 (23.74) | $y = 1.074x + 2.60$ | $R^2 = 0.896$ | |
| | $b$ | 402.77 (11.41) | | $(N = 245)$ | | | $b$ | −3.28 (0.14) | | $(N = 123)$ | |
| | $x0$ | **1163.03** | | | **923.60** | | $x0$ | **1385.63** (79.62) | | | **842.25** |
| | $y0$ | * | | | $(r^2 = 0.9908)$ | | $y0$ | 0.044 (0.24) | | | $(r^2 = 0.9213)$ |
| | $R^2$ | 0.9847 | | | | | $R^2$ | 0.9945 | | | |
| **2009 (C)** | $a$ | 123.01 (2.05) | $y = 0.991x + 3.89$ | $R^2 = 0.985$ | | **2009 (C)** | $a$ | 114.20 (1.00) | $y = 1.066x - 13.13$ | $R^2 = 0.926$ | |
| | $b$ | −1.67 (0.04) | | $(N = 245)$ | | | $b$ | −3.53 (0.06) | | $(N = 123)$ | |
| | $x0$ | **768.28** (17.47) | | | **736.34** | | $x0$ | **721.70** (4.91) | | | **682.0** |
| | $y0$ | 3.33 (0.30) | | | $(r^2 = 0.9582)$ | | $y0$ | 0.22 (0.13) | | | $(r^2 = 0.9731)$ |
| | $R^2$ | 0.9965 | | | | | $R^2$ | 0.9984 | | | |
| **2005–2009** | $a$ | 137.42 (6.72) | | | | **2005–2009** | $a$ | 93.65 (1.27) | | | |
| | $b$ | −1.18 (0.06) | | | | | $b$ | −12.94 (0.66) | | | |
| | $x0$ | **726.74** (63.03) | | | **636.55** | | $x0$ | **801.08** (3.49) | | | **754.64** |
| | $y0$ | 0.8499 (0.71) | | | $(r^2 = 0.8608)$ | | $y0$ | 1.06 (0.38) | | | $(r^2 = 0.7807)$ |
| | $R^2$ | 0.9231 | | | | | $R^2$ | 0.9176 | | | |

*Boltzmann equation $Y(x) = a/1 + e^{((-x-xo)/b)}$.

As in other agroecosystems, in the cultivation of stone fruits, the joint action of different factors (variety, agricultural choices, fertilizer, etc.) can influence the development rates and population dynamics of pests. Among other climatic factors that may affect the adaptability of pests, it is possible to consider photoperiod, but in this case, the difference of this factor between the areas was very low. Moreover, factors such as moisture availability, competition, and extreme weather events could affect phenology, potentially modulating the effects of cumulative heat units. Even the effect of heat on development rate might be nonlinear [30, 31] or can vary according to the life stage of the insect [23].

In early July, the new generation of adults causes more adverse effects on vegetation (decrease of photosynthetic activity and disorders) in accordance with the negative action of the beetle on leaves and buds. As these effects are added, the opportunity for adults to reach sexual maturity before winter is reduced (C. P. Bonsignore, unpubl. data). This advantage of early onset, however, may be accompanied by the inability to survive a second winter, as for some individuals in a temperate climate. In fact, emergence of the new generation in late summer could lead to a second overwintering (C. P. Bonsignore, pers. observation). Also, the average time between the appearances of each generation (TBG) showed in the temperate area for the three years yielded a greater value (DD = 858), while the semiarid area yielded the lowest value (DD = 631). This difference showed a shorter interval between the two generations and a greater overlap of generations in the semiarid area. In confirmation of this species' thermophilic preferences, other activities (e.g., egg laying, egg hatching, and feeding activity) require high thermal optimal condition [12, 32], and some of these events also need to be associated with drought conditions, such as low soil humidity being preferable for newly hatched larvae to reach plant roots [14].

The information collected here suggests new possibilities for the pest to expand its range of distribution under drought and warmer conditions. The various reports of the presence of the species, in areas such as the south of France [8], are generally not considered preferential for the presence of *C. tenebrionis*, which suggests that its expansion may be linked to global warming. In these new areas, the simple, single-parameter degree-day models of phenology may not fit well and could have little power to predict phenology under new conditions. Greater understanding of these possible complexities should permit better forecasting of the phenology of species.

The possibility to recognize, in the orchard, the susceptible stages of the pest and their seasonal predictability is crucial for the application of control measures. This may be even more important if the natural antagonist is unable to exert, either individually or together, a satisfactory control of root-borer populations [33–35].

The values in degree-days calculated for each generation in the five years of study show a close correspondence with the values observed annually for the adoption of a defense strategy that takes into account the emergence of adults. Given the length of adult life of *C. tenebrionis*, it is necessary to take the first action against adults of the overwintering generation when the DD reaches 50% of the value calculated with the equation. Generally, half of this value coincides with the first peaks of the presence of adults, and the first treatment would reduce the number of eggs laid in the field. Understanding the life history of *C. tenebrionis* and its population will be necessary for improving its management and further understanding the spread of this beetle, which is heavily influenced by abiotic climatic factors.

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