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Temperature-Dependent Development of the Pistachio Bark Beetle *Chaetoptelius Vestitus* (Coleoptera: Curculionidae: Scolytinae)

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ABSTRACT

Chaetoptelius vestitus (Mulsant & Rey, 1861) is a major pest of Pistacia vera L. The biology of this species is understudied. Thus, in this study, the effect of temperature on development, survival and reproduction of the pistachio bark beetle was examined at the different fixed temperatures 25, 30 and 35.0°C. The developmental periods for egg, larvae, and pupae stages decreased with increasing temperature. In this study, we determined rates of development of immature stages (larval and pupal) for C. vestitus at each temperature, and we developed linear regression models to describe the influence of temperature on developmental rate. There were significant differences in the rate of development among the temperature for larval development (from oviposition to peak emergence of larvae) and pupal development (from larval to peak emergence of adults). The mean duration of total larval development and pupa were 55.37 ± 7.010 and 14.79 ± 4.857 days at 25°C, 36.69 ± 3.203 and 9.32 ± 1.106 days at 30°C, and 26.74 ± 2.984 and 4.60 ± 0.876 days at 35°C, respectively. Therefore, C. vestitus accomplished its total immature stage development in 128.93 ± 18.797 days at 25°C, 84.651 ± 9.097 days at 30°C, and 52.60 ± 9.524 days at 35°C. These thermal biology data will be a useful key factor to develop control strategies for this important pest.

Keywords: *Chaetoptelius vestitus, Pistacia vera, development time variation, developmental rate, temperature, population, mortality, fecundity.*

INTRODUCTION

Chaetoptelius vestitus (Mulsant & Rey, 1861) is a serious pest of pistachio trees in the world. This insect attacks live trees that suffer from poor growing conditions (e.g. non-irrigated and unmanaged trees surrounded by fallen dry branches, prolonged drought, etc.). In addition to Iran (Ziaaddini *et al*., 2002), the pistachio bark beetle is present in Syria (Lababidi, 1998), Algeria (Chebouti-Meziou *et al.,* 2011), Iraq (Abu Yaman, 2009), Italy (Fabbri and Valenti, 1998), Turkey (Turanli and Sütçü, (2009), Greece (Fragoulis, 2004) and in other pistachio producing countries in the Middle East. Biology and damage of *C. vestitus* were studied by Chebouti-Meziou *et al*., (2009) and also its bio-ecology (Rizk *et al.,* 1981a, b, c). In Tunisia, *C. vestitus* is considered to be a major pest of pistachio trees (Braham and Jardak, 2012). It affects both buds and bark parts of the pistachio tree (Chebouti-Meziou *et al.,* 2006). *C. vestitus* is univoltine, having one generation that emerges in winter and moves to pistachio buds or young twigs in early spring (Braham and Jardak, 2012). *C. vestitus* immature stages and adults overwinter in thickened branches in and around pistachio orchards and become active as temperatures increase in early spring, feeding on buds and young twigs in the orchard.

According to Braham and Jardak (2012), the species has a feeding phase and a reproduction phase. The feeding period begins in March. Adults feed upon tree buds and boring caves along the twigs just beneath the buds. The species always attacks healthy trees for feeding during spring, summer and autumn, destroying either fruits or vegetative buds. The beetles stay inside the tunnel until early October, then they begin to abandon their feeding location seeking oviposition sites. They choose the pruned pistachio branches or twigs, the damaged trunks or unhealthy branches or trees, inside or outside the pistachio orchard to reproduce. The reproduction period covers the whole year except the summer period owing to the rapid desiccation of branches and twigs. Mating occurs at the entrance of maternal gallery. Females typically oviposit in thickened branches. After hatching, the larvae move perpendicularly to the maternal gallery, undergo five instars, and after pupation the newly emerging adults exit the branches from tiny holes and go towards the buds and young twigs to initiate a feeding phase to complete their maturation. According to Braham and Jardak (2012), up to 80 % of newly formed pistachio shoots can be destroyed by adults feeding.

The protection of the pistachio orchards is currently carried out by the use of costly pesticides. Thus, alternative strategies are needed. However, despite the economic importance of pistachio trees in the region, factors that affect the biological and ecological traits of *C. vestitus* are unknown. The timing of adult emergence and duration of different stages is only approximate and additional information on population dynamics of *C. vestitus* are needed to predict adult emergence time more precisely. Thus, predicting the emergence time of a particular life stage is required to optimize the timing of population monitoring and control tactics by the use of pesticides or mass trapping. Identifying and understanding the factors that affect the development, survival, and oviposition behavior of pistachio bark beetle is important in designing and implementing successful control strategies.

Overall, the internal temperature of the body of Insects varies with the ambient temperature, as in other poikilothermal animals. Their metabolism is closely related to it and grows at the same time. However, the life of insects is only possible between certain limit values of temperature characteristic of the species and even of the stage at which it is studied as is the case of *Ips typographus* (Wermelinger and Seifert, 1999). Any biological character varies as a function of temperature so as to pass through an optimum at a given temperature. This is the case, for example, with the rapidity of development, growth, survival, longevity and fertility. Several studies documented a pronounced influence of temperature on development and activity of Curculionid beetles (Soltani, 2012; Son *et al.*, 2005; Schebeck and Schopf, 2017; Dolezal and Sehnal, 2007; Faccoli, 2009). Temperature-based models using laboratory rearing data have been developed for numerous coleopteran species. For example, Soltani (2012) studied the immature stages of the date palm beetle (*Oryctes agamemnon* arabicus) at three constant temperatures and determined thermal time requirements for various life stages. Son *et al*. (2005) studied the stage-specific effects of temperature on development time and survival of *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) on horticultural crops. The development times of *O. sulcatus* decreased with increasing temperature up to 27°C for eggs and 24°C for larvae and pupae. According to Schebeck and Schopf (2017) the mean developmental time of one generation of *Ips cembrae* was about 120, 64 and 37 days at 15°C, 20°C and 25°C, respectively. Dolezal and Sehnal (2007) and Faccoli (2009) investigated the possible weather effect on the biology and damage caused by *Ips typographus* L. in the southeastern Alps and showed that spring drought increases damage caused by *I. typographus* in the following year, whereas warmer spring affects insect phenology.

Models of temperature- dependent development are useful in predicting insect activity and population dynamics in the field. Effects of temperature alone and in relation to other abiotic and biotic factors are not documented for predicting bark beetle overwintering, survival, spring emergence, diapause induction, and bark beetle mortality in pistachio branches. Also, no studies on the effects of temperature on pistachio bark beetle development, reproduction, and survival during the pistachio growing season were available. The studies of Braham and Jardak (2012) were conducted only at one temperature.

Quantification of the relationship between stage development and temperature is required to predict seasonal occurrence of particular life stages and to optimize the timing of monitoring and control tactics. The results will be a valuable contribution for monitoring and risk assessment models to estimate the beetle’s phenology and its potential impacts on agriculture ecosystems under changing climate conditions.

Therefore, the objective of this paper was to study the effects of temperature on the development, reproduction and survival of *C. vestitus* under three controlled temperature conditions.

MATERIAL AND METHODS

Insect rearing

Adults of *C. vestitus* used in this study were originally collected from infested area of Elguettar in Gafsa governorate (34°21'N; 8°58'E), located on the southwest of Tunisia during the end of summer 2014. Infested shoots by the insect during their feeding phase were cut and transported to the laboratory. Branches were dissected and adults were collected. These adults are used to mass rearing of insect in laboratory conditions, which are 27°C, 75% relative humidity, and a photoperiod of 16: 8 (L/D). When the majority of the larvae begin to pupate, the pupae were collected and placed in boxes with some humidified vermiculite. Pupae were collected and maintained individually in the breeding chamber to ensure that the newly emerged adults will be virgins. These insects where later used for artificial rearing in fresh branches at different temperatures. The newly emerged adults were harvested and sexed according to the method of Ghrissi *et al*. (2018). Males have a 7th tergite with circular form, while females are distinguished by their sharp 7th tergite. Only healthy adults were used for this study, i.e. not damaged by the sexing procedures.

Constant temperature experiment

C. vestitus development, reproduction and survival rate were studied at three constant temperature of 25 ± 1°C, 30 ± 1°C and 35°± 1°C. These temperatures were recorded in the region in spring summer and autumn periods. All experiments started with virgin adults emerging in the laboratory. Relative humidity was fixed in each chamber at an average of 70 ± 5%. We used thickened branches (diameter between 2 and 3 cm) as breeding medium. This substrate was collected directly from pistachio orchards. The first part of the study consists of putting one couple in individual boxes, to induce mating and oviposition at the same time. Fifty couples were used. Branches were inspected daily for the presence of insect perforations (morning and afternoon). Then, the branches were dissected and the eggs, larvae, and nymphs were counted in each box to determine the development time of each stage at each temperature. Living and dead males and females were counted during each observation and placed in new boxes with healthy branches to maintain breeding. The Sex ratio of *C. vestitus* was also examined. In addition, the number of eggs laid during the first week was determined as well as the oviposition period. The number of eggs was divided by the number of females as a proxy of maximum daily fecundity.

Statistical analyses

Data showed a normal distribution and thus they were subjected to a one-way analysis of variance (ANOVA) using R.3.2.2 software (R Core Team 2015). This test was applied to each stage to determine the significance of temperature effects on incubation period, larval and pupae development, fecundity, total development time and survival of *C. vestitus*. Means being separated by Fisher's least significant differences (LSD) test.

RESULTS

Temperature-dependent development

Table1. Average time of development (in days [d] ±SE) and relative [%] of eggs, larvae and pupae of *C. vestitus*, fecundity (eggs per female) and adult longevity under long-day conditions (L:D 16:8) under laboratory conditions at 25°C, 30°C and 35°C.

Eggs/female = mean number of deposited eggs per female beetle (±SD) at the three temperature regimes under long-day conditions.

	25°C			30°C			35°C				
	d	%	n	d	%	n	d	%	n	p	LSD
Egg	10.97±2.37	9	33	8.30±1.2	10	28	5.39±0.97	10	42	<0.0001	a
Larva	55.37±7.01	43	30	36.69±3.2	43	25	26.74±2.98	51	30	<0.0001	b
pupa	14.79±4.85	11	15	9.32±1.1	11	18	4.60±0.87	9	20	<0.0001	c
Total	128.93±18.7	100		84.65±9.09	100		52.60±9.52	100		<0.0001	
Eggs/female	50.63±1.2			58.16±1.8			51.16±2.6			<0.0001	

n = number of studied individuals; p = p-values for differences between the mean developmental times at the various temperature conditions and for differences between the mean number of eggs/female at the three temperature regimes.

The mean duration of development of immature stages (measured in days) at various constant temperatures with standard deviation is given in Table 1. Incubation period was the shortest stage (Table 1). The analysis of variance showed significant differences between the incubation periods under the temperatures tested. The analysis of variance (ANOVA) also revealed an effect of temperature on the larval stage (Table 1).

The larval period varied between (minimum and maximum) 44-69, 30-42 and 20-37 days at 25, 30 and 35°C, respectively. These results indicated that the larval stage the total development. In fact, it represented 68.24%, 67.55% and 72.78% of immature stage duration at 25°C, 30°C and 35°C, respectively.

Pupa took (minimum and maximum) 7-24, 7-12 and 3-6 days at 25°C, 30°C and 35°C, respectively, to complete their development (Table 1).

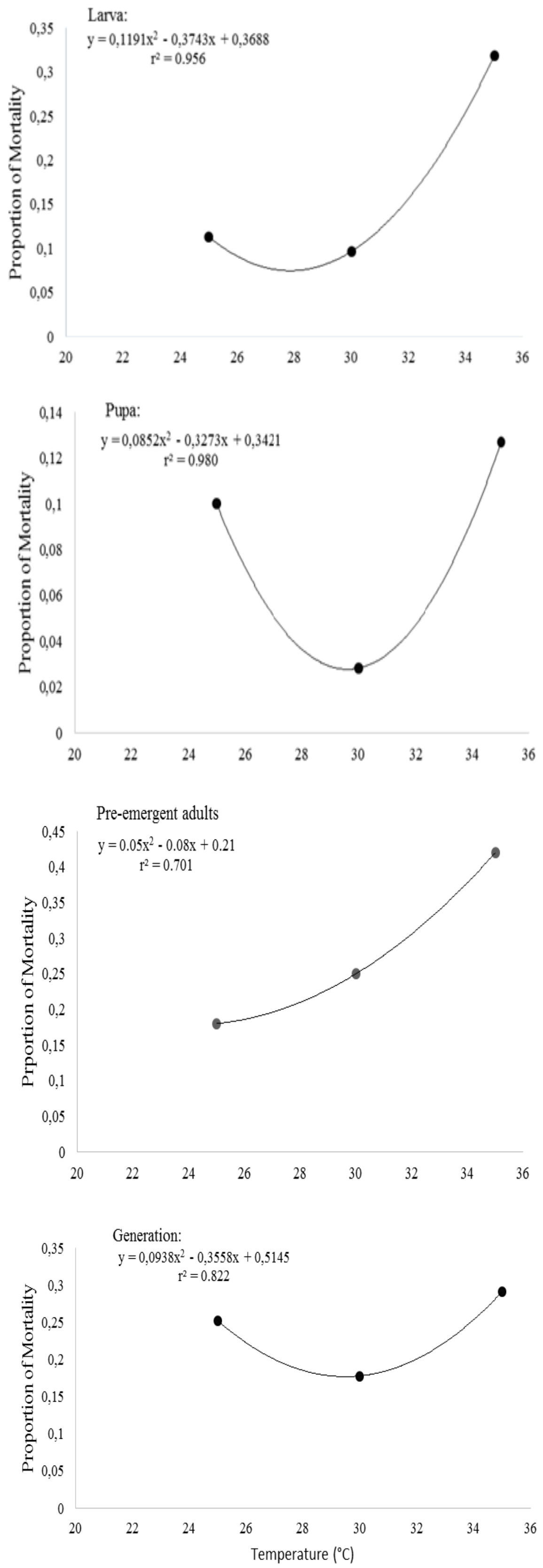
Finally, the immature stage developmental duration varies with temperature (Table 1). Significant differences were observed for the total life cycle of immature stages of *C. vestitus* developed under three constant temperatures. The duration was longer when the insect was reared at 25°C and 30°C compared with 35°C.

The mean developmental time (DTm) of one generation at 25°C lasted about 129 days, at 30°C about 85 days and at 35°C about 53 days (Table 1). On average, the egg stage took about 10% of the total development time, (between 5 and 11 days). The larval stage comprised about 46% of the entire developmental time, (between 27 and 55 days). The development of the pupa took about 10% of the total development, (between 5 and 15 days). *C. vestitus* oviposition was calculated by the number of eggs per female beetle. A high deviation in the mean number of eggs per female at the three temperature regimes was found (table 1). On average, females deposited slightly more eggs at 30°C than at the two other temperatures (ANOVA: $p<0.0001$).

Mortality of immature stages

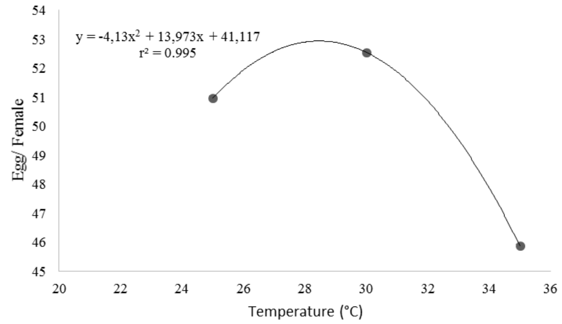
Overall, mortality increased gradually as temperature increased from 25°C to 35°C. The egg stage was more vulnerable to high temperatures, and mortality was lower for the other stages. At temperatures 25°C and 30°C, few eggs failed to hatch, while at 35°C the oviposition rate of females was higher but hatching was more difficult. The effect of temperature on mortality at each immature life stage was described by a second-order polynomial function (Fig 1).

Fig1. *Age-specific mortality (Frequency) of pistachio bark beetle stages at different temperatures.*



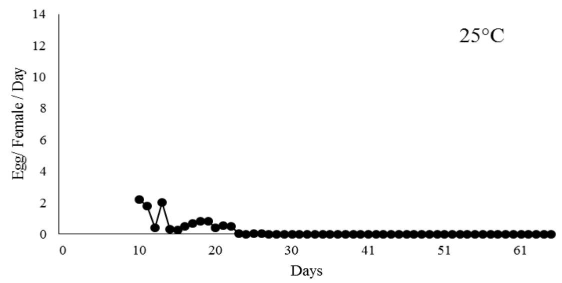
Sex-ratio, longevity and oviposition

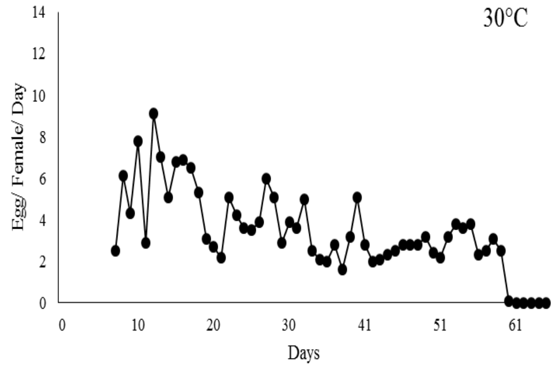
Fig2. *Mean oviposition per female of C. vestitus at three constant temperatures. Mean of each temperature (the initial no. of couple for each replicate contained 30 couples).*

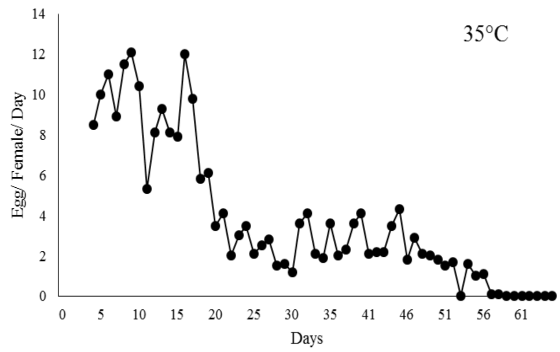


The fertility of females was found to be 50.96, 52.54, 45.86 eggs at 25, 30 and 35 °C, respectively (Fig. 2). The number of eggs oviposited per female per day was affected by temperature. The lowest number of eggs per day was observed at 25 ° C and 30 ° C, while the highest number of eggs per female per day was recorded at 35 ° C (Figure 3).

Fig3. *Lifetime profiles of daily mean (±SE) oviposition activity of C. vestitus females reared at three constant temperatures*







Temperatures significantly affect the sex of the offspring ($p<0.0001$) (Table 2). The pre-ovipositional period decreased significantly from 10.8 ± 0.3 to 4.4 ± 0.3 days when the temperature rose from 25°C to 35°C ($F = 491.06$, $df = 2$, $P < 0.0001$) (Table 2). The longevity of adult females also tended to decrease with temperature. Females maintained at 25° C lived much longer than at 30°C or more ($P< 0.0001$) (Table 3). Lifetime oviposition also increased with temperature, with the highest (58.3±8.6 eggs) at 30°C ($P< 0.0001$) Table 3).

Table2 Effects of different temperatures on development time (days) of *C. vestitus* females and males (Mean ± SE)

Temperature (°C)	Development time (days)		
	Females	*Males*	t-test
25	131.1 ± 3.5	120.3 ± 2.9	t= 5.30 ; df= 58 ; p< 0.0001
30	88.5 ± 3.1	84.4 ± 2.2	t= 3.02 ; df= 58 ; p= 0.0037
35	58.1 ± 2.5	57.7 ± 2.8	t= 3.15 ; df= 58 ; p= 0.0025

Table3 Effects of temperature on *C. vestitus* preoviposition period (days), lifetime oviposition (Mean ± SE), sex-ratio, and longevity (days)

Temperature (°C)	Preoviposition period (days)	Oviposition (egg punctures)	Percentage female	Longevity (days)
25	10.8±0.3	50.4±2.8	46.6±2.1	43.8±1.0
30	6.2±0.4	58.3±8.6	50.1±3.1	38.3±7.7
35	4.4±0.3	51.6±13.7	51.8±1.8	13.6±3.4

Discussion

Several environmental factors (e.g., relative humidity and temperature) influence the development and survival of insects, which in turn affect their population dynamics and distribution (Chararas, 1959). Knowledge on such parameters that affect biology of insect pests will be a basis for launching a management program against the pest in the future. An environmentally sound pest management should be based on a thorough study of pest ecology, which can be described and understood in part by mortality table statistics and phenology. Estimation of mortality table parameters at a constant temperature, taking into account the life history (e.g. the rate of development, mortality and fertility), makes it possible to estimate growth potential as function of temperature.

Development, reproduction and survival of coleopteran species greatly depending on temperature (Soltani, 2012; Son and Lewis, 2005; Greenberg *et al*., 2005; Schebek and Schopf, 2016; Dolezal and Sehnal, 2007; Faccoli, 2009; Bentz, 1991; Shimoji, 2011; Li *et al.*, 2010; Spurgeon and Raulston, 1998; Lapointe, 2000; 2001). In this study, we describe the effect of temperature on development, survival, fecundity and mortality of *C. vestitus*. Regarding bark beetles in general and C. vestitus in particular, the performance is improved at higher temperatures. Higher temperatures can promote earlier flying, faster preimaginal development and have a negative effect on oviposition of the insect. By Contrast, lower temperatures favors good oviposition in *C. vestitus* females.

According to our study, *C. vestitus* can complete development from egg to adult during feeding at temperatures ranging from 25 to 35°C in the laboratory with best development time at 35°C among the 3 tested temperatures. But at this same temperature, the adult mortality rate is maximal. The highest survival rate to adulthood for *C. vestitus* was obtained at 25°C (37%), whereas lower survival was obtained at 30°C (35%) and 35°C (30%). The study region (Elguettar) has an arid climate which is characterized by an average temperature of 30°C during the dry season, which is adequate for the establishment of this species. Therefore, we recommend maintaining this species at 30°C during mass rearing in the laboratory. In fact, this temperature was the optimum temperature for oviposition, permitting the maximum oviposition rate of *C. vestitus*. The temperature showed a strong correlation with the developmental speed indicating that the studied temperature regimes are still below the value of the maximum developmental rate. Dolezal and Sehnal (2007) and Faccoli (2009) studied the effects of weather in general and temperature in particular, on the development and mortality of immature stages of *Ips typographus*. Fertility increased with increasing temperatures up to 30°C and decreased significantly thereafter.

Temperature affects both the time of development as well as fecundity; consequently, the appearance and dynamics of insect populations in the field are dictated by ambient temperature (Rattle, 1984). Spurgeon and Raulston (1998) reported a high thermal dependence on boll weevil reproductive development, with low and high temperature inhibition at observed temperatures. There is no other study on the effect of temperature on *C. vestitus* development, oviposition or survival to compare with our own results. However, the results appear to be close to those estimated for another bark beetle species, *I. typographus* (Wermelinger and Seifert, 1998), *I. sexdentatus* (Pineau *et al*., 2016) and *I. cembrae* (Schebeck and Schopf (2017). The effect of temperature on development were similar. In fact, both the development rate and productivity of all of these bark beetles species increased with temperature.

The duration of development of immature stages of *C. vestitus* varies significantly with temperature. Indeed, the optimum temperature for reproduction, allowing the shortest development time, is located at 35 ° C. It is clear that the immature stages and in particular the larval development dominate the life cycle of this pest. In general, this cycle is similar to that of other bark beetles characterized by the predominance of larval development time in relation to total cycle time.

In this study, it is important to mention that the pest life cycle was completed in less than 5 months at 25 ° C and less than 3 months at 30 ° C and 35 ° C. It is clear that more than one generation can occur under laboratory conditions under high photoperiod and resource available all time. However under field conditions, the insect undergoes aestivating period in summer and hibernating period in winter (Braham and Jardak, 2012).

CONCLUSION

The present work on the immature stages of *C. vestitus* is a fundamental step in understanding the species. On the one hand, it confirmed the mastery of the techniques of breeding and, on the other hand, provided important and fundamental information on its cycle of development and immature stage under three constant temperatures. Now, it becomes possible to start management experiments on this parasite under laboratory conditions before applying in the oasis ecosystem.

The information presented is the basis for developing a more complete understanding of the development of the pistachio bark beetle. These data will also be useful in creating a temperature-based degree-day model.

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