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Degree days and phenological synchrony in Western tussock moth and coast live oak

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DEGREE DAYS AND PHENOLOGICAL SYNCHRONY IN WESTERN TUSSOCK MOTH AND COAST LIVE OAK

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ABSTRACT

DEGREE DAYS AND PHENOLOGICAL SYNCHRONY
IN WESTERN TUSSOCK MOTH AND COAST LIVE OAK

by Ingrid Graeve

Western tussock moth populations fluctuate cyclically in the San Francisco Bay Area, and this study developed a degree day model as a tool to manage damaging populations. I raised tussock moths in the lab at fixed temperatures and found the lower threshold temperatures for egg, larval, and pupal development to be 10, 7.2, and 9.8°C. Egg hatch occurred after 160DD accumulation, pupation 360DD after hatch, and adult emergence 151DD after pupation. Because moth populations may also be affected by degree of synchrony between caterpillar hatch and oak budburst, I monitored buds and egg masses in Palo Alto, CA, every 2-3 days. Hatch and budburst during outbreak years were synchronized within an area but not at the level of individual tree. Since hatch preceded budburst and extended over a longer time in most trees, the degree of synchrony may not have strong fitness consequences for oaks or tussock moths in urban areas.

ACKNOWLEDGMENTS

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The staff at Stanford University, where the field studies took place, supplied data, observations, and my introduction to the questions this research attempts to answer. Thanks to Karen Stidd in Grounds and Craig Barney in Environmental Health and Safety. Another thank you goes to Mark Rodriguez and Lifescan, for permission to study the tussock populations in Milpitas.

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INTRODUCTION

In California's foothill woodlands, coast live oak (*Quercus agrifolia*) is a keystone species providing food and habitat for many organisms. Several kinds of caterpillar feed on oak leaves in spring, including Western tussock moth (*Orgyia vetusta gulosa*). In developed areas, tussock larvae are also found in gardens and orchards, where they extend their feeding to roses, plums and other ornamental species. Large numbers of larvae cause plant dieback and are considered a nuisance. To manage damaging populations, an integrated pest management approach uses degree day modeling to predict the appearance and disappearance of eggs, larvae, pupae and adults, as a tool in scheduling monitoring and control activities.

One objective of my research was to determine the parameters of a degree day model for Western tussock moth. The first chapter covers the laboratory studies I conducted to describe the temperature-dependent development of tussock eggs, larvae and pupae. I compared my lab findings to observations of tussock development in the field.

Degree day modeling is often coupled with plant phenology monitoring. Phenology is the timing of life cycles, and plants are used as indicators of certain insect behavior. For example, past observations indicate that oak buds bursting in springtime may coincide with tussock larvae hatching. Yet there is much tree-to-tree variation in budburst. The second chapter covers my field studies assessing variation in budburst and caterpillar hatch. I explored whether tussock hatch is synchronized with oak budburst at the individual tree level, a possible isolating mechanism that might lead to speciation.

Chapter 1 Degree Day Model for Western Tussock Moth

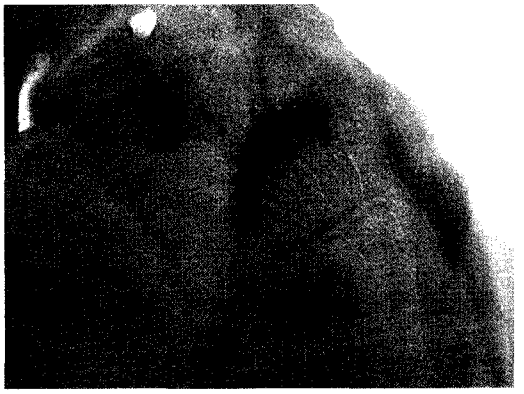
INTRODUCTION

Both plant and insect development are affected by photoperiod and temperature (Larcher, 2003; Saunders, 1982). This knowledge is the basis for developing degree day models that tie insect development to ambient temperatures and are used to manage potentially damaging insect populations. These models are also increasingly used to predict plant and insect responses, such as those of abundance and distribution, to climate change.

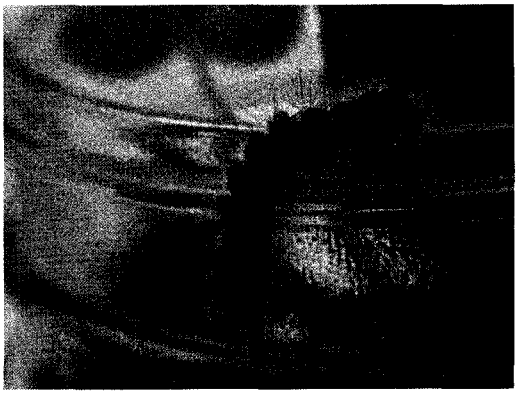
In the coastal mountains of California, the larvae of native Western tussock moth, *Orgyia vetusta gulosa* Boisduval (syn *O. gulosa* Henry Edwards; Lepidoptera: Lymantriidae) feed primarily on coast live oak (*Quercus agrifolia* Née; Fagaceae). In urban areas of the San Francisco Bay Area, the host range extends to roses and several species of ornamental trees, shrubs and groundcovers (Dreistadt, 1994). Larval feeding causes defoliation and weakens stressed plants, particularly in years of population booms. Large numbers of dispersing larvae are considered a nuisance. In Southern California orange orchards, larvae feed on the new spring flush of growth as well as newly set and maturing fruit, causing fruit drop and surface-scarring (Atkins, 1958). They are also considered a common pest of apple, cherry, prune, walnut and avocado trees in commercial orchards (Dreistadt, 1994).

Orgyia vetusta gulosa is univoltine, having one generation a year, with larvae hatching from overwintering egg masses on or near the host tree (Furniss and Knopf, 1971). Eggs hatch in early spring and larvae feed on succulent new growth as they develop through five (male) or six (female) instars. Larvae often congregate on the trunk of the host tree to pupate

EARLY INSTAR LARVA



LATE INSTAR LARVA



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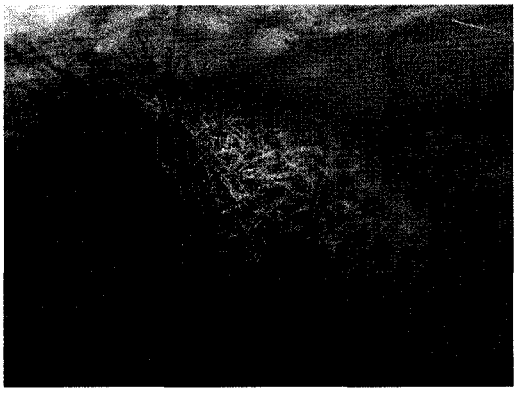
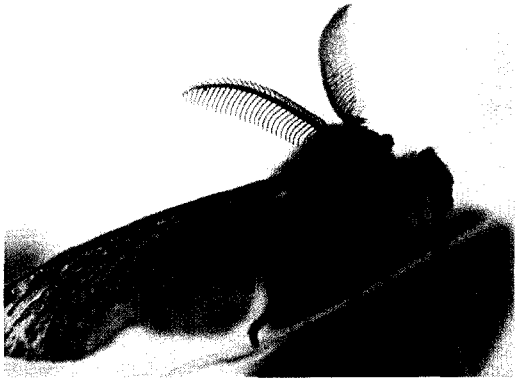
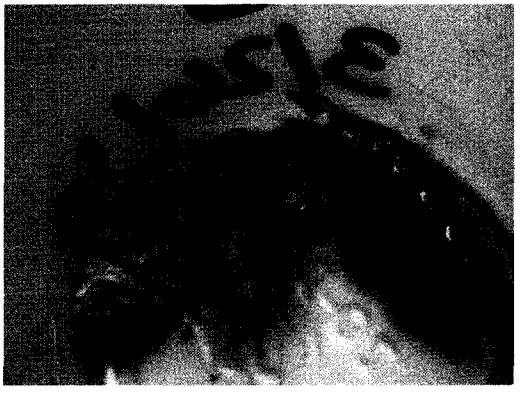


MALE MOTH



FEMALE MOTH



EGG MASSES

FIGURE 1.1 Life stages of Western tussock moth, *Orgyia vetusta gulosa* (photos courtesy of Carol Selter)

in groups in late spring. After several weeks, adults emerge. Females have only vestigial wings and cannot fly. They release pheromones to attract winged males and, following mating, lay 100-300 eggs in a mass, often on top of the cocoon from which they emerged. Activity ceases as the insects survive the hot dry summer and cold winter in egg form.

A degree day model for *O. v. gulosa* can be used to predict the appearance of each life stage, as a tool in scheduling monitoring and control activities as part of an integrated pest management (IPM) program. The degree day model for a particular insect is characterized by three variables, each of which is defined below: the biofix date, the lower-threshold or base temperature (LBT), and the upper-threshold temperature (UTT). In practice, a simplified degree day model may use one biofix date and one lower and upper threshold temperature for all life stages and genders.

The biofix date indicates when an overwintering insect becomes responsive to favorable temperatures. The biofix date for *O. v. gulosa* larva, pupa and adult stages is straightforward; degree days begin accumulating when that stage appears. For example, the date on which a larva hatches from an egg is the larval biofix date. The biofix date for the *O. v. gulosa* egg stage is more complicated because eggs undergo diapause, when little or no development takes place. This diapause is likely obligate.

The chosen egg biofix date would indicate when development within the egg resumes. The amount of development after diapause can vary from fully developed (fully formed larva within egg enters diapause) to slightly developed (young embryo enters diapause, and most embryo development takes place post-diapause). No studies have been done on *O. v. gulosa* embryology, so it is unclear which stage of the embryo undergoes diapause.

The lower and upper threshold temperatures are those temperatures below and above which no development takes place; these temperatures are determined in the lab by raising the insect at a series of fixed temperatures. Current recommendations for improving the reliability of degree day models include 1) observing insect development at a minimum of five temperatures within the linear range; 2) including temperatures at the borders of the linear range; 3) observing enough individuals to dampen the effects of differences in body mass; and 4) raising insects on foods of varying nutritional quality (Bergant and Trdan, 2006).

To use the model, degree days are calculated by comparing the daily high and low temperatures in an area to the upper and lower threshold temperatures, starting on the biofix date. Each life stage (egg, larva, pupa, adult) can be associated with a specific range of accumulated degree days following the biofix date for that stage.

No published degree day model exists for *O.v. gulosa.* While both nonlinear and linear models exist to describe insect development at constant temperatures, nonlinear modeling is considerably more complex, though fit is not always sufficiently improved (Jones, *et al.*, 2005). The goal of this study was to determine the biofix date and the lower and upper threshold temperatures for eggs, larva and pupa of *O. vetusta gulosa* in the lab using standard methods for linear modeling of development. The resulting degree day model was then validated by comparing it to field data.

MATERIALS AND METHODS

The controlled temperature studies were conducted in Conviron E7 growth chambers (Winnipeg, Canada) set to photoperiods of 12L:12D. Maximum and minimum temperatures in each growth chamber, measured by Fisher Scientific 15-077-80 monitoring thermometers (Pittsburgh, PA, USA), were recorded daily. Two chambers had Hobo ProTemp temperature loggers (Onset Computer Corporation, Bourne, MA, USA) that recorded hourly humidity as well as temperature. Growth chamber temperatures were rotated for each group to prevent pseudoreplication.

Egg Diapause Termination and Biofix date

To determine when overwintering eggs become responsive to favorable temperatures, the methods of Judd and Gardener (1993) were followed. Five egg masses were collected at two week intervals from September 2006-February 2007. Egg masses were randomly chosen from the Stanford University (Palo Alto, CA) campus, placed individually in glass vials stoppered with cotton, and placed at 20°C. Egg masses were checked daily, and hatching larvae were recorded and removed.

To determine whether embryo development occurred, between 5 and 25 eggs were dissected from each of the collected egg masses in June 2007, following the methods of Du Merle (1999). Eggs containing yolk versus those containing embryos (up to fully formed larvae) were tallied, and the percent of larvae developed was calculated for each sampling date.

Egg development as a function of temperature

Three groups of egg masses, representing different stages of post-diapause development, were placed at a series of fixed temperatures to determine embryo development rate as a function of temperature (Table 1.1). Group 1 egg masses were presumed to have

undergone no post-diapause development. Because Group 1 hatching occurred at only 3 temperatures (15, 20, 23°C) over a long duration, two more groups were studied to increase temperature resolution. Egg masses in Groups 2 and 3 were naturally out of diapause when collected and had probably undergone some embryo development in the field.

All egg masses were randomly collected at Stanford over two seasons and placed individually into glass vials stoppered with cotton. Three to five egg masses were placed at each of the following temperatures: 10, 12.5, 15, 17.5, 20, 23, 27, 30°C. Egg masses were checked daily, and hatching larvae were recorded and removed.

TABLE1.1. Temperatures and egg mass sources for *O. v. gulosa* egg development studies.

Group	Source of egg masses	Temperatures (°C) (bold=unreplicated)
Summer 2006	Collected in October 2005 and kept in the dark at 2-4°C to delay development. In June 2006, egg masses were removed from cold storage and 3 were placed at each temperature.	15, 20, 23, 27, 30
January 15 2007	Collected at Stanford on Jan 15 07 and immediately transferred to growth chambers, 5 at each temperature.	15, 17.5, 20, 23, **25**, 27
February 15 2007	Collected at Stanford on Feb 15 07 and immediately transferred to growth chambers, 5 at each temperature.	**10, 12.5**, 15, 17.5, 20, 23, **26**

Egg development as a function of temperature – data analysis

Separate regressions were performed for each group, since they represented different stages of development. The number of days to hatch was converted into a development rate (1/days) and this was plotted against mean actual growth chamber temperature. The linear portion of the curve was visually determined, and least-squares linear regression was used to determine the slope and intercept of this line (parameters m and b of the line y=mx+b). The lower base temperature was found by extrapolating the best-fit line through the x-axis (at y=0,

x=-b/m). The thermal constant, or number of degree-days above the base temperature required for 50% of the population to complete development, was calculated as the reciprocal of the slope of the best-fit line (1/m).

The best-fit lines for the 3 groups were compared using ANOVA. All statistical testing was done using SYSTAT (v. 10.0, San Jose, CA). Standard errors of the lower base temperatures and thermal constants were manually calculated as reported in Campbell *et al* (1974).

Comparison of models to egg hatch in the field

All three models for egg hatch were compared against field observations from 2006 and 2007 (see complete description of field study in Chapter 2). Degree days were calculated online using UC Davis' IPM degree day calculator (www.ipm.ucdavis.edu), single sine method with horizontal upper cutoff. Daily minimum and maximum temperatures came from the Stanford University Grounds Department's weather station. The number of days between predicted and observed hatch described the degree of fit.

Group 1 regression parameters were applied to different start dates at two-week intervals beginning September 1 2006, to determine the best fit between predicted and observed hatch. For Groups 2 and 3, January 15 and February 15 were used as start dates, since collected eggs had undergone some development in the field. Additionally, different combinations of lower base temperature and start dates suggested by the diapause termination study were tested.

Larval and pupal development as a function of temperature

In preliminary studies, larvae failed to develop on artificial diet (see Appendix), so larvae were raised instead on new growth of *Q. agrifolia*. Four groups were raised to increase sample size, add temperatures, eliminate growth chamber effects and expose larvae to a

spectrum of young leaf qualities (Table 1.2). In each group, between 30 and 60 larvae were raised at each temperature. Six to ten larvae were placed on oak leaf bouquets (young leaves from the current season's growth placed in a flask of water) into a wide-mouth 1-L glass jar covered with two 5 x 5 cm gauze squares secured by rubberbands. Leaves came from both irrigated planted trees and naturally occurring unirrigated trees. Leaves were refreshed every 2-7 days, as they were consumed. Pupae were transferred to individual 30 gram paper cups capped with a plastic lid. Pupation date, pupal weight and adult eclosion were recorded. Adults were collectively placed into paper bags and allowed to mate.

TABLE 1.2. Temperatures and sources of larvae and oak leaves in controlled temperature studies.

Group	**Season**	**Temperatures (°C)** (bold=unreplicated)	**Source of newly hatching larvae**	**Oak leaf supply**
1	Early Spring 2006	**10**, 15, 20, 23, 27, 30	Hatching egg masses collected in the field	Fresh cuttings
2	Late Spring 2006	20, 23, 27, 30	Egg masses removed from cold storage and placed on lab bench at room temperature	Fresh cuttings and new growth held fresh in cold storage
3	Summer 2006	15, 20, 23, 30	Egg masses removed from cold storage and placed directly into growth chambers (20, 23°C) or onto lab bench at room temperature (15, 30°C)	Fresh cuttings and new growth (lammas shoots) held fresh in cold storage
4	see Appendix			
5	Early Spring 2007	**12.5**, 15, **17.5**, 20, 23, **26**	Egg masses collected at 2 week intervals and placed at 20°C	Fresh cuttings

Larval and pupal development – data analysis

To compare the effects of temperature and gender on development times, two-way ANOVA was performed on each group of larvae and pupae. Temperature dependence of development time was tested using one-way ANOVA for each gender within each group, and temperatures were compared using Tukey pairwise comparisons.

As with egg development, plots of larval and pupal development as a function of temperature were generated, and a best-fit line determined. The lower threshold temperature and thermal constant were calculated from the equation of the best-fit line. Separate regressions were performed for males and females of each group, for use in future studies modeling responses to climate change. The slopes and intercepts of the best-fit lines were compared using ANOVA. Data were also pooled for a final regression, to represent development across a range of leaf qualities.

Validation of larval and pupal development models

The degree day models for larval and pupal development were also compared against field observations from 2006 and 2007. Because the Grounds Weather Station malfunctioned in April 2007, daily maximum and minimum temperatures were obtained from the Wcampus LogTag temperature recorder used in the field studies described in Chapter 2 and which closely matched 2006 Grounds weather data. The observed median hatch date each year was used as the starting point for larval development, and the median pupation date as the starting point for pupal development. Degree days were calculated online using UC Davis' IPM degree day calculator.

Predicted median pupation and adult emergence dates were generated from each regression, and these were compared to observed data. The difference between predicted and observed days was used to determine which regression most accurately matched field conditions over two years.

Ecological implications: mortality, development time and pupal weight as functions of temperature and diet

To compare the effects of temperature and diet on larval and pupal development times and pupal weight, two-way ANOVA was performed comparing groups 1, 2 and 3 at 20, 23 and 30°C. Groups 1 and 5 were also compared at 15, 20 and 23°C.

RESULTS

Egg Diapause Termination and Biofix Date

Biweekly sampling suggests that some eggs break diapause by mid-December, with all eggs out of diapause by mid-January (Table 1.3, Figure 1.2). Most egg masses showed some hatching when transferred from the field to 20°C and 12L:12D. However, few (< 25) larvae hatched from each egg mass collected before early December. The number of emerging larvae fluctuated in egg masses collected in September and October, then gradually increased with increasing collection date after Oct 30. The largest number of larvae hatched from egg masses collected on January 15, then declined in subsequent collections.

TABLE 1.3. Embyro development revealed by dissection of *O. v. gulosa* egg masses collected in the field at 2 week intervals and placed at 20°C and 12L:12D.

Egg collection date	% yolk	% larvae
Sept 4 2006	54%	46%
Sept 18 2006	75%	25%
Oct 1 2006	62.50%	37.50%
Oct 15 2006	38%	62%
Oct 30 2006	39%	61%
Nov 14 2006	29%	71%
Dec 2 2006	4%	96%
Dec 15 2006	0%	100%
Dec 31 2006	0%	100%
Jan 15 2007	0%	100%
Feb 3 2007	0%	100%
Feb 15 2007	0%	100%

While the number of eggs hatching from an egg mass varies greatly, my simultaneous field study (Chapter 2) suggests that roughly 100 eggs hatch on average from each egg mass. This level of hatching was achieved by egg masses collected after January 15 in this study. By mid-December, an average of more than 50 larvae (representing 50% of average hatch of 100 tussock moth larvae) had completed development.

Egg dissections further support the timing of the end of diapause (Table 1.3). Eggs contained either thick yellow liquid or embryos at various stages of development, up to fully formed larvae. The latter in some cases had created exit holes but not left the egg shell. The percentage of embryos completing development increased gradually with collection date. All embryos completed development when collected after December 1, though not all left the eggshell.

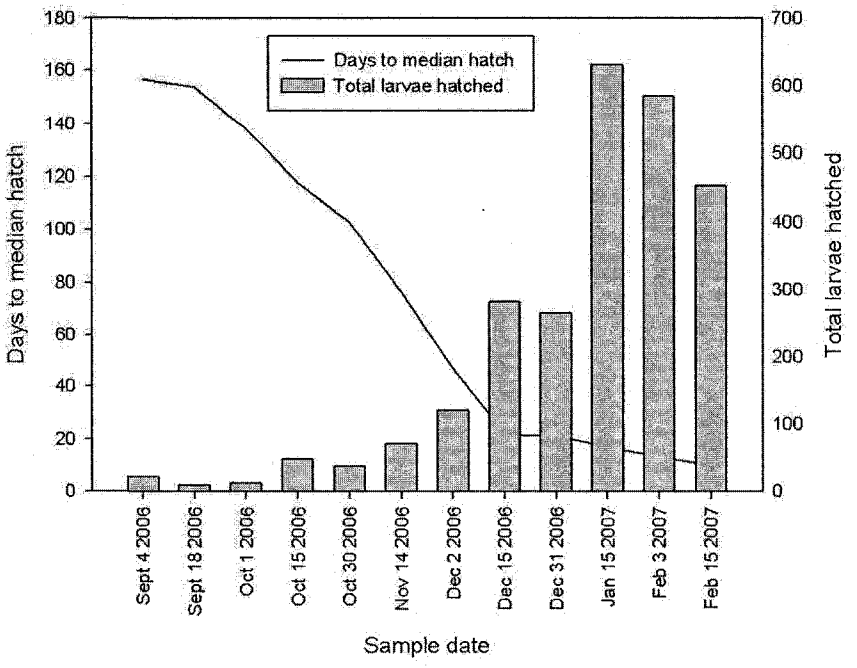


FIGURE 1.2. Diapause termination in *O. v. gulosa* egg masses collected at 2 week intervals and placed at 20°C and 12L:12D.

Median days to hatch decreased as sampling occurred later (Figure 1.2). There appeared to be a slope change after December 15, and the time to hatch appeared to level off, indicating a change in embryo development rate. However, when days to hatching is converted to embryo development rate (1/days to hatch), and this is plotted against time (collection date), embryo development rate is not a linear function but rather rises exponentially over time.

Egg development as a function of temperature

Three groups of egg masses were placed at fixed temperatures (Table 1.4). For all three groups, the duration of the egg stage decreased as temperature increased from 12.5 to 23°C, but the number of larvae hatched was noticeably lower at 23°C than at lower temperatures. No hatching took place above 23°C. The duration of hatching decreased fairly linearly with increasing temperature, from 15°C to 23°C. The relative humidity inside two growth chambers decreased fairly linearly with increasing chamber temperature, as measured in two chambers only (Table 1.5).

Because the three egg mass trials represent different stages of egg development, a separate regression was performed for each trial (Table 1.4, Figure 1.3). The regression using the summer 2006 data showed a decrease in development time with increasing temperature. No larvae hatched from egg masses placed at 27 or 30°C. Very few larvae hatched from egg masses held at 15 °C. Hatching at 20 and 23°C was typical in terms of number of larvae hatched but took place over a prolonged period of time. This regression yielded a lower threshold temperature of 11°C and a thermal requirement of 285 DD.

TABLE 1.4. Descriptive statistics and estimated regression parameters for 3 groups of *O. v. gulosa* egg masses held at constant temperatures and 12L:12D. (* Data at this temperature were beyond linear range and not included in regression.)

Group	Actual temp (°C)	Mean days to hatch (±SD)	Duration of hatch (mean days ± SE)	# larvae hatched	r^2	Intercept ± SE	Slope ± SE	Lower base temperature ± SE (°C)	Thermal Constant ± SE (DD)
June	15.2	32.25 (2.14)	8.5 (2.12)	12	0.57	-0.038873	0.003504	11.09	285.4
06	19.79	41.25 (18.58)	37.67 (15.14)	185		±0.005773	±0.000274	±0.27	±22.3
	22.91	24.29 (6.18)	20.00 (5.20)	151					
	29.93	-		0					
Jan 07	15	37.2 (6.05)	25.40 (5.73)	445	0.84	-0.063592	0.006301	10.09	158.7
	17.9	20.21 (3.14)	11.20 (2.28)	505		±0.001772	±0.000098	±0.13	±2.47
	19.5	17.36 (2.84)	11.00 (3.46)	632					
	22.6	13.93 (1.35)	3.40 (1.95)	114					
	25.67	-		0					
Feb 07	12.5*	20.77 (6.06)	20.80 (3.49)	548	0.60	-0.122988	0.011976	10.27	83.5
	15	20.79 (4.83)	21.80 (1.79)	688		±0.006582	±0.000368	±0.22	±2.57
	17.9	10.93 (3.16)	13.40 (3.29)	530					
	19.5	9.95 (2.35)	11.80 (1.30)	476					
	22.6	7.69 (2.1)	9.00 (0.71)	190					

TABLE 1.5. Conditions inside each of six growth chambers used to rear *O.v.gulosa* at San Jose State University, San Jose, CA. Relative humidity was measured in chambers E and F only.

Photoperiod	Intended temperature (°C)	Actual temperature (°C)	Group		Chamber	Relative humidity (%)
12L:12D	10	-	1	E Spr 06	B, F	85
12L:12D	12.5	12.5	5	Spr 07	F	
12L:12D	15	14.64	1	E Spr 06	C	
12L:12D	15	15.2	3	Sum 06	E	75
12L:12D	15	15	5	Spr 07	D	
12L:12D	17.5	18.7	5	Spr 07	E	53
18L:6D	20	-	1	E Spr 06	F	40
12L:12D	20	20	2	L Spr 06	D	
12L:12D	20	19.8	3	Sum 06	G	
12L:12D	20	19.5	5	Spr 07	B	
12L:12D	20		4	Fall 06		
12L:12D	23	-	1	E Spr 06	A	
12L:12D	23	23.2	2	L Spr 06	E	41
12L:12D	23	22.9	3	Sum 06	C	
12L:12D	23	22.6	5	Spr 07	C	
12L:12D	26	25.7	5	Spr 07	H	
12L:12D	27	-	1	E Spr 06	D	
12L:12D	27	26.3	2	L Spr 06	F	34
18L:6D	30	-	1	E Spr 06	E	21
12L:12D	30	29.71	2	L Spr 06	A	
12L:12D	30	29.9	3	Sum 06	D	

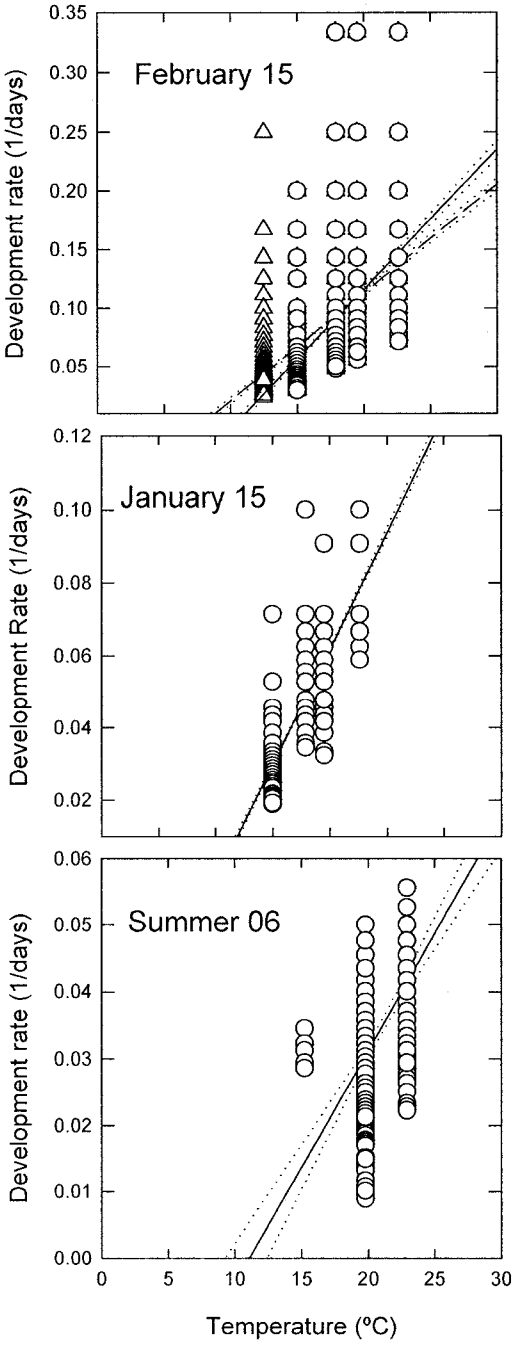


FIGURE 1.3. Egg hatch as a function of temperature for 3 groups of *O. v. gulosa* egg masses placed at controlled temperatures and 12L:12D. Regression line and 95% confidence interval shown.

The regression on the second group of eggs showed the same relationship between development time and temperature, but provided a better fit of the data. However it yielded a similar lower threshold temperature to the first regression. The number of larvae hatched was highest at 19.5°C, lowest at 22.5°C, and similar at 15 and 17.9 °C. The regression line was linear for data from four temperatures; the lower threshold temperature was 10.36°C, and hatching occurred after an accumulation of 156DD.

In the third group of eggs, development took considerably longer at the lowest temperatures, and when plotted, the 12.5°C data may be below the linear range. Because of this, egg masses at 10°C were removed before hatching had begun. The highest number of larvae hatched at 15°C, and the number hatching dropped off gradually at higher temperatures. Slightly fewer larvae hatched at 12.5°C. The linear regression line yielded a considerably lower threshold temperature of 7.8 °C, compared to the previous two regressions. If 12.5°C data are excluded, the lower threshold temperature becomes 10.3°C, similar to that of the first two regressions. Hatching occurred after 83.5 accumulated DD.

A comparison of the three regression equations showed significant differences in slopes ($F_{2,3922}=121.88$, $p < 0.0001$) and intercepts ($F_{2,3924}=1678.89$, $p < 0.0001$).

Evaluation of models: comparison to egg hatch in the field

Models using the three lower threshold temperatures above were used to generate predicted hatch dates. These dates were then compared to hatch dates in the field (Table 1.6). Hatch in the field was characterized as follows: egg hatch in spring 2006 was spread over a 10 week period (Jan 23 – Apr 7) though most hatching took place over three weeks. The 25%, median and 75% hatch dates were February 20, March 2 and March 12, respectively.

TABLE 1.6. Comparison of model predictions to *O. v. gulosa* hatch in the field over two seasons.

Start date	Lower threshold temp (°C)	Thermal Constant (DD)	2006 Model prediction 50% hatch	2006 Observed 50% hatch	Diff 06 predicted vs obs (days)	2007Model prediction 50% hatch	2007 Observed 50% hatch	Diff 07 obs vs predicted (days)	Mean diff (days)
from Regression 1:									
15-Oct	11	285	12/31/2005	3/2/2006	61	1/29/2007	3/7/2007	37	49
1-Nov	11	285	2/11/2006	3/2/2006	19	3/9/2007	3/7/2007	-2	8.5
15-Nov	11	285	3/18/2006	3/2/2006	-16	3/21/2007	3/7/2007	-14	-15
from Regression 2:									
15-Jan	10	150	-	-	-	3/11/2007	3/7/2007	-4	-
from Regression 3:									
15-Feb	10	83.5	-	-		3/12/2007	3/7/2007	-5	-
15-Feb	7.8	107	-	-		3/10/2007	3/7/2007	-3	-
other combinations of start date and lower threshold temperature:									
1-Dec	10°C	150	1/21/2006	3/2/2006	40	2/9/2007	3/7/2007	26	33
15-Dec	10°C	150	2/5/2006	3/2/2006	25	2/21/2007	3/7/2007	14	19.5
1-Jan	10°C	150	2/26/2006	3/2/2006	4	3/5/2007	3/7/2007	2	3
1-Jan	10°C	160	3/2/2006	3/2/2006	0	3/5/2007	3/5/2007	0	0

Egg hatch in the field in spring 2007 was spread over 8 weeks, and most hatching took place over a 9 day period. The 25%, median and 75% hatch occurred on March 2, March 7 and March 11, respectively.

All models predicted a later hatch date than observed in the field. The lower threshold temperature and thermal requirement from regression 1 were compared to 2006 and 2007 hatch data using several start dates. No combination provided a good fit for both years. At such high base temperature and thermal requirement, hatch is predicted to occur much later than it actually does. The second regression results were compared to the spring 2007 hatch. The model's thermal accumulation was reached five days later than actual mean hatch. The third regression results were compared to the spring 2007 hatch and were also five days off. The third regression results that included 12.5°C data provided a better fit; the predicted median hatch date was only three days later than the actual median hatch date.

When models are fit to two years of data (2006 and 2007) and various combinations of start date and lower threshold temperature are tried, there is relatively good fit between predicted and observed hatch with a start date of January 1 and a lower threshold temperature of 10°C. Median hatch is predicted to occur after an accumulation of 160DD.

Larval development as a function of temperature

Female larvae took longer to develop than male larvae at all temperatures in all groups, except at 10°C (Tables 1.7, 1.8). Males and females followed similar trends across temperatures.

Larval development decreased linearly with increasing temperature above 10°C, was fastest at 26-27°C, then slowed at higher temperature. Within each group, differences between some temperatures were not statistically significant, though this varied with group.

TABLE 1.7. Development of 4 groups of male and female *O. v. gulosa* larvae at constant temperatures. Number of individuals in parentheses. Values followed by a different letter in the same column are significantly different (Tukey test, $p<0.05$).

Intended temperature (°C)	Group	MALE Days as larvae	FEMALE Days as larvae
10	1	143.5 ± 10.01 (6)a	137 (1)a
12.5	5	52.36 ± 2.17 (14)b	62.63 ± 3.9 (16)b
15	1	47.25 ± 3.33 (12)c	53.9 ± 2.2 (10)c
15	3	53.81 ± 8.31 (21)d	62.1 ± 9.7 (11)d
15	5	50.18 ± 4.87 (11)c	57.3 ± 2.7 (8)c
17.5	5	26.25 ± 1.82 (24)e	30.8 ± 1.6 (20)e
20	1	26.25 ± 3.28 (8)f	29.3 ± 2.7 (6)f
20	2	26 ± 1.62 (14)f	30.1 ± 2.1 (20)f
20	3	29.95 ± 1.92 (39)g	32.8 ± 2.1 (20)g
20	5	25.36 ± 1.68 (36)f	29.6 ± 1.1 (34)f
23	1	20.21 ± 2.42 (14)h	23.0 ± 1.0 (7)h
23	2	21.05 ± 1.13 (19)h	25.2 ± 1.9 (9)h
23	3	23.2 ± 2.29 (40)i	28.5 ± 3.7 (55)i
23	5	19.08 ± 1.25 (24)h	23.2 ± 0.8 (17)h
26	5	16.75 ± 1.34 (16)j	19.2 ± 1.1 (20)j
27	1	17.55 ± 1.51 (11)k	20.6 ± 0.7 (12)k
27	2	18.24 ± 0.70 (21)k	21.9 ± 2.1 (15)l
30	1	17.64 ± 1.39 (14)l	19.2 ± 1.0 (6)m
30	2	20.7 ± 2.98 (10)m	24.5 ± 1.6 (15)n
30	3	22.32 ± 3.87 (22)m	26.1 ± 2.8 (13)n

TABLE 1.8. F values in ANOVA comparing development times of groups of *O. v. gulosa* raised at similar temperatures in the lab. Significance levels are: * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

Temp (°C)	Female larvae		Female pupae		Male larvae		Male pupae	
	df	F	df	F	df	F	df	F
15	2,26	4.45*	2,25	24.85****	2,41	4.02*	2,38	112.47****
20	3,76	15.14****	3,74	14.98****	3,93	39.31****	3,85	42.37****
23	3,84	17.23****	3,81	NS	3,93	25.56****	3,89	NS
27	2,44	16.67****	2,41	5.22**	2,45	7.61**	2,42	3.86*
30	2,31	22.09****	2,31	NS	2,43	9.59***	2,38	4.82*

For example, development times for larvae raised in spring and summer 2006 decreased as the temperature increased from 15 to 23°C (Group 1: $F_{4,90}=817.56$, $p<0.0001$; Group 3: $F_{3,213}=451.81$, $p<0.0001$), but there was no difference in development time from 27 to 30°C for

both male ($p<1$) and female ($p=0.44$) larvae. For Group 2 larvae raised in late spring 2006 however, development at 27°C was faster than at 23 or 30°C ($F_{3,115}=118.4$, $p<0.0001$), which did not differ from each other (Males: $p=0.9$, Females: $p=0.8$). Similarly, for group 5 larvae raised in spring 2007, development was faster at 26°C than at 23°C ($F_{5,228}=2024.21$, $p<0.0001$), but there was no significant difference in development times between 12.5 and 15°C in males ($p=0.12$), or between 18.7 and 19.5°C in males ($p=0.61$) and females ($p=0.24$).

Least squares linear regression was performed on data from each group of larvae raised, and also on data from all groups combined, in order examine development under a range of leaf qualities (Figure 1.4, Table 1.9). A comparison of regression equations among each group of larvae showed significant differences between groups in slope ($F_{3,604}=6.05$, $p=0.0005$) and intercept ($F_{3,607}=85.85$, $p < 0.0001$).

When least-squares linear regression was performed on all data in the linear range (excluding 30°C data, all groups together), the lower threshold temperatures for larval development was 6.1°C for females and 7.1°C for males. When data from males and females were pooled, the resulting lower temperature threshold for larval development was 7.2°C and the thermal constant was 360 DD.

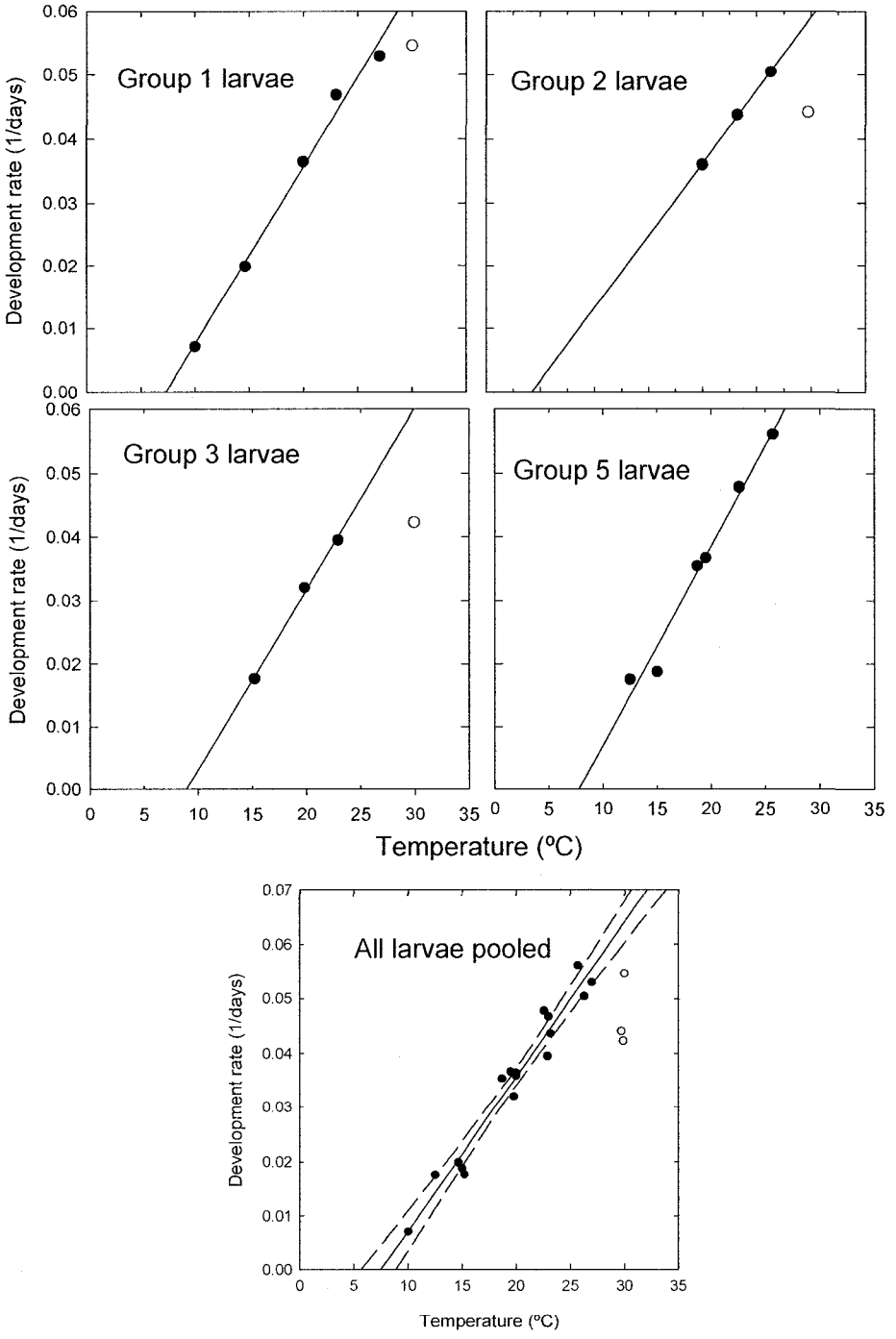


FIGURE 1.4. Development rates of 4 groups of male and female *O. v. gulosa* larvae at constant temperatures. (Open data point are beyond linear range and not included in regression. Dotted lines show 95% confidence interval.)

TABLE 1.9. Estimated values of parameters in models of *O. v. gulosa* development using least-squares linear regression. Data at 30°C were beyond the linear range and not included in each regression.

Developmental Stage	Group	Intercept ± SE	Slope ± SE	Thermal constant ± SE	LBT ± SE	r^2
Male	1	-0.021027 ± 0.002175	0.002829 ± 0.000103	353.48 ± 12.87	7.43 ± 0.51	0.9
&	2	-0.007287 ± 0.003794	0.00219 ± 0.000161	456.62 ± 33.57	3.33 ± 1.27	0.66
Female	3	-0.019804 ± 0.002317	0.00257 ± 0.000111	389.11 ± 16.81	7.71 ± 0.57	0.74
Larvae	5	-0.019186 ± 0.001501	0.002896 ± 0.000075	345.3 ± 8.94	6.63 ± 0.35	0.86
	All	-0.019928 ± 0.001189	0.002775 ± 0.000057	360.36 ± 7.4	7.18 ± 0.29	0.8
Female	1	-0.017943 ± 0.001954	0.002527 ± 0.000089	395.73 ± 13.94	7.1 ± 0.53	0.96
Larvae	2	-0.006538 ± 0.003928	0.001999 ± 0.000171	500.25 ± 42.79	3.27 ± 1.69	0.76
	3	-0.018366 ± 0.003342	0.002377 ± 0.000156	420.7 ± 27.61	7.73 ± 0.9	0.73
	5	-0.021062 ± 0.000964	0.002838 ± 0.000048	352.36 ± 5.96	7.42 ± 0.22	0.97
	All	-0.014446 ± 0.001360	0.002351 ± 0.000064	425.35 ± 11.58	6.14 ± 0.42	0.83
Male	1	-0.024092 ± 0.002422	0.003108 ± 0.000117	321.75 ± 12.11	7.75 ± 0.5	0.94
Larvae	2	-0.012926 ± 0.002996	0.002591 ± 0.000126	385.95 ± 18.77	4.99 ± 0.91	0.89
	3	-0.029611 ± 0.002059	0.003189 ± 0.000101	313.58 ± 9.93	9.29 ± 0.35	0.91
	5	-0.025741 ± 0.001656	0.003385 ± 0.000083	295.42 ± 7.24	7.6 ± 0.31	0.93
	All	-0.020921 ± 0.001382	0.002947 ± 0.001382	339.33 ± 7.60	7.10 ± 0.31	0.86

Pupal development as a function of temperature

Female pupae took less time to develop than male pupae (Tables 1.8, 1.10), and adult moths emerged at similar times. Males and females followed similar trends across temperatures. The development rate for both male and female pupae increased linearly in the 10 to 27°C temperature range (Figure 1.5). No adults emerged from pupae held at 10°C, which may be near the lower threshold temperature for development.

TABLE 1.10. Development (mean days ± SD) of 4 groups of male and female *O.v.gulosa* pupae at constant temperatures. Number of individuals in parentheses. Values followed by a different letter in the same column are significantly different (Tukey test, $p<0.05$).

Intended temp (°C)	Group	MALE Days as pupae	FEMALE Days as pupae
10	1	-	-
12.5	5	51.71 ± 2.05 (14)[a]	37.81 ± 1.22 (16)a
15	1	35.82 ± 2.56 (11)[b]	25.80 ± 1.75 (10)b
15	3	36.71 ± 1.49 (21)[b]	26.64 ± 2.29 (11)b
15	5	48.56 ± 2.88 (9)[c]	33.57 ± 3.26 (7)c
17.5	5	22.00 ± 0.87 (22)[d]	16.32 ± 1.06 (19)d
20	1	14.20 ± 1.79 (5)[e]	11.67 ± 1.86 (6)e
20	2	15.00 ± 1.00 (13)[e]	12.10 ± 0.72 (20)e
20	3	17.05 ± 0.73 (38)[f]	12.90 ± 0.64 (20)f
20	5	17.33 ± 0.65 (33)[f]	13.53 ± 0.84 (32)f
23	1	13.09 ± 2.02 (11)[g]	10.71 ± 0.76 (7)g
23	2	12.79 ± 0.71 (19)[g]	11.00 ± 1.58 (9)g
23	3	12.85 ± 0.83 (40)[g]	10.21 ± 0.87 (52)g
23	5	12.52 ± 0.51 (23)[g]	10.29 ± 0.69 (17)g
26	5	10.38 ± 0.50 (16)[h]	9.12 ± 0.93 (17)h
27	1	9.78 ± 0.97 (9)[i]	8.08 ± 1.00 (12)i
27	2	10.45 ± 0.51 (20)[h]	8.73 ± 0.59 (15)i
30	1	10.82 ± 1.25 (11)[j]	7.83 ± 0.75 (6)j
30	2	9.60 ± 1.07 (10)[k]	8.27 ± 0.46 (15)j
30	3	9.65 ± 0.99 (20)[k]	8.15 ± 0.55 (13)j

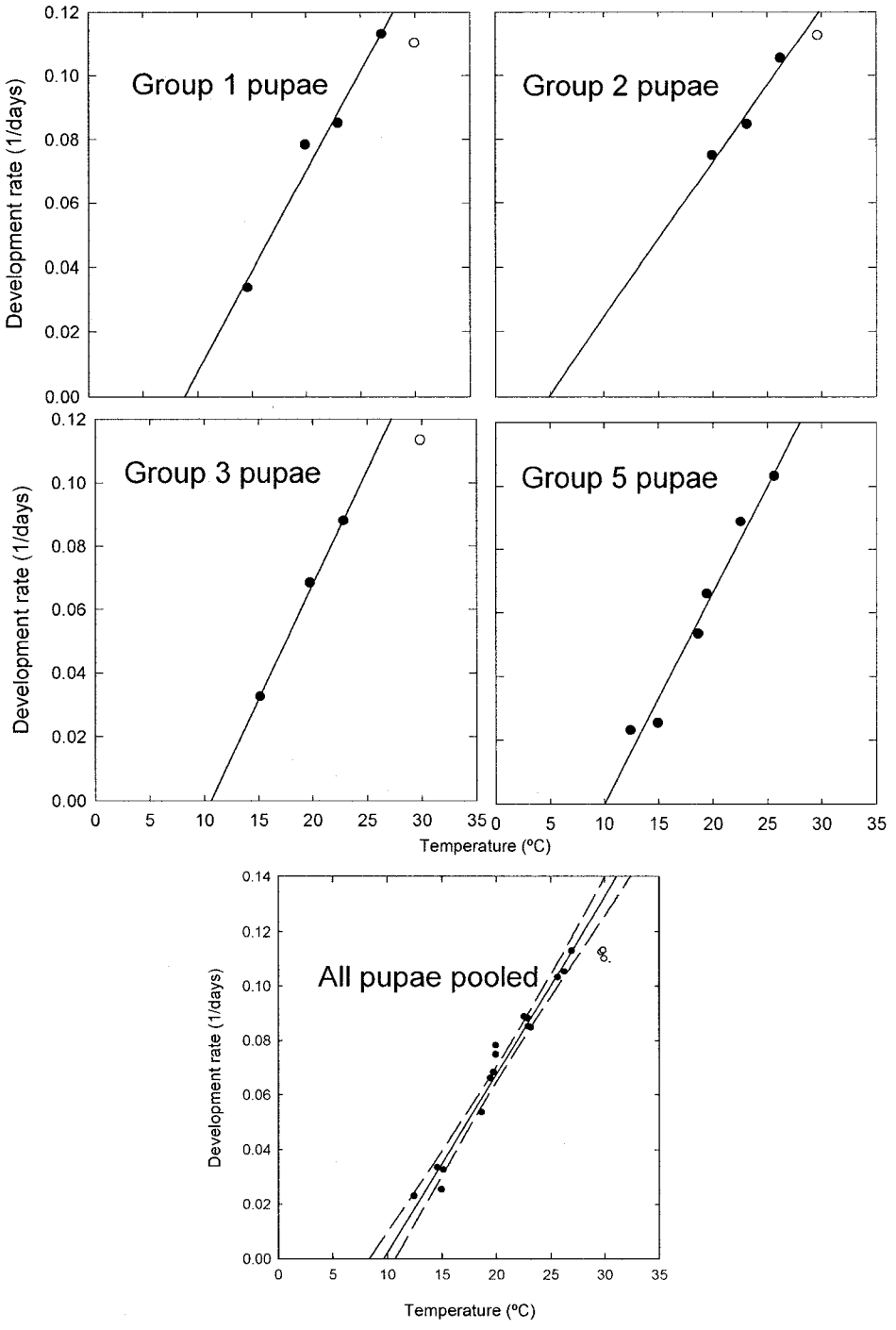


FIGURE 1.5. Mean development rates of 4 groups of male and female *O.v.gulosa* pupae at constant temperatures. A. Group 1 B. Group 2 C. Group 3 D. Group 5. E. all groups pooled.

As with larval development, sometimes there were no statistically significant differences between temperatures within groups. While pupal development time decreased as the temperature increased from 15 to 27°C in most groups, two exceptions were found. Group 1 pupae did not differ between 20 and 23°C ($p_{♂}$=0.80, $p_{♀}$=0.69), and group 5 females did not differ between 23 and 26°C (p=0.06). At high temperatures, there was no significant difference in development time between 27 and 30°C for both male and female pupae (Group 1 $p_{♂}$=0.72, $p_{♀}$=1; Group 2 $p_{♀}$=0.42) except in Group 2 males, which developed faster at 30 than at 27°C ($F_{3,58}$=124.17, p=0.04).

Comparing regression equations for the four groups of pupae (Table 1.11), there were significant ($F_{3,566}$=14.36, $p < 0.0001$) differences between slopes, but not between intercepts ($F_{3,569}$=0.53, p=0.66). The slope of group 2 was shallower than that of groups 1,3 and 5; it did not include larvae raised at temperatures below 20°C, which may explain this result.

When using all data in the linear range (below 30°C, all groups combined), the lower threshold temperatures for pupal development was calculated as 8.6°C for females and 10.0°C for males. When data from males and females were pooled, the resulting lower temperature threshold for pupal development was 9.4°C and the thermal constant was 157.6 DD.

Comparison of degree day model to larval and pupal development in the field

Agreement between predicted and observed pupation date varied by group (Figure 1.6). Most models predicted pupation 3-20 days earlier than it actually occurred, except for the Group 2 model, which predicted pupation nine days too late. The most accurate models, the Group 1 model and the model based on pooled data from all groups, were off by roughly three days each year.

TABLE 1.11. Estimated values of parameters in models of *O. v. gulosa* development using leasat-squares linear regression. Data at 30C were beyond linear range and not included in each regression.

Develop-mental Stage	Group	Intercept ± SE	Slope ± SE	Thermal constant ± SE	LBT ± SE	r^2
Male	1	-0.065138 ± 0.007791	0.006697 ± 0.000356	149.32 ± 7.94	9.73 ± 0.67	0.84
&	2	-0.005413 ± 0.008584	0.004008 ± 0.000364	249.5 ± 22.66	1.35 ± 2.02	0.56
Female	3	-0.081241 ± 0.005563	0.007405 ± 0.000267	135.04 ± 4.87	10.97 ± 0.36	0.81
Pupae	5	-0.058661 ± 0.002982	0.006273 ± 0.000149	159.41 ± 3.79	9.35 ± 0.26	0.89
	All	-0.059613 ± 0.002492	0.006347 ± 0.000118	157.55 ± 2.93	9.39 ± 0.22	0.84
Female	1	-0.063997 ± 0.010152	0.007045 ± 0.000459	141.94 ± 9.25	9.08 ± 0.87	0.88
Pupae	2	-0.019534 ± 0.010292	0.005061 ± 0.000448	197.59 ± 17.49	3.86 ± 1.69	0.75
	3	-0.078206 ± 0.005933	0.007743 ± 0.000278	129.15 ± 4.64	10.1 ± 0.94	0.91
	5	-0.056188 ± 0.00311	0.006531 ± 0.000156	153.12 ± 3.66	8.6 ± 0.28	0.94
	All	-0.057235 ± 0.002806	0.006661 ± 0.000132	150.1 ± 2.98	8.59 ± 0.26	0.9
Male	1	-0.062143 ± 0.007001	0.006158 ± 0.000324	162.39 ± 8.54	10.09 ± 0.63	0.91
Pupae	2	-0.028582 ± 0.006387	0.004702 ± 0.000269	212.68 ± 12.17	6.08 ± 1.01	0.86
	3	-0.072941 ± 0.002667	0.006614 ± 0.000131	151.19 ± 2.99	11.03 ± 0.19	0.96
	5	-0.061451 ± 0.001953	0.006061 ± 0.000097	164.99 ± 2.64	10.14 ± 0.17	0.97
	All	-0.059680 ± 0.001669	0.005967 ± 0.000079	167.59 ± 2.22	10.00 ± 0.15	0.95

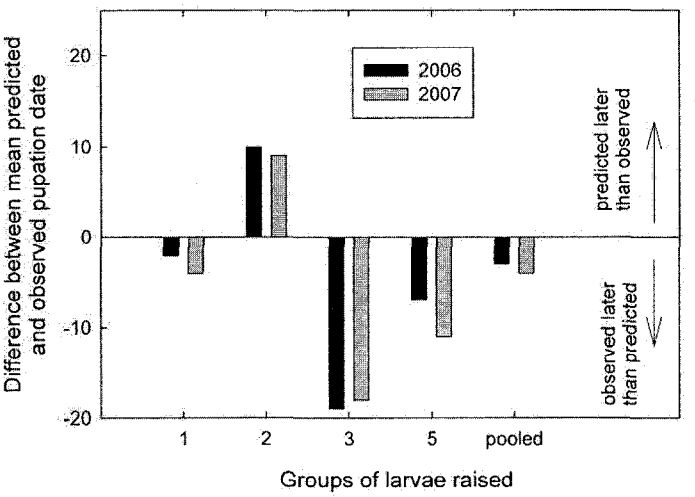


FIGURE 1.6. Comparison of predicted to observed *O. v. gulosa* pupation date over 2 years.

There was less variation between groups in predicting adult emergence (Figure 1.7). Most models predicted adult emergence 1-5 days later than it actually occurred. The Group 2 model was most accurate, off by one day both years. The resolution of observed moth emergence was limited by once-a-week field monitoring.

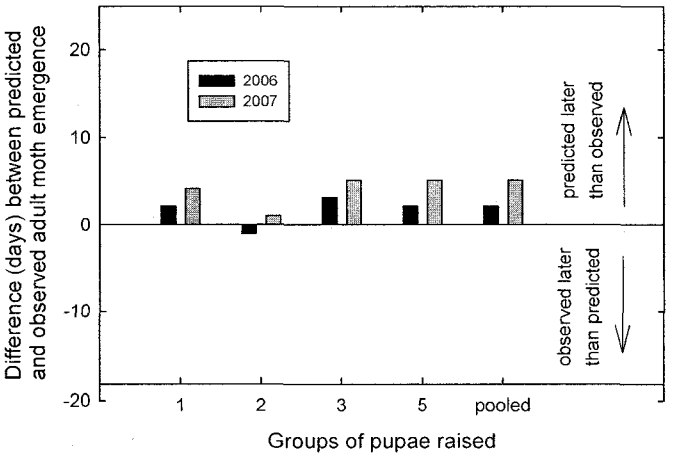


FIGURE 1.7. Comparison of predicted to observed *O.v.gulosa* eclosion over 2 years.

Ecological implications: Mortality as a function of diet and temperature

Cold temperatures and leaves produced later in the season affected larvae detrimentally (Figures 1.8 and 1.9). Larval mortality was higher at 10°C (Group 1: $F_{5,13}=25.42$, $p<0.0001$) than at higher temperatures, which did not differ from one another ($p>0.2$). Larvae fed summer leaves had significantly higher mortality than those fed spring leaves in 2006 ($F_{2,45}=13.77$, $p<0.0001$). There were no differences between early and late spring leaves in 2006 ($p<1$). Larval mortality was slightly higher in early spring 2007 than in early spring 2006 at 15, 20 and 23°C ($F_{1,17}=7.45$, $p=0.014$).

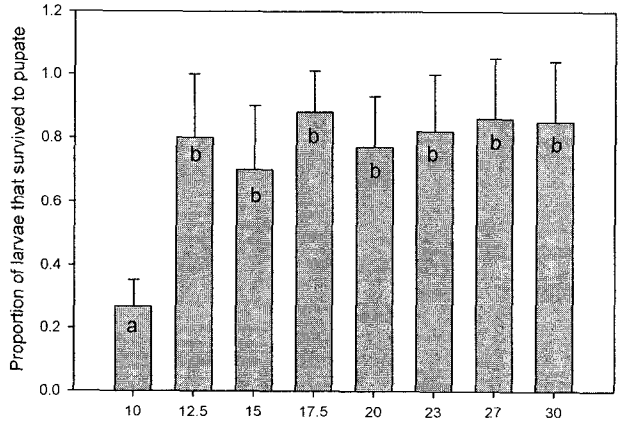


FIGURE 1.8. Mortality of *O.v.gulosa* larvae, averaged for all groups, as a function of temperature. Letters imply significant differences ($p<0.05$).

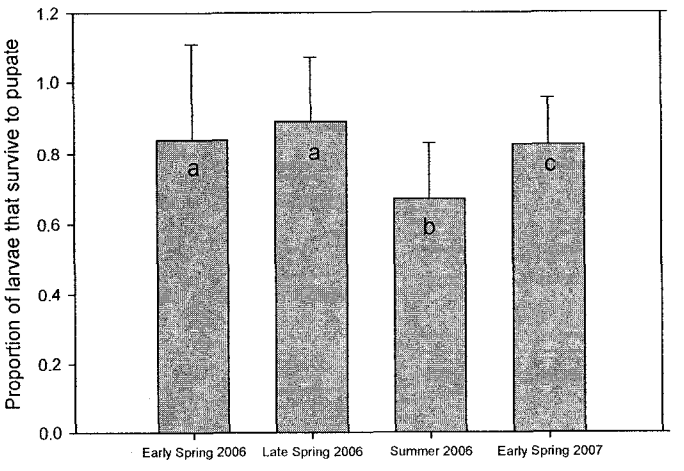


FIGURE 1.9. Mortality of *O.v.gulosa* as a function of diet. Letters imply significant differences ($p<0.05$).

Pupae were more sensitive to cold temperatures than larvae but were not affected by diet (Figure 1.10). Pupal mortality was higher at 10°C (Group 1: $F_{5,13}=16.56$, $p<0.0001$) and 12.5°C (Group 5: $F_{5,22}=3.84$, $p=0.012$) than at higher temperatures, which did not differ from one another ($p>0.2$). There were no differences in pupal mortality from early spring 2006 to early spring 2007 ($p= 0.53$). There was a weak difference in pupal survival between groups in 2006 ($F_{2,45}=4.15$, $p=0.03$); fewer Group 1 pupae survived than in Groups 2 and 3.

Larval mortality was higher than pupal mortality in all groups (larvae 21%; pupae 9%; $t=5.67$, $p<0.0001$).

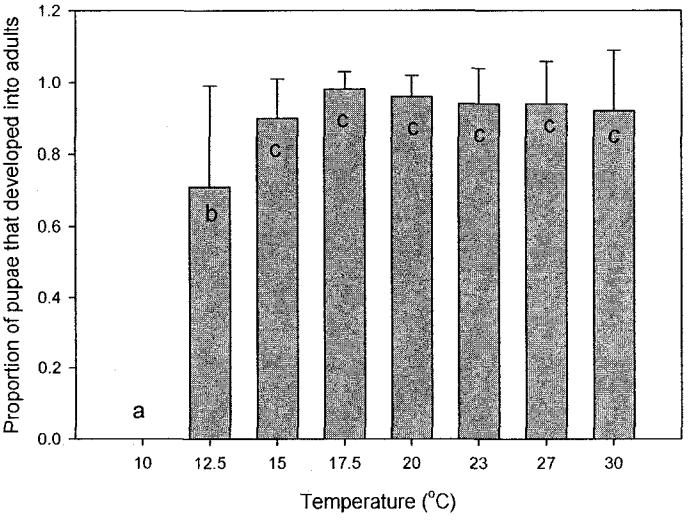


FIGURE 1.10. Mortality of *O. v. gulosa* pupae as a function of temperature. Letters imply significant differences ($p<0.05$).

Ecological implications: development time as a function of diet and temperature

Larvae took longer to develop on new growth produced later in the year, with this effect more pronounced at higher temperatures (Figure 1.11). Comparing groups 1, 2 and 3 at 20, 23 and 30°C, there were significant differences between groups depending on the temperature considered ($F_{4,313}=7.57$, $p<0.00001$). At all temperatures, group 3 larvae raised on summer leaves took longer to develop than group 1 and 2 larvae raised on spring leaves, though there was no difference in development time between groups 1 and 2 at 20 and 23°C. At the highest temperature of 30°C, group 2 larvae raised on late spring leaves took longer to develop than group 1 larvae raised on early spring leaves. There was no difference in larval development time between larvae raised on early spring leaves in different years (groups 1 and 5) ($p=0.92$).

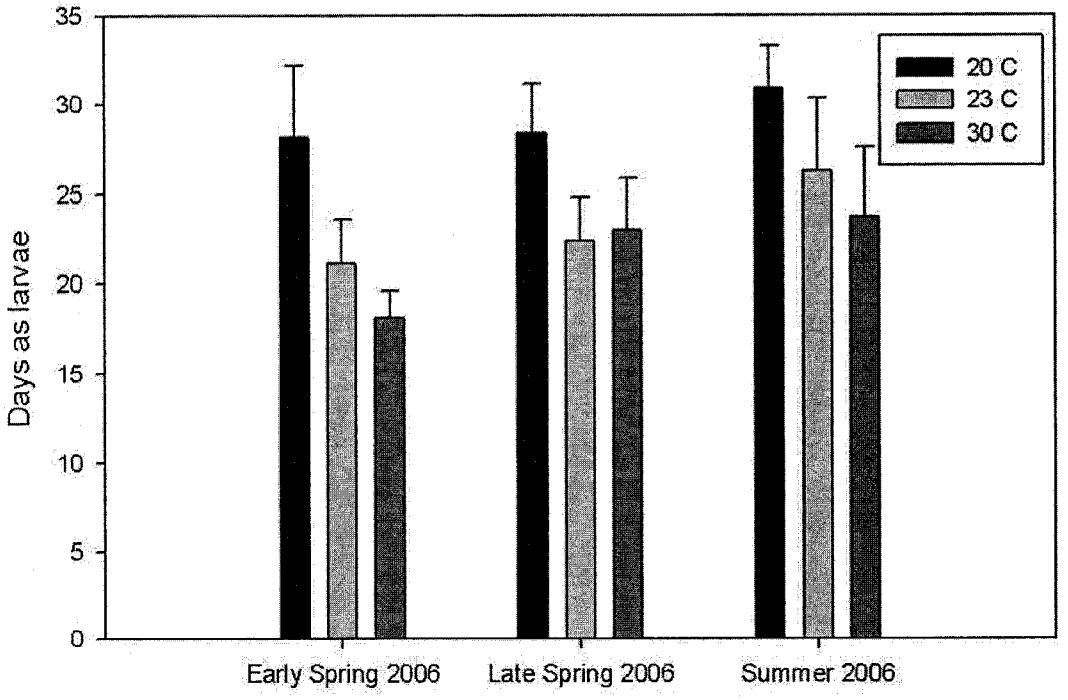


FIGURE 1.11. Development time of *O.v.gulosa* larvae as a function of diet.

Larval development time was more variable than pupal development time (larval cv = 9.0%; pupal cv = 7.3%). Unlike larvae, trends in pupal development time were inconsistent across groups. A three-way interaction between group, gender and temperature was found comparing Groups 1, 2 and 3 at 20, 23 and 30°C ($F_{4,297}$=3.68, $p<0.01$), and a two-way interaction between group and temperature comparing Groups 1 and 5 at 15, 20 and 23°C ($F_{2,175}$=11.25, $p<0.0001$).

Ecological implications: pupal weight as a function of diet and temperature

Female pupae weighed three times more than male pupae on average, though female pupal weight was considerably more variable than male pupal weight (Figure 1.12) (Temperature x gender interaction: Group 1 $F_{5,92}$=10.80, $p<0.0001$; Group 3 $F_{3,212}$=7.50, $p<0.0001$; Group 5 $F_{5,218}$=48.83, $p<0.0001$). Pupae raised at 15°C and below were lighter

than those raised at higher temperatures. The slight differences in pupal weight within these two temperature categories were inconsistent between groups. Comparing groups, both male and female group 1 pupae weighed more than group 3 pupae ($F_{2,231}$=8.47, p<0.00001) (group 2 pupae were not weighed).

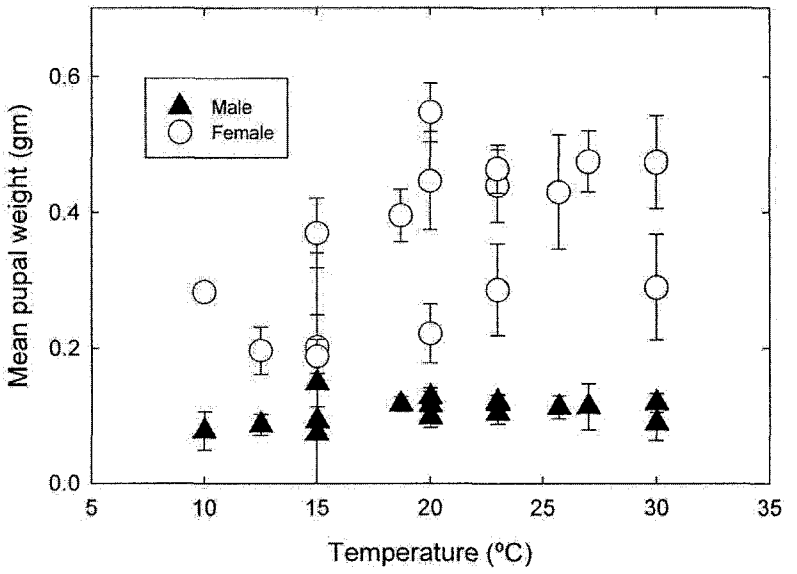


FIGURE 1.12. Weight of *O. v. gulosa* as a function of gender, temperature and diet. Females were heavier but showed more variation/sensitivity than males.

DISCUSSION

Egg diapause termination and biofix date

To determine the most appropriate biofix date, I considered a number of factors: 1) current understanding of diapause termination in related species; 2) results of biweekly egg mass collections; 3) dissections of collected egg masses; and 4) comparison of degree day model predictions to observed hatch in the field.

Egg mass dissections showed that most embryo development takes place post-diapause in *O. v. gulosa*. This is similar to green oak tortrix (Du Merle, 1999) and silkworm *Bombyx* (Goldsmith and Wilkins, 1995), but different from gypsy moth, which undergoes egg diapause as a fully formed larva (Lyons and Lysyk, 1989).

An appropriate biofix date may correspond to the termination of diapause, when eggs can resume development under favorable conditions. Diapause development is still not well-understood and varies from insect to insect. The end of diapause may vary between years and among individuals (Koštál, 2006). Egg masses are laid over several weeks, and the date on which they are laid may affect hatch date, as in the winter moth (van Dongen *et al*, 1997). Differences exist between geographically disparate populations; for example, Harrison (1997) reported late-hatching populations along the central coast of California. My results apply only to *O. v. gulosa* in the San Francisco Bay Area.

Diapause is controlled by both genetic and environmental factors. In silk worms, a diapause hormone produced by the female causes egg diapause early in embryogenesis (Yamashita, 1996). Diapause development and termination depend on environmental factors such as temperature and photoperiod, though sensitivity to these factors changes over time (Chapman, 1998; Gillott, 1995;Tauber et al, 1986).

Cold exposure has been shown to decrease diapause intensity and duration in the lab, but chilling should not be presumed to hasten diapause termination in nature (Tauber and Tauber, 1976). Rearing programs for several tussock relatives (*Orgyia cana*, Peterson, 1978; *Orgyia pseudotsugata*, Beckwith and Stelzer, 1979) recommend placing eggs at 2-4°C for several months to overcome diapause. While extremely cold temperatures are a rarity in the Palo Alto area, exposure to temperatures as high as 8°C may provide enough chilling for some species,

such as green oak tortrix (Du Merle, 1999), and occur consistently in Palo Alto by the second half of October (National Climatic Data Center, www.ncdc.noaa.gov). It is generally recognized that in temperate regions, overwintering diapause may terminate in fall or early winter, though cold temperatures inhibit development (Hodek, 1996).

Cool winter temperatures may synchronize hatching (Hodek, 2002). Hatch patterns of *O. v. gulosa* in the field in 2006 and 2007 support this. January 2006 was relatively warm, and field hatch occurred over 6 weeks on average within an egg mass. By contrast, January 2007 was unusually cold, and field hatch within an egg mass took 2 weeks on average. These differences in duration are also temperature-dependent, as March 2006 was much cooler than March 2007.

As diapause progresses, the time to hatch decreases and becomes more synchronous (Tauber and Tauber, 1976); this pattern was seen in the lab studies of *O. v. gulosa* egg masses collected through January 15. A small number of larvae hatched from egg masses collected in September and October; these may have been weakly- or non-diapausing individuals, as have appeared in studies of Douglas-fir tussock moth (Beckwith and Stelzer, 1979) and other moths.

Humidity and changing photoperiod may also be important factors not considered in this study. In egg mass dissections, the proportion of larvae that had developed increased steadily in egg masses collected between mid-September and mid-December. However, larvae developed in over half of the egg masses collected after mid-October, but these larvae failed to eclose. Du Merle (1999) determined that oak tortrix eggs need 80% relative humidity to hatch. Humidity was not controlled in the chambers in this study, decreased fairly linearly with increasing chamber temperature, and may have prevented hatching in some circumstances.

Controlled temperature studies in the lab on this population of *O. v. gulosa* determined a lower threshold temperature of 10°C and a degree day accumulation of 160DD for egg development. When these parameters were applied to various start dates between December 1 and January 15, the best fit between predicted and observed hatch was found with a biofix date of January 1.

Egg development as a function of temperature

Three trials considering *O. v. gulosa* egg development between 15 and 23°C generated a lower threshold temperature (lower base temperature, or LBT) of 10-11°C. This temperature is high within the range for forest defoliators, from 3°C (37°F) for spruce budworm to 11°C (51°F) for fall webworm. For the related Douglas-fir tussock moth, 5.56°C (42°F) has been arbitrarily chosen for degree day modeling, having been used in many biological studies as the base temperature for the onset of vegetative growth (Wickman, 1976). Fruittree leafroller, which co-occurs with *O. v. gulosa* in Palo Alto, has an LBT of 4.95°C in British Columbia (Judd and Gardener, 1993). However, scientists have warned that degree day parameters are valid only in the area of the study, so that fruittree leafroller in Palo Alto may behave differently. No published degree day models were found for Bay Area Lepidoptera with life histories similar to *O.v.gulosa.*

The limited range of temperatures at which hatching occurred affected regression development and interpretation. Bergant and Trdan (2006) recommended using at least five temperatures within the linear range when raising insects for degree day models. Regression 1 (Summer 06) contained data from only 3 temperatures. Regression 2 (January 15) involved egg masses at 6 temperatures, but no hatching took place at the two highest temperatures, so the regression is based on four temperatures only. It is unclear whether hatching was inhibited by

high temperature or low humidity. Temperatures above 23°C (73°F) are not common in Palo Alto during the time of hatching (NCDC-NOAA). Regression 3 contained data at an additional lower temperature, 12.5°C, which appeared beyond the linear range of the curve and supports the idea of a relatively high LBT.

The LBTs for several life stages (egg, larva, and pupa) of an insect are often similar to one another. For obliquebanded leafroller, these are 9.5, 10, and 9.6 °C, respectively (Jones *et al*, 2005). For grape berry moth, the LBT for egg, larva and pupa are 8.8, 7.9, and 8.5°C (Tobin *et al*, 2001). For tea tortix, they are 9.8, 9.3 and 11.5°C (Nabeta *et al*, 2005). Given that the LBTs for *O. v. gulosa* larvae and pupae are 7.2 and 10.2°C, an LBT of 10-11°C for egg development seems reasonable.

Comparison of models to egg hatch in the field

The thermal constant for egg development was different for each trial, likely reflecting different stages of diapause and/or embryo development at the time of egg collection. Eggs removed from cold storage in during summer 2006 had the highest thermal constant, 285DD; they were collected in October 2005, probably before any development in the field had taken place. Yet this large thermal constant does not fit observed field hatch in 2006 and 2007 well.

Eggs collected on January 15 had a thermal constant of 159DD. It is unclear whether any development had occurred in the field by this date. Given a biofix date of January 1 and a lower threshold of 10C, it's possible that 20-30DD had accumulated in the field before egg masses were collected. Eggs collected on February 15 had certainly undergone some development in the field, and this is reflected in the decreased thermal constant of 83.5DD.

The thermal constants from the January 15 and February 15 regressions support one another: both predict a field hatch on March 12 or March 14, depending on the weather

station used. This is 5 or 7 days later than observed hatch. A better fit would occur if a biofix date of January 1 is used with a LBT of 10° C and a thermal constant of 160DD; predicted hatching matches observed hatching on March 2, 2006 and March 7, 2007.

It is difficult to predict mean hatching to an accuracy of a single day that will apply across years. Weather station data (from an exposed site 1.5 m above ground) probably do not reflect the temperatures insects in trees are experiencing. Both Stanford weather stations predict hatching later than it actually occurs, probably because temperatures in the tree canopies are warmer than in either weather station and accumulate heat more rapidly. The thermal constants are determined in the lab, where the temperatures the insects are subject to are carefully controlled.

As climate changes over time, insect populations also change. Temperatures in 2006 and 2007 were warmer than the 30 yr average. Van Asch *et al* (2007) showed that winter moth hatching occurred earlier over a 10 year time period, in conjunction with warmer winter temperatures. Mild temperatures without periods of extreme cold seem to result in prolonged hatching, though warmer temperatures may inhibit hatching. Further studies are needed to understand the effects of climate change on diapause development.

Larval and pupal development as a function of temperture

The linear range for larval development was 10 to 27°C. At temperatures of 30°C, larvae developed more slowly, so their upper threshold temperature (UBT) appears to be approximately 27°C. This matches temperatures present in spring in Palo Alto and also observations of the larvae's appearance and feeding patterns. Early instar larvae, present in February and March, fed during the day; later instar larvae, present in April and May, switched to feeding at night, when temperatures were cooler (Graeve, personal observation). Larvae

also change color with each instar, possibly affecting thermoregulation (Bryant *et al*, 2000). Earlier instars are darker, to gather more heat at a cooler time of year; later instars are lighter, when temperatures are warmer.

The lower threshold temperature (LBT) for larval development was determined by extrapolation to be between 7 and 8°C for trials 1, 3 and 4. The importance of raising larvae at more than three temperatures, some of which are below 20°C, was highlighted in Trial 2. When larvae were raised at relatively warm temperatures only, the LBT was lower than other trials and does not appear to correspond to field observations.

In light of this result, egg development studies that did not include hatch at temperatures above 23°C may also have incorrectly determined the LBT. Controlling humidity may permit hatching at higher temperatures, which may result in a lower LBT by extrapolation.

Pupae had higher upper and lower threshold temperatures for development than larvae. The UBT was not determined, since development did not slow down above 27°C. The LBT was 9.78°C, and may explain why no adults emerged from pupae held at 10°C for over 3 months. These pupal developmental thresholds make sense in terms of temperatures encountered in May and June in Palo Alto. The differences in pupal development time between Groups 1, 2 and 3 don't follow a pattern, perhaps because pupal development is relatively independent of diet (Jones *et al*, 2005).

Validation of larval and pupal development models

Repeating the controlled temperature studies over several seasons resulted in a better-fitting model of larval development. While the model developed from Group 1 data was as accurate as the pooled model, those for Groups 2, 3 and 5 were less accurate. Groups 2 and 3,

raised in late spring and summer, represent leaf qualities not normally encountered by larvae that hatch in late winter. However, these may be similar to leaves produced during drought years or under warmer spring temperatures. The Group 5 model was surprisingly less accurate than that of Group 1, though both were developed using early spring leaves, and Group 5 had a larger sample size. A virus in the 2007 population increased mortality and may explain slower development at 15°C. This could have affected the regression parameters and the predictive ability of the model.

The pupal development models for the different groups had similar predictive value. This supports earlier work showing that pupal development is independent of diet (Jones *et al*, 2005).

Ecological implications of laboratory studies

These temperature studies have implications for the distribution and life history (voltinism) of *O. v. gulosa.* Degree day accumulations indicate that temperatures could support two generations, although *O. v. gulosa* has an obligate diapause and only one generation per year in Palo Alto. Warming temperatures may affect female moth production of diapause hormone and could be further investigated.

Mortality, larval development time and pupal weight were not extremely sensitive to differences in new growth collected at different times of year. This is similar to studies of the related *O .v. vetusta*, in which plant quality, as measured by repeated defoliation, did not affect pupal weights (Harrison, 1994). While female pupal weight varied with temperature and season, these differences were inconsistent between groups, and the ecological implications are unclear.

In conclusion, my results provide a field-tested model of *O. v. gulosa* development in the

inland areas of the San Francisco Bay Region. More work needs to be done on *O.v.gulosa* diapause development and possible changes in response to global warming. These lab studies also provide insight into population dynamics sensitive to temperature and diet, such as local adaptation and outbreak occurrences.

Chapter 2 Phenological Synchrony between Coast Live Oak and Western Tussock Moth: Are moths adapted to individual trees?

INTRODUCTION

Mediterranean climate regions are characterized by cool moist winters and hot dry summers, and the plants and animals living in such regions have adapted to this seasonality (Dallman, 1998). Spring is the favorable time for above-ground growth, so it is also the active time for herbivores such as leaf-eating caterpillars. Phenology is defined as the timing at which a particular stage of a life cycle occurs, for example tree budburst or caterpillar hatch, and it varies from year to year.

As caterpillar fitness depends on food quality, many studies have looked at synchronization of caterpillar hatch and host plant budburst (Crawley and Akhteruzzaman., 1988; Wickman, 1976). Some studies have focused on the physiological mechanisms affecting caterpillar hatch or tree budburst (Du Merle, 1999; Hunter and Lechowicz, 1992). Others have looked at the fitness consequences of synchrony on herbivores (Ivashov *et al,* 2002; Tikkanen and Julkunen-Tiitto, 2003), or for herbivore adaptation to individual hosts (Edmunds and Alstad, 1978; van Dongen *et al*, 1997). A third group of studies has been concerned with the effects of synchrony on population dynamics of herbivores, many of which are outbreak species with widely fluctuating population densities (Hunter and Elkinton, 2000).

Studying synchrony between a particular insect and its host first starts with an assessment of the amount of variation in budburst and hatching. Next, the effects of temperature on budburst and hatching are investigated. Finally, it should be determined if and at what level (branch, tree or population) budburst and hatching are synchronized and how this varies over time.

Variation in budburst can be studied at different levels: between individual trees, between years, and within individual trees in a particular year. Tree budburst varies greatly among individuals in a population. The date of budburst in full siblings of English oak (*Quercus robur*) can range over three weeks (Scotti-Saintagne *et al.*, 2004). Current studies in California also show variation in valley oak (*Quercus lobata*) flowering dates (Koenig, 2005).

When comparing among trees, tree phenology is consistent from year to year, as early trees are consistently early, and late trees are consistently late (Crawley and Akhteruzzaman, 1988; Koenig, 2005; van Dongen *et al.*, 1997). This phenology is probably genetically controlled, though maternal effects may also be involved. Budburst in English oaks is likely controlled by at least 12 unique genes or chromosomal regions; each probably has a low to moderate effect but the overall result is high genetic variance (Scotti-Saintagne *et al.*, 2004).

One overlooked area of study is the amount of variation in budburst *within* a tree. Studies of phenological synchrony between herbivores and hosts considered variation at the level of the individual tree, and trees were scored as "budbursting" when more than 50% of buds had opened (Crawley and Akhteruzzaman, 1988; Hunter, 1992; Tikkanen *et al.*, 2003; van Dongen *et al.*, 1997). Other studies looking at variation within a tree have focused on chemical and physical properties of leaves, finding that the level of variation depends on the trait under

consideration. Leaf weight and water content varied widely within individual tree crowns of English oak, while leaf phenolic content varied substantially among individuals but not among stands (Roslin *et al.*, 2006). Similarly, water content and toughness of birch leaves were more variable within trees than among trees (Suomela and Ayres, 1994). Neither study looked at variation in leaf phenology within a tree.

Variation in hatching is both genetically and environmentally determined. Many studies of insect hatching have modeled development of a population by combining some average development rate with terms to account for environmental fluctuations and genetic differences between individuals (Johnson *et al*, 2007; Kontodimas *et al*, 2004). Few studies have looked at variation among individuals within a cohort (Son *et al*, 2007).

Individual tree phenology varies over time with the weather; budburst occurs earlier in warm winters and later in cold winters. While budburst and hatching are functions of temperature, trees and insects are not always receptive to favorable temperatures. Growth in certain plants and insects alternates between active and rest phases, and the transition between phases is affected by temperature and photoperiod. In Mediterranean oaks, such as coast live oak (*Quercus agrifolia* Née; Fagaceae), winter chilling may release buds from dormancy and initiate the change from the resting phase to the active growth phase. In late winter, shoots elongate, new leaves are produced and stores are depleted. After a period of rest, growth may resume in summer, when additional shoots (lammas) may be produced. Trees are dormant in fall and winter until growth resumes the following year.

Insects in overwintering eggs may function similarly to their host trees. Diapause is a hormonally induced condition that prevents development at unfavorable times of year. Photoperiod is commonly the cue that breaks diapause, following which development is

temperature-dependent. Both plant and insect development have been modeled using degree days, or units of development that are functions of temperature. While insect models are developed in the lab, tree models are often generated by finding the best model that corresponds to long-term field observations. If a particular model describes both insect and tree phenology well, then the mechanisms that underlie development in each particular plant and insect may be similar and contribute to synchrony.

Synchrony in hatch and budburst at the level of individual tree has important evolutionary consequences. The adaptive deme formation hypothesis predicts that short-lived insect herbivores with long-lived hosts can adapt to the traits of individual plants and form genetically differentiated sub-populations (Edmunds and Alstad, 1978). This may be one isolating mechanism leading to speciation. Following Edmunds' and Alstad's research on pine scale, a number of studies looked for similar examples of coevolution; about half the studies presented evidence for adaptive deme formation, whereas the other half showed no evidence of local adaptation (Van Zandt and Mopper, 1998).

Subsequent studies have shown synchronization between winter moth, *Operophthera brumata*, and English oak at the level of individual tree, with recent focus on disruption of this synchrony by global climate change. Larval fitness depended on synchronization of egg hatch with English oak budburst (Tikkanen *et al,* 2003). A mismatch of 4.4 days between larval emergence and budburst decreased fitness of moths by 50%. This synchrony may be strengthened by the limited dispersal ability of female winter moths, which have only vestigial wings and cannot fly. Flightlessness in insects may allow for resource allocation to egg production instead of wings (Roff, 1990), and the resulting limitation in dispersal ability may contribute to synchrony with the host tree. Variation in budburst may help trees escape

defoliation, and early and late-flushing trees would be maintained in the population to limit successful colonization by larvae ballooning on threads (Roff,1990).

In California, western tussock moth, *Orgyia vetusta gulosa* Boisduval (syn. *O. gulosa* Henry Edwards; Lepidoptera: Lymantriidae), has a similar life history to winter moth. Eggs are laid in summer but are in a state of low metabolic activity, or diapause (Furniss and Knopf, 1971). Changes in photoperiod and/or exposure to chilling may release eggs from diapause, and development resumes at a rate dependent on temperature. Eggs hatch in late winter and larvae feed on new growth of *Q. agrifolia* over a two month period. Larvae pupate in late spring for several weeks; this is followed by adult emergence, mating and egg laying that ends tussock activity for the year. Female moths also have only rudimentary wings and cannot fly, and lay their eggs where they emerged from their cocoons.

The nutritional quality of coast live oak leaves changes quickly over time (Mauffette and Oechel 1989), which may affect larval fitness. Nitrogen and phosphorous levels drop rapidly over the first month of a leaf's life, while percent dry weight of cellulose, lignin, and acid detergent fiber increase. Total phenolic content was highest at budburst for new leaves while tannin concentrations increased as leaves matured. The sensitivity of larvae to these changes affects the importance of degree of synchrony.

The goals of this study were to assess the amount of variation in budburst in *Q. agrifolia* and hatching in *O. v. gulosa*, to look at the effect of weather and microclimate on hatching and budburst, and to look for synchrony in budburst and hatch at the individual tree level.

MATERIALS AND METHODS

Study sites

Two urban populations of *O. v. gulosa* on *Q. agrifolia* were monitored in 2006. Three *Q. agrifolia* were monitored on the campus of the biotechnology firm LifeScan (Milpitas, CA), and thirteen *Q. agrifolia* were monitored on the Stanford University (Palo Alto, CA) campus. At Stanford, ten trees were randomly chosen in December 2005, then three more were added in February 2006, to include early and late developing trees that represent a variety of phenologies. The Stanford trees were also monitored in 2007. As the trees are growing in various environments that may affect their phenology, the following characteristics were noted: DSH (diameter at standard height); tree size (small=DSH<25cm, medium=25<DSH<40cm or large=DSH>40cm); irrigation level (none, low = drip irrigation, medium=overhead spray for shrubs within dripline or high=sprinklers in lawn); vigor (Stanford Grounds Tree Inventory vigor ratings); distance to buildings (urban heat island effects) and distance to other trees.

Foliage Sampling and Egg Mass Examinations

To measure bud development and identify budburst in each tree, four branches located at cardinal directions on each tree were flagged in early February 2006. Oak buds go through several stages in their development, and the following scale, modified from Crawley and Akhteruzzaman's study of deciduous *Quercus robur* (1988), was used to score marked terminal buds on individual trees every 2-3 days. Stage 2 was called "budburst", instead of stage 1 as in their study, because of difficulty in consistently identifying budstages 0 and 1 in 2006. *Quercus agrifolia* go through a long period of bud swelling, with no green visible, which

seemed to fall in between stages 0 and 1, but budstage 2 was more easily recognized and represented food available for herbivores.

	Bud stage:
0	Bud tight, no development
1	Bud swelling – green first visible between brown bud scales
2	"Big bud" – buds elongated and predominantly green or pink
3	"Shaving brush" – leaves and flowers protrude beyond tip of bud
4	"Leaf extension" – individual leaves and anthers hang separately
5	"Anthesis" – pollen is shed
6	"Full leaf extension" – foliage adopts dark green color

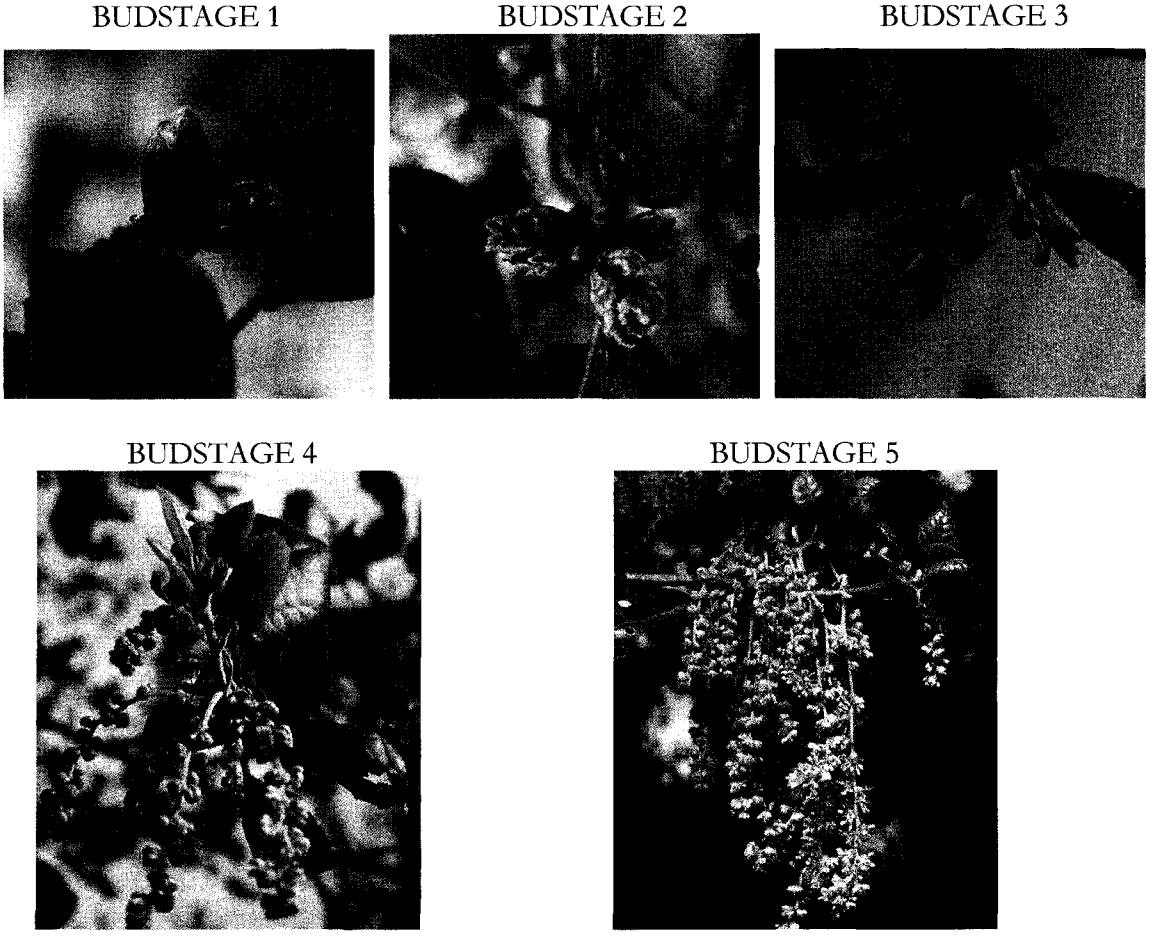


FIGURE 2.1. Budstages identified in *Q. agrifolia*.

The overall growth of the trees was observed, and observed budburst was recorded as the first day when more than 50% of all buds had burst and some shoot elongation and leaf unfolding (budstage 2) had occurred. While shoot development continued to be monitored after budburst, data were incomplete for several trees. Both 2006 and 2007 were outbreak years for *O. v. gulosa* at Stanford, and most new growth was eaten, sometimes as early as stage 2.

To characterize *O. v. gulosa* hatch at each tree, four to six egg masses were randomly selected in the canopy of each tree. Every 2-3 days, the number of larvae present on each egg mass was recorded. At the end of the season, mean and median hatch dates for each egg mass were calculated.

The Stanford field monitoring in Spring 2007 was slightly modified from the 2006 protocols. New buds were identified in each cardinal direction for bud development monitoring, and four egg masses per tree were flagged. No readily observed egg masses were found on three of the trees, so these were monitored only for budburst. An additional *Q. agrifolia* was added for monitoring.

Temperature and microclimate monitoring

Because plant and insect development rates are temperature-dependent, the microclimates of the trees were compared. One LogTag temperature recorder (LogTag Recorders Ltd., Kowloon, Hong Kong) was placed in the north quadrant of each tree, ~ 3 m above the ground and halfway between the edge of the canopy and the trunk. Temperatures were recorded hourly from February 1, 2006 to June 12, 2007. To see how temperatures vary within a canopy, a second logger was installed 180° from the first logger in four trees (Terman, W. Campus, Cypress and Escondido) in February 2007.

To eliminate microenvironment as a factor affecting egg hatch, four egg masses were collected from each monitored tree at Stanford on October 1, 2006. These were placed individually into glass vials stoppered with cotton, then weighed and stored outdoors together over the winter. Egg masses were checked daily beginning in January and emerging larvae were removed. The duration and number of larvae hatching from each collected egg mass was recorded and then compared to hatching in the field. A median hatch date for each egg mass and a mean hatch date for each tree were calculated. The trees' mean hatch dates (field vs collected) were compared using ANOVA and by calculating Pearson's correlation coefficient. Egg masses from which fewer than 10 larvae hatched were excluded because they were not representative of a typical hatch.

Data Analysis

The "day of year" (Julian date) was assigned to each bud and hatch observation to begin the analysis. Hartley's test for equality of variances in budburst and hatching at various levels (branch (=egg mass), tree and area) was manually calculated. Budburst was normally distributed with equal variance between areas, between trees and between branches within trees, but variances were not equal between years. Cool weather in 2006 extended budstage 2 over a longer time than in 2007, leading to unequal variances between the years and between individual trees over time. To prepare data for analysis of variance, a median budburst date was calculated for each N, E, S and W bud in a tree's canopy, to use as raw data in all of the analyses.

Hatch was normally distributed, but because of the large number of larvae that hatched from each egg mass, variances were not equal between egg masses within trees, between trees and between years. Similar to budburst, the raw data were converted into a

median hatch date for each egg mass; this led to equal variances between trees, areas and years, and these median dates were used as the raw data for all analyses.

Separate ANOVAs were performed to look for differences in the following: 1) among areas in 2006; 2) among years and trees at Stanford; and 3) from 2006 to 2007, for each tree at Stanford. Stanford trees were compared to one another within a year using Tukey tests. Each of these ANOVAs was performed on bud data and on hatch data. Percent variance explained by buds/egg masses and trees within a year was manually calculated. To compare relative phenologies of trees from year to year, I calculated Pearson's correlation coefficients for 2006 and 2007 *observed* budburst, and for 2006 and 2007 mean hatch.

To look for microclimate effects, UC Davis' IPM degree day calculator (IPM 2007), single sine method with horizontal upper cutoff, was used to calculate daily DD above a base temperature of 10°C. I looked at degree days accumulated between February 4 and March 4, when insect and plant development were occurring and complete data were available for both years. For the four trees with 2 loggers in 2007, daily degree day accumulations between loggers within and among trees were compared. Tree data were compared to 2006 and 2007 data from two local weather stations, Stanford Grounds and Stanford EH&S. These were also compared to several long-term averages: the 10-year average data for the Stanford Grounds weather station and the 30-year averages for Palo Alto from the WRCC (Western Regional Climate Center) and NOAA-NCDC (National Oceanic and Atmospheric Administration – National Climate Data Center). I used Pearson's correlation coefficient to examine the relative degree day accumulation in the trees over time.

To examine microclimate effects another way, the correlation in hatch between field-observed eggs and collected eggs masses overwintered together was calculated.

To examine the synchrony between budburst and hatching, trees were considered both collectively and individually. While many studies rank trees (separate ranks in order of budburst and insect hatch) to compare rank between years or to look for a correlation between budburst and insect hatch, I used mean dates for correlation analyses. Rather than being distinctly early, average or late, the trees in this study differ from one another along a continuum, and some trees are not significantly different from one another. I checked for associations of the following variables for individual trees using Pearson's correlation coefficients: mean observed Stanford budburst 2006 vs. mean Stanford hatch 2006; mean observed Stanford budburst 2007 vs. mean Stanford hatch 2007; observed budburst vs. mean hatch for individual trees in both 2006 and 2007.

To explain consistent differences in budburst between trees, I looked for an association between observed budburst and degree days accumulated by March 1, 2007. To look for a nonlinear relationship, trees were ranked in order of observed budburst and DD accumulated, and the Spearman rank coefficient was calculated. I also looked for associations between tree budburst date and tree DSH, proximity to buildings and other trees, and irrigation level, using a separate regression for each of these characteristics.

Canonical correlation analysis was used to see if phenology (mean hatch and budburst in 2006) was associated with site characteristics (irrigation level, distance to buildings, distance to trees, and accumulated degree days). Log linear analysis was used to look for a relationship between tree phenology (early, middle, late) and tree size (small, medium, large), irrigation (yes/no), whether trees were surrounded by pavement (yes/no), or in a grove (yes/no). All analyses were performed using Systat (v.10.0, San Jose, CA), except for the canonical correlation and log linear analyses, which used SPSS (v. 14.0, San Jose, CA).

The number of days separating hatch and budburst varied from tree to tree, and this varying degree of synchrony may affect female moth fitness. I performed a one-way ANOVA looking for significant differences in egg mass weights between trees, to see if heavier egg masses were found on more synchronous trees.

RESULTS

Variation in budburst within and among trees

I found large variation in observed budburst among trees at Stanford (Table 2.1). Budburst in the earliest tree preceded that in the latest tree by 29 days in 2006, and by 25 days in 2007. Budburst occurred 4-10 days later for 9 trees in 2007 than in 2006. Two trees showed no difference in observed budburst date. The earliest tree (Wcampus) was 8 days later in 2007 than in 2006, while the latest trees (Terman and Packard) were 4 days later in budburst from year to year. Differences between trees in 2006 were greater than in 2007. In 2007, observed budburst occurred on the same date (March 12) in over half of the trees.

TABLE 2.1. Year-to-year consistency in observed budburst among 11 *Q. agrifolia* at Stanford University.

Tree	2006 observed budburst	2007 observed budburst
Wcampus	Feb 10 06	Feb 18 07
Cypress	Feb 15 06	Feb 21 07
Ecampus	Feb 28 06	Mar 10 07
GSB	Mar 4 06	Mar 12 07
Early Oval	NA	Mar 12 07
Cogen	Mar 4 06	Mar 12 07
Meadow	Mar 4 06	Mar 12 07
Packard	Mar 11 06	Mar 15 07
Late Oval	Mar 11 06	Mar 12 07
Escondido	NA	Mar 11 07
Dink	Mar 8 06	Mar 12 07
Law	Mar 11 06	Mar 12 07
Terman	Mar 11 06	Mar 15 07
Average for all trees	*Mar 4 06*	*Mar 9 07*

Individual trees were consistently "early" or "late" when observed budburst dates were compared between the two years (Figure 2.2). Two trees ("early") produced new growth several weeks earlier than the other trees observed over two years. These remaining ten were similar to one another, separated in budburst by 12 days in 2006 and by 5 days in 2007.

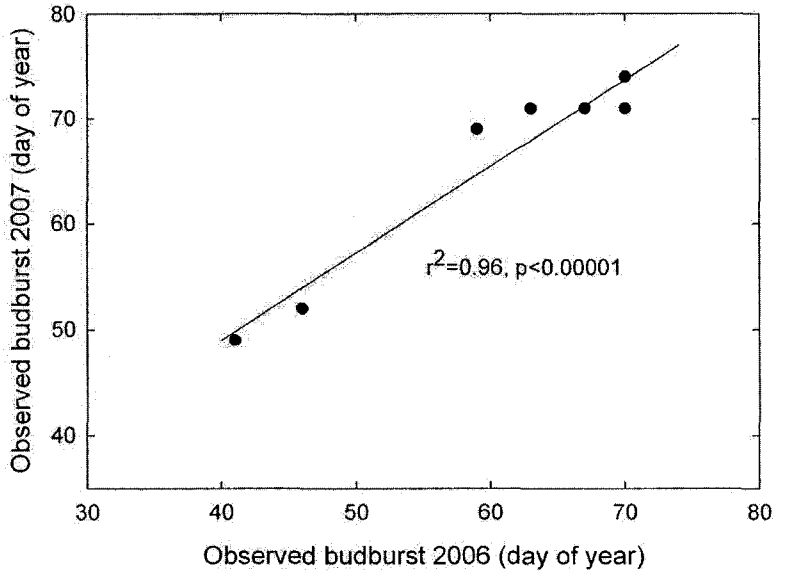


FIGURE 2.2 Year-to-year consistency in observed budburst among 11 *Q. agrifolia* at Stanford University (4 points overlying one another).

When the Stanford trees were considered collectively, observed budburst in 2007 occurred 5 days later than in 2006. Similarly to the analysis of individual trees, bud development generally started earlier but occurred over a longer period of time in 2006 than in 2007 (Figures 2.3 and 2.4). In 2006, mean bud development (stages 1-5) for all trees was spread over a 4 to 5 week period (Feb 24-Apr 1, 2006). In 2007, median bud development for all trees was spread over 2.5 weeks, from Mar 7 to Mar 24.

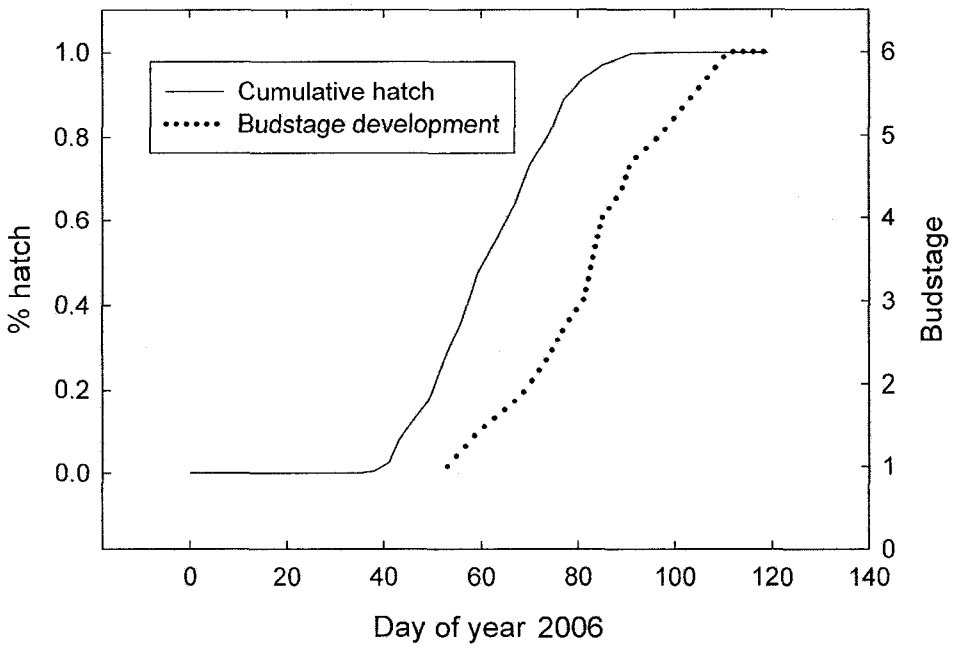


FIGURE 2.3 Mean *Q. agrifolia* budstage dates and *O.v.gulosa* hatch in 2006.

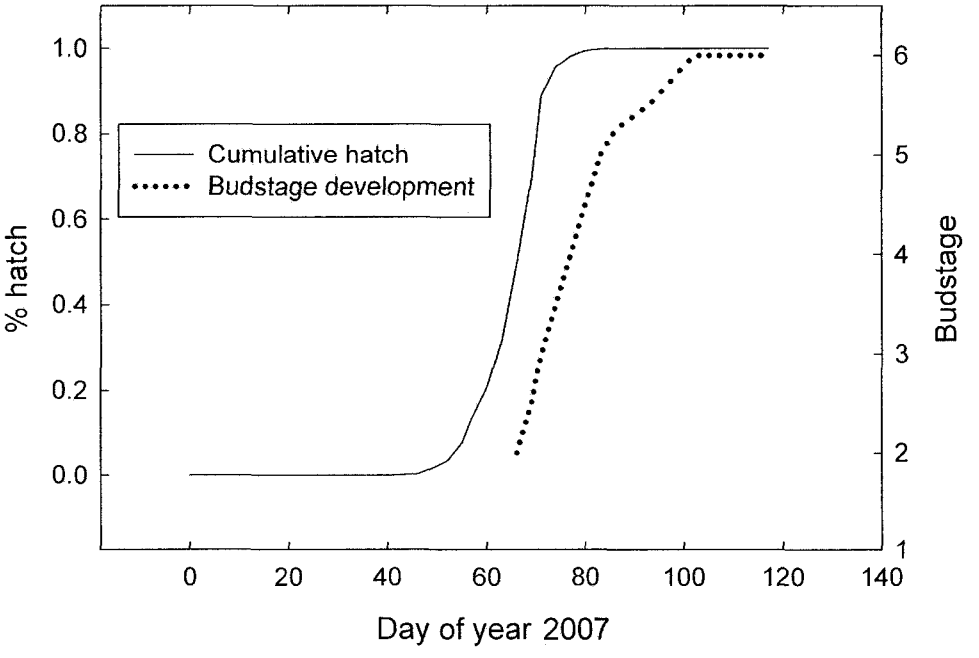


FIGURE 2.4 Mean *Q. agrifolia* budstage dates and *O.v.gulosa* hatch in 2007.

Taking into account duration of budstage 2, few differences were discernable between trees and years. There was a significant tree × year interaction ($F_{12,71}$=1.97, p=0.04) at Stanford: of the two trees in which budburst differed from 2006 to 2007, one (Cogen) had later mean budburst in 2007, while the other (Law) had earlier budburst in 2007. There was no significant difference between years for the remaining 10 trees. Within 2006, while there was a significant difference between trees ($F_{11,31}$=3.87, p=0.001), this was only between the earliest and latest trees (Cypress was significantly earlier than Escondido, Law and Terman; similarly, Wcampus was significantly earlier than Escondido). In 2007, significant differences between trees ($F_{13,40}$=52.6, p<0.0001) separated the 3 early trees from the remaining 11 trees observed.

The amount of variation in 2006 mean budburst was roughly twice that in 2007. There was more variation within trees (55.4%) than among trees (44.6%) in 2006. However, in 2007, 93% of variation in mean budburst was between trees, while 7% was within trees.

There was a significant difference ($F_{1,51}$=4.09, p=0.048) in mean budburst between areas in 2006: Milpitas was 7 days ahead of Stanford. There was no significant difference (p=0.3) in mean budburst date between 2006 and 2007 at Stanford.

Variation in tussock hatch within and among trees, years and areas

I found a significant difference ($F_{1,118}$=13.05, p<0.001) in mean hatch date from 2006 to 2007 (Table 2.2). Mean hatch occurred on March 2 2006 but was 5 days later in 2007. There was a significant tree × year interaction ($F_{11,96}$=3.94, p<0.0001); while there was no difference in mean hatch across years for 9 trees, hatching occurred later in 2007 at two trees, Wcampus ($F_{1,9}$=19.25, p<0.01) and Law ($F_{1,8}$=29.94, p<0.001).

TABLE 2.2 *Orgyia v.gulosa* hatch in *Q. agrifolia* at Stanford in 2006 and 2007 (day 1 = Jan 1).

Area	Tree	Hatch date 2006 (mean ± SD)	Hatch date 2007 (mean ± SD)
Stanford	GSB	49.9 ± 10.7 (Feb 19)	missing
Stanford	Meadow	54.5 ± 11.1 (Feb 24)	64.6 ± 6.9 (Mar 6)
Stanford	E. Campus	54.7 ± 13.6 (Feb 24)	61.9 ± 6.6 (Mar 3)
Stanford	W Campus	56.4 ± (Feb 25)	65.9 ± 5.0 (Mar 7)
Stanford	Terman	58.9 ± 10.8 (Feb 28)	63.4 ± 6.0 (Mar 4)
Stanford	Law School	59.1 ± 11.0 (Mar 1)	70.3 ± 5.9 (Mar 11)
Stanford	Early Oval	61.7 ± (Mar 3)	62.6 ± 6.6 (Mar 4)
Stanford	Late Oval	66.2 ± (Mar 7)	NA
Stanford	Packard	63.5 ± 11.0 (Mar 5)	70.3 ± 3.5 (Mar 11)
Stanford	Escondido	66.1 ± 11.5 (Mar 7)	65.5 ± 4.6 (Mar 7)
Stanford	Dinkelspiel	66.7 ± 11.8 (Mar 7)	69.1 ± 4.0 (Mar 10)
Stanford	COGEN	67.0 ± 11.5 (Mar 8)	58.3 ± 6.0 (Feb 27)
Stanford	Cypress	73.9 ± 11.1 (Mar 15)	70.1 ± 4.9 (Mar 11)
Stanford	*Average*	*61.4 (Mar 2)*	*65.9 (Mar 7)*
Milpitas	South Oak	57.3 ± 10.5 (Feb 26)	
Milpitas	North Oak	59.6 ± 10.9 (Mar 2)	
Milpitas	Lone Oak	64.8 ± 12.4 (Mar 6)	
Milpitas	*Average*	*60.6 (Mar 2)*	

While there were significant differences between trees in 2006 ($F_{12,45}$=3.75, $p<0.001$) and in 2007 ($F_{11,50}$=3.66, $p<0.001$), these were only between the earliest and latest trees each year. Similar to, but less pronounced than budburst, there was a moderate but nonsignificant correlation (Figure 2.5; r^2=0.59) in mean hatch by tree, from 2006 to 2007.

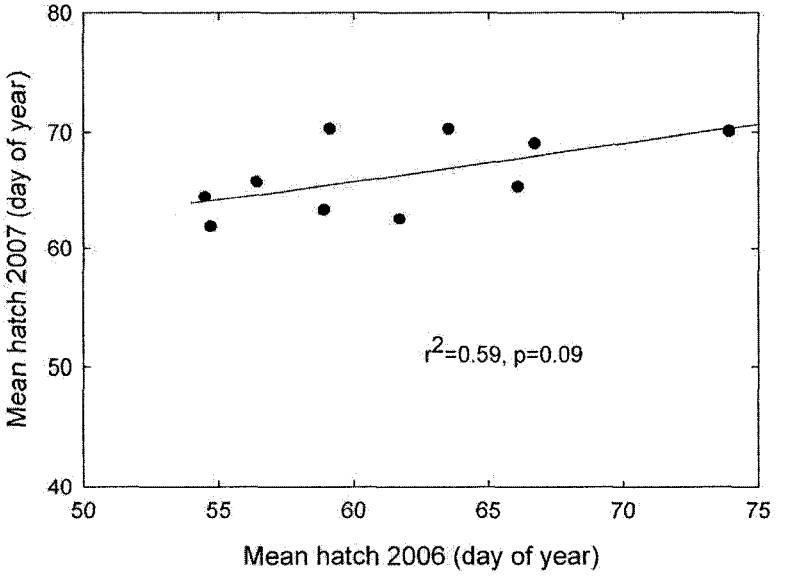


FIGURE 2.5 Year-to-year consistency in *O.v.gulosa* hatch among *Q. agrifolia* trees at Stanford University.

The duration of hatch in 2006 was roughly twice that in 2007, with about 1.5 times as many total larvae counted on egg masses. In both years, relatively more variation occurred within trees (egg masses within trees) than among trees. In 2006, the proportion of variation among egg masses was 61.8%, while that among trees was 38.2%. Similarly, in 2007, the percent variation within trees was 65.9% while that among trees was 34.1%.

Unlike budburst, there was no significant difference in hatch between Stanford and Milpitas in 2006 ($t=-0.35$, $df=68$, $p=0.73$).

Field hatch vs collected eggs

Mean hatch differences between trees were consistent for field-monitored and collected egg masses (exclude Packard and Cogen, with small N), indicating little or no effect of microclimate within an area (Figure 2.6). While there were significant differences in mean hatch between monitoring areas ($F_{1,64}=10.73$, $p<0.01$) and trees ($F_{22,64}=2.54$, $p<0.01$), there

was a strong correlation ($r=0.76$, $p<0.01$) among trees between mean field hatch date and mean collected hatch date.

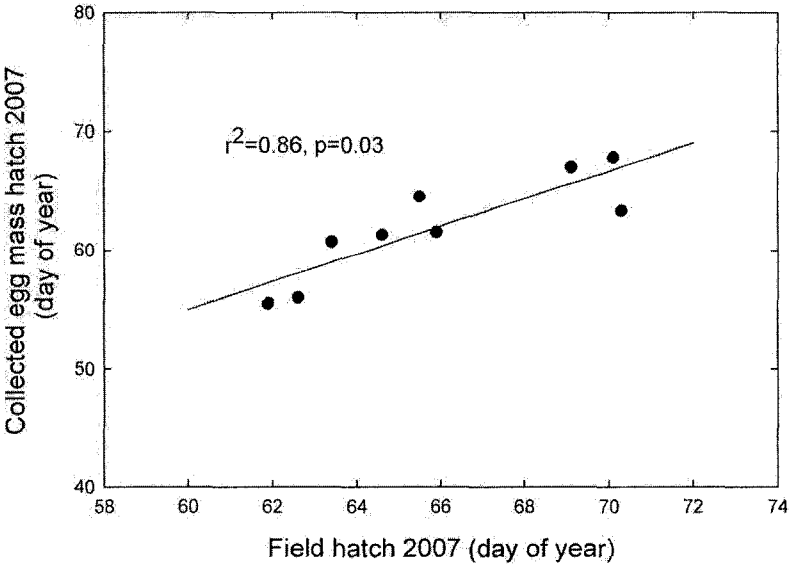


FIGURE 2.6. Consistency in mean hatch date between field-monitored and collected egg masses in 2007.

Variation in temperature within and among years

Although 2006 and 2007 had similar three-month averages, they differed in week-to-week winter temperature fluctuations (Figures 2.7c,2.8c). January 2006 was about one degree warmer than the 30 year average (WRCC), while January 2007 was about one degree colder than average. Both February 2006 and 2007 were very close to average. March 2006 was 2.3°C colder than average, while March 2007 was 1.3°C warmer than average.

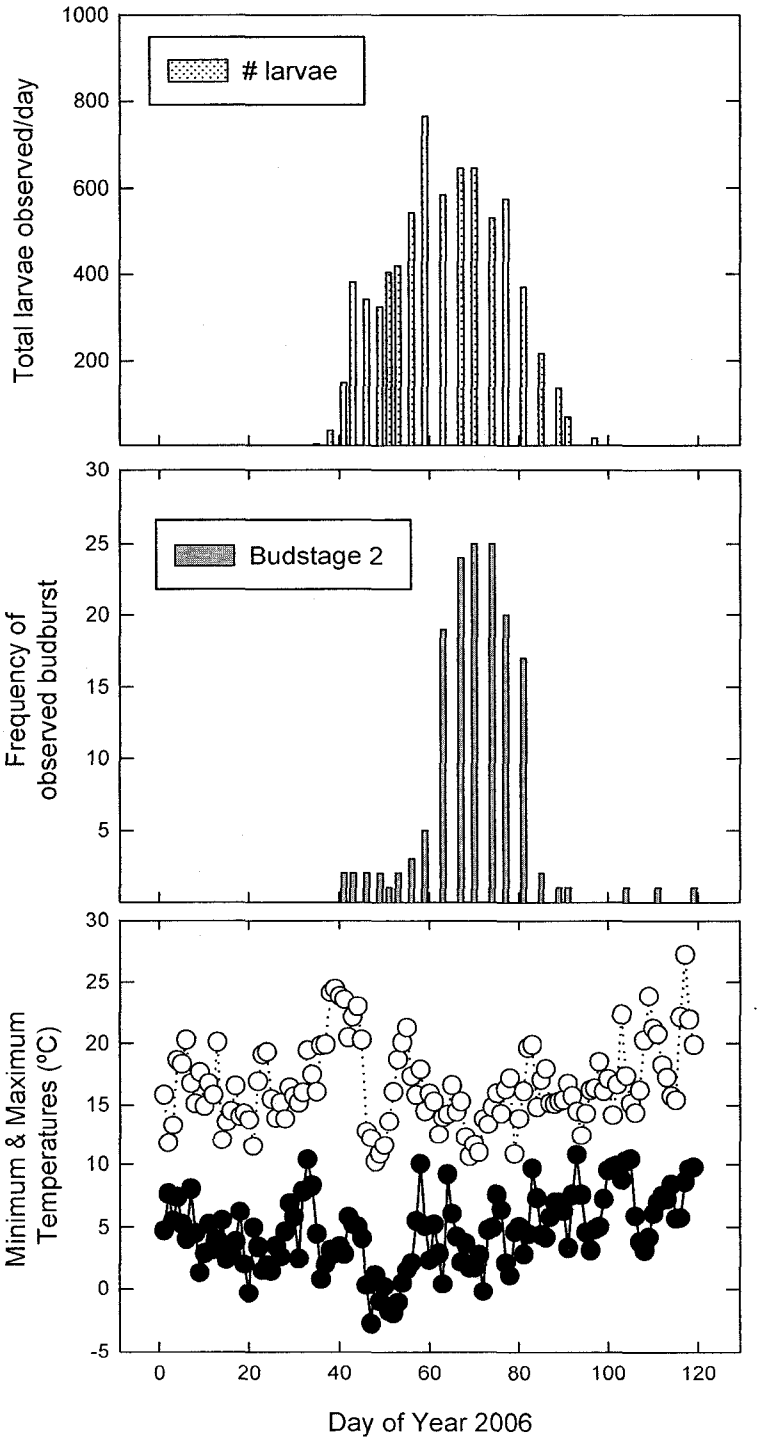


FIGURE 2.7 a. Hatch b. Budburst c. Temperatures in 2006.

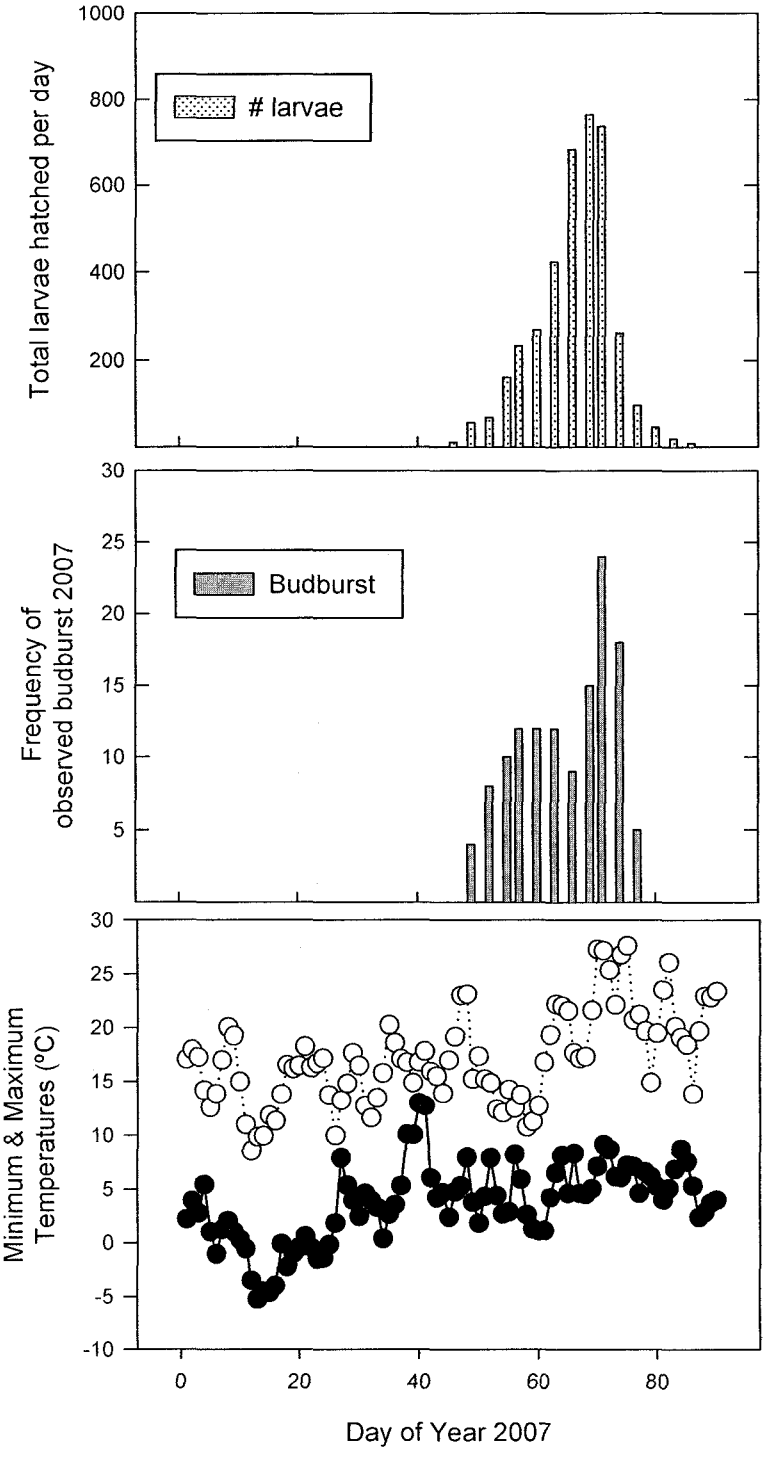


FIGURE 2.8 a. Hatch b. Budburst c. Temperatures in 2007.

Variation in degree days within and among trees

In daily degree days accumulated from February 1 to 28, there was a significant difference between years ($F_{1,567}$=11.98, p<0.001) but no significant difference between trees ($F_{10,567}$=2.05, p=0.026) nor any tree by year interaction ($F_{9,567}$=0.42, p=0.93). More daily degree days accumulated in February 2006 than in February 2007 or over the 30 year average, which did not differ from each other. For the four trees with two temperature loggers in 2007, there were no significant differences either between trees ($F_{3,192}$=1.47, p=0.22) or between loggers within trees ($F_{4,192}$=0.40, p=0.81).

Effect of temperature on budburst and hatching

Both bud development and hatching followed periods of warmer temperatures. In 2006, temperatures climbed above 20°C for a week beginning on February 7. In the earliest trees, W Campus and Cypress, budburst occurred on February 10 and 15, respectively. Hatching in 2006 also began around February 7. In 2007, temperatures first climbed above 20°C for several days starting on February 16; budburst at W Campus and Cypress occurred on February 18 and 21. Hatching in 2007 began around February 15.

March temperatures account for differences in duration of hatch between years (Figures 2.7, 2.8). Temperatures from the end of February to the end of March were relatively cool in 2006. Larvae were not removed from egg masses as they were counted, and it is possible that the same larvae were counted on subsequent visits, as hatching lasted roughly six weeks within an egg mass. The number of larvae counted on the latest-hatching egg masses was about twice that of the earliest hatching egg masses. In contrast, temperatures in March 2007 were warm, and hatching lasted two to three weeks within an egg mass.

Translating these temperatures to degree days (DD) using the parameters developed in Chapter 1, the rate of DD accumulation varied within each year and between years. In 2006, there appeared to be a slope change around February 14; degree days accumulated more rapidly before that date, then the rate of accumulation slowed through the month of March. In 2007, there appeared to be a slope change around March 2; degree days that had been accumulating slowly began accumulating more rapidly after that.

Correlation between hatching, budburst and degree days

When the trees were considered collectively, there was considerable synchrony in observed budburst and hatching; mean hatch preceded mean observed budburst by 2 days in both 2006 and 2007. Hatching began (Stanford weather stn, start 1/1, T_b =10°C) with an accumulation of roughly 100DD and finished after 200DD, with median hatch occurring at 160DD (March 2, 2006 = 161DD, March 7, 2007 = 162DD). Mean observed budburst occurred after roughly 165 DD (164DD on March 4, 2006 and 167DD on March 9, 2007; Grounds weather station, start 1/1, T_b=10°C). When the duration of budstage 2 is considered, mean hatch preceded mean budstage 2 by 11 days in 2006 and by 6 days in 2007. Tussock hatch was roughly twice as variable as budburst in both years, though hatch and budburst in 2006 were twice as variable as in 2007.

However, when the trees were considered individually, there was no synchrony between budburst and hatching. In fact, the difference between observed budburst date and mean hatch date was as much as 21 days within one tree in 2006, and 19 days in 2007. In most cases, hatch preceded budburst (Figure 2.9).

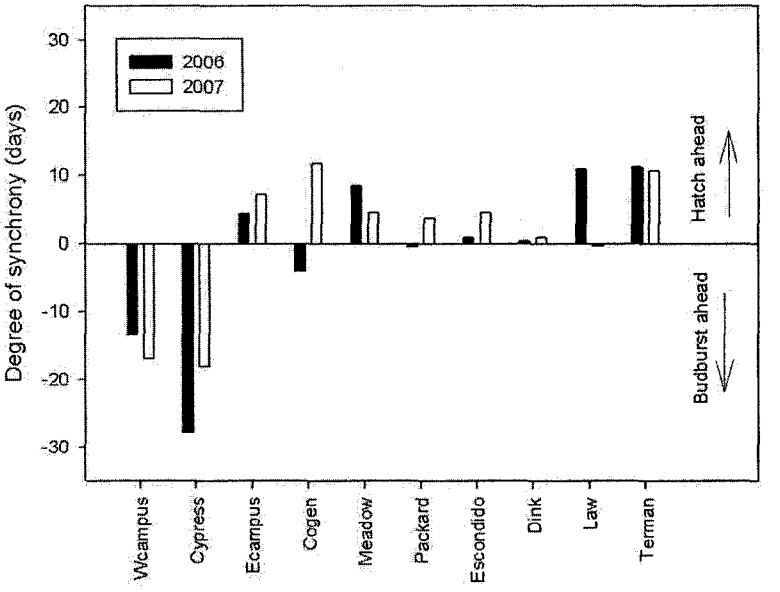


FIGURE 2.9 Variation in synchrony of hatch and budburst over 2 years.

Peak hatching coincided with different budstages each year within a tree. For example, at Cypress, in 2006 hatching peaked during budstage 2 but was skewed right, with continuing hatch through budstages 3 and 4. In 2007 most hatching at Cypress coincided with budstage 4, though the distribution was skewed left, with earlier hatching during budstages 2 and 3.

Timing of hatch relative to budburst affected resource loss in individual trees but did not affect female moth fitness, measured by weight of egg mass produced. "Late" trees (developing new growth later than average) did not lose as many resources to herbivory, since buds instead of shoots and leaves were devoured. Egg masses collected in fall 2006 showed no significant difference in mass between egg masses from different trees ($p<=3.72$). While peak hatch coincided with different budstages in individual trees, this difference did not adversely affect egg mass weight.

Weather station choice affected degree day accumulation greatly (Figure 2.10). The two stations at Stanford differed by 30DD for accumulations between January 1 and March 7

2007. The Stanford Grounds station more closely matched the average degree day accumulation for all trees. The EH&S data more closely matched data from 30 yr averages.

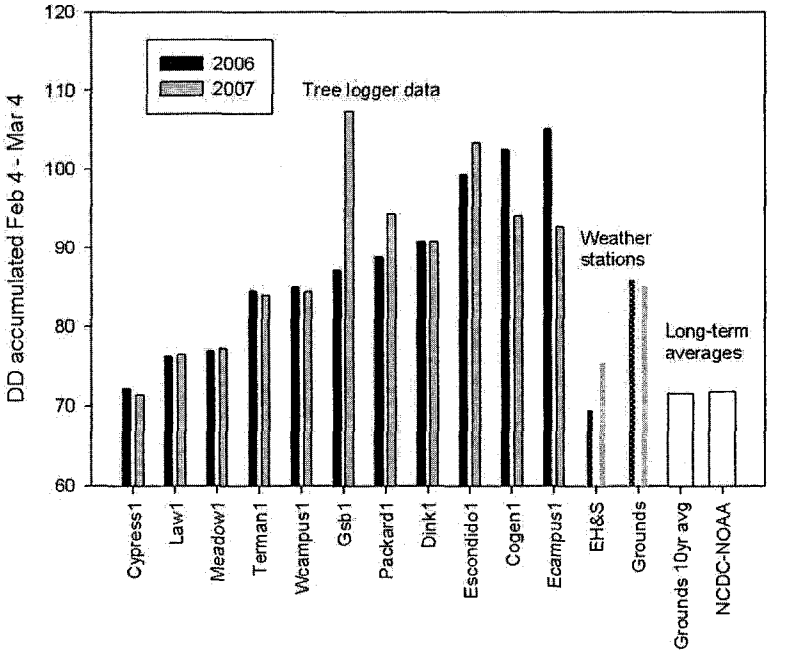


FIGURE 2.10 Variation in degree day accumulations as a function of temperature data source.

When accumulated degree days were calculated using each tree's temperature logger in 2007, there were no patterns to explain hatch or budburst. For median hatch in each tree, (using T_b=10°C, start January 1), there was a 65DD spread across all trees in 2007. Median hatch occurred between 135 and 200DD, depending on the tree. When the same was done for observed budburst date, the spread was almost twice that, between 106 and 230DD. Only for the trees at the temperature extremes was hatch correlated with degree day accumulation in 2007. Cypress, the tree with the lowest recorded temperature, had among the latest hatch both years. Two of the three trees with the warmest temperatures, Ecampus and Cogen, had the earliest hatch in 2007, though Cogen had a late hatch in 2006. For most of the trees, there was no correlation with temperature and hatch rank.

Microclimate temperature differences between trees did not explain observed budburst patterns. When DD accumulated by March 1, 2007 were plotted against observed budburst date, there was no correlation between the two. Similarly, when trees were ranked in order of DD accumulation and observed budburst, there was no correlation. Most tree were consistent in DD accumulation rank in February from 2006 to 2007 (Figure 2.11, $r=0.78$, $p<0.01$), with the exception of Cogen, Dink, Ecampus and Gsb.

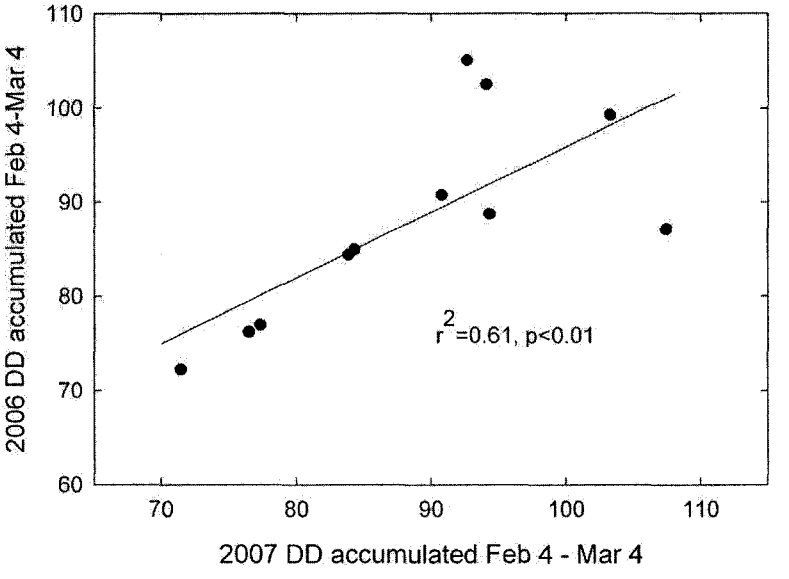


FIGURE 2.11 Consistency in degree day accumulation from 2006 to 2007 among trees.

Other variables

There was a weak correlation between tree size and budburst ($r=0.34$, $p=0.31$); small trees leafed out earlier than medium or large trees. None of the other variables considered (irrigation level, vigor, proximity to buildings and trees) were strongly correlated with observed budburst or median hatch differences between trees.

DISCUSSION

Overall, my results show differences in how variation is distributed in *Q. agrifolia* budburst and *O. v. gulosa* hatch. Eggs and buds both respond to winter temperatures and show synchrony between moth and tree collectively within an area, but not at the level of individual tree. The degree of synchrony may not have strong fitness consequences for either *Q. agrifolia* or *O. v. gulosa* populations in urban areas.

The long-lived oaks in this study varied in individual phenologies that were consistent from year to year. This is similar to European studies of English oak *Quercus robur* (Crawley and Akhteruzzaman, 1988; Van Dongen et al, 1997). While budburst rank may be genetically determined, bud development is a temperature-dependent process. Differences in budburst between trees were more pronounced in a cooler spring with less winter chilling.

Quercus agrifolia behave like islands when it comes to budburst. Variation in budburst was largely at the tree level and not at the bud level, though that depended on the year considered. This is similar to variation in leaf phenolic content in English oak, which is also relatively consistent within a tree but varies between trees (Roslin *et al*, 2006). These characters may be strongly genetically controlled, whereas characters such as leaf toughness or water content, which vary more within a canopy than between trees, may reflect differences in root environment and resource availability. Temperature affects the rate of bud development, and greater within-canopy variation in budburst occurs in cool springs.

Studies of *Q. agrifolia* have found variation at the tree level, both in genetic structure and in resistance to disease. *Quercus agrifolia* susceptibility to the pathogen *Phytophthora ramorum* has shown to vary between individuals within a population, but exhibits little variation between

populations. This pattern of variation is consistent with genetic structure in *Q. agrifolia* (Dodd et al, 2005).

Unlike budburst, most variation in *O. v. gulosa* hatch was at the level of egg mass, not individual tree. *Orgyia vetusta gulosa* egg masses hatched larvae over a relatively long period of time, though this varied from 2006 to 2007. Duration of egg hatch is a function of temperature (see chapter 1) and variation between individuals in a population. It has often been modeled using a cumulative Weibull function (Bryant et al, 2002; Hunter, 1993; Wagner et al, 1984). In this study, the long duration of hatch masked differences between all but the very earliest and latest hatching egg masses.

Orgyia vetusta gulosa hatch was more prolonged than that reported in studies of other forest defoliators; these other studies often reported field hatch occurring over a 1- to 2-week period (3-7 days in Douglas-fir tussock moth, Wickman, 1976; 5 days in gypsy moth, Hunter, 1993; 7 days in fruittree leafroller in Judd and Gardiner, 1993. However, prolonged hatching was reported in *Orgyia vetusta vetusta*, the lupine-feeding form of tussock moth along the California coast. Hatching occurred over 5-6 weeks, from mid-April to early June, coinciding with new leaf production (Harrison and Maron, 1995; Maron et al, 2001). Coastal temperatures are relatively mild, and egg masses go through less pronounced cold exposure that may synchronize development within an egg mass.

My method for measuring egg hatch did not account for larvae lingering on egg masses, as larvae were not removed after counting. After hatching, larvae of Douglas-fir tussock moth, *Orgyia pseudotsugata*, remain congregated on egg masses for 3-7 days, feeding on egg chorion (Wickman, 1976), then disperse rapidly within 1-2 days. Dispersal, like hatching, is probably temperature-dependent, with little dispersal occurring during cool weather and

rapid dispersal with warmer temperatures. Cooler weather in March 2006 may have caused larvae to linger and be counted more than once; the total number of larvae counted in 2006 was 7148 from 69 egg masses, compared to 3836 from 71 egg masses in 2007.

Synchrony between tussock hatch and oak budburst was present on an area-wide basis but not on a tree-by-tree basis. This is typical of a polyphagous moth with some dispersal ability. Young larvae can balloon on threads to reach neighboring hosts, though the related *O. v. vetusta* do so infrequently unless trapped on dead plants (Harrison, 1997). Last instar larvae often crawl onto adjacent structures, such as buildings or trees, to pupate. However, the related *O. v. vetusta* had a median displacement of only 2 m over one generation (Harrison, 1997). While male moths can fly to reach mates, female moths are flightless, and limited dispersal in *O. v. vetusta* is thought to limit spatial distribution of populations (Harrison, 1997).

Some scientists have argued that strong selection pressure overrides dispersal ability (see section below on fitness consequences of a/synchrony) in creating local adaptation. Winter moth, *Operophtera brumata*, is one of the few polyphagous moths with flightless females shown to be adapted to individual trees. Two of its relatives with similar life histories, *O. fagata* and *Erannis defoliaria*, did not show such fine-scale adaptation, perhaps because of higher tolerance for starvation and leaf maturation (Tikkanen et al, 2006).

Research in the 1970s found that peak egg hatch in Douglas-fir tussock moth occurred once 77-97% of white fir buds had burst (Wickman, 1976). In this study, *O. v. gulosa* hatch preceded budburst in both years. Recent studies of winter moth have also found earlier hatching in recent years, perhaps in response to global warming (Van Asch *et al*, 2007). The timing and duration of hatch are functions of heat accumulation (see Chapter 1, physiological studies), and more degree days above 10° C accumulated from January 1 to March 1 in both

2006 and 2007 than over the 10-yr average at Stanford or the 30-yr average at NCDC-NOAA. The physiological mechanisms responsible for hatching may be more sensitive to warmer temperatures than those in oak budburst.

The level of synchrony between hatch and budburst has fitness consequences for both *Q. agrifolia* and for tussock larvae. Both 2006 and 2007 were outbreak years for *O. v. gulosa*, and trees that leafed out early lost all new growth to defoliation, whereas trees that leafed out late lost buds to defoliation and supported smaller numbers of larvae (personal observation). Late trees did not produce new leaves during 2007, perhaps because heavy feeding removed buds; this may have delayed budburst in 2008. While past studies concluded that early trees suffer higher levels of defoliation, Crawley and Achteruzzaman (1988) found no relationship between budburst phenology and degree of herbivory, other than the same trees being defoliated consistently year after year. Hunter (1992) concluded that tree budburst/insect density relationships vary, perhaps with differences in habitats (tree density: parkland vs woodland) and evolutionary histories of particular insects and their host plants.

For *O. v. gulosa*, hatching too early brings the risk of starvation, as larvae cannot feed on mature oak leaves. Starvation tolerance in *O. v. gulosa* is temperature-dependent (see Appendix). At temperatures of 15°C and below, larvae had a relatively high starvation tolerance, similar to that shown by gypsy moth, *Lymantria dispar* (Hunter, 1993). These cool temperatures are common in late winter in the South Bay, decreasing the negative effects of hatching too early.

Hatching after budburst has been shown to decrease fecundity in winter moth, but *O. v. gulosa* does not seem to be as sensitive to changes in leaf quality. Larvae raised in the lab at 20°C on spring oak leaves showed little difference in pupal weight when started over a three

week (or 36.5 DD) period (see Appendix). This is similar to *O. v. vetusta* tolerance of variable lupine quality (Harrison, 1997). There is a limit to food tolerance, in that *O. v. gulosa* larvae raised on new growth produced in summer had longer development times and lower pupal weights (see Chapter 1).

In nature, tussock larvae may have few alternative hosts to oaks. Furniss and Knopf's (1971) description of *O. vetusta* lists plants found in Southern California as important hosts. These are not common in the San Francisco Bay Region, where *Q. agrifolia* is the main host, and it is possible that Furniss and Knopf describe a different species. [The taxonomy of *Orgyia* is still under development, though Ferguson (1978) recognized several subspecies of *O. vetusta*, including an oak-feeding form (*O. v. gulosa*) distinct from a lupine-feeding form (*O. v. vetusta*).] In this study, first instar larvae could not survive on mature oak leaves and were dependent on new growth for survival. In oak woodlands, alternate host plants are few: *Ceanothus, Prunus* and *Rosa* spp. The other dominant trees (buckeye, bay, madrone) are probably not appropriate for first instars. California buckeye, *Aesculus californica*, produces new growth earlier than *Quercus*. A small test study (see Appendix) here found 50% mortality in first-instar larvae fed buckeye leaves, although those that survived developed to a similar pupal weight as those fed oak leaves.

In urban areas, the number of alternate hosts increases both early and late in the season. Purple leaf plums (*Prunus cerasifera* cvs.) and roses (*Rosa* sp.) develop leaves earlier than *Q. agrifolia*, while *Liquidambar, Celtis* sp., *Arctostaphylos* and others develop later. In some areas, tussock larvae have become pests of fruit trees (*Prunus* spp. and *Citrus* cvs.). The large diversity of ornamental species in proximity to *Q. agrifolia* in urban areas seems to sustain *O. v. gulosa* at higher population densities than in the wild.

Synchrony has been suspected to affect population dynamics in outbreak species, predicting population booms in years of close synchrony. In this study, both 2006 and 2007 were outbreak years at Stanford, when hatching and budburst were synchronized at the population level, not the individual tree level. This synchronization at the population level occurs most years (Graeve, personal observation) and probably leads to gradual population increase over time. *Telenomus californica*, a small wasp which parasitizes tussock egg masses, may slow tussock population growth. In 2000, when the tussock population density was low, most collected egg masses had between 5 and 30 wasps present. In outbreak years, the large number of tussock egg masses in the field may swamp the *Telenomus* population; *T. californicus* was rare in egg masses collected in 2005 and 2006 (see Appendix).

Tussock population decrease may occur abruptly. In 2007, no female tussock larvae were observed to survive pupation because a virus swept through the population. Both males and females were affected, though females, with their longer larval development period, seemed particularly hard-hit, and no egg masses were observed. Nuclear polyhedrosis viruses have been widely studied as a pest control measure in Douglas-fir tussock moth, and high densities of larvae foster spread of the disease.

In conclusion, *Q. agrifolia* budburst and *O. v. gulosa* hatch are both temperature-dependent processes that occur around the same time in late winter each year. *Orgyia vetusta gulosa* hatch is not synchronized with individual host tree budburst but extends over a longer period of time. The relatively high starvation tolerance of first-instar larvae during cool weather and an abundance of alternate hosts in urban areas may decrease the fitness consequences of asynchrony and lead to gradual increases in population size. Disease caused a population crash in 2007. Longer-term studies covering years of low tussock population

density may give more insight into synchrony between moth and tree, and may reveal whether *Q. agrifolia* budburst and *O. v. gulosa* hatch respond in similar ways to global warming. Wildland populations may be more sensitive to asynchrony due to lack of alternate hosts, and comparison of wildland populations to urban populations may indicate other important factors in population regulation.

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APPENDIX A: STARVATION TOLERANCE

Introduction

Leaf-eating caterpillars that hatch before the budburst of their host are at risk of starvation. Sensitivity to starvation varies among insects; winter moth larvae are very sensitive, while gypsy moth larvae are not. I followed the methods of Hunter (1993) to determine the starvation tolerance of western tussock moth as a function of temperature.

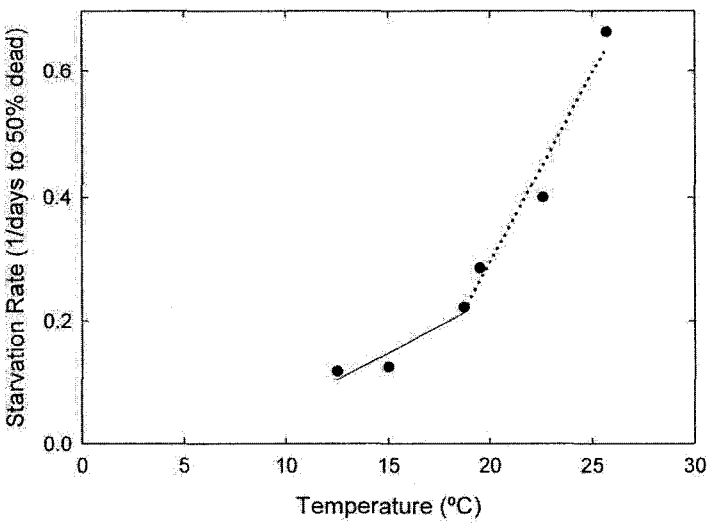
Materials and methods

As larvae hatched from egg masses incubated at 20°C and 12L:12D, they were transferred to test tubes covered with parafilm. Three tubes containing ten larvae each were placed at each of six temperatures: 12.5, 15, 17.5, 20, 23 and 26°C. I checked the tubes daily, counting and removing larvae that had died.

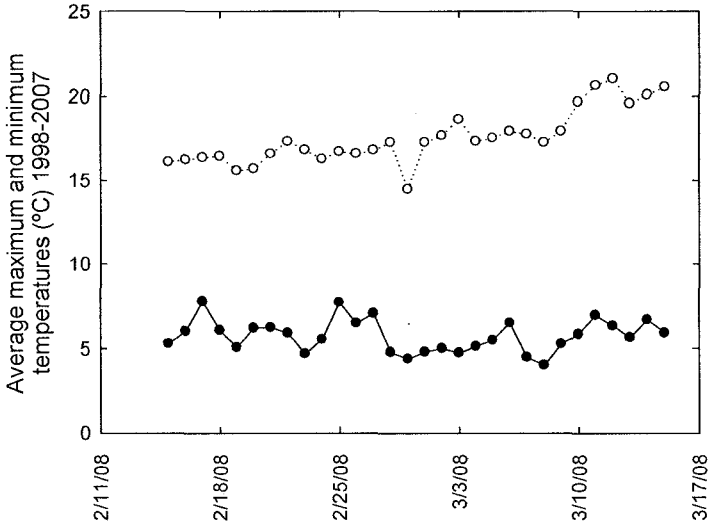
I averaged the number of days to 50% dead for the three test tubes at each temperature, then plotted median starvation rate (1/days) as a function of temperature.

Results

The larval starvation rate increased linearly at temperatures above 15°C (Figure below). At 12.5 and 15°C, there was little difference in starvation rate, so I assumed the rate was constant. Larvae can survive without food for eight days at and below 15°C.



Appendix A 1. Median starvation rate of newly hatched larvae as a function of temperature.



Appendix A 2. Ten-year average maximum and minimum temperatures at Stanford.

Discussion

Starvation tolerance is temperature-dependent, and average spring temperatures are cool enough that tussock larvae could survive hatching before budburst for about one week.

APPENDIX B: FITNESS AS A FUNCTION OF START DATE

Introduction

The degree of synchrony between hatching and budburst may affect moth fitness, since leaf quality changes rapidly as leaves mature. In *Q. agrifolia,* nitrogen and phosphorous levels drop rapidly over the first month of a leaf's life, while percent dry weight of cellulose, lignin, and acid detergent fiber increases. Total phenolic content is highest at budburst for new leaves while tannin concentrations increase as leaves mature (Mauffette and Oechel, 1989). It is unknown how sensitive *O.v.gulosa* is to these nutritional changes.

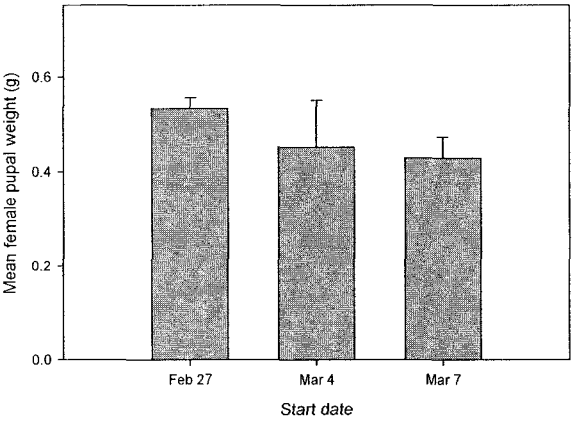
Materials and methods

Newly hatched larvae were transferred to oak leaves three times over an eight day period, to simulate different degrees of synchrony between hatch and leaf development. The first group of 30 larvae was started just after oak shoots reached budstage 2, or budburst, on February 27. The second group of 30 larvae was started 5 days later, on March 4, when the same tree was still at budstage 2. The last group of larvae was started 3 days later, on March 7, when oak shoots were at budstage 3. The larvae were removed within a day of hatching from egg masses held at 20°C, 12L:12D, used in the diapause termination study (see Chapter 1). They were randomly transferred to three jars containing oak cuttings, ten larvae to a jar, and placed back at 20°C 12L:12D.

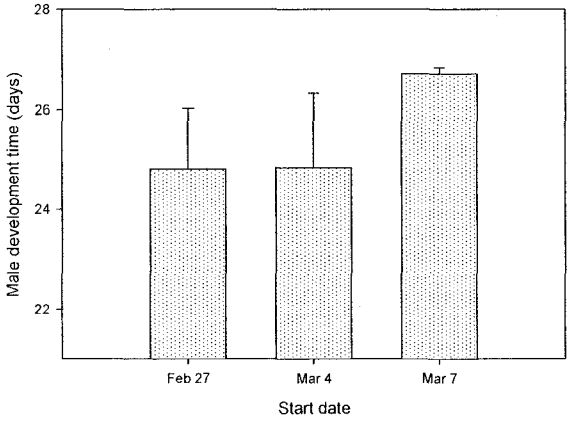
To determine if more synchronous female larvae were heavier (leading to bigger egg mass and higher fitness) I performed a one-way ANOVA of pupal weight as a function of start date. To determine if more synchronous larvae developed faster, I performed another one-way ANOVA of larval development time as a function of start date.

Results

There was a weak but nonsignificant difference ($F_{2,29}$=2.84; p=0.075) in female pupal weight as a function of start date. Female larvae that hatched later after budburst attained progressively lower pupal weights (see Figure 1 below). There was no difference in male pupal weights across groups.



Appendix B 1. Females that hatch more synchronously with budburst weigh more when they pupate.



Appendix B 2. Male larvae take longer to develop when hatch coincides with budstage 3, instead of budstage 2.

There was a significant difference ($F_{2,33}$=5.57, $p<0.01$) in male development times as a function of start date. Group 3 males (budstage 3) took longer to develop than those of Groups 1 and 2 (both budstage 2) (Figure 2). There was no difference in development time of females as a function of start date ($p=0.33$).

Discussion

The small number of female larvae in Group 1 makes it difficult to know whether there is a real difference in pupal weight as a function of start date. Female pupal weight translates directly to egg mass size in flightless moths and is an important component of population dynamics. Further studies are needed. There may be no advantage to heavier weight in males, who fly to reach mates.

The significant difference in male development time may have important consequences. Males that pupate later will emerge as adults later. This may put them out of synchrony with females on the host tree on which they emerged, although they can fly to another tree to mate.

APPENDIX C: ARTIFICIAL DIETS AND ALTERNATIVE HOST PLANTS

Introduction

Artificial diets are often used in developing degree day models, to eliminate diet as a variable affecting development. In various published studies, western tussock moth larvae were reared on western spruce budworm diet (*Hemerocampa vetusta* Boisduval in Page and Lyon, 1973), or standard gypsy moth laboratory diet (*Orgyia cana* Henry Edwards in Schaefer and Barth, 2006), and one diet developed specifically for western tussock moth (*Orgyia cana* Edwards in Peterson, 1978). *Orgyia vetusta gulosa* larvae in the San Francisco Bay Area are polyphagous but naturally occur on or near *Quercus agrifolia* in urban areas. Performed as a series of preliminary studies, I compared larval development (pupal weight, 1st instar mortality, and larval development time) on gypsy moth diet and western tussock moth diet to that on coast live oak (*Quercus agrifolia)* and buckeye (*Aesculus californica*) leaves collected over various seasons.

Materials and methods

I prepared reduced volumes of Peterson's western tussock moth diet by combining individual ingredients. I prepared standard gypsy moth diet (GMD) from a mix supplied by Bio-Serv (Frenchtown, NJ, USA). Leaves of *Aesculus californica* and *Quercus agrifolia* came from planted and irrigated trees on the San Jose State University campus.

As larvae hatched, they were transferred to diet:

Western tussock moth diet (hereafter WTMD): In early spring 2006, as larvae hatched, they were placed individually into plastic 30 gm cups ("soufflé cups", Smart & Final) on 1x1 cm blocks of diet, covered with a lid in which I had punched holes. I placed the cups in growth chambers at 20 and 27°C, 18L:6D. I checked the larvae everyday and replaced the food every

2-3 days. Because these cups collected condensation quickly, I experimented with placing the food on paper towels inside the cups, swabbing the cups daily, switching to paper cups, first with plastic lids, then with paper lids. Because larvae were not eating much or growing, I experimented with other containers: Petri dishes (standard size and mini size) and 6-well tissue culture plates (BD Falcon), sealed with Paraffin tape to prevent tiny larvae from escaping. Because of continued problems with condensation, I added filter paper (various amounts) and/or silica gel packets to the Petri dishes. I also increased the amount of agar in the diet by 25% (by mass).

Standard Gypsy Moth Diet (SGMD): In fall 2006 (Group 4), I placed hatchlings onto 2x2 cm blocks of diet in 0.5L Mason jars covered with aluminum foil, held in place with a rubber band. I placed ten larvae at 20°C, three larvae at 27°C, and five larvae at 15°C. All had photoperiods of 12L:12D. I checked the larvae daily and replaced the food once a week.

California Buckeye leaves (AECA): In early spring 2006, ten hatchlings were transferred to buckeye leaves kept fresh by immersing the petioles in a flask of water. The larvae and leaf were placed inside a 1 L Mason jar covered with a square of gauze secured with a rubberband, and the jar was placed at 20°C, 18L:6D. The leaves were refreshed as needed, usually twice a week.

Coast live oak leaves (QUAG): See Chapter 1 Group 1 for methods used to raise larvae on *Q. agrifolia* leaves.

Results

WTMD: All larvae at both 20°C (n=100) and 27°C (n=15) died in either the 1st or 2nd instar stage of development, either from drowning or from starvation. Food spoiled quickly and was unpalatable to the larvae. Condensation was a problem in all containers, though the multiwell

containers had the least and the plastic soufflé cups had the most condensation. No satisfactory method was found to raise larvae on this diet.

SGMD: At 20°C, first instar mortality was high (42%), but those that survived to the second instar developed successfully into pupae. Males (n=4) took an average of 36.75 days to develop into pupae, while females (n=2) took 42 days. Ten larvae were started (on Oct 23) on spruce budworm diet; 1st instar mortality was 30%, four grew to 2nd instar after 10 days but were transferred to gypsy moth diet after Dec 2. Development took longer than SGMD, 61 days on average for 4 females (no males survived). At 27°C, there was no 1st instar mortality, but larvae could not pupate and died after reaching their final instar. At 15°C, 1st instar mortality was 33%, and those that survived took 20-31 days to develop to 2nd instar. None survived to pupate.

AECA: First instar mortality was 50%, but those that survived developed successfully into pupae. One male took 30 days to develop into a pupa, while females (n=4) averaged 39.5 days. The male weighed 0.089g while the females weighed 0.5g on average.

QUAG: Group 1 first instar mortality was very low (6%) at 20°C and took roughly seven days to complete (development time to 2nd instar). Total larval development time was very similar to that in Groups 2 and 5, about 26 days for males and 30 days for females. Group 3 larvae raised on summer lammas shoots took slightly longer to develop. At 15°C, there was no 1st instar mortality, and development from 1st to 2nd instar took 10-14 days to complete.

Discussion

Larvae raised on oak leaves developed more quickly than those on buckeye leaves or standard gypsy moth diet. First instar mortality was also significantly lower on oak leaves than

on buckeyes or either artificial diet. However, pupal weight was not significantly different between females raised on buckeye and oak leaves.

Orgyia v. gulosa can be raised on artificial diet, though the development time may be longer. Both the spruce budworm and standard gypsy moth artificial diets showed promise, though western tussock moth diet did not. Sample sizes in these preliminary studies were small, and it is unclear why larvae raised on artificial diet did not pupate at higher temperature, or why development from 1st to 2nd instar was so much slower (took twice as long) at 15°C.

APPENDIX D: PURPLE-LEAF PLUM

Introduction

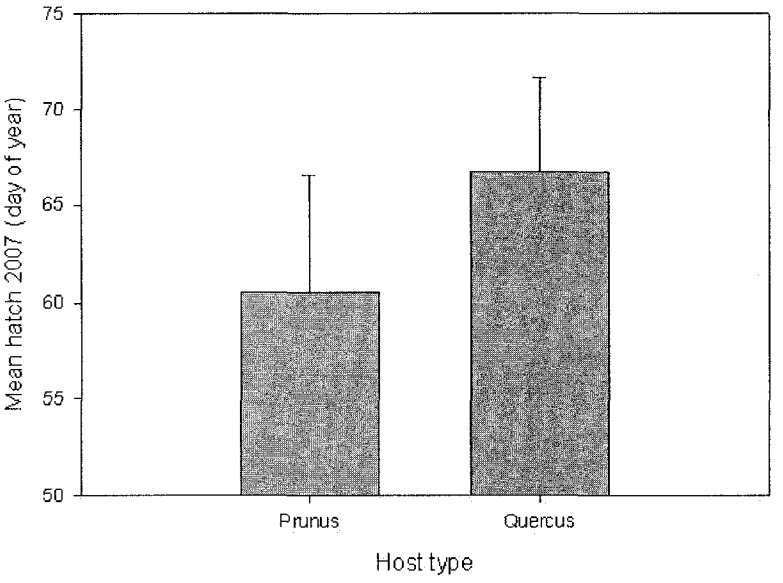
Polyphagous herbivores with limited dispersal may become adapted to the traits of their individual hosts (see Tikkanen *et al*, 2006). This may be one mechanism leading to speciation. Western tussock moth larvae on the Stanford University campus feed primarily on coast live oak but are also found on Liquidambar (*Liquidambar styraciflua*), Chinese hackberry (*Celtis sinensis*) and purple-leaf plum (*Prunus cerasifera* cvs). Purple-leaf plum trees develop new leaves earlier in the season than coast live oaks and represent a food source for larvae that hatch before oak budburst. This small study examined whether mean hatch of egg masses found on purple-leaf plum occurs earlier than that of egg masses found on coast live oak.

Materials and methods

Three purple-leaf plums growing next to one another were monitored on the Stanford campus in 2007. The same methods from Chapter 2 (phenological synchrony studies) were used to monitor egg masses and prepare data for analysis. Median hatch dates were assigned for each egg mass, and a t-test was used to compare mean hatch in purple-leaf plums to that in oaks.

Results

There was a significant difference in mean hatch dates between purple-leaf plums and coast live oaks ($t=-3.52$, $df=69$, $p<0.001$). Mean hatch occurred six days earlier on purple leaf plums than on coast live oaks.



Appendix D. 1 Mean hatch occurred 6 days earlier on purple leaf plum than on coast live oak in 2007 at Stanford.

Discussion

Western tussock moth larvae hatch earlier on purple-leaf plum than on coast live oak, corresponding to earlier budburst in purple-leaf plum compared to coast live oak. Over time, moths on plums may diverge from those on coast live oaks and form a separate plum-eating variety. Several forces may counter this: the plums at Stanford are surrounded by oak stands, and gene flow presumably occurs between male moths from coast live oak and female moths on purple-leaf plum. However, strong selection pressure may override this gene flow, and maternal effects may be very important in determining hatch date. Long-term studies of larger numbers of individuals could add information, as could reciprocal transplant and laboratory experiments.

APPENDIX E: TELENOMUS CALIFORNICUS

Introduction

Telenomus californicus is a highly mobile native wasp that parasitizes egg masses of western tussock moth and may play a role in regulating tussock populations. Tussock moth is a cyclically outbreaking herbivore, spending a few years at high density followed by a number of years at low density. Tussock moth females are flightless, which limits dispersal and produces spatial variation in abundance. Widely-dispersing wasps may limit the spatial spread of tussock populations but not their density over time (Wilson et al, 1999). Harrison (1997) reported high-density populations of the lupine-feeding form of western tussock moth (*O.v.vetusta*) that completely defoliated their host plants for more than ten consecutive years. This has not been reported in the oak-feeding form (*O.v.gulosa*), whose populations may be regulated by different factors. This paper reports differences in *Telenomus* densities in eggs collected during outbreak and nonoutbreak years.

Materials and methods

Tussock egg masses were collected at two-week intervals over a six week period, from mid-August through the end of September, during a non-outbreak year (2000) on the Stanford campus. Roughly 10 egg masses were randomly chosen at each collection date, and 55 egg masses in total were collected. The egg masses were placed individually into glass vials stoppered with cotton and placed at room temperature and ambient light conditions. The egg masses were checked daily and the number of wasps and larvae present was recorded.

During a tussock outbreak, forty-four randomly chosen egg masses were collected on October 1 (2006), placed individually into glass vials as above, but stored outside over the winter. The egg masses were weighed and number of wasps present was recorded on

November 5. In late winter, the egg masses were checked daily and the number of larvae present was recorded.

Results

During the non-outbreak year, 72% of collected egg masses were parasitized. An average of 13 wasps and 92 larvae emerged from each egg mass. There was an inverse relationship between wasps and larvae: the higher the number of wasps that emerged, the lower the number of caterpillars hatched.

During the outbreak year, 18% (8 of 44) of collected egg masses showed parasitism by November 1. Of those parasitized, an average of two wasps and 32 larvae emerged from each egg mass.

Discussion

I don't have careful records of the number of wasps emerging from egg masses collected at two week intervals, from mid-September through mid-February during the outbreak, but the number was small. Few egg masses had wasps emerging.

During non-outbreak years, the number of wasps in an egg mass is small relative to the number of larvae that hatch, but most egg masses have been parasitized.

During outbreak years, fewer wasps emerge from egg masses, and fewer egg masses are parasitized. *Telenomus* does not quickly bring down the tussock population during an outbreak. However, nucleopolyhedrovirus does.

Because the tussock population crashed as a result of disease (nucleopolyhedrovirus) in 2007, few egg masses were produced. Widely dispersing wasps could probably fly beyond the infected area, to where the tussock population is less dense and conditions are less conducive to viral attack, to find egg masses to parasitize.