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Effects of temperature on development of vedalia beetle, *Rodolia cardinalis* (Mulsant)

E.E. Grafton-Cardwell*, P. Gu, G.H. Montez

Department of Entomology, University of California, Riverside, CA 92521, USA

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Abstract

The effect of temperature on the development of the vedalia beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), fed *Icerya purchasi* Maskell (Homoptera: Margarodidae) under controlled laboratory conditions was studied. Adults exposed to temperatures of 25, 28, 31, 34, and 37 °C for 72 h showed 95–100% survival, however egg production was significantly reduced at 34 and 37 °C. In addition, eggs maintained at 34 °C showed reduced hatch and survival of larvae, and eggs held at 37 °C failed to hatch. The duration of each developmental stage and survival of each stage were measured at 10, 14, 18, 22, and 25 °C. There was no egg eclosion at 10 °C. The developmental time from egg to adult emergence decreased from 79 to 18 days for temperatures from 14 to 25 °C. The sex ratio was unaffected by these temperatures. The lower developmental temperature threshold of *R. cardinalis* was estimated to be 10.8 °C and the degree–day accumulation was calculated as 279 for development from egg to adult eclosion. These results will guide further research designed to optimize management of vedalia populations in the San Joaquin Valley of California.

Keywords: Temperature; Lower developmental threshold; Cottony cushion scale

1. Introduction

Vedalia beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), has been a primary natural enemy regulating populations of cottony cushion scale, *Icerya purchasi* Maskell (Homoptera: Margarodidae) in California since it was introduced in the winter of 1888–1889 from Australia (Caltagirone and Doutt, 1989; Doutt, 1964). It provides excellent biological control of cottony cushion scale because of its high reproduction rate, rapid development, and host specificity (Quezada and DeBach, 1973). During the late 1800s, the vedalia beetle saved the southern California citrus industry from devastating yield losses caused by cottony cushion scale, and since this early success, it has been transported to other areas of the state and exported to many other parts of the world, with equally successful results.

Periodically, vedalia populations have been disrupted by the use of insecticides and outbreaks of cottony cushion scale have resulted. For example, when DDT was introduced in the 1940s, organophosphate insecticides were first used in the 1950s, and carbamates were introduced in the 1970s, vedalia populations were affected and outbreaks of cottony cushion scale were noted (DeBach and Bartlett, 1951; Ebeling, 1959). Since that time, the primary insecticide classes used to control key pests of citrus, citrus thrips *Scirtothrips citri* Moulton (Thysanoptera: Thripidae), and California red scale *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae) have remained organophosphates and carbamates. Thus, for a number of years, vedalia beetle was not exposed to new classes of broad spectrum insecticides. During 1997–2002, six new insecticides, toxic to vedalia

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* Corresponding author. Fax: +1 559 646 6593.
E-mail address: bethgc@uckac.edu (E.E. Grafton-Cardwell).

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(Grafton-Cardwell and Gu, 2003), including the neonicotinoids imidacloprid and acetamiprid, the pyrethroids cyfluthrin and fenpropathrin, and the insect growth regulators pyriproxyfen and buprofezin, were registered and used in citrus in the San Joaquin Valley of California. Both widespread and localized outbreaks of cottony cushion scale have occurred since the introduction of these insecticides. Mendel et al. (1994) in Israel and Hattingh and Tate (1995) in South Africa also noted that insect growth regulators were disruptive to *R. cardinalis*.

Cottony cushion scale outbreaks were most serious in 1999, following the first use of pyriproxyfen in 1998 on 48,000 acres of San Joaquin Valley citrus (Grafton-Cardwell, 1999). Buprofezin was introduced that same year, however usage was limited (<5000 acres) due to its longer preharvest interval (60 days) and higher cost. Vedalia beetle disappeared from the San Joaquin Valley for nine months (July–March) after pyriproxyfen treatments began. Since that time, vedalia populations have been disrupted by insecticide use, but not for such an extended period of time. Uses of insect growth regulators have diminished since the first year and growers have learned to delay treatments of disruptive insecticides until after vedalia activity has diminished naturally, in June. Citrus growers respond to localized cottony cushion scale outbreaks by waiting for vedalia to arrive naturally or by moving them from orchard to orchard during the early spring (March and April). When vedalia does not completely eliminate cottony cushion scale, then an organophosphate such as methidathion is applied.

Cottony cushion scale outbreaks are likely to continue because of pesticide disruption, especially in the San Joaquin Valley where insecticide use is heavier than in other regions of the state. The situation is especially serious because of the wide variety of newly introduced insecticide classes (pyrethroids, IGRs, and neonicotinoids) that are needed for key pests (citrus thrips and California red scale) and invasive pests [glassy-winged sharpshooter, *Homalodisca coagulata* (Say)] of citrus. Citrus growers need a greater understanding of the factors that influence vedalia beetle arrival and abundance in citrus, specifically for the San Joaquin Valley. Prior studies of *R. cardinalis* biology focused on southern California citrus where the environment is fairly uniform and vedalia and the parasitic fly *Cryptochaetum iceryae* (Williston) are found during various times of the year (Quezada and DeBach, 1973). The San Joaquin Valley of California experiences greater extremes of winter and summer temperatures. In that region, vedalia beetle populations decline in June or July even in the presence of adequate food (Grafton-Cardwell, unpublished data). The decline appears to be correlated with increasing temperature and the use of disruptive insecticides. In addition, the arrival of vedalia beetle in citrus orchards in the springtime varies widely from year to year, possibly affected by winter temperature. The parasitic fly, *C. iceryae*, is not a significant mortality factor in the San Joaquin Valley region. Natural arrival of vedalia is critical for initiating colonies in cottony cushion scale-infested orchards in the spring, because control exerted by vedalia beetle predation surpasses chemical control in most situations. The purpose of the proposed project was to determine the effect of temperature on the development of vedalia stages.

2. Materials and methods

Vedalia beetle stages used for testing were from a colony initiated from a collection in 1999 from an orange orchard *Citrus sinensis* (L.) Osbeck in Exeter, CA. The vedalia were reared in a room maintained at 25 ± 3 °C with supplemental lighting 14:10 L:D. Vedalia adults and larvae were fed adult female cottony cushion scale from a colony initiated in 1999 from a navel citrus orchard in Orange Cove, CA. The cottony cushion scale were reared on *Pittosporum tobira* L. (Thunb.) in a greenhouse using natural lighting and temperatures of 25 ± 5 °C.

2.1. Effects of high temperatures

To examine the effect of high temperature on vedalia survival and reproduction, 200 pupae were collected from the vedalia colony and the emerging adult beetles were provided an excess of adult female cottony cushion scale adults as food. Males and females were allowed to mate for two days before use in the experiment. One hundred adult female beetles were individually caged in 1 oz plastic containers with 5 g of adult female cottony cushion scale. Twenty of the 1 oz containers were placed in each growth chamber with temperatures set for 25, 28, 31, 34 or 37 °C, and 69–83% RH, in the absence of light for 72 h. The adults were removed after 72 h and the eggs that had been deposited during that period were returned to the growth chamber for 10 days. Additional food in the form of adult female cottony cushion scale (3–4 scales) was provided every 2 days. The entire experiment was replicated two times for a total of 40 adult beetles/treatment.

At the end of 72 h of exposure, the percentage of surviving adults was calculated and at the end of 10 days of exposure, the mean number of eggs produced, the percentage egg hatch, and the percentage larval survival were calculated for each temperature regime. The percentage survival of adults, egg hatch, and larval survival were transformed using arcsin ($x^{1/2}$) before ANOVA was conducted (Statistical Graphics Corp., 2000). The mean number of eggs was transformed $\log_{10}(x+1)$ to stabilize variances before ANOVA was conducted. Mean separation was conducted using the least significance difference test (LSD).

2.2. Lower developmental temperature threshold

To determine the lower developmental temperature of vedalia beetle, 200 adult beetles, 1–5 days after emergence were placed in 12 containers for 12 h in groups of 15–20 with an excess of adult female cottony cushion scale and allowed to deposit eggs. Vedalia eggs attached to the cottony cushion scale females were separated into groups of 10 and placed in 1 oz plastic cups in a growth chamber set for one of five temperatures (10, 14, 18, 22, 26 ± 2 °C) in the absence of light. Each growth chamber contained a 46 × 30 × 18 cm plastic container with a saturated solution of D-glucose anhydrous that maintained the relative humidity of the growth chamber in the range of 40–55%. There were 5 cups/temperature for a total of 50 eggs tested/temperature. Each growth chamber contained a 17 × 9 cm cold pack equilibrated to the temperature of the growth chamber (Super Ice, San Leandro, CA). Every 24 h, the cups were opened and placed on the cold pack from that chamber to help maintain the life stage at uniform temperature, and vedalia survival and stages were recorded. When eggs hatched, larvae were placed in individual cups with adult female cottony cushion scale as food. Approximately, 0.1 g fresh adult female cottony cushion scales (3–4 scales) was added every 2 days until pupation. When an individual reached the adult stage, it was removed from the experiment and sexed.

The percentage egg hatch, larval survival, and development to adulthood were calculated for each individual for each temperature tested. Developmental rate was calculated using the reciprocal of the number of days to complete a stage (1/D, D = days to complete a life stage) multiplied by 100 to give an integer value. The developmental rate for each individual in each temperature regime was analyzed and linear regression equations were calculated ($y = a + bx$, where y is the developmental rate and x is the temperature) using a general linear models procedure (Statistical Graphics Corp., 2000). The point of intercept of the regression line with the x axis corresponding to the lower developmental threshold DT_L was calculated for each life stage. The degree–days required to complete each phonological stage were calculated as the 1/slope of the regression line for each stage (Arnold, 1959).

3. Results

3.1. Effects of high temperatures

Table 1 shows the effects of constant temperatures of 25–37 °C on adult vedalia beetle survival, egg production, and successful emergence and survival of larvae. Adults exhibited 95–100% survival of 72 h of exposure to test temperatures (25–37 °C). Egg production over the 72 h period ranged from. 22.0 to 26.8 eggs/female for the 25, 28, and 31 °C temperatures, but was significantly reduced to 9.3 and 1.2 eggs/female in the 34 and 37 °C regimes, respectively ($F = 68.5$; $df = 4, 195$; $P < 0.0001$). Percentage egg hatch ranged from 98.0 to 99.9% for the 25–31 °C temperature regimes and was not significantly different between these treatments, but was significantly reduced to 71.4 and 0% in the 34 and 37 °C regimes, respectively ($F = 104.1$; $df = 4, 162$; $P < 0.0001$). Finally, percentage larval survival ranged from 94.7 to 99.3% for temperatures of 25–31 °C. Percentage larval survival was significantly reduced by the treatment of 34 °C ($F = 113.5$; $df = 3, 142$; $P < 0.0001$) and since no eggs hatched in the 37 °C test regime, larval survival was not measured.

3.2. Lower developmental temperature threshold

Fig. 1 shows the survival of vedalia as it developed from egg to adult eclosion at constant temperatures ranging from 14 to 26 °C. None of the eggs hatched in the 10 °C regime. No mortality occurred for the 22 and

Table 1
Effects of 25–37 °C temperatures on adult vedalia beetle survival and egg laying over a 72 h period and the percentage egg hatch and larval survival during the subsequent 10 days

Temperature °C	Mean ± SE			
	% Adult survival	# Eggs/female	% Egg hatch	% Larval survival
25	100	22.0 ± 1.90a	99.7 ± 0.27a	99.3 ± 0.53a
28	100	26.8 ± 2.29a	99.9 ± 0.13a	98.8 ± 0.51a
31	97.5	23.7 ± 1.87a	98.0 ± 0.55a	94.7 ± 1.08a
34	97.5	9.3 ± 1.63b	71.4 ± 6.61b	35.9 ± 5.79b
37	95.0	1.2 ± 0.30c	0.0c	—
F		68.51	104.13	113.48
df		4, 195	4, 162	3, 142
P		<0.0001	<0.0001	<0.0001

Means in the same column followed by the same letter are not significantly different according to the least significant difference test ($P < 0.05$). Number of eggs transformed $\log_{10}(x + 1)$ and percentages transformed arcsin ($x^{1/2}$) before ANOVA. Untransformed means listed. $N = 40$ adult beetles/treatment.

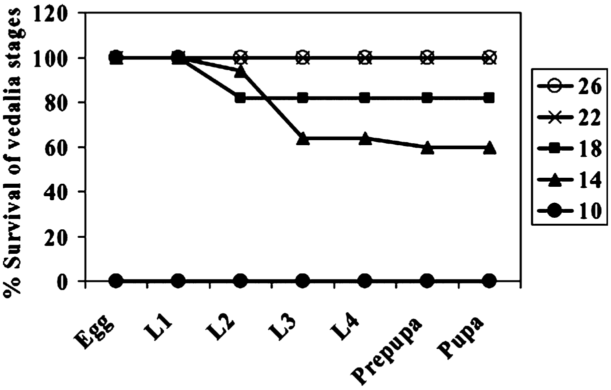

Fig. 1. Percentage survival of *R. cardinalis* stages reared under constant temperatures (°C).

26 °C temperatures. Survival was reduced to 79% at 18 °C and 63% at 14 °C. The majority of mortality occurred during the second and third larval instars.

The mean duration of the development of each immature stage of vedalia beetles reared on cottony cushions scale is shown in Table 2. Eggs did not eclose at 10 °C. Eggs showed decreasing periods of days to eclosion from 15.7 days at 14 °C to 4.6 days at 26 °C. At 14 °C, larvae required 11.8, 6.7, 6.8, and 4.6 days for 50% completion of each of the four larval instars. In contrast, at 26 °C larvae required 3.6, 1.5, 1.1, and 1.2 days to 50% completion of each of the four larval instars. Development of the prepupal and pupal stages declined from 14.4 and 19.8 days at 14 °C to 1.7 and 4.5 days at 26 °C. The total development from egg through to adult eclosion decreased from 79 days at 14 °C to 18.4 days at 26 °C. The result of 18.4 days of development from egg to pupa at a constant temperature of 26 °C was similar to the 19.7 days reported by Matsuka and Watanabe (1980) for a constant temperature of 25 °C.

The lower temperature thresholds ($\mathrm{DT_L}$), the linear regression for each stage of vedalia, that describes the relationship between developmental rate (y) and temperature (x), and the coefficient of correlation (r^2), are shown in Table 2. The lower development thresholds differed according to life stage and ranged from 6.58 °C for the first instar to 13.39 °C for the prepupa. The smallest slopes (0.013 and 0.015) corresponded with the egg and pupal stages, which had the longest durations of development at all temperatures. Analysis of the development of vedalia from egg to adult eclosion ($y = 0.0063x - 0.038$, $r^2 = 0.97$) resulted in an estimated lower developmental threshold of 10.8 °C.

Table 3 shows the observed degree–day accumulations for 50% of individuals to complete egg, larval, prepupal, and pupal stages for the tested temperatures. Development from oviposition to adult eclosion for vedalia required an average of 279 degree–days above a threshold of 10.8 °C. Over a 14-year period (1990–2003), the yearly total accumulated degree–days for the Exeter, CA. weather station averaged 2717. This would allow for nine generations of vedalia beetles if they were not limited by upper temperatures as well.

The proportion of males (n = number of beetles examined) that developed in the 14, 18, 22, and 26 °C treatments were 0.59 ($n = 29$), 0.51 ($n = 41$), 0.58 ($n = 50$), and 0.60 ($n = 50$), respectively. Thus, while sex ratio was

Table 2
Development of *R. cardinalis* stages under constant temperatures (°C)

	Days (± SEM) required for 50th percentile to complete each stage					$\mathrm{DT_L}$ (°C)	Regression equations	R^2	N
	Temperature (°C)								
	10	14	18	22	26				
Egg	—	15.66 ± 0.20	11.40 ± 0.26	6.76 ± 0.07	4.64 ± 0.08	9.74	$y = 0.0129x - 0.125$	0.91	186
First instar	—	11.81 ± 0.61	5.07 ± 0.21	4.20 ± 0.18	3.64 ± 0.13	6.58	$y = 0.0158x - 0.104$	0.49	173
Second instar	—	6.69 ± 0.30	4.56 ± 0.22	2.32 ± 0.12	1.52 ± 0.08	12.01	$y = 0.0520x - 0.624$	0.55	173
Third instar	—	6.81 ± 0.32	2.66 ± 0.20	1.40 ± 0.08	1.14 ± 0.05	10.52	$y = 0.0638x - 0.671$	0.59	173
Fourth instar	—	4.63 ± 0.38	2.51 ± 0.15	1.20 ± 0.06	1.24 ± 0.06	8.48	$y = 0.0553x - 0.469$	0.52	173
Prepupa	—	14.37 ± 1.22	5.34 ± 0.21	2.90 ± 0.13	1.72 ± 0.09	13.39	$y = 0.0503x - 0.673$	0.62	173
Pupa	—	19.83 ± 0.87	10.73 ± 0.18	6.48 ± 0.14	4.52 ± 0.12	11.24	$y = 0.0151x - 0.170$	0.86	171
Total egg to adult eclosion		79.00 ± 1.31	41.49 ± 0.43	25.26 ± 0.26	18.42 ± 0.17	10.79	$y = 0.0036x - 0.038$	0.97	171

$\mathrm{DT_L}$, lower developmental threshold; y, developmental rate; x, temperature; R^2, coefficient of determination; N, no. tested.

Table 3
Degree–day accumulations for the 50th percentile to complete each stage at different temperatures using $\mathrm{DT_L}$ of 10.8

°C	Eclosion	1st instar	2nd instar	3rd instar	4th instar	Prepupa	Pupa
14	50.27	87.49	116.47	138.64	153.49	189.94	253.60
18	82.20	113.08	145.96	165.13	183.24	221.76	299.13
22	75.78	122.86	148.87	164.57	178.02	210.53	283.17
26	70.58	125.94	149.06	166.40	185.26	211.42	280.17
Avg.	69.71	112.34	140.17	158.68	175.00	208.41	279.02

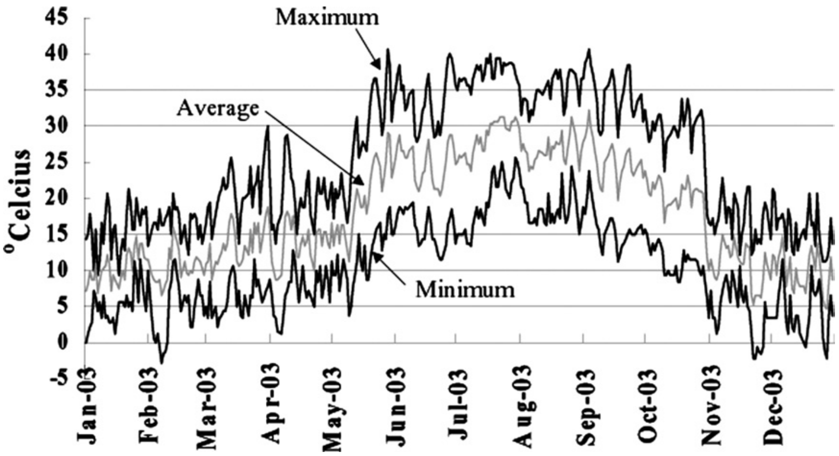

Fig. 2. Maximum, average, and minimum daily air temperatures (°C) for Exeter, CA during 2003.

biased toward males in this colony, exposure to low temperatures did not seem to affect the ratio.

4. Discussion

Fig. 2 shows that from late November through early February (using 2003 temperatures), average daily temperature frequently fell below the lower developmental threshold of 10.8 °C. During this period of time, little growth and development of vedalia would occur. Vedalia eggs and larvae apparently also have an upper temperature threshold. The reduction in fecundity, egg hatch, and larval survival at 34 °C, and the severe reduction in egg laying and complete lack of egg hatch at 37 °C suggest that the upper temperature threshold is between 34 and 37 °C. During June–September of 2003 (Fig. 2), the maximum daily temperature frequently exceeded these temperatures and during August and September, the average daily temperature approached 34 °C. The results of our study explain, at least in part, why vedalia beetle activity has been observed to be greatly reduced during the summer months in the San Joaquin Valley. Xia et al. (1999) showed that the aphidophagous coccinellid, *Coccinella septempunctata* L. had reduced egg laying at 35 °C and suggested that hot summers are a detriment to performance of this biological control agent.

On average, Exeter accumulates more than 2700 DD above a threshold of 10.8 °C from January through December. This would allow development of nine generations of vedalia per year. However, this calculation does not include reduced development due to an upper threshold, so the actual number of vedalia generations in California's San Joaquin Valley is likely to be fewer than nine. For comparison, 12 generations/year of vedalia were observed at Riverside in southern California (Quezada and DeBach, 1973) where average minimum and maximum temperatures are less extreme.

Our data suggest that maximum reproduction and growth of vedalia beetle is achieved in the early spring (March–May) and fall (October–November) months in Exeter, CA (Fig. 2). Collection and release of vedalia beetle stages by citrus growers would be most effective during these time periods. Insecticides that are toxic to the vedalia should be avoided during the months of peak vedalia activity. If vedalia are not available for release, degree–day units could be used to predict how many generations would develop from the time of natural arrival of adults until increasing temperatures limit their population growth. A minimum of two generations of vedalia beetle population growth are needed to reduce cottony cushion scale below an economically damaging level (unpublished data). Field studies are needed to determine the number of days above 34 °C that reduces vedalia development and to validate the degree–day estimations of vedalia development for the Exeter, CA region.

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