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PUPATION AND EMERGENCE OF BLUEBERRY GALL MIDGE, DASINEURA OXYCOCCANA (DIPTERA: CECIDOMYIIDAE), UNDER VARYING TEMPERATURE CONDITIONS

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ABSTRACT

Temperature-based development models have been used in pest management for many years to predict emergence and other life history events of insect pests. Blueberry gall midge, Dasineura oxycoccana (Johnson), is an early-season pest of rabbiteye blueberries, Vaccinium virgatum Aiton, in Florida. The ability to predict emergence of adults at the beginning of the season would improve monitoring and control activities. Blueberry gall midge larvae were collected from an organic blueberry farm in Gainesville, FL from Jan through Mar in 2008 and 2009. Late third instars were reared to adults in either 5-mL vials or 947mL cups with soil substrate. The duration of the pupal stage was determined under 6 constant temperatures: 10, 15, 20, 25, 30, and 35°C. More midges survived to adult emergence in the 5-mL vials than the large cups. The developmental threshold was estimated with linear and nonlinear regression models. Both models fit the data well, but the nonlinear model was limited due to the few data points at the extreme high and low temperatures. Based upon the linear model and data from the 5-mL vials, the developmental threshold for pupation was estimated as 9.8°C and the thermal constant as 134 degree-days. These experiments provide useful information on the biology of blueberry gall midge, but estimates of the thermal requirements for the other life stages will be needed before it will be possible to forecast blueberry gall midge infestations.

Key Words: Dasineura oxycoccana, rabbiteye, blueberries, degree-days, pupation

RESUMEN

Modelos de desarrollo basado en la temperature han sido utilizados por años en el manejo de plagas para predecir la emergencia y otros eventos del ciclo de vida de plagas insectiles. La mosquita de agalla de arándano (blueberry), Dasineura oxycoccana (Johnson), es una plaga de estación temprana del arándano, Vaccinium virgatum Aiton, en la Florida. La habilidad de predecir la emergencia de adultos en el principio de la estación mejoraria el monitoreo y las actividades de control. Se recolectaron larvas de la mosquita de agalla de arándano de una finca organica de arándano en Gainesville, Florida de enero a marzo del los años 2008 y 2009. Se criaron instares de tercer estadio larval hasta el adulto en viales de 5mL o tazas de 947-mL con sustrato de suelo. La duración de los estados pupales fue determinada bajo 6 temperaturas constantes: 10, 15, 20, 25, 30 y 35°C. Mas mosquitas sobrevivieron hasta la emergencia de adulto en los viales de 5-mL que en los frascos grandes. Se estimó el umbral de desarrollo con modelos de regresión lineal no linear. Ambos modelos concordaron bien con los datos, pero el modelo no lineal fue limitado debido a los pocos puntos de datos para las temperaturas extremadamente altas y bajas. Basado en el modelo lineal y datos de los frasquitos de 5-mL, se estimó el umbral de desarrollo para la pupacion a 9.8°C a el constante termal de 134 grados-dias. Estos experimentos proveen información útil sobre la biología de la mosquita de agalla de arándano, pero se necesesitaria estimados de los requisitos termales para los otros estadíos de vida antes de que sea posible el pronosticar las infestaciones de la mosquita de agalla de arándano.

Environmental factors, such as temperature and humidity, influence rate responses of development. The effect of temperature on development rate is a key component of insect population dynamics and can be used to develop forecasting models for predicting insect activity (Diaz et al. 2007). Forecasting models can indicate the appropriate time to start monitoring and implement control measures, significantly improving pest management programs (Diaz et al. 2007). Temperature development models have been calcu-

lated for many important insect pests, such as the Hessian fly *Mayetiola destructor* (Say), fall armyworm *Spodoptera frugiperda* (Smith), and codling moth *Cydia pomonella* (L.) (Foster & Taylor 1975; Barfield et al. 1978; Rock & Shaffer 1983).

Blueberry gall midge, *Dasineura oxycoccana* (Johnson), is an early-season pest of blueberries, *Vaccinium* spp. In north-central Florida, it emerges as early as Jan and can cause serious damage to developing blueberry flower buds (Dernisky et al. 2005). Rabbiteye blueberries, *V.*

virgatum Aiton, are more susceptible to flower bud damage than southern highbush blueberries, $V. corymbosum L. \times V. darrowi Camp (Dernisky et$ al. 2005). In Florida, there are several overlapping generations of blueberry gall midge each year (Sarzynski & Liburd 2003). Monitoring blueberry gall midge is challenging because, like many other gall midge species, adults are short lived (2-3 d) and larvae are hidden within plant tissues (Gagné 1989). Sarzynski & Liburd (2003) developed a method for sampling blueberry gall midge larvae in flower buds. Unfortunately, by the time larvae can be detected, damage is already being done. Furthermore, eggs and larvae within blueberry buds are sheltered from foliar insecticides (Lyrene & Payne 1995). The most vulnerable stages of blueberry gall midge are mature larvae that leave blueberry buds to pupate in the soil and adults after emerging from the soil. A temperature-based development model could predict the onset of these events and tell us when to begin monitoring or implement control strategies. In addition, we could estimate the number of generations of blueberry gall midge possible in a given season.

The objectives of this experiment were to determine the developmental threshold and thermal constant for blueberry gall midge pupation, and apply this information to field trap data to estimate optimal times for control measures. No artificial diet has been developed for blueberry gall midge and that makes it difficult to rear in the laboratory. As a result, calculations for temperature development models were made for only the pupal stage

MATERIALS AND METHODS

Development of Midges in the Laboratory

Larvae were collected from infested rabbiteye blueberry flower buds from an organic blueberry farm in Gainesville, Florida. Flower buds were taken to the Small Fruit and Vegetable Integrated Pest Management Laboratory at the University of Florida, Entomology and Nematology Department (Gainesville, FL). All flower buds collected were in development stages 2 or 3 according to the scale developed by Spiers (1978). Buds were placed in 9-cm plastic Petri dishes with moistened filter paper, sealed, and kept in a growth chamber (Percival Model I-35 LL, Percival Mfg. Co., Boone, IA) at $30 \pm 2^{\circ}$ C (day) and $20 \pm 2^{\circ}$ C (night) with a photoperiod of 14:10 (L:D). Petri dishes were checked twice a week for 2 weeks, and larvae that had exited buds were removed for use in the experiments. Only late third-instars (sternal spatula present and dark orange color) were used in the experiment. This was determined by examination under a dissecting microscope (Olympus SZ60, Olympus America, Center Valley, PA) at 10x magnification.

Blueberry gall midge pupates in the soil; therefore, containers were prepared with substrates into which larvae could burrow and pupate. Two experiments were conducted, each with a different type of pupation container: cups or vials. Cups were 947 mL (32 oz.) plastic deli cups (Fabri-Kal Corp., Kalamazoo, MI) filled with a 4cm deep layer of potting soil (Jungle Growth Products, Statham, GA) and vermiculite (1:1 by volume) moistened with approximately 83 mL deionized water. Twenty larvae were placed in each cup. Vials were 5-mL polystyrene round-bottom tubes (Falcon 2054, Becton Dickenson Labware, Lincoln Park, NJ) filled with a 2-cm deep layer of vermiculite moistened with deionized water. One larva was placed in each vial. The pupal stage was determined to start when mature larvae were introduced to the pupation containers and burrowed into the substrate, and the pupal stage ended when adults emerged from the substrate (Weston & Diaz 2005).

The temperature chambers used in the study were Florida Reach-In Chambers (Walker et al. 1993). Six constant temperatures were used: 10, $15, 20, 25, 30, \text{ and } 35 (\pm 0.1) \,^{\circ}\text{C}$, with a photoperiod of 16:8 (L:D). These experiments were conducted in 2008 and repeated in 2009. Data from both years were pooled for each container type to increase the number of replications and, consequently, the accuracy of the analysis. Pooling the data was justified because experimental conditions in both years were the same. For each temperature, 6 cups were prepared (120 larvae total, 20 larvae per cup) and 180 vials (180 larvae total, 1 larva per vial). The number of adults emerging at each temperature and time to emergence, measured in days from the time containers were placed in temperature chambers, was recorded. Containers were checked daily up to 28 d. The 28d limit is 3 to 4 times the length of the pupal stage under laboratory conditions as determined by Bosio et al. (1998).

Temperature Models

Two models were used to estimate the lower temperature threshold, c, and the thermal constant, K, for the blueberry gall midge pupal stage. The first method was the x-intercept method, which is based on the linear regression equation:

$$r(T) = a + bT \tag{1}$$

where r(T) is the rate of development (1/d to adult emergence), T is the temperature (°C), a is the intercept and b is the slope of the line. The lower temperature threshold (c) was found by extrapo-

lating the regression line to the point where it intercepts the *x*-axis (Campbell et al. 1974).

The second method was the Lactin model which is a modified version of the Logan model, a nonlinear regression model (Logan et al. 1976). The Lactin model equation is:

$$r(T) = e^{\rho T} - (e^{\rho T_{\text{max}} - T_{\text{max}} - T/\Delta} + \lambda)$$
 (2)

where r(T) is the rate of development, T is the temperature and, Tmax (the upper temperature threshold), ρ , Δ , and λ are fitted parameters (Lactin et al. 1995). The modified Logan model can estimate upper and lower temperature thresholds, but it cannot estimate the thermal constant.

Once the lower temperature thresholds were estimated, the thermal constants were calculated with the following equation:

$$K = D_{\mathrm{T}_{i}}(T_{i} - c) \tag{3}$$

where K is the thermal constant, D_{Ti} is the duration of the pupal stage at temperature T_{i} in days and c is the lower temperature threshold (Van Kirk & AliNiazee 1981).

Trapping Midge Adults in the Field

Blueberry gall midge adult emergence data in the field were collected with emergence traps placed at a blueberry farm in Gainesville, FL. All blueberry bushes at the farm were rabbiteye, *Vaccinium virgatum* Aiton. The trap design was based on descriptions from Smith & Chapman (1996). The emergence trap was constructed from a 3-L plastic food container that was painted white (Krylon Interior-Exterior, 1502 Flat White, Krylon Products Group, Cleveland, OH). The opening at the top of the trap was covered by a 14-cm Petri dish lid, the underside of which was coated with Tangle-Trap® (The Tanglefoot Company, Grand Rapids, MI). Traps were placed beneath blueberry bushes approximately 0.3 m from the trunk. One trap was placed in each sample row and sample rows were separated by 2 unused rows of blueberry bushes (3.7) m between rows). Traps were placed at different distances (up to 40 m) from the edge of the plot to eliminate bias cased by potential edge effects. Traps were checked once a week throughout the period of blueberry flower bud development in 2007 and 2008. Eight emergence traps were used each year.

Soil temperature at 10 cm below the surface were measured on site with a data logger with external soil temperature sensor (WatchDog™ Model 450, Spectrum® Technologies, Plainfield, IL). In the case of missing data points, the data set was supplemented with readings from a University of Florida weather station at the Dept. of Agronomy Forage Research Unit through the Florida Automated Weather Network (http://www.fawn.ifas.ufl.edu). Daily maximum and minimum temperatures (recorded at hourly intervals) were used in degree-day calculations. Degree-days were calculated by the following formula:

$$degree - days = [(Tmax + Tmin)/2] - c$$
 (4)

where Tmax is the maximum daily temperature, Tmin is the minimum daily temperature and c is the lower developmental threshold (Arnold 1960).

Statistical Analysis

Linear regressions of development rates on temperature were calculated with PROC REG (Littell et al. 2002). Lactin model curves were fit by iterative nonlinear regression (PROC NLIN, SAS Institute 2003). The Marquardt algorithm was used in fitting the nonlinear model because parameter estimates were highly correlated (Lactin et al. 1995).

RESULTS

For both pupation containers, the greatest number of adults emerged at 20 and $25^{\circ}\mathrm{C}$ (Tables 1 and 2). No adults emerged at $10^{\circ}\mathrm{C}$ and only 1 adult emerged at $35^{\circ}\mathrm{C}$ in both years. These temperatures were, therefore, not included in the analysis. A higher percentage of adults emerged in the vials than in the cups. Minimum and mean days for development were similar (± 1 day) for both containers, but the range of days over which adults emerged was longer in the vials.

Linear Model

Estimates of the lower developmental threshold and thermal constant were calculated by linear regression for both the vials (Fig. 1) and cups (Fig. 2). Development rates were linear between 15 and 30°C for the vials [$y=0.006\ T-0.037$; $r^2=0.628\ (F=371.0; df=221; P<0.001)$] and the cups [$(y=0.007\ T-0.045; r^2=0.749\ (F=221.0; df=75; P<0.001)$]. Excluding 30°C from the regression improved the fit of the line for the vial data ($r^2=0.753; F=604.5; df=199; P<0.001$) but not for

Table 1. Time to emergence of <i>Dasineura Oxycoccana</i> adults reared in 5-ml vial pupa	TION CONTAINERS

			Days t	o adult eme	ergence	Devel	opment rate ^b
Temperature	No. emerged ^a	% emerged	Min	Max	Mean	Mean	95% CI
10	0	0.0	_	_	_	_	_
15	60	33.3	14	28	22.0	0.047	0.045, 0.048
20	71	39.4	8	26	11.8	0.089	0.085, 0.093
25	69	38.3	6	15	8.3	0.125	0.120, 0.131
30	22	12.2	6	16	9.1	0.119	0.105, 0.132
35	1	0.6	8	8	8.0	0.125	_

^aFor each temperature, total number of larvae evaluated = 180.

TABLE 2. TIME TO EMERGENCE OF DASINEURA OXYCOCCANA ADULTS REARED IN 947-ML DELI CUP PUPATION CONTAINERS.

			Days t	o adult eme	ergence	Devel	opment rate ^b
Temperature	No. emerged ^a	% emerged	Min	Max	Mean	Mean	95% CI
10	0	0.0	_	_	_	_	_
15	15	12.5	14	23	20.1	0.051	0.047, 0.055
20	32	26.7	9	13	11.2	0.091	0.087, 0.094
25	26	21.7	7	14	8.6	0.122	0.112, 0.131
30	3	2.5	7	7	7.0	0.143	<u> </u>
35	0	0.0	_	_	_	_	_

^aFor each temperature, total number of larvae evaluated = 120.

^bDevelopment rate is the inverse of the number of days to adult emergence.

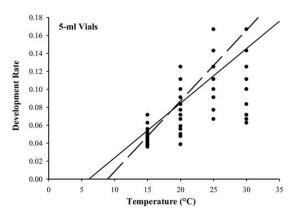


Fig. 1. Development rates of *Dasineura oxycoccana* pupae in 5 mL vials at 4 constant temperatures. Rates are expressed as the inverse of the duration of the pupal stage in days. Regression on 4 temperatures (solid line): $r^2 = 0.628$, root mean square error (RMSE) = 0.022. Regression on 3 temperatures (dashed line): $r^2 = 0.753$, RMSE = 0.018.

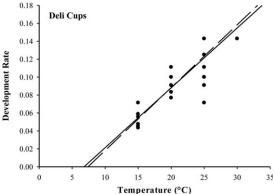


Fig. 2. Development rates of *Dasineura oxycoccana* pupae in deli cups at 4 constant temperatures. Rates are expressed as the inverse of the duration of the pupal stage in days. Regression on 4 temperatures (solid line): $r^2 = 0.749$, root mean square error (RMSE) = 0.016. Regression on 3 temperatures (dashed line): $r^2 = 0.731$, RMSE = 0.016.

the cup data ($r^2 = 0.731$; F = 192.8; df = 72; P < 0.001). From the vial data with all 4 temperatures, c was estimated as 6.1° C, and based on 3 temperatures (15, 20, and 25°C) c was estimated

as 8.9° C. From the cup data with all 4 temperatures, c was estimates as 4.7° C, and from 3 temperatures c was estimated as 7.4° C. Values of K for each temperature are reported in Table 3.

^bDevelopment rate is the inverse of the number of days to adult emergence.

				Thermal Co	onstant (K)ª	
Model	Method	Threshold (°C)	15°C	20°C	$25^{\circ}\mathrm{C}$	30°C
Linear	5-mL vial 4 ^b	6.1	195.8	164.0	165.9	217.5
	5-mL vial 3°	8.9	134.2	131.0	133.6	192.0
	Deli cup 4 ^b	4.7	207.0	171.4	174.6	177.1
	Deli cup 3°	7.4	152.8	141.1	151.4	158.2
Nonlinear	5-mL vial	6.9	178.2	154.6	150.2	210.2

Table 3. Estimates of the thermal constant for *Dasineura oxycoccana* pupation under constant temperatures, with thermal constants reported in degree-days.

Nonlinear Model

Parameter estimates and statistics from the Lactin regressions are reported in Table 4. The plot of observed values of r(T) and fitted curve for the vial data are shown in Fig. 3. There were not enough data from the cups at the high temperatures to justify fitting a curve. The lower temperature threshold was calculated by solving equation 2 for rate equal to zero. Based on the vial data, c was estimated as 6.9° C. This threshold temperature was used in calculating estimates of K (Table 3). For the cup data, no reasonable temperature threshold could be calculated based on the estimated parameters. All estimates of model parameters based on cup data had high standard errors.

The accuracy of c was determined by comparing the K values (equation 3) calculated for each temperature. The correct c should give the same K at each temperature (Howell & Neven 2000; Weston & Diaz 2005). K values calculated for c equal to 8.9°C had the least variation over the temperature range 15-25°C (Table 3). These were the temperatures used in calculating the threshold.

Field Data

Degree-day approximations were applied to first and peak adult midge emergence observed in 2007 and 2008 from emergence traps. Estimates based on cup data were not used due to concerns over accuracy. The K values for 15°C (134.2 and 178.2, from linear and nonlinear models, respectively) were used because this was close to the average soil temperature over the sampling period in both years. In 2007, the first adults were collected on 24 Jan and emergence peaked on 21 Mar. Based on the 2 models, it was estimated that the first midges collected began pupation around 6 Jan. Both models predicted the same starting time for pupation. For peak emergence, midges began pupation around 9 Mar (linear) or 7 Mar (nonlinear). In 2008, the first adults were collected on 16 Jan; adult emergence peaked on 2 dates, 5 Mar and 2 Apr. It was estimated that the first midges collected began pupation around 28 Dec (linear) or 27 Dec (nonlinear). For peak emergence, midges began pupation around 16 Feb (linear) or 14 Feb (nonlinear) for the first peak and 19 Mar (linear) or 17 Mar (nonlinear) for the second peak.

DISCUSSION

Campbell et al. (1974) cautioned that determining the threshold temperature by extrapolation of the regression line is inaccurate. To compensate for this inaccuracy they recommended that in constant temperature experiments at least 50 individuals should be reared (Campbell et al. 1974). Dent (1997) recommended that for accurate estimates a minimum of 30 to 40 insects should survive to the end point of each temperature treatment. The number of adults emerging in the vials exceeded these levels, but this was not the case for the cups. Only 3 adults emerged at 30°C in the cups. It is unclear why mortality was higher in the cups than the vials, but it is possible that the soil used may have been contaminated with fungal spores. Higher moisture content in the cups may have promoted fungal growth. The number of replications would need to be increased to cope with mortality in the cups. This calls the accuracy of the estimates from the cup data into question.

Baxendale & Teetes (1983) and Baxendale et al. (1984) used the same 6 temperatures in these experiments, plus 40°C, to model temperature development of sorghum midge, *Contarinia sorghicola*. The small number of *D. oxycoccana* adults emerging at 30°C and failure of any adults to emerge at 35°C justify the decision to omit 40°C. The maximum temperature for *D. oxycoccana* development in the pupal stage appears to be between 30 and 35°C.

Determining development rates at extreme temperatures provides useful information about

^a Formula for calculating $K = D_{T}(T_i - c)$.

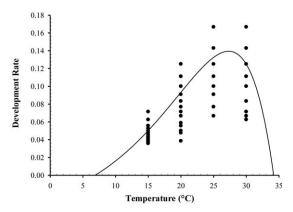
^bFour temperatures used (15-30°C).

[°]Three temperatures used (15-25°C).

Table 4. Parameter estimates (mean \pm SEM) and statistics from the nonlinear lactin regression

Pupation Container	df	φ	Tmax	Δ	γ	Lower Threshold (°C)	${f R}^2$	${f RSS}^a$
5-ml vial	221	0.1298 ± 0.03	34.9882 ± 1.71	7.6624 ± 1.71	-0.0481 ± 0.05	6.9	0.7194	0.0861
Deli cup	75	0.0514 ± 0.09	50.4300 ± 36.63	17.9644 ± 25.76	-0.2492 ± 0.77	l	0.7525	0.0168

*residual sums of squares



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Fig. 3. Lactin model curve for development rates of *Dasineura oxycoccana* pupae in 5-mL vials at 4 constant temperatures. Rates are expressed as the inverse of the duration of the pupal stage in days.

midge biology, but they should not be used in a linear temperature model. In this case, omitting 30°C from the analysis was justified because it was outside the linear part of the development curve. Removing these data improved the fit of the linear model for the 5-mL vial pupation container data. The rate of development is approximately linear above the threshold, but at high temperatures, the rates decline from linearity (Campbell et al. 1974). In nature D. oxycoccana does not encounter these high temperatures. Soil temperatures do not reach 30°C in the spring. In fact, soil temperatures at the blueberry farm rarely exceeded 25°C during the sampling period in 2007 and 2008.

All estimates of c were below 10°C, but no adults were observed emerging at this temperature. At 15°C, adults emerged up to the 28-d limit. It is possible that this limit was too short and the experiment was terminated before pupation was complete at the lower temperatures. In future phenology model experiments, the time limit should be extended to determine if blueberry gall midge can pupate at temperatures below 15°C. Larvae that fail to emerge at low temperatures should be checked to determine if the low temperature is lethal. The thermal unit comparison indicated that 8.9° C was the best estimate of c. The accuracy can only be determined over the range of temperatures used in calculating the lower threshold. This threshold was close to the minimum thresholds for the gall midges Feltiella acarisuga (Vallot) (8.4°C) and Cystiphora schmidti (Rübsaamen) (10.0°C) (Moore 1987; Gillespie et al. 2000). The threshold for Hessian fly, Mayetiola destructor, puparia development was considerably lower at 1.6°C (Foster & Taylor 1975).

The linear model was the best fit for the data. Fitted parameters from the nonlinear model could not be accurately estimated for the cup data. Only 3 adults emerged in cups at temperatures above 25°C. Therefore, the rate does not remain constant at the upper temperature limit. More temperatures with a shorter interval are required at the upper and lower limits to determine the shape of the curve and fit the model.

The challenge of using a temperature model for forecasting pest events in Florida is setting a biofix, the time to begin adding degree-days. In colder climates, winter temperatures are below threshold for a prolonged period of time. Degreeday calculations would begin at some point during this period. In Florida, however, daily temperatures are often above threshold throughout the winter. Data from emergence traps were used to approximate the duration of the pupal stage. It was estimated that for blueberry gall midge adults to emerge in mid Jan 2008, pupation would have to begin no later than late Dec 2007. This does not necessarily mean that blueberry gall midge is developing all winter long, but that temperature is not the limiting factor. Other climate factors such as rainfall may also affect the timing of emergence as observed for other midge species (Fisher & Teetes 1982; Chen & Shelton 2007). The time immediately preceding and following pupation are the periods of greatest vulnerability for blueberry gall midge. Unfortunately, this information cannot be used to forecast these events.

This was the first experiment looking at the effect of temperature on the development rate of blueberry gall midge. The lack of effective laboratory rearing methods for blueberry gall midge limited the stage that could be studied to the pupa. Therefore, the thresholds calculated can only be applied to the pupal stage. Gillespie et al. (2000) were able to rear a cecidomyiid, *Feltiella acarisuga*, from egg to adult, but this was a predatory midge that feeds on spider mites (Acari: Tetranychidae) so the food source was not an issue. Development rates for all life stages would be needed before temperature data can be used to forecast blueberry gall midge infestations.

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