Prediction of Egg and Nymphal Developmental Times of the Squash Bug (Hemiptera: Coreidae) in the Field

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ABSTRACT Egg and nymphal developmental times of the squash bug, *Anasa tristis* DeGeer, were measured at five and six constant temperatures, respectively, in the laboratory. A linear equation was used to describe the effect of constant temperature on the mean rate of egg development. A degree-day model derived from the linear equation adequately predicted rates of egg development at two variable temperatures in the laboratory and in the field. A nonlinear equation, the two-parameter form of the enzyme kinetic model of Sharpe & DeMichele (1977), was used to describe the effect of constant temperatures on median nymphal development (egg hatch to adult eclosion). Although the nonlinear model underestimated developmental rates at two variable temperatures in the laboratory by 12 and 14% and in the field by 25–32%, its predictions were consistent enough to permit accurate predictions of developmental times. Nymphal survivorship, teneral adult weights, and adult mesothoracic femur length decreased with decreasing rearing temperatures.

KEY WORDS Insecta, temperature, development, Heteroptera

ACCURATE PREDICTION OF the appearance and duration of certain stages of an insect has many applications in efforts to manipulate and understand the insect's population dynamics. Part of the information needed for accurate prediction concerns the effects of temperature on rates of growth and development of the immature stages.

There are many potential complications in predicting rates of development in the field. The temperature experienced by an insect within its characteristic microhabitat may differ from the temperature within meteorological enclosures (Baker 1980). Thermoregulatory behavior also may result in internal temperatures of the insect higher than ambient, thus speeding development (Woodburn et al. 1978). Basking and simple movement to a warmer microhabitat are two of the most common forms of thermoregulatory behavior (Casey 1981). Another potential source of error lies in the selection of the proper model to describe the relationship between temperature and development; i.e., a linear degree-day model versus a nonlinear rate-summing model (Wagner et al. 1984). Photoperiod also may affect developmental rates through its influence on the neuroendocrine system. Photoperiodic effects on developmental rates are usually associated with diapause (Beck 1980).

Squash bugs (SB), *Anasa tristis* DeGeer, are serious pests of *Cucurbita* spp. throughout North America. In central Illinois, one and a partial second generation of SB occur each summer, with nymphs present in the field from late June to late October. Prediction of developmental times for SB can be used to schedule sampling, to increase the efficiency of insecticide applications, to estimate mortality, and to study life histories. The objective of our study is to determine the effect of temperature on egg and nymphal developmental times to predict the appearance and duration of the immature stages of the squash bug under field conditions. To provide further insights into the effects of temperature on development, the weights and sizes of adults reared under different regimes are compared.

Materials and Methods

Laboratory Studies. Laboratory observations of egg and nymphal developmental times were conducted in controlled environment chambers accurate to ±0.6°C. Temperatures within the chambers were monitored with mercury thermometers and thermographs. Relative humidity within the chambers varied between 40 and 80%. Photoperiod was 16:8 (L:D) for all temperature treatments. Photophases coincided with thermophases in the alternating temperature treatments.

Eggs were obtained from a greenhouse colony where temperature varied diurnally anywhere between 20 and 37°C. Eggs were placed in the experimental temperatures within 12 h of oviposition. The egg clusters were maintained in plastic Petri dishes with a moist paper towel to maintain humidity. Twenty to 25 egg clusters at each temperature treatment were observed daily between 1200 and 1400 hours (CST) for hatch. Because all eggs within a cluster usually hatched within 24 h of one another, each egg cluster was treated as a single observation in estimating mean hatching time for each temperature treatment. Hatching time for

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Table 1. Temperature regimes for the four sets of field cohorts

Cohort	Dates	Mean temp (°C)	
		Max	Min
1985 field	2 Aug.–18 Sept.	28.8	18.5
1986 field 1	2 July–28 July	31.9	20.8
1986 field 2	5 Aug.–10 Sept.	27.6	15.9
1986 field 3	5 Sept.–30 Oct.	21.6	11.8

an egg cluster was taken as the date when any eggs within the cluster were observed to have hatched. Eggs were reared at 21.1, 23.9, 26.7, 28.9, and 35.0°C constant temperatures and at rectangular thermoperiods of 16 h, 25.6°C : 8 h, 15.6°C and 16 h, 23.9°C : 8 h, 10.0°C.

Nymphs were reared in cohorts confined to pumpkin seedlings, *Cucurbita moschata* Duchesne var. Libbey's Select, within bags of nylon mosquito netting at the three- to five-leaf stage. Cohorts consisted of siblings hatched from a single egg cluster; they varied from 15 to 21 individuals. Eggs were allowed to hatch at the experimental temperature, and measurements of developmental times began with the first observation of hatched eggs. Observations of the numbers of nymphs within each stage were made at daily intervals. Nymphs were reared from egg hatch to adult at 21.1, 23.9, 26.7, 28.9, 32.2, and 35.0°C constant temperature and at 16 h, 23.9°C : 8 h, 10.0°C; 16 h, 25.6°C : 8 h, 15.6°C; and 16 h, 32.2°C : 8 h, 21.1°C rectangular thermoperiods.

Field Studies. Field measurements of egg and nymphal developmental times were made during the summers of 1985 and 1986 at Urbana, Ill. The location of newly laid, feral eggs was marked and the eggs were observed daily until hatching. Cohorts of 10–25 nymphs were confined to leaves of pumpkin plant by enclosing them within a bag made of nylon mosquito netting. The number of nymphs within each stage was recorded daily. Cohorts were observed in August and September 1985. In 1986, three sets of cohorts were observed during July, August, and September, and September through October, to provide data over a variety of temperature conditions (Table 1).

Temperatures within a standard meteorological enclosure 7.5 cm above the ground were recorded with a hygrothermograph calibrated with a mercury thermometer. Temperature measurements within the meteorological enclosure, the crop canopy, and the rearing bags were made on several occasions during the daylight hours using a Bailey BAT-4 battery-operated, portable thermometer. Also, internal thoracic temperatures of three fifth-instar nymphs exposed to full sunlight were made on four occasions between 0830 and 0930 hours (CDT) using a copper–constantan thermocouple inserted in a hypodermic needle.

Analysis of Data. A linear equation regressing the mean egg development rate (1/developmental time) versus constant temperature was fitted to the laboratory egg hatch data (Fig. 1). From this equation, the base temperature (intercept of the abscissa) and the number of degree-days (DD) (1/slope) required for egg hatch was calculated. The number of DD required at the variable temperatures in the laboratory was calculated by multiplying the average number of degrees above the threshold temperature for both temperatures of the thermoperiod (weighted by the proportion of the thermoperiod occupied by each temperature) by the developmental time in days.

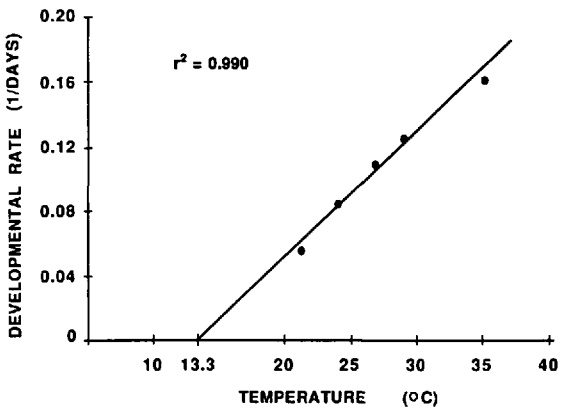

Fig. 1. Relationship of mean rate of egg development to constant temperatures. $r(T) = 0.00784T - 0.10403$, where $r(T)$ = mean rate of egg development at constant temperature T (°C).

The number of DD accumulated in the field from the day of oviposition to the day of egg hatch for each egg cluster observed was calculated from the recorded daily maximum and minimum temperatures using the method of Baskerville & Emin (1969).

Median times and confidence intervals for the probability distribution for each molt (in the laboratory and field studies alike) were determined using probit analysis (Bliss 1935, SAS Institute 1986). Probit analysis was not performed for the first molt; the observational interval was not small enough to provide enough data points for analysis because of the short duration of the first instar.

A linear DD model was first used to attempt to predict nymphal developmental times in the field, as with the egg stage, but proved to be inadequate. Nymphal development required fewer DD as rearing temperatures declined, indicating that the nymphal developmental rates were not in a linear relationship to temperature over the range of temperatures experienced in the field. A nonlinear equation was then used to describe nymphal developmental rates (Fig. 2). The enzyme kinetic model of Sharpe & DeMichele (1977), as modified by Schoolfield et al. (1981), was used to describe the effects of temperature on rates of total nymphal development (egg hatch to adult eclosion). The two-parameter form of the model (without high or

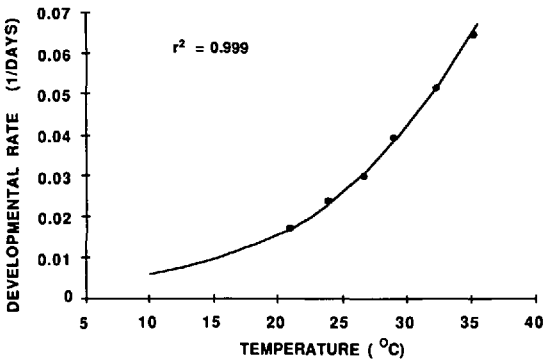

Fig. 2. Relationship of median rate of nymphal development (egg hatch to adult eclosion) to constant temperature. $r(T)\ \rho_{25}T/298.15\ \exp[HA/R(1/298.15 - 1/T)]$ where $r(T)$ is the mean daily rate of development at constant temperature T (°K), R is the universal gas constant (1.987 cal/degree/mole), ρ_{25} is the development rate at 25°C (0.02688), and HA is the enthalpy of activation (15,942.25).

low temperature inhibition) was chosen based on the criteria of Wagner et al. (1984). The model's predictions of the daily increment of development under the variable temperatures in the laboratory were obtained by averaging the predicted rates of development for each temperature of the thermoperiod (weighted by the proportion of the thermoperiod at each temperature). The median time for each molt was then multiplied by the predicted daily increment of development to obtain the accumulated development at each molt. Data for nymphal developmental rates in the field were treated similarly. The predicted rates of development were calculated on a 2-h basis. Temperatures in the field at 2-h intervals were obtained from the thermograph recordings. Rates were summed over the period of nymphal development, and median accumulated rates at each molt were determined by probit analysis.

When available, newly molted adults were weighed (to the nearest 0.1 mg) on a Mettler balance. Mesothoracic femur lengths were measured to the nearest 0.05 mm using an ocular micrometer.

Table 2. Observed degree-day (DD) requirements for egg hatch under various temperature regimes[a]

Temp treatment (°C)	DD	SD	*n*
35.0	134.9	12.45	10
28.9	124.0	13.01	15
26.7	122.5	12.23	15
23.9	124.9	13.21	23
21.1	139.6	12.67	15
25.6–15.6	115.7	12.78	15
23.9–10.0	118.0	13.55	15
Field 1985	125.2	12.31	19
Field 1986	128.1	12.89	15

[a] Predicted DD for egg hatch = 127.0 above a base temperature of 13.3°C.

Table 3. Estimated percent cumulative nymphal development (±95% CI) attained at each molt under various temperature regimes as predicted by nonlinear model[a]

Temp treatment (°C)	Median % cumulative development				Median total developmental times (d)
	Molt				
	II	III	IV	V	
35.0	33.9 (8.2)	50.7 (1.6)	67.7 (2.27)	100.7 (1.4)	15.1
32.2	34.7 (2.0)	60.5 (3.1)	73.7 (2.3)	100.1 (2.0)	19.3
28.9	33.9 (0.7)	61.6 (1.9)	74.0 (2.5)	97.5 (2.6)	25.3
26.7	34.5 (2.0)	63.3 (3.1)	82.6 (2.3)	102.9 (2.0)	32.7
23.9	32.1 (0.6)	59.6 (1.8)	72.5 (1.7)	98.9 (2.7)	40.8
21.1	39.5 (1.8)	54.4 (2.0)	71.6 (2.4)	107.5 (2.8)	57.8
32.2–21.1	33.7 (0.4)	52.9 (2.0)	72.5 (3.9)	100.4 (1.0)	24.6
25.6–15.6	30.8 (0.4)	49.5 (0.9)	61.3 (1.1)	87.8 (1.0)	38.9
23.9–10.0	29.3 (0.5)	39.6 (1.5)	56.3 (1.8)	86.3 (1.6)	47.4
1985 field	21.5 (0.9)	36.9 (2.3)	52.1 (1.8)	75.8 (2.1)	33.7
1986 field 1	25.8 (1.5)	35.4 (0.4)	48.4 (2.9)	73.5 (0.1)	24.5
1986 field 2	24.4 (0.5)	35.9 (3.2)	47.8 (2.6)	68.6 (0.8)	33.2
1986 field 3	30.4 (1.6)	37.7 (1.2)	51.9 (0.9)	73.3 (1.1)	53.0

[a] Actual cumulative nymphal development at molt V was 100%.

Results

Daytime temperatures within the canopy and within the rearing bags averaged 0.5 and 1.1°C warmer, respectively, than in the weather station (mean of 20 measurements taken between 0800 and 1800 hours [CDT] on 10 different dates in August 1985). Internal body temperatures of fifth-instar nymphs in full sunlight for 10 min rose an average of 6.3°C above ambient.

Egg Development. The relationship of egg development to constant temperatures was nearly linear over the range of temperatures examined (Fig. 1). From the intercept of the abscissa of the linear equation, a base temperature was obtained (13.3 degrees), and the predicted number of DD required for egg development was calculated as the inverse of the slope (127 DD). The observed number of DD required for egg development under variable temperatures in the laboratory and in the field were within 8% of the predicted DD (Table 2).

Nymphal Development. The nonlinear equation gave an accurate description of the effects of constant temperatures on nymphal developmental rates over the range of temperatures tested (Fig. 2). This model accurately estimated cumulative develop-

Table 4. Survival, weights, and sizes of squash bugs reared under various temperature regimes

Temp treatment (°C)	% survival	Teneral adult wt (mg) $\bar{x}$ ± SE		Mesothoracic femur length (mm) $\bar{x}$ ± SE		No. cohorts
		♀	♂	♀	♂	
35.0	54.5	133.7 ± 6.04	97.9 ± 4.08	—	—	4
32.2	35.6	—	—	—	—	8
28.9	36.7	137.6 ± 5.47	98.3 ± 3.70	4.07 ± 0.094	3.70 ± 0.074	5
26.7	44.6	139.0 ± 5.54	98.2 ± 4.20	—	—	3
23.9	28.6	124.9 ± 4.73	87.5 ± 3.38	3.82 ± 0.030	3.62 ± 0.077	6
21.1	23.7	80.4 ± 1.80	76.3 ± 3.16	3.43 ± 0.072	3.12 ± 0.076	3
32.2–21.1	52.2	123.7 ± 4.46	92.1 ± 3.68	3.89 ± 0.034	3.49 ± 0.053	5
25.6–15.6	48.1	125.3 ± 2.39	95.0 ± 1.38	3.94 ± 0.098	3.58 ± 0.097	7
23.9–10.0	21.5	106.6 ± 4.71	74.9 ± 2.58	—	—	3
1985 field	58.6	—	—	—	—	9
1986 field 1	93.6	124.9 ± 3.01	93.2 ± 2.68	3.92 ± 0.063	3.41 ± 0.056	6
1986 field 2	66.2	112.1 ± 1.81	88.8 ± 2.41	3.83 ± 0.057	3.50 ± 0.094	4
1986 field 3	64.6	87.9 ± 2.32	72.8 ± 1.87	3.50 ± 0.044	3.26 ± 0.051	5

ment under the 32.2–21.1°C variable temperature regime but not under the other two variable regimes nor under field conditions (Table 3), although it was quite consistent under a variety of field conditions. The greatest variation in estimated cumulative development among the four field tests was 8.9% for the second molt. Estimated cumulative development at the final molt varied from 68.6 to 75.8% under field conditions (Table 3).

Nymphal survival rates tended to decline with decreasing rearing temperatures and increasing developmental times (Table 4). Teneral adult weights reached a maximum at constant temperatures of 26.7°C and higher. Adult size, measured as femur length, was proportional to teneral weight. Bugs reared at the 32.2–21.1°C thermoperiod weighed less than those reared at constant 26.7°C, even though developmental times were shorter under the variable regime than at the constant temperature (Tables 3 and 4).

Discussion

Prediction of the duration of the egg stage in the field is relatively straightforward. The egg stage DD model had an error <10% under fluctuating temperatures in the laboratory and in the field (Table 2), indicating that development was close to linear over the range of temperatures experienced in the field. The daily minimum temperature in the field during the oviposition season in central Illinois (mid-June through August) seldom and only briefly falls below the theoretical threshold for embryo development, so that possible subthreshold development is not a concern. Eggs cannot move to warmer microclimates or bask in sunshine.

Prediction of nymphal developmental times is not so straightforward. The nonlinear model provided a good fit to the data (Fig. 2), but estimated cumulative nymphal development at the fifth molt varied from 68.6 to 75.8% under field conditions (Table 3). Even if temperatures inside the rearing bags were consistently 1°C above ambient, day and night, developmental rates would be increased by a maximum of 10%.

It has been suggested that, for some insects, rates of development under fluctuating temperatures are not equivalent to rates of development at constant temperatures (Parker 1930, Messenger & Flitters 1959). In this case, it appears that fluctuating temperatures produce an acceleration of rates, at least at the rectangular thermoperiods in the laboratory. Under the two fluctuating temperatures in the laboratory where the low portion of the thermoperiod was 15.6 and 10.0°C, developmental rates were 12 and 14% faster, respectively, than estimated by the nonlinear equation. This implies either that development is accelerated or that the extrapolation of the equation beyond the data is wrong. The enzyme kinetic model of Sharpe & DeMichele (1977) is based on biophysical theory and so, according to Wagner et al. (1984) can be confidently extrapolated for some small distance beyond the data to predict developmental rates below the viability threshold. Analyzing the data from the 25.6–15.6°C variable regime, one can estimate the rate of development occurring at 15.6°C by subtracting the known rate of development at 25.6°C from the observed daily rate under the variable regime, thus obtaining an estimated daily rate of 0.0196 for 15.6°C. This rate is higher than the observed rate of 0.0175 at 21.1°C, indicating that acceleration is taking place. The validity of the observed rate at 21.1°C is shown by the results of the 32.2–21.1°C treatment, which are in complete agreement with the model's estimations (Table 3).

Sharpe & DeMichele (1977) claim that their model should give an accurate description of developmental rates over a variety of temperature regimes, whether one or more enzymes are rate controlling over the entire range of temperatures examined or whether or not they even share similar temperature responses. This may be true if the enzymes are not part of the same metabolic pathway and there is no time lag between the switch

from one rate-controlling enzyme to another. But if the enzymes were part of the same metabolic pathway in which one reaction produces the substrate for subsequent reactions, then under fluctuating temperatures, some apparent acceleration of rates may be observed. For example, in squash bug nymphs, an enzyme which is not rate controlling above 20°C may be partially or totally inactivated below 20°C (i.e., become the rate-controlling enzyme). This would account for the inability of the nymphs to develop at constant temperatures below this threshold. However, there may be a time lag of several hours after inactivation of this enzyme when enough of the product of this enzyme's reaction is available as a substrate for the dependent enzymes (which are not inactivated at this temperature), so there may be a period of several hours after the temperature drops before the switch is effectively made to this rate-controlling enzyme. This would result in an apparent acceleration of rates over constant temperature measurements where the rates will equilibrate.

Other environmental factors could also influence developmental times. Adults reared in the field in 1986 weighed less and were smaller (Table 4) as developmental times increased in response to cooler temperatures later in the season. This effect was also noted by Balduf (1950). However, this effect on size does not seem to have influenced developmental rates because the model predictions are consistent. Photoperiod also may influence developmental rates (Beck 1980). Again, developmental rates in the first cohorts (July) of 1986 (which did not enter diapause) were similar to rates in the later cohorts (which did diapause), indicating that photoperiod and diapause induction did not influence the developmental rates. Hori (1986) has demonstrated that decreasing photoperiods decrease the developmental times of nymphs of *Palomena angulosa* Motschulsky (Heteroptera: Pentatomidae); the squash bugs in the field were exposed to decreasing photoperiods in every case. Whether this could have had any impact on their developmental times remains to be discovered.

Thermoregulatory behavior could be important in determining developmental rates. Squash bug nymphs may be observed on the tops of the leaves or on the fruit on cool, clear days, especially later in the season. Measurements of fifth-instar nymphs in the sun showed body temperatures several degrees above ambient. These measurements were taken with isolated individuals. When basking takes place with a large group as is often observed, then convection cooling would be reduced and heating efficiency would be even greater. However, quantifying the impact of basking on developmental times would be very difficult, requiring measurements of time spent basking and rates of heat loss and gain by the nymphs at different wind speeds, radiation intensities, body masses, and nymphal aggregation sizes.

Although the model underestimated developmental rates in the field, it was consistent enough in its predictions over a wide variety of field conditions to be useful on an empirical basis. The model should prove valuable for both research and pest management applications.

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