Physiological Entomology (1992) **17**, 309–315

A dynamic model of water gain and loss in larval Mexican bean beetle

PING-CHU CHU,* K. J. GIROUX† and R. E. STINNER
Department of Entomology, North Carolina State University, Raleigh, North Carolina, U.S.A.

Abstract. Quantitative effects of temperature, vapour pressure deficit, host, and larval body size were experimentally determined. A simulation model for dynamic water balance in the Mexican bean beetle, *Epilachna varivestis* Mulsant, is presented and parameters are estimated from laboratory data for water gain/loss equations. The model is based on water loss through the cuticle, spiracles and frass, and water gain through ingestion.

Key words. Water balance, simulation model, population dynamics.

Introduction

Many studies point to the importance of body water balance as a factor in survival of the Mexican bean beetle, *Epilachna varivestis* Mulsant. Regardless of humidity, temperatures from 22 to 27°C were favourable to Mexican bean beetle survival, but 37°C killed all stages of Mexican bean beetle within 40 h (Sweetman & Fernald, 1930). However, on *Phaseolus* in the field, Eddy & McAlister (1927) and Howard (1921) noted that high temperature and low humidity reduced survival. Marcovitch & Stanley (1930) found that large larvae and adults survived *c.* 25 h at 37.5°C and 80% relative humidity (r.h.) without access to host foliage, whereas small larvae died within 8 h. At both 40 and 100% r.h., these survival times were halved.

Miller (1930) investigated survival of adults and larvae on *Phaseolus* under extreme temperatures (37.5–42.5°C) and varying r.h. (0–100%) without food or water for 3 h. They measured 100% survival at 37.5°C, 88% at 39.5°C, and less than 10% survival at 41.5°C, regardless of humidity.

Sprenkel & Rabb (1981) modified the micro-climate of field cages containing soybean and found that small differences in mean daily maximum temperature from 34.2 to 38.1°C significantly decreased the survival of eggs, larvae, and adults.

In glasshouse and laboratory experiments, adult longevity was affected by temperature, humidity, and their interaction. Longevity increased with increasing humidity and decreasing temperature (Kitiyama *et al.*, 1979). Further studies by Wilson (1979) and Wilson *et al.* (1982) demonstrated strong effects of host phenology, host, temperature and humidity, as well as many interactions, on larval survival. Mexican bean beetle reared on soybean compared to those on *Phaseolus* species were less tolerant of high temperature and low humidity conditions. Water loss rates are higher and water gain rates lower for larvae feeding on soybean, with recuperation of partially desiccated larvae more rapid on *Phaseolus* (Wilson *et al.*, 1982).

Wilson (1981) compared the percentage of body weight gained per minute for Mexican bean beetle larvae, after a partial desiccation, on soybean and lima bean during a 30 min recuperation period. She found a significantly higher rate on lima bean than on soybean. She also demonstrated that percentage body weight gain per minute on lima bean was influenced by temperature.

If one considers water loss in general, the rate of evaporation from any wet surface is theoretically proportional to the vapour pressure deficit (VPD) of the air near the source, known also as the saturation deficit (Ramsay, 1935; Cloudsley-Thompson, 1959). However, Ramsay (1935) and Loveridge (1968) investigating water loss in *Periplaneta americana* and *Locusta migratoria*, respectively, found that as vapour pressure deficit increased, the rate of evaporation did not increase linearly, but approached an asymptotic limit, perhaps the result of spiracular closure (Bursell, 1974).

Diet and rearing temperature affect the body lipids of many species of insects (Fraenkel & Hopf, 1940; Schaefer, 1968; Ahearn, 1970). Ramsay (1935) found that temperature has little effect on water loss in insects when below a specific temperature, the transition point. When above

* Present address: Fayetteville State University, Department of Mathematics and Computer Science, Fayetteville, North Carolina, U.S.A.

† Present address: Biokentics, P.O. Box 13333, Research Triangle Park, North Carolina, U.S.A.

Correspondence: Dr R. E. Stinner, Department of Entomology, Box 7643, North Carolina State University, Raleigh, North Carolina 27695, U.S.A.

this point, water loss increases rapidly with rising temperature. Wilson (1981) determined that this transition temperature is *c.* 36°C for soybean-fed Mexican bean beetle larvae and 37°C when fed lima bean.

The surface:volume ratio of an individual becomes progressively greater the smaller the size. Wilson (1981) showed that the water loss rate (% per min) increased as body size decreased for Mexican bean beetle larvae feeding on both soybean and lima bean.

The above suggests that the following factors are involved in determining water loss rates for larval Mexican bean beetle: host plant, temperature, vapour pressure deficit and body size (weight). Under conditions of high vapour pressure deficit, Wilson (1981) provided water loss rates for Mexican bean beetle larvae feeding on lima bean and soybean for a range of temperatures from 27 to 42°C.

To accurately model water loss, and also to be more certain of several assumptions, additional data were required at lower vapour pressure deficits. As part of a study on the influence of water balance on the population dynamics of Mexican bean beetle, this paper describes the additional experimentation necessary and the development of dynamic models for water gain rate, evaporative water loss rate and frass water loss rate, the three major components of water balance in larval Mexican bean beetle.

Materials and Methods

Overwintered Mexican bean beetle adults were collected from a lima bean field on the N.C. State University campus, Raleigh, North Carolina, and then established in the glasshouse (20–30°C, 50–90% r.h, LD 14:10 h photocycle), with both larvae and adults maintained separately on soybean (var. Essex) and lima bean (var. Fordhook & Henderson).

Larvae of different sizes were chosen randomly from each glasshouse host. All larvae were given access to their respective host and it was assumed that they were at maximum water content prior to experimentation.

Water gain. A factorial experiment was conducted using the following independent variables: two hosts (soybean, var. Essex, lima bean, var. Fordhook & Henderson), three larval sizes (small, 0–15 mg for lima, and 0–10 mg for soybean; medium, 15–30 mg for lima and 10–25 mg for soybean; large, 30–40 mg for lima and 25–35 mg for soybean), and four temperatures (20, 27, 33, 37°C, all ±20°C).

Relative humidity was maintained at 50–70%, with a L:D 16:8 h photocycle for all treatments. For each larval size, host and temperature, eight larvae were chosen at random. Each larva was weighed, placed on a bouquet of trifoliate leaves held in a water pic in a waxed cardboard container (9.7 diameter × 6.4 cm high) with a plastic lid having numerous pin holes. Similar foliage was also collected for determination of foliage water content. A water pic is a plastic 'test tube' (8 cm long × 1.3 cm diameter), pointed at one end, with a rubber septum at the other. The septum contains a 1.5 mm diameter hole through which a leaf petiole can be pushed into water contained in the pic. These containers are inexpensive and readily available from all florists and florist suppliers in the U.S.A.

To reduce experimental error, a block design was added to the factorial. The experimental chamber was divided into two blocks (upper and lower). One container of each size larva and host was placed in each block randomly; thus, two trials were run concurrently for a given temperature. This procedure was repeated four times per temperature for a total of eight replicates per treatment combination.

After 24 h, each larva was removed and weighed a second time. The area of foliage eaten was then calculated by tracing the area eaten and computing this area using a digitizing table attached to an Apple II computer. Calculation of water ingestion was accomplished by the following procedure.

Seven foliage samples (1.27 × 1.27 cm) were taken from randomly selected leaves of each host in the glasshouse, fresh weighed, dried, and dry weighed to calculate mean foliage water (mg/cm^2) as:

$$\text{foliage water} = (\text{wet weight} - \text{dry weight})/\text{area}.$$

Since larvae leave a lacy network of tissue while feeding, three samples (1.27 × 1.27 cm) of this tissue were also collected and weighed as above to calculate water in remaining leaf tissue. The total water ingested by a larva was then calculated as (foliage water/cm^2 – remaining tissue water/cm^2) times the area eaten (cm^2).

To express this water gain (intake in grams) as a percentage/min, the mean body weight (BW) of the larva was calculated as (initial weight + final weight)/2. The water gain (WG), expressed as %/minute, is

$$\text{WG} = 100^*\text{total water gain per day}/(\text{BW}^*1440\,\text{min/day}).$$

For an initial model of water intake in the Mexican bean beetle, it was assumed that there was no water vapour absorbed directly, and that free water (e.g. dew) was not

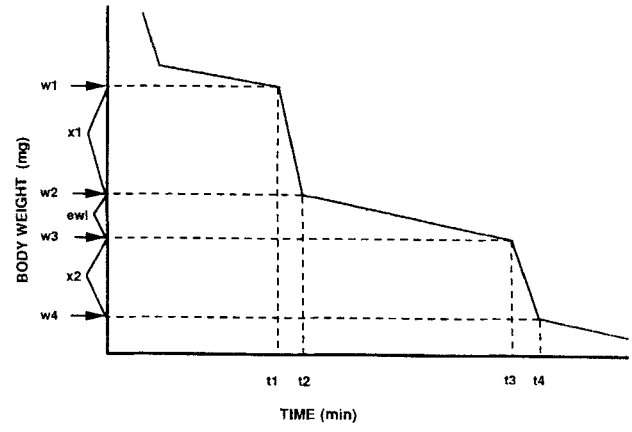

Fig. 1. Example of strip chart recording of water loss for larval Mexican bean beetle. See text for explanation of variables measured.

available. Therefore, food was the only component of water gain considered.

Water loss. Water loss rates were obtained by placing a single live larva on the weighing platform of a Cahn model 27 electrobalance (Wilson, 1981). The balance housing is separate from the electronics in this model, permitting its placement in a constant environment chamber which was free from mechanical vibration (Wilson & Stinner, 1981). The larvae spent most of the 30–40 min weighing interval walking upside down on the outer edge of the aluminium disc. Analogue readings from the balance were recorded to calculate the weight loss per initial body weight per minute over a time interval which included both evaporative and frass water loss. Deposition of liquid frass droplets results in steep decreases in the recorded weight (Fig. 1). The rapid evaporation of the water from these frass droplets provided a measure of the water loss in frass.

From these recordings, it was possible to calculate both evaporative and frass water loss at follows:

(1) EWL = mg water lost per mg initial wet weight per minute × 100
= percentage evaporative water loss per minute
$= (100*ew_1/w_2)/(t_3 - t_2)$

(2) FWL_1 = frass water loss in first frass droplets (per cent/excretion). Since x_1 consists of both frass water loss and evaporative water loss, evaporative water loss during the frass water loss measurement interval must be subtracted to obtain FWL_1.
$FWL_1 = 100*(w_1 - w_2) - [ew_1/(t_3 - t_2)]*(t_2 - t_1)$

(3) FWL_2 = frass water loss in the second excretion (per cent/excretion). For the same argument as in (2),
$FWL_2 = 100*(w_3 - w_4) - [ew_1/(t_3 - t_2)]*(t_4 - t_3)$

(4) Tf = time between excretions
$Tf = t_3 - t_1$

Chamber temperatures of 27, 34, 36, 38 and 40°C were used, with vapour pressure deficits ranging from 10.7 to 36.9 mbars.

Experimental Results

Water gain. At the lowest temperature, larvae on both hosts appeared to eat normally and gained weight during the 24 h experiment (Table 1). At the other temperature extreme (37°C), most of the larvae spent their time actively moving, with little feeding. At this temperature, over 90% of the larvae actually lost weight during the experiment, due to a net water loss.

Water gain from food ingestion is strongly affected by larval size, temperature, and host. Thus, it was necessary to develop a model of water gain as a function of these independent variables.

Water loss. Even at low vapour pressure deficits, the evaporative water loss rate (EWL) decreased as body

Table 1. Mean water gain (%/min) for larvae of three size at four temperatures when fed on lima beans or soybeans. Standard deviations are given in parentheses. Sample size is eight larvae for each treatment combination.

Host larval size (mg)	Temperature (°C)			
	20	27	33	37
Lima beans				
<15	0.1157 (0.0124)	0.1872 (0.0463)	0.2699 (0.0738)	0.0647 (0.0123)
15–30	0.0840 (0.0200)	0.1655 (0.0433)	0.2071 (0.0646)	0.0290 (0.0135)
30–40	0.0574 (0.0148)	0.0751 (0.0318)	0.1203 (0.0345)	0.0237 (0.0110)
Soybeans				
<10	0.0853 (0.0064)	0.1938 (0.0238)	0.2241 (0.0100)	0.0230 (0.0140)
10–25	0.0810 (0.0057)	0.1147 (0.0372)	0.1581 (0.0330)	0.0053 (0.0031)
25–35	0.0465 (0.0106)	0.0850 (0.0208)	0.1328 (0.0480)	0.0037 (0.0038)

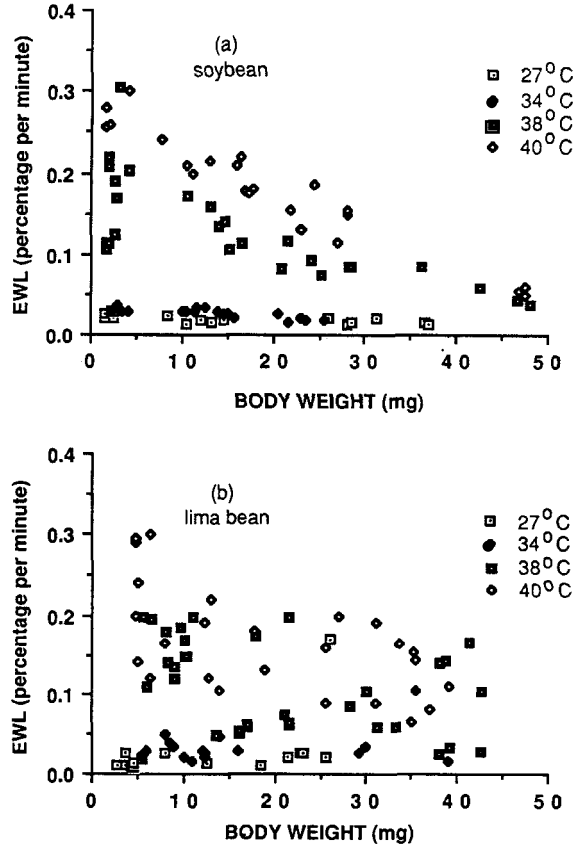

Fig. 2. Evaporative water loss versus larval body weight at different temperatures on (a) soybean and (b) lima bean.

weight increased for all temperatures (Figs 2a, b), regardless of host. These results are in agreement with Wilson (1981) under high vapour pressure deficit. In general, EWL was higher for soybean-reared larvae than for those fed on lima bean. For both hosts there was a transition temperature, above which larvae became agitated, moved around rapidly, and EWL increased rapidly with temperature (Figs 3a, b). EWL was low for temperatures below the transition temperature. The transition temperature for soybean Mexican bean beetle larvae was between 35 and 36°C, while for lima bean it was at 36–37°C. As vapour pressure deficit increased, EWL did not increase linearly, but asymptotically (Figs 4a, b).

One set of questions which must be answered is whether there are relations between any of the frass water loss variables and host plant. Analyses of variance for FWL_1, FWL_2, and Tf all yielded no significant effect of host on these variables.

Another question of major concern was whether the test conditions affected frass water loss as the experiments continued. To test this, water loss in frass from the first (FW_1) and second (FW_2) excretions were compared. A paired t test (44 df) was not significant at the 5% level, thus allowing the use of all measured values for frass water loss (FWL), regardless of timing (first or second excretion). However, it is important to note that there is evidence (Giroux, unpublished data) that FWL is related to body

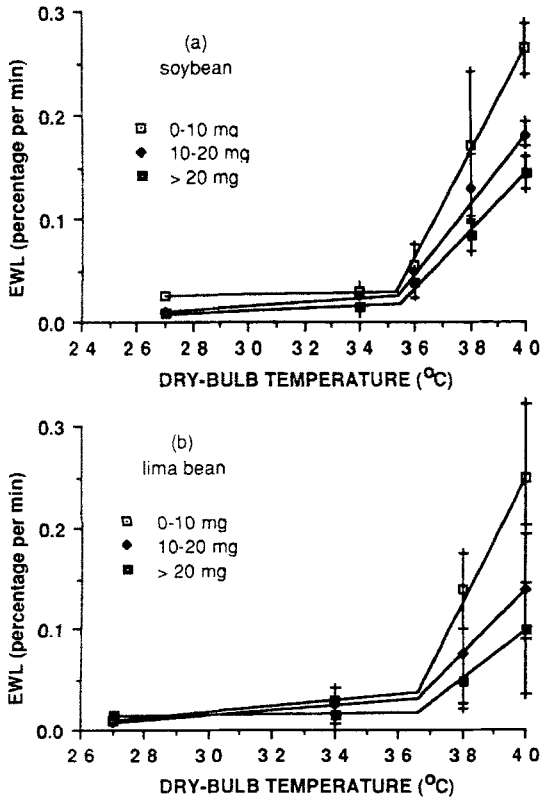

Fig. 3. Mean evaporative water loss versus dry-bulb temperature for Mexican bean beetle larvae of different body weights on (a) soybean and (b) lima bean. Vertical bars are standard deviations.

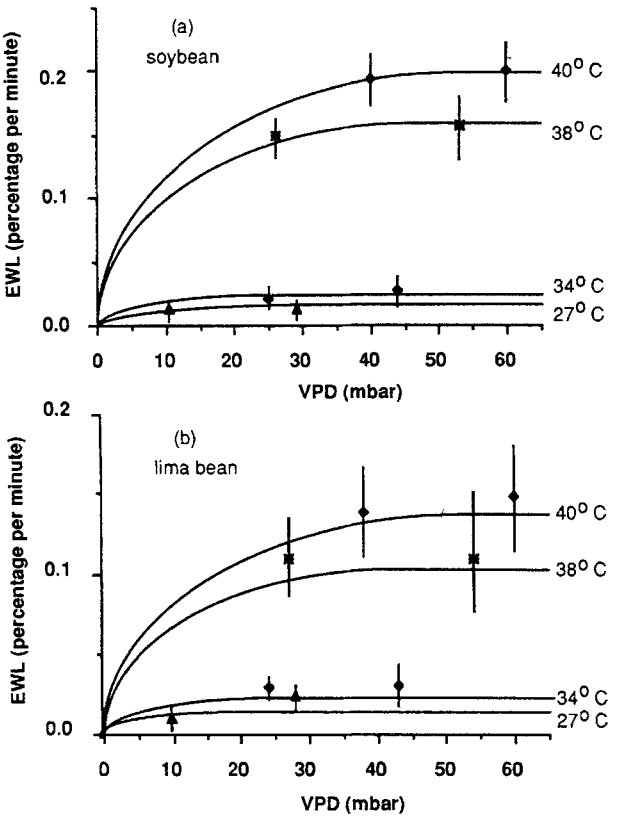

Fig. 4. Mean evaporative water loss versus vapour pressure deficit at different temperatures on (a) soybean and (b) lima bean. Vertical bars are standard deviations.

water content. The experimental times used here were sufficiently short such that this relationship could not be determined.

A final question which arises is whether there is a dependence between FWL and Tf. That is, whether frass water loss is related to the time between excretions. From a correlation analysis for FWL and Tf, the null hypothesis $r = 0$ could not be rejected ($r = 0.0717$, $P = 0.51$). Therefore FWL and Tf can be considered independent.

Model development

Water gain. As a function of temperature for each larval size and host (Figs 5a, b), water gain can be mimicked by a modified Beta function of the form:

$$WG = g^*(z^{**}c)^*[(1-z)^{**}d] \qquad \text{(Eq. 1)}$$

where WG = water gain (percentage/min), g, c, d = estimated parameters, and $z = (37.001 - \text{temperature})/(37.001 - 8.0)$

The values 37.001 and 8.0 were estimated by grid search to be the maximum and minimum temperatures. respectively, at which feeding (water ingestion) could occur. Since it was obvious that parameter g (a multiplier) would be a function of body size (BW), the simplest form,

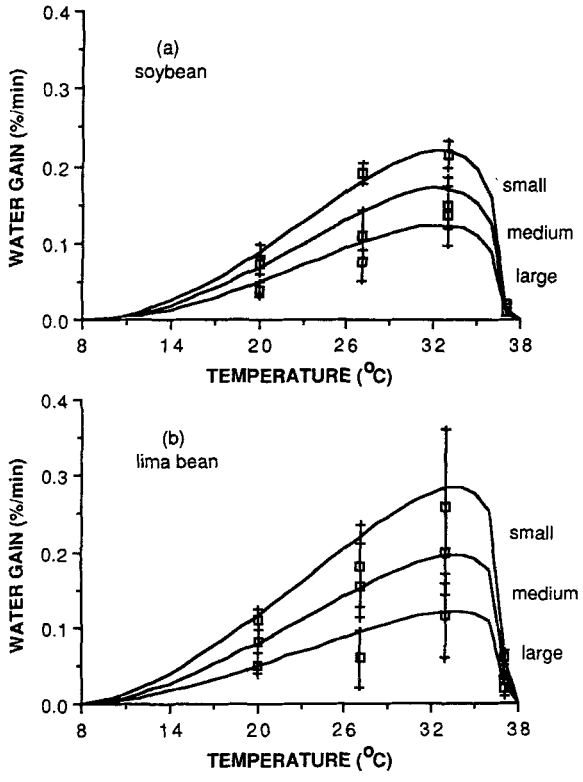

Fig. 5. Water gain versus dry-bulb temperature for Mexican bean beetle larvae of different sizes on (a) soybean and (b) lima bean. Vertical bars are standard deviations.

$g = a + b$*BW, was used. Thus for each host, water gain was described as:

$$WG = (a + b*BW)*(z**c)*[(1 - z)**d] \quad \text{(Eq. 2)}$$

Parameters were estimated initially for each host size by linearization of Eq. 1, followed by simple linear regression. The parameter g was then regressed against body size (mg). With these initial estimates, nonlinear regression (PROC NLIN; SAS Institue, 1985) was then used separately for each host to estimate parameters for Eq. 2. The final equation for each host was as follows:

Lima bean

$$WG = (0.6415 - 0.0116*BW)*(z**0.2150)*[(1 - z)**1.6455] \quad \text{(Eq. 3)}$$

Soybean

$$WG = (0.6891 - 0.0111*BW)*(z**0.3870)*[(1 - z)**2.0045] \quad \text{(Eq. 4)}$$

To test goodness-of-fit, predicted values were regressed for WG against observed values for the lima bean host and the soybean host. In both cases, $r^2 > 0.95$, with the slope not significantly different from 1 and the intercept not significantly different from zero.

Evaporative water loss. The initial model for EWL was based on a body weight of c. 5 mg. An approximation for EWL (Figs 5a, b) is as follows:

$$EWL = f(T)*[1 - \exp(-c*VPD)] \quad \text{(Eq. 5)}$$

where T = temperature (°C), VPD = vapour pressure deficit (mb), and c = estimated curvature parameter.

To determine the shape of $f(T)$, the data for each host were partitioned at the transition temperature. Both above and below the transition point, and for each host, a linear function fit the relationship between EWL and temperature, yielding:

$$EWL = (a + bT)\,[1 - \exp(-c*VPD)] \quad \text{(Eq. 6)}$$

where a, b are parameters estimated independently for each host temperature domains above and below the transition point.

Both our and Wilson's (1981) data indicate that body size also affects EWL. It was assumed that the effect of body weight on EWL is proportional to body weight (B); thus

$$EWL = PL*(a + bT)*[1 - \exp(-c*VPD)] \quad \text{(Eq. 7)}$$

where PL = effect of B, compared with 5 mg larvae.

Since limited data were available, the largest range of EWL versus body weight at 40°C was used. Under identical conditions, for 5 mg larvae, the mean EWL was 0.2140, while for 25 mg larvae the mean EWL was 0.1605, or 0.75 of the EWL for 5 mg larvae. Thus, setting the proportional loss (PL) for 5 mg larvae at 1.0 and the PL for 25 mg larvae at 0.75, and assuming a linear response, the effect of body weight (B) on PL can be expressed as:

$$PL = 1.0625 - 0.0125*B \quad \text{(Eq. 8)}$$

Initial parameter values were then used for nonlinear regression (PROC NLIN: SAS Institute, 1985) to reestimate temperature and vapour pressure deficit associated parameters. The final EWL model is given by:

$$EWL = (1.0625 - 0.0125*B)*(k_1 + k_2*T)*[1 - \exp(-k_3*VPD)] \quad \text{(Eq. 9)}$$

The values for $k_1 - k_3$ for each host and temperature range are given in Table 2. A regression of predicted versus observed EWL for all hosts, temperatures, and vapour pressure deficits yielded an $r^2 = 0.999$, with the intercept not different from zero, and the slope not different from one.

Frass water loss (time between excretions). Although the mean for Tf was 11.1 min, there was wide variability, such that for a stochastic model of water loss, a distribution for Tf is necessary. An easily fit model for the cumulative distribution of Tf (Fig. 6) is given by:

$$P(\text{Tf}) = (1 - z)**(a*z**b)$$
$$\text{and } 0 < P(\text{Tf}) = < 1 \quad \text{(Eq. 10)}$$

where $z = (34 - \text{Tf})/(34 - 0)$ (34 and 0 are the maximum and minimum observed times between excretions); a, b are estimated parameters.

Nonlinear regression (PROC NLIN; SAS Institute, 1985) was used to estimate a and b as 1.0613 and 2.0383, respectively. For comparing predicted and observed values of P(Tf), a simple regression ($n = 9$) was used ($r^2 = 0.995$). The intercept was not significantly different from zero, and the slope was not significantly different from one.

Table 2. Parameter estimates for EWL model.

Host	Temperature	k_1	k_2	k_3
Soybean	<36°C	−0.0245	0.001683	0.103406
	≥36°C	−1.5736	0.044869	0.064569
Lima	<37°C	−0.0182	0.001630	0.050588
	≥37°C	−1.0914	0.030739	0.064476

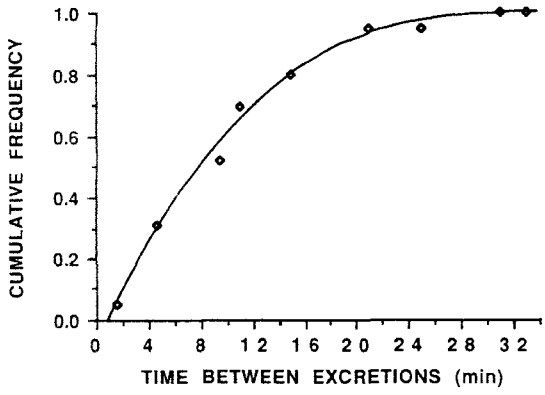

Fig. 6. Cumulative relative frequency of time between two excretions (*Tf*): ◊, observed; ——, predicted.

Frass water loss (loss per excretion). Given no evidence to indicate otherwise, it was assumed that FWL is dependent, at most, on body water content (WC). The data of Wilson (1981) were available and contained, for numerous larvae, the fresh body weight (FWT), dry body weight (DWT), and water loss in frass (expressed as per cent of FWT per minute). With WC calculated as (FWT − DWT)/FWT, a regression was calculated for frass water loss per minute (FWTM) against WC. The regression was nearly significant at the 5% level ($P = 0.074$), and so was accepted, providing the following relationship:

$$\text{FWTM} = -0.0473 + 0.1003*\text{WC} \qquad \text{(Eq. 11)}$$

It was then necessary to calculate FWL (percentage frass water loss per excretion) as:

$$\text{FWL} = \text{FWTM}*(\text{mean Tf}) = \text{FWTM}*11.1 \qquad \text{(Eq. 12)}$$

$$\text{FWL} = -0.5250 + 1.1133*\text{WC} \qquad \text{(Eq. 13)}$$

Given models for water gain (WG) and water loss (EWL and FWL), one can calculate the water balance, or body water content WC, at any time as $\text{WC} = \text{WC}_0 - \text{EWL} - \text{FWL}$.

The range of WC in Wilson's (1981) data was *c.* 0.72–0.84, which is almost identical to the range observed in these experiments. In preliminary measurements, under optimal conditions, a maximum water content in larvae of 0.86 was observed; for this WC, which occurred with larvae fed lima beans, the mean FWL measured was 0.442, compared with a model prediction based on Wilson's data of 0.432.

If dehydrated larvae can conserve water by decreasing the frass water content or defecation frequency, then the accuracy of this model may be reduced.

To use and assess this model of water balance in simulating its effect on survival under variable conditions, it is necessary to first relate body water content to survival and to validate the results against independent data collected under variable temperatures and moistures for both hosts. Such an effort is beyond the scope of this paper and is reported elsewhere (Chu *et al.*, 1992).

Acknowledgment

This research was partially supported by a grant from the North Carolina Soybean Producers Association.

References

Ahearn, G.A. (1970) The control of water loss in desert tenebrionid beetles. *Journal of Experimental Biology*, **53**, 573–595.

Bursell, E. (1974) Environmental aspects – humidity. *The Physiology of Insecta* (ed. by M. Rockstein), vol. II, pp. 44–84. Academic Press, New York.

Chu, P.-C., Stinner, R.E. & Giroux, K.J. (1992) A dynamic model of larval Mexican bean beetle survival as a function of body water balance. *Physiological Entomology*, **17**, 316–324.

Cloudsley-Thompson, J.L. (1959) Studies in diurnal rhythms. IX. The water-relations of some nocturnal tropical arthropods. *Entomologia Experimentalis et Applicata*, **2**, 249–256.

Eddy, C.O. & McAlister, L.C., Jr (1972) The Mexican bean beetle. *South Carolina Agriculture Experiment Station Bulletin*, **236**, 38pp.

Fraenkel, G. & Hopf, H.S. (1940) The physiological action of abnormally high temperatures on poikilothermic animals. I. Temperature adaptation and the degree of saturation of the phosphatides. *Biochemistry Journal*, **34**, 1085–1092.

Howard, N.F. (1921) The Mexican bean beetle in its bearing on Florida citrus growing. *Florida State Plant Board Quarterly Bulletin*, **6**, 15–24.

Kitiyama, K., Stinner, R.E. & Rabb, R.L. (1979) Effects of temperature, humidity and soybean maturity on longevity and fecundity of the adult Mexican bean beetle, *Epilachna varivestis* Mulsant. *Environmental Entomology*, **8**, 458–464.

Marcovitch, S. & Stanley, W.W. (1930) The climatic limitations of the Mexican bean beetle. *Annals of the Entomological Society of America*, **23**, 666–686.

Miller, D.F. (1930) The effect of temperature, relative humidity and exposure to sunlight upon the Mexican bean beetle. *Journal of Economic Entomology*, **23**, 945–955.

Ramsey, J.A. (1935) The evaporation of water from the cockroach. *Journal of Experimental Biology*, **12**, 373–378.

SAS Institute (1985) *SAS User's Guide: Statistics*, 5th edn. SAS Institute, Cary, N.C.

Scheafer, C.H. (1968) The relationship of the fatty acid composition of *Heliothis zea* larvae to that the of its diet. *Journal of Insect Physiology*, **14**, 171–178.

Sprenkel, R.K. & Rabb, R.L. (1981) Effects of micrometeorological conditions on survival and fecundity of the Mexican bean beetle in soybean fields. *Environmental Entomology*, **10**, 219–221.

Sweetman, H.L. & Fernald, H.T. (1930) Ecological studies of the Mexican bean beetle. *Massachusetts Agriculture Station Report*, **261**.

Wilson, K.G. (1979) Effects of temperature, relative humidity and host-plant type on larval survival of the Mexican bean beetle, *Epilachna varivestis* Mulsant. M.S. dissertation, North Carolina State University, Raleigh, North Carolina.

Wilson, K.G. (1981) Aspects of the physiological ecology of the Mexican bean beetle, *Epilachna varivestis* Mulsant. Ph.D. dissertation, North Carolina State University, Raleigh, North Carolina.

Wilson, K.G. & Stinner, R.E. (1981) Inexpensive, multiple-unit environmental chambers with temperature and humidity control. *Annals of the Entomological Society of America*, **74**, 560–562.

Wilson, K.G., Stinner, R.E. & Rabb, R.L. (1982) Effects of temperature, relative humidity, and host plant on larval survival of the Mexican bean beetle, *Epilachna varivestis* Mulsant. *Environmental Entomology*, **11**, 121–126.

Accepted 12 December 1991