

DISCONTINUOUS CARBON DIOXIDE RELEASE IN THE EASTERN LUBBER GRASSHOPPER *ROMALEA GUTTATA* AND ITS EFFECT ON RESPIRATORY TRANSPIRATION

NEIL F. HADLEY and MICHAEL C. QUINLAN

Department of Zoology, Arizona State University, Tempe, AZ 85287-1501, USA

Accepted 16 December 1992

Summary

Ventilatory patterns were examined in the Eastern lubber grasshopper *Romalea guttata* and correlated with respiratory transpiration. Discontinuous release of CO₂ was only observed in quiescent individuals during their scotophase. Interburst periods (spiracles closed) alternated with bouts of CO₂ emission and O₂ consumption (burst phase); no true 'flutter' phase was observed. Cycle duration decreased with increasing temperature in both hydrated and dehydrated individuals. Metabolic rates for this large, sluggish species are lower than those reported for smaller and/or more active grasshoppers. Water loss rates fell within an expected range of values for arthropods from mesic environments. Respiratory transpiration accounted for only 1.9–3.9% of the total water loss between 15 and 30°C and for only 7% of the water loss during the burst phase of the cycle. These data indicate that the cyclic release of CO₂ in this adult insect does not result in substantial savings of water.

Introduction

Terrestrial arthropods lose water through several parallel pathways that include the cuticle, spiracles or book lungs, and oral and anal openings. Although cuticular transpiration is greatly reduced by lipids associated with the outermost layer of the cuticle (epicuticle), the cuticle is still believed to be the primary water-efflux pathway because of the large surface area to body-volume ratio exhibited by insects and arachnids (Edney, 1977; Hadley, 1986, 1989). For most species, the contribution of respiratory transpiration to total water loss is either unknown or uncertain. Until quite recently, investigators typically estimated water loss associated with gas exchange by subtracting water loss in freshly killed animals whose spiracles were blocked (i.e. cuticular transpiration) from rates obtained from untreated live animals. Although conceptually sound, the validity of data obtained in this manner was often tainted by the fact that the cuticular permeability of dead animals was not an accurate reflection of their permeability while alive (Edney, 1977; Loveridge, 1980). Correlations obtained from independent plots of gravimetrically determined transpiration rates and manometrically determined gas exchange suggest that

Key words: discontinuous ventilation, gas exchange, grasshopper, respiration, spiracles, water loss, *Romalea guttata*.

respiratory transpiration becomes an increasingly important contributor to total water loss at temperatures at which metabolic rates also increase dramatically (Ahearn, 1970; Hadley, 1970); however, this observation was never verified experimentally.

The discovery by Kestler (1971, 1978) that the cyclic release of CO₂, initially described for diapausing pupae by Schneiderman and his colleagues (Schneiderman and Williams, 1953, 1955; Schneiderman, 1960; Levy and Schneiderman, 1966*a,b,c*), is also widespread in many adult insects has stimulated renewed interest in respiratory transpiration. The discontinuous ventilation (DV) cycle can be divided into three stages: (1) spiracles closed (C), during which time little, if any, gas exchange takes place; (2) the flutter phase (F), during which the spiracles open slightly on either an intermittent or a continuous basis, allowing some O₂ to enter but little egress of CO₂; and (3) spiracles open (O), allowing a proportionally high rate of CO₂ release. In species capable of abdominal ventilation, pumping supports diffusive release of CO₂ during the burst phase (Miller, 1981; Kestler, 1991). By measuring transpiration during that period of the ventilatory cycle when the spiracles are fully open and comparing it with transpiration measured during the closed and flutter phases, one can calculate the amount of water lost as a result of gas exchange (it is assumed that cuticular transpiration remains constant throughout the ventilatory cycle).

In this study we correlated ventilatory patterns with water loss in the lubber grasshopper *Romalea guttata*, a large species that occurs in mesic/hygic habitats throughout the southeastern United States. Preliminary tests indicated that this species exhibits discontinuous CO₂ release. Using an air-flow system that measures O₂, CO₂ and water loss simultaneously, we show how activity, hydration state and temperature influence ventilatory patterns and, in turn, respiratory transpiration in this species. The ratio of cuticular to respiratory transpiration at various temperatures is also reported.

Materials and methods

Animal collection and maintenance

Romalea guttata Serville were collected as nymphs from Immokalee, Collier County, Florida, in May 1991 and returned to the laboratory by overnight mail. Grasshoppers were reared in a controlled environment room at 30°C and 50% relative humidity (RH) under a 14h:10h L:D cycle. The insects were fed lettuce, collard greens, spinach, sweet potato and oatmeal *ad libitum*. Only male *R. guttata* (mean mass 2.921±0.495g; s.d., *N*=34) were used in the present experiments as the females were too large (approximately 6g) for the test apparatus.

Gas analysis

Evaporative water loss (EWL), \dot{V}_{O_2} and \dot{V}_{CO_2} were determined using a flow-through system described in Hadley *et al.* (1991). Test animals were confined in a small (83ml) temperature-controlled (±0.1°C) respirometer. Air flow (dry, <0.5% RH) through the respirometer was regulated by a mass flow controller (Brooks). Outflow from the

respirometer was directed sequentially to the water sensor (see Hadley *et al.* 1982, for details), the CO₂ analyzer (Anarad 411) and the oxygen analyzer (Ametek S-3A/II).

Rates of O₂ uptake and CO₂ loss were determined using the equations of Hill (1972) and Hadley *et al.* (1991), respectively. EWL was determined from hygrosensor calibration curves such as those discussed in Hadley *et al.* (1982). All volumes were corrected to STPD. Surface area corrections were calculated using the standard equation relating body mass to area ($SA=12M^{0.67}$, where SA is area in cm² and M is body mass in g).

Experimental protocol

The O₂ analyzer is less sensitive and more unstable than either the CO₂ or water analyzers. Flow rates low enough to provide \dot{V}_{O_2} values are often not optimal for the other two instruments, and the O₂ analyzer tends to drift during extended runs. To accommodate these instrument characteristics, each run was divided into two phases: an initial, low-flow phase (approximately 2h at 75mlmin⁻¹) with all three variables recorded, and a second phase (approximately 2h) at high flow rate (187mlmin⁻¹) during which only \dot{V}_{CO_2} and EWL were measured. All experiments were conducted in complete darkness. Test specimens were frequently checked visually with a hand-held infrared viewer (Find-R-Scope, Edmund Scientific).

Two objectives of the study were to assess the effects of hydration state and temperature on gas exchange and water loss in *Romalea guttata*. To facilitate statistical analysis, experiments ('runs') were organized into groups called 'blocks'. Blocks were designed to minimize biases associated with ageing and treatment sequence. Each block included runs from six animals, of comparable age, tested 'hydrated' and 'dehydrated' (see below). Two animals were assigned to each temperature (15, 25 and 30°C), and both members of a temperature subgroup were run each evening during the animals' scotophase (22:00–06:00h). One member of each subgroup was tested hydrated and the other dehydrated. Three days later the grasshoppers were retested in the opposite hydration state. Each block required 6 days to complete and included a total of 12 runs; blocks were repeated as necessary to obtain the required sample size for each temperature.

Animal pretreatment

Grasshoppers to be run 'hydrated' were isolated from culture 12h before testing, but kept at 30°C and 50% RH. Water provided during the fasting period was rarely, if ever, consumed by the insects. 4h before testing, the animals were placed in glass cylinders similar in size to the respirometer and transferred to the run temperature (ambient RH). This 4h adjustment period was included to minimize thermal shock effects. Grasshoppers to be tested 'dehydrated' were removed from culture 36h before the run started and placed in a large desiccator (0% RH). These animals were desiccated for the next 32h; treatment during the final 4h was identical to that of the hydrated animals. A minimum of 30h in culture was allowed for recovery between runs.

Grasshoppers in both hydration groups experienced some mass loss during pretreatment. Animals in the 'hydrated' group showed a mean (\pm s.d.) loss of $4.6\pm 2.13\%$ of their body mass during the pretreatment (excluding faecal loss). 'Dehydrated' animals,

in contrast, lost an average of $15.2 \pm 3.46\%$ of their body mass (sample size for both groups was 24).

Data collection and analysis

Output from the gas analyzers was simultaneously recorded on a stripchart recorder and a personal computer. The latter consisted of a data acquisition board (ADALAB-PC, Interactive Microware, Inc.) running under LABTECH NOTEBOOK software (version 5.0, Laboratory Technologies Corp.). Data were analyzed using the DATACAN acquisition and analysis package (Sable Systems, Salt Lake City, Utah). In cases where data were taken directly from stripchart recordings, a digitizing tablet (Kurta) was used to convert the data into computer-usable form.

All values are expressed as means \pm standard errors. Statistical comparisons were performed with a Student's *t*-test unless otherwise noted.

Results

Gas exchange in *Romalea guttata* is characterized by discontinuous periods of ventilation, especially in animals that are hydrated and tested at lower temperatures (Fig. 1). Periods when the spiracles are apparently closed and there is little or no gas exchange (interburst phase) alternate with periods during which the spiracles are open

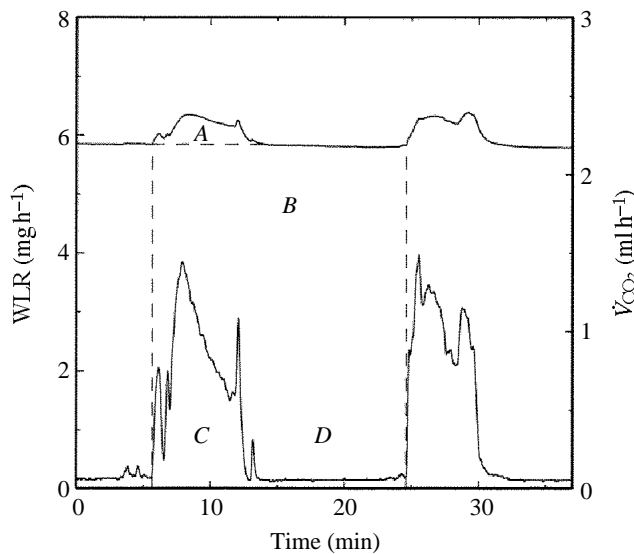


Fig. 1. A simultaneous recording of water loss (upper trace) and discontinuous CO_2 release (lower trace) in *Romalea guttata* at 25°C . The vertical dashed lines enclose one complete ventilatory cycle in which bursts of CO_2 release (C) alternate with periods of little or no CO_2 expiration (D). The dashed line separating the respiratory water loss peak (A) from the cuticular component (B) represents an interpolation between the two adjacent interburst periods.

and respiratory gases are exchanged (burst phase). Simultaneous measurements of O_2 consumption and CO_2 excretion indicate that the two gases exhibit parallel burst and interburst periods (Fig. 2). The respiratory quotient (RQ) for hydrated individuals (mixed diet) for the three test temperatures was 0.83 ± 0.023 ($N=21$). For dehydrated individuals, the mean RQ decreased to 0.71 ± 0.018 ($N=16$), reflecting a shift to fat metabolism when access to food was denied.

No true flutter phase uptake of O_2 is evident; however, tracings (Figs 1 and 2) typically show several smaller peaks caused by the exchange of small amounts of CO_2 and O_2 prior to the onset of the main burst phase. Both CO_2 and O_2 bursts are pronounced and account for approximately 90% of the total excretion/uptake of these two gases, respectively. During the interburst period, neither O_2 consumption nor CO_2 expiration drops to zero (Figs 1 and 2). This suggests that small amounts of these gases diffuse through incompletely closed spiracles during this time.

The duration of one complete cycle (interburst+burst) varies strongly with temperature but is unaffected by hydration state (Table 1). At $15^\circ C$, cycle durations average slightly less than 1h for hydrated animals. In contrast, cycle lengths are shortened by 46 and 73%, respectively, at 25 and $30^\circ C$. At 15 and $25^\circ C$, the duration of the interburst and burst phases for hydrated animals is about the same; however, at $30^\circ C$ the interburst period shows a much greater reduction relative to the duration of the burst period. Similar patterns are evident in dehydrated animals with cycle durations at 15 and $25^\circ C$ comparable to those for hydrated animals.

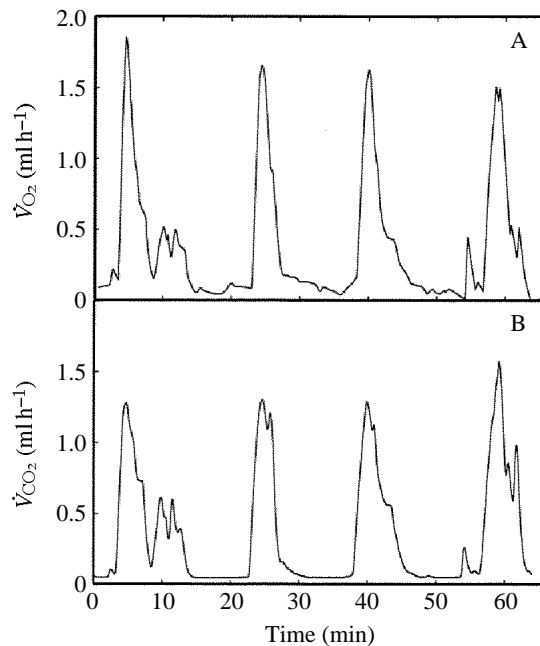


Fig. 2. Simultaneous measurement of oxygen consumption (A) and carbon dioxide excretion (B) in *Romalea guttata* at $25^\circ C$.

Not all individuals exhibit a pronounced discontinuous release of CO₂. The sample sizes given in Table 1 represent how many of the eight animals tested at each temperature exhibited cyclic CO₂ release. The DV cycle is less common in dehydrated animals and was not observed in this group at 30°C. Ventilation is highly variable even in hydrated animals (Fig. 3). At 30°C, patterns range from very regular bursts of CO₂ emission

Table 1. *Effect of temperature and hydration state on duration of phases of the ventilatory cycle in Romalea guttata*

Conditions	N	Interburst (h)	Burst (h)	Cycle (h)
Hydrated				
15°C	5	0.468±0.121	0.470±0.029	0.938±0.144
25°C	7	0.212±0.039	0.202±0.021	0.415±0.045
30°C	5	0.089±0.022	0.163±0.007	0.252±0.017
Dehydrated				
15°C	3	0.500±0.177	0.425±0.152	0.926±0.180
25°C	4	0.194±0.020	0.211±0.026	0.405±0.240
30°C	0	–	–	–

N values indicate the number of animals that showed cyclic CO₂ release (eight animals were tested at each temperature).

Values are mean ± S.E.

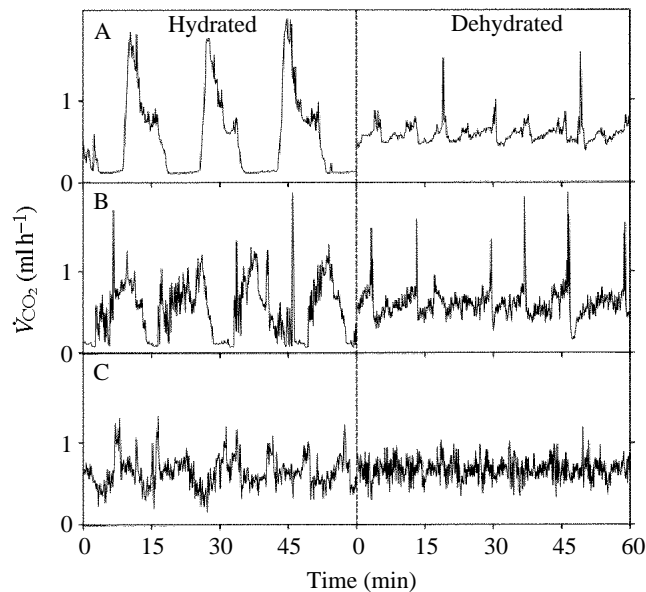


Fig. 3. Individual variation in the respiratory patterns of *Romalea guttata* and the effect of hydration state. Traces for three (A–C) hydrated animals are shown on the left. On the right are the respiratory patterns of animals A–C following a 15% loss of body mass caused by dehydration. All tests were conducted at 30°C.

Table 2. Effect of temperature and hydration state on water loss (mg) from cuticle and respiratory system of *Romalea guttata*

Conditions	N	Cuticular	Respiratory	Total
Hydrated				
15°C	5	4.36±0.725 (97.9%)	0.09±0.007 (2.1%)	4.46±0.727
25°C	7	3.74±0.838 (97.0%)	0.12±0.020 (3.0%)	3.85±0.856
30°C	5	3.29±0.299 (96.1%)	0.13±0.016 (3.9%)	3.42±0.310
Dehydrated				
15°C	3	2.84±0.907 (98.1%)	0.06±0.010 (1.9%)	2.90±0.913
25°C	4	2.19±0.372 (96.5%)	0.08±0.016 (3.5%)	2.27±0.384
30°C	0	–	–	–

Values are mean ± S.E.; numbers in parentheses represent the percentage of total water loss.

Values are 'per cycle'.

N values indicate the number of animals that showed cyclic CO₂ release (eight animals were tested at each temperature).

(Fig. 3A) to emissions that are essentially random (Fig. 3C). Dehydration to approximately 85% of the initial body mass in every case inhibits the magnitude and regularity of the CO₂ bursts (Fig. 3).

The close synchrony between H₂O and CO₂ bursts (Fig. 1) provides a means of separating cuticular and respiratory water loss. During interburst periods, respiratory water loss is assumed to be near zero. Thus, the area beneath the dashed line (labelled B, Fig. 1) represents cuticular transpiration, while the area beneath the curve (A) above the dashed line represents respiratory transpiration.

At all temperatures and hydration states, cuticular transpiration far exceeds respiratory

Table 3. Effect of temperature, dehydration state and hydration–dehydration treatment sequence on water loss rates (WLR) and \dot{V}_{CO_2} for *Romalea guttata* during periods of high air-flow rates

Temperature (°C)	Treatment sequence	N	WLR (mg cm ⁻² h ⁻¹)		\dot{V}_{CO_2} (ml g ⁻¹ h ⁻¹)	
			Hydrated	Dehydrated	Hydrated	Dehydrated
15	Hy De	4	0.200±0.009	0.127±0.015	0.054±0.006	0.047±0.002
	De Hy	4	0.199±0.020	0.160±0.017	0.050±0.006	0.049±0.002
	Pooled	8	0.199±0.010	0.143±0.012	0.052±0.004	0.048±0.001
25	Hy De	4	0.414±0.042	0.271±0.018	0.141±0.013	0.127±0.010
	De Hy	4	0.329±0.035	0.244±0.032	0.124±0.009	0.127±0.007
	Pooled	8	0.371±0.030	0.258±0.018	0.133±0.008	0.127±0.006
30	Hy De	4	0.644±0.068	0.398±0.032	0.204±0.020	0.191±0.005
	De Hy	4	0.604±0.043	0.376±0.049	0.214±0.016	0.205±0.008
	Pooled	8	0.624±0.038	0.386±0.027	0.209±0.012	0.198±0.005

Values are means ± S.E.

Hy, hydrated; De, dehydrated.

transpiration, with cuticular transpiration never accounting for less than 96% of the total water loss per cycle under any condition (Table 2). The respiratory contribution does, however, increase slightly with increasing temperatures in both hydrated and dehydrated animals. The total amount of water lost per cycle decreases with increasing temperature (Table 2) but, because the rate increases, the water loss rates for both groups of animals are higher at higher temperatures (see below).

Mean water loss rates (WLR) and \dot{V}_{CO_2} for hydrated compared with dehydrated animals at the three test temperatures are summarized in Table 3. These values are based on rates during the third and fourth hours of the experiments, at which time flow rates were maximal. WLR increased significantly with temperature [15–30°C; analysis of variance (ANOVA) $P < 0.001$] in both hydrated (3.14-fold) and dehydrated (2.70-fold) animals, with mean values significantly higher (t -test, $P < 0.002$) for hydrated animals at each test temperature. The hydration sequence used to precondition test animals had little effect on final WLR values. \dot{V}_{CO_2} also increased significantly with temperature, with both hydrated and dehydrated animals exhibiting over a fourfold increase between 15 and 30°C (ANOVA, $P < 0.001$). However, hydration state did not significantly affect \dot{V}_{CO_2} at any of the three test temperatures.

Discussion

The Eastern horse lubber grasshopper *Romalea guttata* is a resident of the humid Gulf Coast region of the United States, ranging from central North Carolina southward to the tip of Florida and westward to the eastern edge of Texas. It prefers swamp edges, weedy fields and the borders of cultivated fields. The adults are large, flightless, slow-moving and polyphagous; they require no free water. During the day they are subjected to warm, humid conditions; at night they roost on low-growing bushes and shrubs.

The water and metabolic relationships of *R. guttata* identified in this study are consonant with many aspects of its life history. Resting metabolic rates at 25 and 30°C (0.156 and 0.246 ml O₂ g⁻¹ h⁻¹, respectively) for this large, relatively sluggish grasshopper are lower than those reported for other acridids. For example, *Arphia conspersa* (0.381g) and *A. pseudonietana* (0.328g), both capable of flight, exhibit metabolic rates of 0.823 and 0.774 ml O₂ g⁻¹ h⁻¹, respectively, at 32°C (Forlow and MacMahon, 1988). Oxygen consumption rates for low-elevation populations of *Aeropedellus clavatus*, a small but flightless montane grasshopper, exceed 1.0 ml g⁻¹ h⁻¹ at 30°C (Hadley and Massion, 1985). The locust *Schistocerca gregaria* (1.69g) has a mean oxygen consumption rate at rest (30°C) of 0.414 ml g⁻¹ h⁻¹; however, this increases approximately 30-fold during tethered flight in a wind tunnel (Armstrong and Mordue, 1985). Some of the differences noted between *R. guttata* and the other species can be attributed to the long period that we gave *R. guttata* to adjust to the respirometer vessel and test temperature and to running them during their scotophase. We feel these are truly 'resting' metabolic rates. In addition, any activity during a test was noted and data obtained during these periods were discarded.

Rates of overall water loss in *R. guttata*, when expressed per unit surface area and corrected for saturation deficit, fall within the range of values exhibited by arthropods that

inhabit mesic environments as well as for orthopterans in general (Edney, 1977). At 30°C, hydrated *R. guttata* lose $0.15 \mu\text{gH}_2\text{O cm}^{-2}\text{h}^{-1}\text{Pa}^{-1}$, a rate similar to that reported for the mesic grasshopper *Trimerotropis suffosa* ($0.18 \mu\text{gH}_2\text{O cm}^{-2}\text{h}^{-1}\text{Pa}^{-1}$; Massion, 1983) and the common house cricket ($0.17 \mu\text{gH}_2\text{O cm}^{-2}\text{h}^{-1}\text{Pa}^{-1}$; Hadley *et al.* 1986) when measured between 3 and 6h after the beginning of a test run. There is no apparent change in cuticular permeability between 15 and 25°C ($0.12 \mu\text{gH}_2\text{O cm}^{-2}\text{h}^{-1}\text{Pa}^{-1}$). Unlike metabolic rate, which remains fairly constant despite changes in body water content, water loss rates decrease significantly in dehydrated animals at each test temperature. The amount of water lost per cycle in hydrated *versus* dehydrated animals (Table 2) suggests that the lower rates result from decreased cuticular transpiration rather than from a lowering of water loss associated with gas exchange.

Hamilton (1964) was the first to demonstrate periodic discharge of CO₂ in a grasshopper. Using an infrared gas analyzer, he observed discontinuous CO₂ release in newly emerged male desert locusts, *Schistocerca gregaria*, and again prior to their death. During the intervening period, CO₂ was discharged erratically (cf. Fig. 3C). In the present study, *Romalea guttata* exhibited cyclic CO₂ release throughout its adult life; however, this does not appear to be the primary mode of ventilation. Discontinuous ventilation was most commonly observed during night-time hours and only in quiescent animals maintained at low to moderate temperatures. Kestler (1985) also noted that cyclic CO₂ release in adult cockroaches requires that the animals be quiescent and undisturbed. For *R. guttata*, this time of discontinuous ventilation corresponds to a period when they are roosting on vegetation and are relatively inactive. When disturbed, lubbers quickly switch from a discontinuous mode to an erratic ventilatory pattern. The latter is characteristic of animals during daytime hours, even when tested under darkened and quiet conditions.

The cyclic pattern exhibited by *R. guttata* differs from the classic 'three-phase' DV cycle in several ways. First, during the initial or C phase, at which time the spiracles are supposedly closed, we continued to record a small, but constant, level of both O₂ uptake and CO₂ release. This exchange appears to be real and not to be an artefact of using low flow rates. Although the latter prolong the time necessary to flush the system, the duration of the interburst period is sufficiently long for the slope to decrease gradually towards zero over time. The source or site of the gas exchange is unknown. The spiracles may be incompletely closed during the interburst period, allowing some diffusion of gases to continue.

A second difference is the absence of a true flutter phase in the ventilatory pattern of *R. guttata*. Small peaks corresponding to bouts of O₂ uptake often appear prior to the main bout of CO₂ release. Although these oxygen peaks occur at a time in the cycle when the flutter phase is typically observed, they are always accompanied by comparable volumes of CO₂ release. In a true flutter phase, the uptake of O₂ takes place with little or no CO₂ release. A flutter phase during which short-term uptake of O₂ greatly exceeds CO₂ emission was observed in the tok-tok beetle *Psammodes striatus* (Lighton, 1988). In a subsequent study, Lighton (1991) reported that 10 species of tenebrionid beetles from the Namib Desert all exhibit a series of discrete, small, but regularly spaced CO₂ emissions ('F bursts') that precede the main CO₂ burst (V phase).

He does not provide concomitant data on O₂ flux in his study; however, the appearance of these preliminary CO₂ bursts and the quantity of CO₂ released are comparable to that observed for *R. guttata*.

It is often implied that during the burst or V phase of the ventilatory cycle the spiracles open widely and remain open, releasing large quantities of CO₂ before closing to initiate another interburst period. Tracings of the V phase in *R. guttata* reveal numerous, smaller peaks that represent pulses of CO₂ released during the duration of the overall burst. These individual bursts cannot be completely resolved, although they become more discrete in individuals that position themselves next to the air outlet port in the respirometer chamber. At high flow rates, however, these become discrete, sharp peaks that rise and fall from the baseline, gradually diminishing in height (and hence in the quantity of CO₂ released) as the V phase comes to a close. These variations in CO₂ release during the burst phase probably represent intermittent abdominal ventilation, but we could not see corresponding abdominal movements. Slama (1988), using a highly sensitive anemometric transducer, recorded pulsations in haemocoelic pressure which led to periodic outbursts of tracheal gases in a variety of lepidopteran pupae.

The DV cycle ventilatory model has often been cited as being an adaptive mechanism for conserving water because of the prolonged periods during which the spiracles remain closed (see Kestler, 1985, and earlier studies on diapausing pupae). It is believed that the bulk of respiratory water loss is restricted to the burst phase of the cycle when large amounts of CO₂ are released. It is also thought that even during the flutter phase (when it occurs) the slow inward movement of air inhibits the outward diffusion of water and CO₂.

Only a few studies, however, have actually measured respiratory transpiration during bouts of intermittent CO₂ release and compared these values to rates of total water loss over the same period. Kestler (1978, 1985), who measured respiratory transpiration during the DV cycle by recording changes in body mass with an extremely sensitive electronic balance, found that, in the cockroach *Periplaneta americana*, the rate of mass (water) loss is lowest during the closed phase of the cycle, but rises sharply at the start of ventilation. However, as the ventilation bout continues, the rate of water loss decreases exponentially. His studies on the cockroach were extended by Machin *et al.* (1991), who reported that the mean water loss during the burst or V phase is 1.65 times higher than rates during the closed-flutter (CF) phase in hydrated animals, 2.61 times higher in animals with normal body water content and 1.87 times higher in dehydrated animals. However, when we recalculate these data, taking into account the mean duration of both the CF and V phases over time, water loss during the CF phase amounts to about 87% of the total water loss in hydrated cockroaches and approximately 83% of the total in active animals. In the most recent study, Lighton (1992) reported that in two species of ants, *Camponotus vicinus* and *Cataglyphis bicolor*, respiratory transpiration accounted for 2.1 and 8.0% of their total water loss, respectively.

The data for *R. guttata* are closer to these latter values. Respiratory transpiration in *R. guttata* accounts for only 1.9–3.9% of the total water loss between 15 and 30°C. Even during the burst phase at 30°C in hydrated animals, cuticular transpiration is still responsible for over 93% of the water loss during this portion of the cycle. These data clearly support the dominance of transcuticular water loss in the low- to mid-temperature

range. In fact, at these temperatures respiratory water loss is so small that it can be neglected. Water loss associated with gas exchange may increase with activity and/or at higher temperatures. We do not have data for either of these conditions; however, based on a regression of moles of H₂O lost per mole of CO₂ released at 25°C in hydrated animals, even a fivefold increase in metabolic rate would only increase the respiratory contribution to 14.2% of the total.

Finally, the cyclic release of CO₂ in *R. guttata* does not appear to result in a substantial saving of water. Not only does respiratory transpiration contribute little to the total water loss in this species, but the DV cycle tends to be replaced by continuous, random CO₂ release in dehydrated animals. One would expect just the opposite in the latter group as their need to conserve water is more critical. Furthermore, the DV cycle is only observed during night-time hours, when the grasshoppers are at rest and the drying power of the air is much less.

For the cyclic release of CO₂ to influence a species' water relationships significantly in nature, it should be the predominant ventilatory pattern. This is probably true for diapausing pupae, on which the water savings hypothesis was originally based. Water conservation is of paramount importance for this immobile life history stage as the options available to pupae for replacing lost water are very limited. Most adult insects, in contrast, are extremely active and can replenish body water by feeding or drinking. The DV cycle probably does not operate during these activities and is clearly incompatible with locomotion and other types of movement. Thus, even if it could be demonstrated that the discontinuous release of CO₂ helps to reduce water loss in adults, its occurrence may not be frequent enough or of sufficient duration to affect the overall water budget significantly.

We thank Steve Roberts for providing the grasshoppers. The research was supported by NSF grant DCB-8901356.

References

- AHEARN, G. A. (1970). The control of water loss in desert tenebrionid beetles. *J. exp. Biol.* **53**, 573–595.
- ARMSTRONG, G. AND MORDUE, W. (1985). Oxygen consumption of flying locusts. *Physiol. Ent.* **10**, 353–358.
- EDNEY, E. B. (1977). *Water Balance in Land Arthropods*. Berlin: Springer-Verlag.
- FORLOW, L. J. AND MACMAHON, J. A. (1988). A seasonal comparison of metabolic and water loss rates of three species of grasshoppers. *Comp. Biochem. Physiol.* **89A**, 51–60.
- HADLEY, N. F. (1970). Water relations of the desert scorpion, *Hadrurus arizonensis*. *J. exp. Biol.* **53**, 547–558.
- HADLEY, N. F. (1986). The arthropod cuticle. *Scient. Am.* **255**, 104–112.
- HADLEY, N. F. (1989). Lipid water barriers in biological systems. *Prog. Lipid Res.* **28**, 1–33.
- HADLEY, N. F., MACHIN, J. AND QUINLAN, M. C. (1986). Cricket cuticle water relations: permeability and passive determinants of cuticular water content. *Physiol. Zool.* **59**, 84–94.
- HADLEY, N. F. AND MASSION, D. D. (1985). Oxygen consumption, water loss and cuticular lipids of high and low elevation populations of the grasshopper *Aeropedellus clavatus* (Orthoptera: Acrididae). *Comp. Biochem. Physiol.* **80A**, 307–311.
- HADLEY, N. F., QUINLAN, M. C. AND KENNEDY, M. L. (1991). Evaporative cooling in the desert cicada: thermal efficiency and water/metabolic costs. *J. exp. Biol.* **159**, 269–283.

- HADLEY, N. F., STUART, J. L. AND QUINLAN, M. C. (1982). An air-flow system for measuring total transpiration and cuticular permeability in arthropods: studies on the centipede *Scolopendra polymorpha*. *Physiol. Zool.* **55**, 393–404.
- HAMILTON, A. G. (1964). The occurrence of periodic or continuous discharge of carbon dioxide by male desert locusts (*Schistocerca gregaria* Forskål) measured by an infra-red gas analyzer. *Proc. R. Soc. Lond. B* **160**, 373–395.
- HILL, R. W. (1972). Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *J. appl. Physiol.* **33**, 261–263.
- KESTLER, P. (1971). Die diskontinuierliche Ventilation bei *Periplaneta americana* L. und anderen Insekten. Thesis, Julius-Maximilians-Universität, Würzburg.
- KESTLER, P. (1978). Gas balance of external respiration by electronic weighing in water saturated air. *Eur. J. Physiol. (Suppl.)* **373**, Abstract 36.
- KESTLER, P. (1985). Respiration and respiratory water loss. In *Environmental Physiology and Biochemistry of Insects* (ed. K. H. Hoffmann), pp. 137–186. Berlin: Springer-Verlag.
- KESTLER, P. (1991). Cyclic CO₂ release as a physiological stress indicator in insects. *Comp. Biochem. Physiol.* **100C**, 207–211.
- LEVY, R. I. AND SCHNEIDERMAN, H. A. (1966a). Discontinuous respiration in insects. II. The direct measurement and significance of changes in tracheal gas composition during the respiratory cycle of silkworm pupae. *J. Insect Physiol.* **12**, 83–104.
- LEVY, R. I. AND SCHNEIDERMAN, H. A. (1966b). Discontinuous respiration in insects. III. The effect of temperature and ambient oxygen tension on the gaseous composition of the trachea of silkworm pupae. *J. Insect Physiol.* **12**, 105–121.
- LEVY, R. I. AND SCHNEIDERMAN, H. A. (1966c). Discontinuous respiration in insects. IV. Changes in intratracheal pressure during the respiratory cycle of silkworm pupae. *J. Insect Physiol.* **12**, 465–492.
- LIGHTON, J. R. B. (1988). Simultaneous measurement of oxygen uptake and carbon dioxide emission during discontinuous ventilation in the tok-tok beetle, *Psammodes striatus*. *J. Insect Physiol.* **34**, 361–367.
- LIGHTON, J. R. B. (1991). Ventilation in Namib Desert tenebrionid beetles: mass scaling and evidence of a novel quantized flutter-phase. *J. exp. Biol.* **159**, 249–268.
- LIGHTON, J. R. B. (1992). Direct measurement of mass loss during ventilation in two species of ants. *J. exp. Biol.* **173**, 289–293.
- LOVERIDGE, J. P. (1980). Cuticle water relations techniques. In *Cuticle Techniques in Arthropods* (ed. T. A. Miller), pp. 301–366. Berlin: Springer-Verlag.
- MACHIN, J., KESTLER, P. AND LAMPERT, G. J. (1991). Simultaneous measurements of spiracular and cuticular water losses in *Periplaneta americana*: implications for whole-animal mass loss studies. *J. exp. Biol.* **161**, 439–453.
- MASSION, D. D. (1983). An altitudinal comparison of water and metabolic relations in two acridid grasshoppers (Orthoptera). *Comp. Biochem. Physiol.* **74A**, 101–105.
- MILLER, P. L. (1981). Ventilation in active and in inactive insects. In *Locomotion and Energetics in Arthropods* (ed. C. F. Herreid, II and C. R. Fournier), pp. 367–390. New York: Plenum Press.
- SCHNEIDERMAN, H. A. (1960). Discontinuous respiration in insects: role of spiracles. *Biol. Bull. mar. biol. Lab., Woods Hole* **119**, 494–528.
- SCHNEIDERMAN, H. A. AND WILLIAMS, C. M. (1953). Discontinuous carbon dioxide output by diapausing pupae of the giant silkworm, *Platysamia cecropia*. *Biol. Bull. Mar. biol. Lab., Woods Hole* **105**, 382.
- SCHNEIDERMAN, H. A. AND WILLIAMS, C. M. (1955). An experimental analysis of the discontinuous respiration of the cecropia silkworm. *Biol. Bull. Mar. biol. Lab., Woods Hole* **109**, 123–143.
- SLAMA, K. (1988). A new look at insect respiration. *Biol. Bull. Mar. biol. Lab., Woods Hole* **175**, 289–300.