

## SIMULTANEOUS MEASUREMENT OF WATER LOSS AND CARBON DIOXIDE PRODUCTION IN THE CRICKET, *ACHETA DOMESTICUS*

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The relationship between respiration and its contribution to total water loss in arthropods is based almost entirely on correlations obtained from independent plots of gravimetrically-determined transpiration rates and manometrically-determined gas exchange (Ahearn, 1970; Hadley, 1970). Recently a new system, the 'transpiration monitor' has been developed to detect the amount of water released by an arthropod into a moving air stream (Hadley, Stuart & Quinlan, 1982). The transpiration monitor not only permits the continuous recording of very small and sporadic losses of moisture, but can be interfaced with either an oxygen or carbon dioxide analyser to provide a simultaneous measure of the arthropod's gas exchange. Thus, peaks that correspond to bursts of moisture can be correlated with concurrent increases in respiratory activity. We report here the application of this system to measure water loss and carbon dioxide production in the cricket, *Acheta domesticus*, under isothermal conditions and at increasing temperatures.

The major components of the transpiration monitor (TM) and their function are summarized below. Air containing no detectable water or carbon dioxide is metered into the instrument housing and passes through a large Plexiglass column containing anhydrous calcium sulphate (Drierite) before entering the arthropod test chamber. The latter is a brass cylinder (diameter = 3.2 cm; length = 6.4 cm; vol. = 50.4 cm<sup>3</sup>) surrounded by tightly wrapped preheating coils which are, in turn, surrounded by heating elements and coated with plastic foam insulation. The chamber contains two chromel-constantan thermocouples. One is embedded in the heating elements and monitors chamber temperature for the TM temperature control circuitry; the second is free-floating and may be used to measure chamber air temperature or specimen temperature. The chamber temperature can be held at a fixed level or increased either manually or automatically to a pre-set temperature at ramp rates of 1 °C min<sup>-1</sup> and 6 °C min<sup>-1</sup>. As the dry, pre-heated air passes through the test chamber it acquires moisture transpired by the animal. The humidified air then exits the chamber and returns to the TM via 30 cm of copper tubing which serves as a thermal radiator, cooling the air to the ambient temperature. The moisture content of the air is measured with an aluminium oxide (Al<sub>2</sub>O<sub>3</sub>) moisture sensor (Panametrics). Water vapour penetrates the permeable gold layer which coats the outer surface of the chip and is adsorbed on the pore walls of the oxide layer, altering its impedance

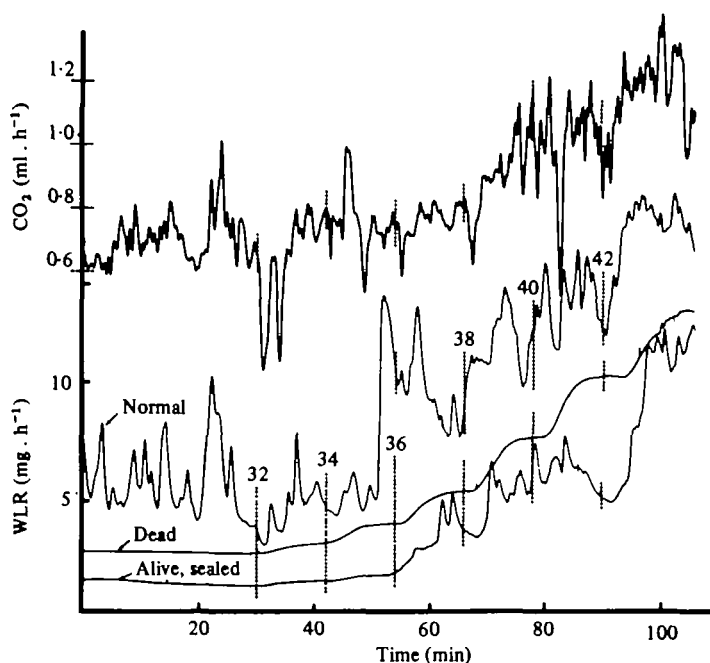


Fig. 1. Effect of increasing temperatures on water loss rates of a normal (untreated) cricket, an alive-sealed cricket (mouth and anus), and a dead cricket. The  $\dot{V}_{\text{CO}_2}$  was recorded concurrently for the normal cricket. Dotted vertical lines through the tracings are the points at which temperatures were increased to the level indicated by the numerals.

in a manner proportional to the ambient vapour pressure. The impedance change is converted into a DC signal, amplified, and plotted on a strip-chart recorder. The sensor can detect vapour densities ranging from  $5.98 \times 10^{-4} \mu\text{g ml}^{-1}$  to  $8.97 \mu\text{g ml}^{-1}$  or 1 to 15000 ppm<sub>v</sub>, respectively, and responds to a 63% step change in moisture content in less than five seconds (see Kovac, Chleck & Goodman, 1978 for details). The air next passes through a rotameter-type flowmeter and is finally vented to an infrared  $\text{CO}_2$  gas analyser (Anarad, Model AR-411) whose output is recorded simultaneously on the strip-chart recorder. The  $\text{CO}_2$  analyser can detect from 0 to 500 ppm  $\text{CO}_2$  with an accuracy of  $\pm 1.0\%$  and compensates for deviations from linearity.

The TM was calibrated using atmometers constructed from segments of disposable micropipettes with cotton wicks. The atmometers produced a tracing which included a period of relatively constant evaporation. The vapour density ( $\mu\text{g ml}^{-1}$ ) during this equilibrium phase was calculated using the equation:

$$\text{vapour density} = \frac{A_b \times M}{A_t \times F \times T}$$

where  $A_b$  is the area ( $\text{cm}^2$ ) beneath the curve during the equilibrium period,  $A_t$  is the total area of the curve,  $M$  is the mass (water) lost by the atmometer during the run ( $\mu\text{g}$ ),  $F$  is the flow rate ( $\text{ml min}^{-1}$ ), and  $T$  is the duration (min) of the equilibrium period. The  $\text{CO}_2$  analyser was calibrated using a certified span gas. Output from the TM and  $\text{CO}_2$  analyser during an actual run was quantified by measuring the areas

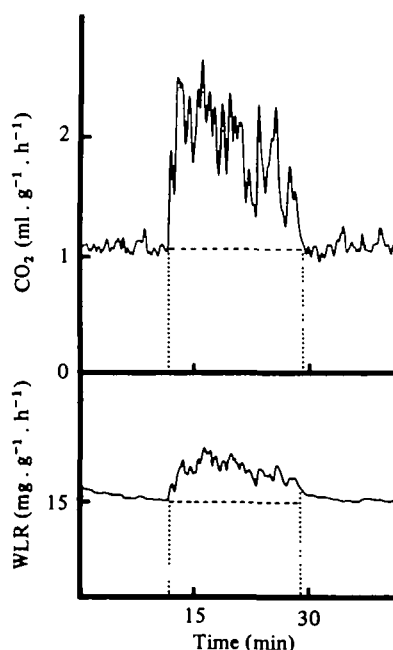


Fig. 2. Simultaneous recording for water loss and  $\text{CO}_2$  production in an alive-sealed cricket (270 mg) at  $40^\circ\text{C}$ . The horizontal dashed lines represent 'basal' water loss rates and  $\dot{V}_{\text{CO}_2}$ . The areas beneath the clusters of large peaks and within the boundaries formed by the dotted vertical lines were integrated to quantify moisture loss associated with ventilatory gas exchange.

under the curves on the recorder with a Numonics digitizer for a specific time interval. Water loss rate ( $\mu\text{g min}^{-1}$ ) was calculated from the flow rate ( $\text{ml min}^{-1}$ ) and vapour density. The  $\text{CO}_2$  concentration registered by the analyser and the flow rate (STP) were used to calculate the  $\dot{V}_{\text{CO}_2}$ .

The water loss rate (WLR) at increasing temperatures for a normal (untreated), an alive-sealed (mouth and anus; paraffin), and a dead (HCN) cricket, as well as the  $\dot{V}_{\text{CO}_2}$  for the normal cricket, are shown in Fig. 1. The experimental crickets were adult males (4–6 days post-moult) and had been fasted (water only) for 48 h before testing. Each cricket was housed in a hardware cloth restraining cage and placed inside the test chamber for 1.5 h at  $30^\circ\text{C}$  before increasing the temperature  $2^\circ\text{C}$  once every 12–15 min. Although air temperature ( $T_a$ ) inside the chamber reached the command temperature within a minute of a temperature change, thermocouples inserted into the cricket's rectum ( $T_b$ ) and attached to the cuticle ( $T_c$ ) indicated that approximately eight minutes were required for  $T_b$  and  $T_c$  to stabilize about  $0.5^\circ\text{C}$  below  $T_a$ .

The normal cricket exhibited numerous fluctuations in both its WLR and  $\dot{V}_{\text{CO}_2}$  during the isothermal ( $30^\circ\text{C}$ ) and temperature ramp phase of the run (Fig. 1). These fluctuations, believed to be caused primarily by oral fluid loss and variation in activity, make it difficult to discern the relationship between  $\dot{V}_{\text{CO}_2}$  and WLR as temperatures increased from 30 to  $42^\circ\text{C}$ . The WLR of the dead cricket and the alive-sealed cricket also rose with the temperature increase, with rates for both groups considerably lower than those for the normal cricket. As often noted in studies of

arthropod water relations (Edney, 1977), the WLR for the dead cricket was somewhat greater than for the alive-sealed cricket at each temperature. A similar relationship between WLR for the three treatment groups and  $\dot{V}_{\text{CO}_2}$  was observed in three other experimental runs.

When the mouth and anus are sealed, the bursts of moisture due to oral discharges are eliminated, and the correlation between WLR and  $\text{CO}_2$  is clearly evident. Figure 2 shows a portion of an isothermal run (40 °C) using an alive-sealed cricket. A basal rate of  $\dot{V}_{\text{CO}_2}$ , indicated by the dashed line, is interrupted by a cluster of large  $\text{CO}_2$  peaks, which probably represent ventilatory movements associated with increased activity inside the holding cage. Water loss associated with these respiratory bursts is mirrored in the WLR tracing below. The  $\dot{V}_{\text{CO}_2}$  during this period of increased respiratory activity was  $1.94 \text{ ml g}^{-1} \text{ h}^{-1}$ , of which  $0.85 \text{ ml g}^{-1} \text{ h}^{-1}$  was represented by the large peaks. The WLR during the same interval rose from a 'basal' rate (cuticular + respiratory transpiration) of  $15.3 \text{ mg g}^{-1} \text{ h}^{-1}$  to  $19.5 \text{ mg g}^{-1} \text{ h}^{-1}$  due to the increased ventilation. Thus, although  $\dot{V}_{\text{CO}_2}$  increased by approximately 78%, the concomitant increase in WLR was only 27.5%. A higher proportion of total WLR, however, may be associated with increased gas exchange as lethal temperatures are approached.

Because the WLR of dead crickets does not accurately reflect the cuticular permeability of living individuals, the system as presently designed does not permit a more precise quantitative partitioning of cuticular versus respiratory water loss. It does, however, permit a more detailed examination of the relationship between these two components and of extra-transpiratory avenues of water loss than previously possible.

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#### REFERENCES

- AHEARN, G. A. (1970). The control of water loss in desert tenebrionid beetles. *J. exp. Biol.* **53**, 573-595.  
 EDNEY, E. B. (1977). *Water Balance in Land Arthropods*. New York: Springer-Verlag.  
 HADLEY, N. F. (1970). Water relations of the desert scorpion, *Hadrurus arizonensis*. *J. exp. Biol.* **53**, 547-558.  
 HADLEY, N. F., STUART, J. L. & QUINLAN, M. (1982). An air-flow system for measuring total transpiration and cuticular permeability in arthropods. Studies on the centipede, *Scolopendra polymorpha*. *Physiol. Zool.* **55**, (in the Press).  
 KOVAC, M. G., CHLECK, D. & GOODMAN, P. (1978). A new moisture sensor for in-situ monitoring of sealed packages. *Solid State Technol.* **21**, 1-6.