WATER BALANCE ACROSS
THE CUTICLE OF A SOIL INSECT

By R. A. GALBREATH*

Zoology Department, University of Canterbury, New Zealand

SUMMARY

1. The water regime in soil commonly approaches equilibrium of water potential with the insects living there.

2. Even under these conditions, non-equilibrium processes have a significant effect on water movement through the cuticle of soil insects.

3. Measurements of water potential on either side of the cuticle of *Costelytra zealandica* larvae showed that equilibrium is not reached while the insect is alive. There is an active outward flow of water by thermo-osmosis associated with the flow of heat from the insect.

INTRODUCTION

Insects living in the moist soil environment do not need to control and limit diffusion of water through their cuticle to the extent required in the much drier environment above the ground, nor in general do they have the specialized adaptations (such as spiracle closing mechanisms) which would allow them to do so. Consequently the flow of water between a soil insect and its environment is usually assumed to be a relatively uncomplicated process and little attention has been given to it. Results such as those of Evans (1943) suggest that in some soil insects it can be described simply as passive diffusion across the cuticle, leading to equilibrium. In this paper some of the processes involved are expressed in thermodynamic terms and measurements are made on a soil insect, *Costelytra zealandica* (White), to discover whether such a simple model is generally applicable. This analysis borrows heavily from that used in the fields of plant and soil water relations, where it has been found useful to employ thermodynamic concepts and terminology to describe similar systems and processes (Slatyer, 1967; Taylor, 1968).

The water potential

The physical forces promoting diffusion of water across membranes between phases of a system can be expressed in terms of the chemical potential of the water in the system, defined as the rate of change of free energy of the system with change in quantity of water (all other properties of the system remaining constant). Provided no other process affecting water movement is occurring, water tends to diffuse spontaneously 'downhill' from areas of higher potential to areas of lower potential until an equilibrium is reached when the potential becomes uniform throughout the system. The force behind this spontaneous flow is proportional to the potential difference involved.

* Present address: Entomology Division, D.S.I.R., Private Bag, Auckland, New Zealand.

Other processes, such as the flow of other chemical components or of heat acr⬛ the same membrane, may interact with water movement down a potential gradient and displace it from equilibrium. These interactions are thermodynamically irreversible processes and may reach a steady state which, unlike equilibrium, is maintained only by a continuing flow of the interacting component. In biological systems such processes are called active transport. The most familiar of these form part of regulating mechanisms such as those maintaining a constant internal environment in animals, but active transport as strictly defined (by irreversible thermodynamics; Kedem, 1961) includes many other processes which are not controlled in this sense, or which may occur in non-living systems.

A practical measure derived from the chemical potential of water is the Water Potential defined by Slatyer & Taylor (1960) as the difference between the chemical potential of water in the system of interest, and that of pure water at the same temperature, free of any solid matrix and at atmospheric pressure. It can be partitioned into components corresponding to the conventional measures of hydrostatic, osmotic and matric pressures (Noy-Meir & Ginzburg, 1967). By definition, pure free water at atmospheric pressure has a water potential of zero, while drier conditions have negative potentials.

Water potential has also been defined with the dimensions of pressure rather than energy (Slatyer, 1967), but this 'equivalent pressure' terminology can be confusing, particularly when both notional and real (hydrostatic) pressures are involved. In terms of the more familiar pressure units a water potential of -100 J/kg at 25 °C is equivalent to a 'suction pressure' or 'diffusion pressure deficit' of 1 bar or 0·987 atmosphere, to a pF of 3·01, and also to a relative humidity of 99·927%. However, it should be noted that the relationship between water potential and relative humidity is logarithmic, so that apparently insignificant depressions of humidity below saturation correspond to significant changes in potential. This applies particularly to the soil environment, where the water potential commonly fluctuates within the range from about -20 J/kg ('field capacity', 99·985% r.h.) down to -1500 J/kg ('wilting point', 98·915% r.h.). Although this range is small compared with that experienced above ground, it is misleading to regard it as negligible and assume that 'the air in the soil is almost always saturated with water vapour' (Ghilarov, 1959; see also Laughlin, 1957; Kühnelt, 1963; Davidson, Wiseman & Wolfe, 1972). Under this assumption the water regime in soil has been likened to that in a freshwater environment. It may be more accurately compared to that in an estuary, where the water potential can vary over a similar range during each tidal cycle of salinity, although a soil animal is likely to be surrounded by a layer of air which provides extra resistance to diffusion of water not available to an estuarine animal.

MATERIAL AND METHODS

C. zealandica (Coleoptera, Melolonthinae) is a common pasture insect in New Zealand which lives in the soil except for brief excursions by the adult beetle. Samples of each stage were collected from the field at intervals during the year, and kept in soil. Most work was done on third instar larvae. In order to find whether diffusion of water across the cuticle of *C. zealandica* proceeds towards equilibrium, measurements were made of the water potential inside and outside its cuticle.

▶The water potential inside the cuticle was estimated by measuring the freezing point of the haemolymph, using the method of Ramsay & Brown (1955). Although generally used as a measure of osmotic pressure only, the freezing point depression of an aqueous solution is a function of its total water potential. Haemolymph was collected by cutting a leg from the insect and drawing haemolymph from the wound into glass capillary tubes. Removal of the haemolymph from the insect eliminates the hydrostatic pressure component of its water potential but, judging by measurements from other insects (Cottrell, 1962; Bayer, 1968), this contributes probably only about 1 J/kg and can be neglected.

If the insect is confined in a closed chamber and left until a steady state is reached, then the humidity inside the chamber is determined by diffusion of water from the insect, provided that processes such as adsorption at the walls of the chamber have no continuing effect. This formed the basis of the technique used to measure the water potential outside the cuticle, unmodified by external processes. Both living and dead larvae were used, the latter killed by freezing in order to stop all metabolic processes with the minimum of other changes. Humidity was measured by the thermocouple psychrometer method of Spanner (1951), simplified after Rawlins & Dalton (1967), with timing and switching controlled electronically. The chromel-constantan thermo-couple of the psychrometer was mounted in the cap of a 5 ml glass vial containing the insect and the vial sealed and suspended in a water bath. The system was calibrated by placing solutions of known water potential in the vial in place of the insect. At first, equilibration was very slow, but treating the vial with dimethyldichlorosilane to minimize adsorption of water vapour onto the glass reduced the equilibration time to a few hours. All water potential measurements were adjusted to refer to a standard temperature of 25 °C.

RESULTS AND DISCUSSION

The mean water potentials measured by the freezing point method and the thermo-couple psychrometer method are listed in Table 1.

The small variation within the freezing point measurements for each instar suggest some form of regulation of the internal water potential of *C. zealandica*, which would require active control of water balance, but not necessarily a direct effect on water movement across the cuticle. Comparison of the different water potentials obtained for third instar larvae showed that the thermocouple psychrometer measurements from living larvae were significantly higher (*t*-test; $P < 0.01$) than those from dead larvae or the freezing point measurements from haemolymph. The water potential around the living larvae was apparently raised about 340 J/kg above the potential of the haemolymph, implying that there was a continual flow of water out of the living insect shifting the balance of passive diffusion away from equilibrium. This process apparently ceased with the death of the larvae, as the psychrometer measurements from dead larvae fell to a level not significantly different from the freezing point measure-ments.

A similar effect was found in terrestrial isopods from humid environments by Lindqvist, Salminen & Winston (1972), who measured the water potential of detached pieces of cuticle from four isopod species and in each case recorded a value higher than that of the corresponding haemolymph potential. The difference (recalculated

Table 1. *Water potential of* C. zealandica

(a) Freezing point depression method

	Water potential in J/kg (mean ± S.E.)
First instar larva	−719 ± 28 (n = 8)
Second instar larva	−816 ± 31 (n = 4)
Third instar larva	−867 ± 21 (n = 13)
Pupa	−893 ± 11 (n = 6)
Adult	−1007 ± 39 (n = 10)

(b) Thermocouple psychrometer method

Third instar larva	
Living	−526 ± 19 (n = 20)
Killed by freezing	−810 ± 70 (n = 6)

from their Table 1) varied from 280 to 540 J/kg and again disappeared after the death of the animal. They concluded that there was 'an active mechanism regulating the cuticular water'. Active regulation of water in the cuticle has also been proposed to explain the reverse effect – reduction of cuticular water potential in *Periplaneta americana* and *Locusta migratoria*, both insects from dry environments (Winston & Beament, 1969). Similarly, the ability of some terrestrial arthropods to absorb water from the air, thus lowering the humidity in a sealed vessel far below the level in equilibrium with their haemolymph, has also been attributed to active uptake (Beament, 1965). In each case an anomalous water potential gradient has been shown to exist across at least two of the three phases: haemolymph – cuticle – air. This indicates active transport but it does not necessarily imply regulation of water movement. In *C. zealandica* the observed water potential gradient could be produced by a purely physical process, thermo-osmosis, although this does not appear to explain the other cases.

Thermo-osmosis is the flow of water coupled with a flow of heat across a membrane: the reverse of the familiar process of evaporative cooling, which is a flow of heat coupled with a flow of water. It is a thermodynamically irreversible process which may reach a steady state if, due to the flow of water, a pressure gradient ΔP (or equivalent osmotic pressure gradient) develops across a membrane with a small temperature gradient ΔT. Spanner (1954) showed that at steady state ΔP is given by:

$$\Delta P = \frac{-Q^* \Delta T}{T V_w},$$

T is the mean absolute temperature and V_w the partial specific volume of water. Q^* is the heat of transfer of the membrane: the heat flow associated with flow of unit quantity of water across the membrane.

Thermo-osmosis across the cuticle of an insect could occur when heat (produced by metabolic processes) flow from the insect across the 'membrane', which in this case comprises two parts: the cuticle and its boundary layer (the region of water potential and temperature gradients in the air around the cuticle). This applies not only in the experimental conditions in which the water potential measurements were made, but also in the soil environment, for scarabaeid larvae such as those of *C. zealandica* maintain an air space about themselves in soil by their mode of burrowing

Schwerdtfeger, 1939). The air boundary layer must be taken into account because it forms the major resistance to heat flow (since it is both thicker and has a lower thermal conductivity than the cuticle) and therefore most of the resulting temperature gradient develops across it. As the heat of transfer of an air space is simply the heat of vaporization of water (Spanner, 1954), it can be calculated using the equation given above that if a steady state is reached, a temperature gradient of 1 °C maintained across the air boundary layer results in a pressure gradient building up to $8 \cdot 17 \times 10^6$ Pa (80·6 atmospheres), or its equivalent in terms of water potential: an apparent gradient of 8170 J/kg, with the higher potential on the cooler side. The potential gradient must be described as apparent only, for it refers to potentials at different temperatures which are therefore not strictly comparable.

Thermo-osmosis across the cuticle itself cannot be estimated so simply. An equation derived by Spanner (1954) enables calculations of the heat of transfer from the temperature coefficient of permeability of the cuticle, which has been measured in many investigations into the critical temperature of insect cuticle. Results from soil insects and others with abraded cuticle suggest that the heat of transfer of their cuticle is very low (Beament, 1959) or perhaps as high as that of an air layer (Holdgate & Seal, 1956). Hence, as only a small part of the temperature gradient from the insect to the surrounding atmosphere is across the cuticle itself, thermo-osmosis in the cuticle probably adds little to the gradient calculated above.

The possible effect of thermo-osmotic flow from the *C. zealandica* larva was estimated from the temperature gradient in the air adjacent to its cuticle measured by using the thermocouple psychrometer as a differential thermometer. Its thermocouple, which has an output of 60 μV/°C, is normally cooled (by the Peltier effect) until water condenses on it, in order to measure wet-bulb depression. By touching the thermocouple against the cuticle it was used instead to measure the difference between ambient and cuticle surface temperatures. The psychrometer was set up in the usual way and when the humidity around the larva had settled to a steady level, the thermocouple was pushed down from its normal position (about 3 cm above the larva) and the change in its output recorded as its 0·1 mm diameter junction approached and touched the cuticle of the larva. The thermocouple was too fragile for this procedure to be repeated very often, but measurements were made on four active third instar larvae which indicated a mean surface temperature $0 \cdot 22 \pm 0 \cdot 02°$ C above ambient temperature, which was about 20 °C.

Thermo-osmosis across an air space with this temperature gradient produces (at steady state) an apparent difference in water potential of 1800 J/kg, with the higher potential on the cooler side. The observed gradient of water potential across the cuticle (and air boundary layer) of living *C. zealandica* larvae was in the same direction but only 340 J/kg in value. The discrepancy suggests that there are processes, either intrinsic or extrinsic to the insect, which have not been taken into account. Certainly the real situation is more complex than the model used for these calculations. However, while other processes may also be involved, the thermo-osmosis which inevitably follows the flow of heat across the cuticle, and particularly its associated air boundary layer, provides a physical explanation for this case of active transport. The effect is small, but significant in conditions of high water potential, as are found in soil.

At first sight it may appear that the above treatment represents an over-complicated

view of an essentially simple effect, namely the evaporation of water from an insect
that is warmer than its surroundings. When the water is evaporating into a dry
atmosphere, i.e. down a gradient of water potential, then this may indeed be an
adequate description. But when this flow continues even up a potential gradient,
induced by an independently maintained flow of heat, then the description of the
phenomenon as thermo-osmosis is a necessary one. Simpler analyses of irreversible
systems by the methods of classical thermodynamics are conceptually incorrect, and
generally unreliable.

REFERENCES

BAYER, R. (1968). Untersuchungen am Kreislaufsystem der Wanderheuschrecke (*Locusta migratoria migratorioides* R et F., Orthopteroidea) mit besonderer Berucksichtigung des Blutdruckes. *Z. vergl. Physiol.* **58**, 76–135.

BEAMENT, J. W. L. (1959). The waterproofing mechanism of arthropods. I. The effect of temperature on cuticle permeability in terrestrial insects and ticks. *J. exp. Biol.* **36**, 391–422.

BEAMENT, J. W. L. (1965). The active transport of water: evidence, models and mechanisms. *Symp. Soc. exp. Biol.* **19**, 273–98.

COTTRELL, C. B. (1962). The imaginal ecdysis of blowflies. Observations on the hydrostatic mechanisms involved in digging and expansion. *J. exp. Biol.* **39**, 431–48.

DAVIDSON, R. L., WISEMAN, J. R. & WOLFE, V. J. (1972). Environmental stress in the pasture scarab *Sericesthis nigrolineata* Boisd. II. Effects of soil moisture and temperature on survival of first-instar larvae. *J. appl. Ecol.* **9**, 799–806.

EVANS, A. C. (1943). Value of the pF scale of soil moisture for expressing the soil moisture relations of wireworms. *Nature, Lond.* **152**, 21–2.

GHILAROV, M. S. (1959). Adaptations of insects to soil dwelling. *Proc. Int. Congr. Zool.* **15**, 354–7.

HOLDGATE, M. W. & SEAL, M. (1956). The epicuticular wax layers of the pupa of *Tenebrio molitor* L. *J. exp. Biol.* **33**, 82–106.

KEDEM, O. (1961). Criteria of active transport. In *Membrane Transport and Metabolism* (ed. A. Kleinzeller and A. Kotyk), pp. 87–93. New York: Academic Press.

KÜHNELT, W. (1963). Soil-inhabiting Arthropoda. *A. Rev. Ent.* **8**, 115–36.

LAUGHLIN, R. (1957). Absorption of water by the egg of the Garden Chafer *Phyllopertha horticola* L. *J. exp. Biol.* **34**, 226–36.

LINDQVIST, O. V., SALMINEN, I. & WINSTON, P. W. (1972). Water content and water activity in the cuticle of terrestrial isopods. *J. exp. Biol.* **56**, 49–55.

NOY-MEIR, I. & GINZBURG, B. Z. (1967). An analysis of the water potential isotherm in plant tissue. I. The theory. *Aust. J. biol. Sci.* **20**, 695–721.

RAMSAY, J. A. & BROWN, R. H. J. (1955). Simplified apparatus and procedure for freezing-point determinations upon small volumes of fluid. *J. scient. Instrum.* **32**, 372–75.

RAWLINS, S. L. & DALTON, F. N. (1967). Psychrometric measurement of soil water potential without precise temperature control. *Proc. Soil Sci. Soc. Am.* **31**, 297–301.

SCHWERDTFEGER, F. (1939). Untersuchungen über die Wanderungen des Maikäfer-engerlings (*Melolontha melolontha* L. und *Melolontha hippocastani* F.). *Z. angew. Ent.* **26**, 215–52.

SLATYER, R. O. (1967). *Plant–water Relationships*. London: Academic Press.

SLATYER, R. O. & TAYLOR, S. A. (1960). Terminology in plant- and soil–water relations. *Nature, Lond.* **187**, 922–24.

SPANNER, D. C. (1951). The Peltier effect and its use in the measurement of suction pressure. *J. exp. Bot.* **2**, 145–68.

SPANNER, D. C. (1954). The active transport of water under temperature gradients. *Symp. Soc. exp. Biol.* **8**, 76–93.

TAYLOR, S. A. (1968). Terminology in plant and water soil relations. In *Water Deficits and Plant Growth.* (ed. T. T. Kolowski), vol. 1, pp. 49–72. New York: Academic Press.

WINSTON, P. W. & BEAMENT, J. W. L. (1969). An active reduction of water level in insect cuticle. *J. exp. Biol.* **50**, 541–6.