J. Exp. Biol. (1968), **49**, 1–13
With 7 text-figures
Printed in Great Britain

THE CONTROL OF WATER LOSS IN *LOCUSTA MIGRATORIA MIGRATORIOIDES* R. & F.

I. CUTICULAR WATER LOSS

BY J. P. LOVERIDGE
Department of Zoology, University College of Rhodesia, Salisbury

(*Received* 12 *January* 1968)

INTRODUCTION

Without a relatively waterproof integument terrestrial insects would be limited in distribution to moist microclimates (as are woodlice, Edney, 1954). Early studies by Kühnelt (1928) established the importance of a lipid in the epicuticle as the waterproofing mechanism, and this work was elaborated by Ramsay (1935). Ramsay not only first demonstrated the existence of a 'critical' or 'transition' temperature at which the evaporation of water from the cuticle of *Periplaneta* was abruptly increased, but was responsible for emphasizing that physical laws are invaluable in the interpretation of phenomena associated with insect transpiration. Wigglesworth (1945) confirmed that transition temperatures existed in various other species of insects with blocked spiracles, and showed that the transition temperature is highest in those insects most resistant to desiccation. Beament (1945) showed the existence of a transition temperature (below the melting-point of extracted cuticular waxes) using isolated pieces of insect cuticle or with wax deposited on a tanned gelatin membrane. Reviews by Beament (1961*a*, 1964*a*, *b*) have developed the theory that a monolayer of polar wax molecules orientated at 24·5° to the vertical are arranged on the tanned cuticulin of the protein epicuticle, and confer upon the cuticle, as a whole, most of the resistance to the outward passage of water molecules. The explanation for the transition temperature lies in the abrupt disruption of Van der Waal's forces between the molecules at a temperature several degrees below the melting-point of the wax, increasing the vibration of the molecules and thus the frequency with which gaps in the monolayer appear (Beament, 1964*a*). Independent evidence for the existence of the orientated monolayer comes from studies of electrical properties of *Periplaneta* cuticle (Beament, 1961*b*) and from electron microscope studies of insect cuticle (Locke, 1964).

Studies on cuticle permeability of *Schistocerca gregaria* by Beament (1959), on *Melanoplus bivittatus* by Chefurka & Pepper (1955) and on *Gastrimargus* by Koidsumi (1934–5) have shown that there is no reason to expect that Acrididae depart from the typical insect pattern with regard to the relation between temperature and cuticle permeability. The effect of relative humidity on permeability will, however, be just as important, and has not often been investigated. In some cases a rectilinear relationship exists (Koidsumi, 1934–5). Malek (1958) has investigated the histology of the cuticle of adult *Schistocerca*, which is similar in all essentials to that of adult *Locusta* (Loveridge, 1967). The present paper examines the contribution of the cuticle to the control of water loss in the African migratory locust.

MATERIAL AND METHODS

Adult male and female *Locusta migratoria migratorioides* R. & F. were used throughout the study. They were reared in cages in a constant-temperature room according to the methods of Hunter-Jones (1961). The temperature in the room was kept at 29·5 ± 0·5° C. and a photophase of 16 hr. in the 24 hr. cycle was maintained. The locusts were fed with fresh grass (*Pennisetum clandestinum*) and bran daily.

Apparatus. The apparatus used for humidity control was a closed Perspex box in which air was circulated by a magnetically driven fan. Boxes of 660–2000 ml. with fan speeds of 500–800 rev./min. were used, but there were no differences in rates of water loss from box to box. The locusts were introduced into the box through a hatch and were exposed on a false floor made of perforated zinc. Water loss was measured by weighing to 0·1 mg. before and after exposure to controlled conditions. Relative humidity was controlled by means of KOH solutions (Buxton, 1931) or by P_2O_5 or saturated salt solutions (Winston & Bates, 1960) and measured with a humidity tester (American Instrument Co.) or by a calibrated Edney hygrometer. In most experiments temperature inside the box was maintained at 30 ± 1° C. by installing the apparatus in a constant-temperature room. In the experiment where a range of temperatures was required a temperature accurate to within ± 1° C. of the setting was obtained by immersing the apparatus in a water bath and driving the fan by a motor. In this case the shaft from the motor entered by a gland in the roof of the box. Air temperatures were measured with a mercury thermometer but cuticle temperatures (Beament, 1958) were not measured. To follow the time course of water loss from dead individuals, and to measure the hygroscopic properties of the surface of whole locusts, the animals were suspended from the hook of a balance pan inside a glass tube containing a humidity-controlling solution. The tube was sealed except for a small hole for the glass capillary on which the locust was suspended, and in this case the air was not stirred.

Experimental methods. In all experiments the locusts were carefully handled by the wings using forceps, to minimize abrasion of the epicuticular waxes. They were prestarved at 29·5 ± 0·5° C., 96% R.H. for 24 hr. to empty the gut (Uvarov, 1948) and were then anaesthetized in CO_2 to prevent, as far as possible, struggling and damage to the cuticle. They were then killed by exposure to HCN or H_2S; chloroform was unsuitable as it increased the rate of transpiration (Wigglesworth, 1945). After death the spiracles were blocked and the locusts were exposed for 15–30 min. to ambient relative humidity (about 50% R.H., 29·5° C.) to ensure that the cellulose paint was dry and to minimize the loss of adsorbed cuticular hygroscopic water during the actual experiment.

Spiracles were blocked with cellulose paint (Brushing Duco-United Paints Ltd.) which was found to run into the atrium and effectively block the entrance to the trachea. No difference could be detected in the rate of water loss from locusts with intact spiracles and with blocked spiracles (Table 1) which confirms the results obtained by Chefurka & Pepper (1955) for *Melanoplus*. It would seem that the spiracle valves, when closed, are so efficient as to permit no water loss, or else that when spiracles are tampered with the cuticular waterproofing mechanism is damaged (Beament, 1959). If the second conclusion is correct (and this seems likely in view of Beament's

Table 1. *The effect of blocking the spiracles on water loss from dead locusts*

(Females and males of the same age, data taken from hourly readings during 6 hr. exposure to 50% R.H., 30° C.)

	Spiracles blocked	Spiracles not blocked
No. of readings	120	42
Mean water loss ±2×S.E. (mg./g./hr.)	3·01 ± 0·16	2·96 ± 0·20

The mean water losses are not significantly different from each other ($P < 0{\cdot}001$)

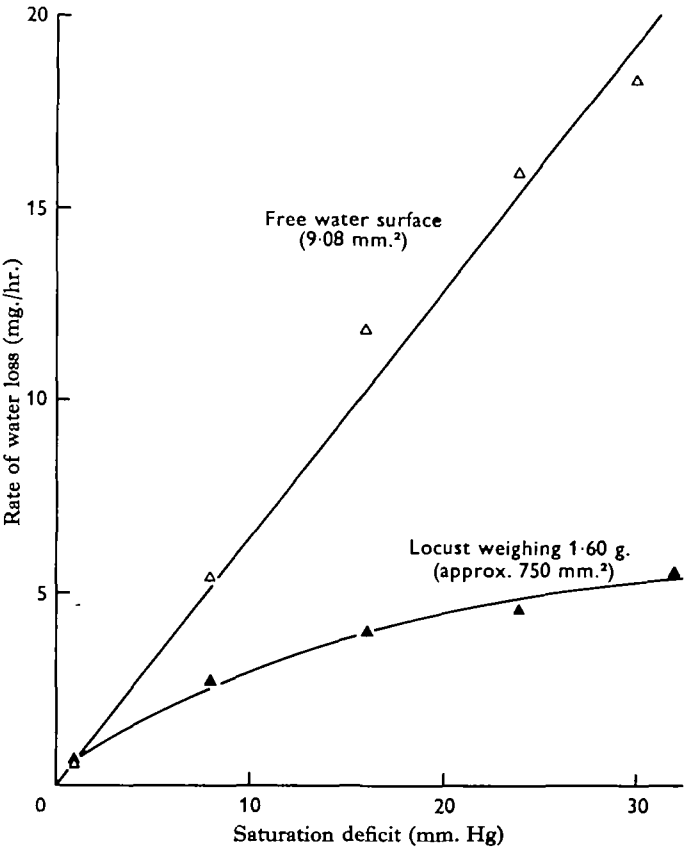

Fig. 1. Evaporation from a free water surface of 9·08 mm.² and a standard locust (surface area of head, thorax and abdomen approximately 750 mm.²) in the same apparatus.

findings on *Schistocerca*) then all estimates of cuticular water loss will be high by a factor of unknown magnitude. This factor will be the amount of water loss resulting from damage (if the spiracles are blocked) or the rate of escape of water vapour through the spiracle valves (if the spiracles are left untouched).

Locusts between the ages of 1–40 days from the final moult were used. No trends of increasing or decreasing water loss which could be associated with age were observed

between these limits. The abrasion of the epicuticle associated with ageing in *Schistocerca* (Malek, 1958) would seem to be effectively repaired (Wigglesworth, 1945).

To check that the humidity-controlling solutions and apparatus used were capable of handling the amounts of water vapour evaporated from locusts, free water surfaces of 9·08 mm.^{2} were exposed in the same apparatus and water loss measured. It is clear from Fig. 1 that the potential evaporation in this apparatus is far in excess of that obtained with the locusts.

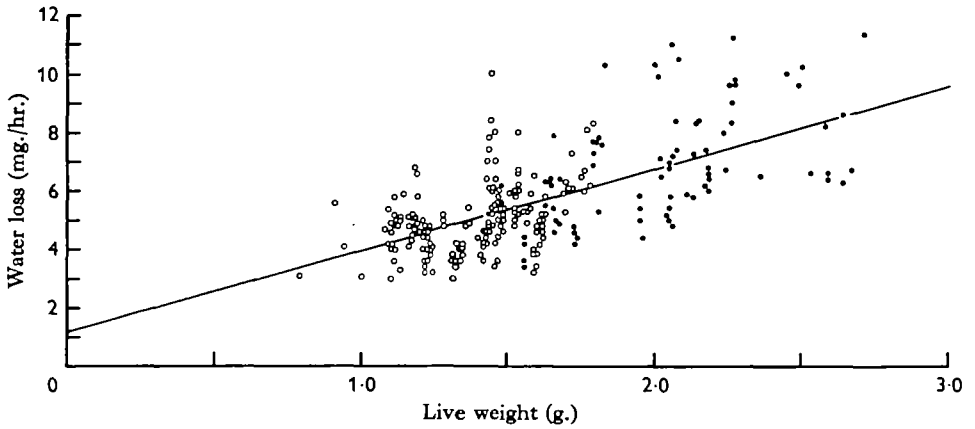

Fig. 2. The relationship between live weight and cuticular water loss at 30° C., 0% R.H. Solid circles represent ♀♀, open circles ♂♂. Coincidental points represented by one circle only.

Table 2. *Correlations between live weight and cuticular water loss at* 30° C.

(Y = rate of water loss in mg./hr.; X = live weight in g.)

R.H. %	Regression equation, $Y = a+bX$	S.E. of a	No. of values	t	P
96±4	$Y = -1{\cdot}7531+1{\cdot}6423X$	0·1141	90	7·302	< 0·001
75±2	$Y = 0{\cdot}7577+1{\cdot}2379X$	0·0809	144	5·951	< 0·001
50±2	$Y = 1{\cdot}5439+1{\cdot}6580X$	0·0811	307	8·374	< 0·001
25±2	$Y = 1{\cdot}1824+2{\cdot}1960X$	0·0974	175	9·211	< 0·001
0	$Y = 1{\cdot}0185+2{\cdot}8834X$	0·0774	271	14·127	< 0·001

THE EFFECT OF SIZE ON WATER LOSS

The rate of water loss from an insect is related to its size, and most workers apply some form of size correction to their results (e.g. Wigglesworth, 1945; Jakovlev & Krüger, 1953). Adult *Locusta* range in live weight from 1 to 3 g. and it is clearly necessary in this case to apply size corrections, particularly as different routes of water loss are to be compared (Loveridge, 1967) to provide an over-all picture of water balance. The most convenient correction for size, and one which is meaningful in most contexts (spiracular water loss, faecal water loss, oxygen consumption) is weight. The relation between live weight and cuticular water loss of locusts exposed to 0% R.H., 30° C. is given in Fig. 2. The relationship can be calculated by regression analysis for each treatment (Table 2), and gives a significant correlation at all five relative humidities used. Where enough data are available, rates of water loss are corrected to

a 'standard locust' of 1·6 g. live weight using such regression equations, and rates are expressed as mg./locust/hr. Where single animals or a few animals were used, the results are expressed as mg./g./hr. To provide comparative figures of water loss in mg./cm.^{2}/hr., the relationship between the surface area of cleaned cuticle (head, thorax and abdomen = Y) and live weight (X) was calculated to be $Y = 393{\cdot}44 + 249{\cdot}31X$ ($P < 0{\cdot}001$).

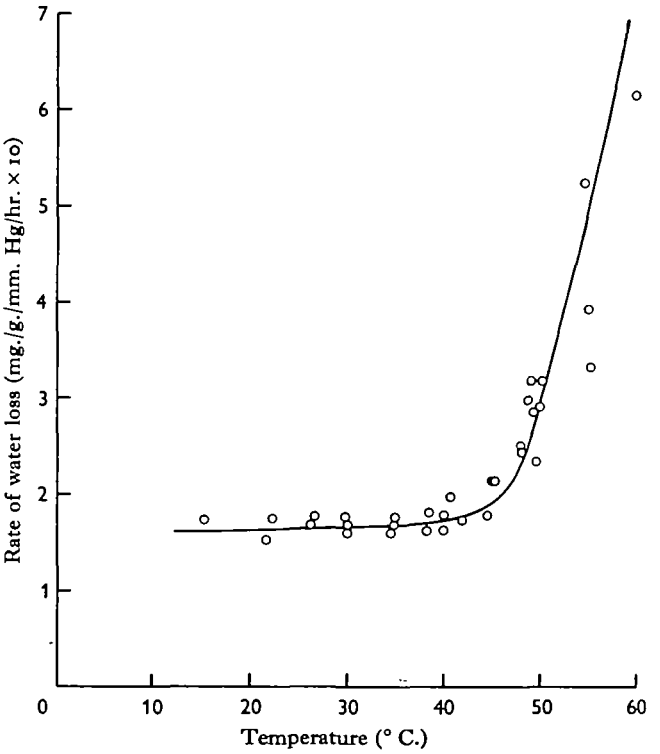

Fig. 3. The effect of temperature on the permeability of the cuticle of *Locusta*. Air, not cuticle, temperatures were measured.

THE EFFECT OF TEMPERATURE ON CUTICULAR TRANSPIRATION

Dead locusts with intact spiracles were exposed to a series of progressively higher constant temperatures. Rates of water loss were determined at each temperature and results are expressed as mg./g./mm. Hg/hr. × 10 to give values independent of the different saturation deficits experienced at different temperatures (Beament, 1958).

The temperature at which the rate of water loss from the cuticle of *Locusta* abruptly increases is 46–48° C. (Fig. 3). This compares with a transition temperature of 48° C. for the cuticle of *Schistocerca* (Beament, 1959), and 43–50° C. for *Melanoplus* (Chefurka & Pepper, 1955).

THE EFFECT OF RELATIVE HUMIDITY ON CUTICULAR TRANSPIRATION

The time course of cuticular water loss. Wigglesworth (1945) stated that it was possible to obtain several readings of cuticular transpiration using the same insect, provided its water content was not substantially reduced. It was discovered in experiments with *Locusta*, however, that weight loss over the first hour was greater than over the second hour; in subsequent 1 hr. periods, too, the rate of water loss showed a steady decline. This apparent decrease in cuticular permeability was seen at 0, 25 and 50% R.H. but not at 75% R.H.

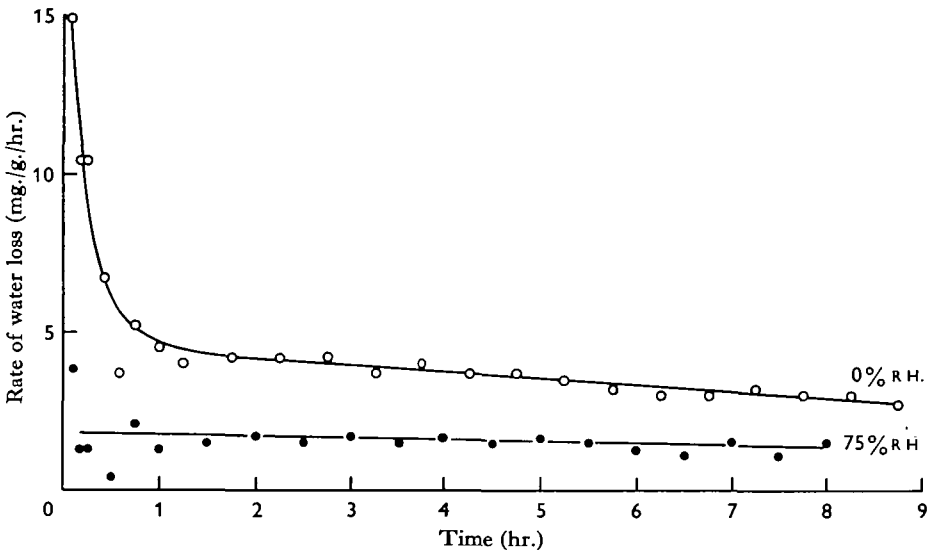

Fig. 4. Decrease in rate of cuticular water loss with time. Temperature 29·5° C. Open circles 0% R.H., solid circles 75% R.H.

Confirmation of this phenomenon was sought using single locusts with blocked spiracles suspended from a balance inside a tube containing a humidity-controlling substance. Weighing at frequent intervals provided an estimate of water loss which was plotted against time for two humidities—0 and 75% R.H. (Fig. 4). The differences are at once apparent: cuticle exposed to low relative humidity appears to undergo a rapid decrease in permeability during the first hour, but this does not happen at high relative humidities (75% R.H.). After the first hour there is a more gradual decrease in water loss which is greater at 0% than at 75% R.H.

Three possible explanations can be given for this phenomenon. The first is that a loss of hygroscopic water is implicated in high rates of water loss during the first hour. This hypothesis was at first thought doubtful in view of the fact that in locusts pre-exposed to approximately 50% R.H. (as all these experimental animals were), the phenomenon was observed in low humidity treatments, but the converse was not found in high humidity treatments (i.e. an apparently low rate of loss at first). Hygroscopic considerations may, however, account for at least some of the large initial water loss, since the loss of hygroscopic water in dry air may well take place faster than gain by the same hygroscopic material in moist air. A second possibility is that there is some

change in the cuticle or within the body of the locust after death that results in this phenomenon. This cannot reasonably be excluded, although the speed of the change makes this explanation unlikely. Experiments lasting 10 hr. and involving exposure of killed locusts to alternating 25% and 0% R.H. reinforce this view (see page 8). The third possibility is that there is some permeability change which might be regarded in effect as a mechanism of water conservation, since it appears at low relative humidities only.

The hygroscopic properties of locust cuticle. A freshly moulted (1-day old) adult locust was starved for 16 hr. at 29·5° C., 96% R.H. and killed in HCN. Mouth, anus

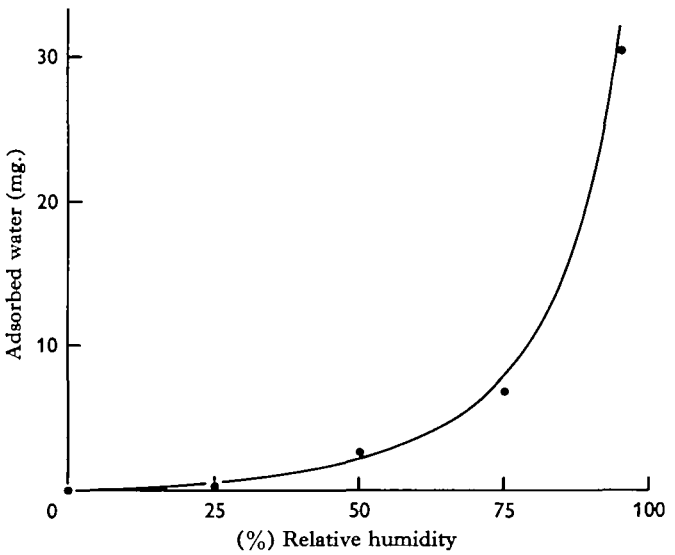

Fig. 5. The amount of hygroscopic water adsorbed on to the dry cuticle of a whole locust (live weight 0·86 g.).

and spiracles were carefully blocked with cellulose paint, and the locust was weighed. It was then gently dried at room temperature over concentrated H_2SO_4 for 8 hr. and then further dried at reduced pressure over concentrated H_2SO_4. A weighed nickel ring was placed in the wing tips for suspension and the locust was stored over P_2O_5 for a further 24 hr. to complete the drying. The locust was weighed at 5 min. intervals to 0·1 mg. over P_2O_5 until five constant consecutive readings were obtained. The tube containing the P_2O_5 was then replaced by one containing a KOH solution to give 25% R.H. and the weighing was repeated. The same procedure was observed using KOH solutions to give 50, 75 and 95% R.H. and the amount of water adsorbed at each of these relative humidities at a temperature of 29·5° C. was measured. The hygroscopic curve of a dry locust of live weight 0·86 g. is given in Fig. 5.

From the finding that 30·5 and 2·6 mg. of water are adsorbed on to the surface of a locust at 95% and 50% R.H. respectively it would appear likely that at least some of the phenomenon of high initial transpiration (Fig. 4) can be explained on hygroscopic grounds.

The demonstration of permeability change. The third possibility, that a real perme-

ability change is responsible for some of the initial and most of the secondary decrease in the rate of water loss from the cuticle exposed to dry air was investigated. Again, single dead locusts with blocked spiracles were used, suspended from a balance inside tubes with relative humidity controlled at 0% or 25% and temperature at 29·5 ± 0·5° C. At first the freshly killed locust was weighed at 10 min. intervals over 2 hr. while exposed to 0% R.H. Then a tube giving 25% R.H. was substituted and the weighings continued for a further 2 hr., when a fresh tube of P_2O_5 was substituted. This procedure was repeated six times in all to give alternating 2 hr. periods of 0% and 25% R.H.

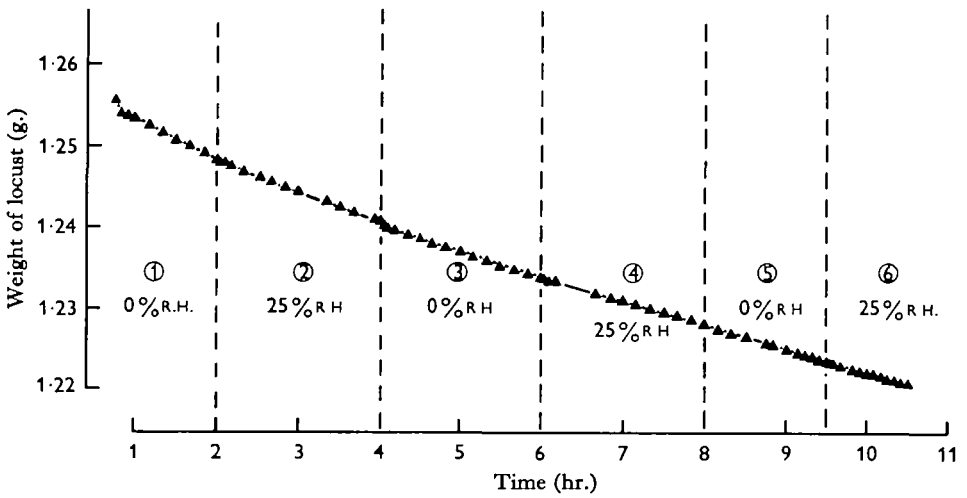

Fig. 6. Rates of cuticular water loss from a single locust at 29·5° C. and alternate 0% and 25% R.H. Figures encircled represent slope number (see text).

Table 3. *Slopes from calculated regressions giving rate of cuticular water loss of whole locusts exposed to alternating* 25% R.H. *and* 0% R.H. *at* 29·5° C.

Slope No.	R.H. (%)	Sat. def. (mm. Hg)	Total water loss (mg./g./hr.)	Water loss calculated from slope* (mg./g./hr.)	Slope *b*	Water loss (mg./g./mm. Hg/hr.)
3	0	30·8	2·7	2·5	−0·0561	0·083
4	25	23·1	2·1	2·2	−0·0451	0·097
5	0	30·8	2·4	2·4	−0·0516	0·079
6	25	23·1	2·0	2·2	−0·0439	0·097

* Water loss read over the straight portion of the slope (see Fig. 6).

Significance of the difference between slopes: between 3 and 4, P 0·4–0·2; between 3 and 5, P 0·4–0·2; between 3 and 6, $P > 0·5$; between 4 and 5, P 0·1–0·05; between 4 and 6, $P > 0·5$; between 5 and 6, P 0·2–0·1.

It can be seen from Fig. 5 that the amount of hygroscopic water on the cuticle at 29·5° C., 25% R.H. is 0·2 mg. so that the effect of loss or gain of hygroscopic water between 0% and 25% R.H. will not materially affect weight loss determination, and can be ignored. The weight of the locust plotted against time (Fig. 6) clearly shows differences in the rate of water loss between 25 and 0% R.H. The slopes represent the

rate of water loss, and for the purposes of discussion slopes 1 and 2 are ignored so that the phenomenon of high initial water loss (due in the main to loss of hygroscopic water) is excluded. Inspection of Fig. 6 and the corresponding regression analysis summarized in Table 3, show that there is no significant difference in the rate of water loss at the two different humidities, despite the great difference in evaporating power. The most likely explanation of this apparent discrepancy is that cuticular permeability is affected by the humidity of the air to which the cuticle is exposed. If the rates are corrected to allow for the drying power of the air, and expressed as mg./g./mm. Hg/hr. a measure of permeability can be obtained which is 0·079–0·083 mg./g./mm. Hg/hr. in dry air as compared with 0·097 mg./g./mm. Hg/hr. in air at 25% R.H. (see Table 3).

The implication of this experiment is that the permeability to water of locust cuticle is lower at 0% R.H. 29·5° C. than it is at 25% R.H. 29·5° C., and provides an answer to the secondary (slow) permeability decrease with time shown in Fig. 4. It seems likely therefore that the apparent rapid initial decrease in permeability of the cuticle in dry air is due mainly to loss of adsorbed water and partly to a real permeability change. The secondary decrease in water loss with time is due wholly or mainly to the continuing permeability change.

The relation between cuticular water loss and saturation deficit

Using the standard Perspex box readings of water loss from dead locusts at a range of five different relative humidities at 30 ± 1° C. were obtained. Size correction was to a standard locust of 1·6 g. live weight as a large number of readings were available (Table 2). It can be seen that the relation between cuticular transpiration and saturation deficit at this temperature is curvilinear (Fig. 7). At high saturation deficit (low relative humidities) the rate of water loss is considerably lower than it would have been if the values obtained at 96–50% R.H. were extrapolated in a linear fashion. If this happens in the living insect, a considerable saving of water is effected under conditions where water would be at a premium. If the phenomenon is not an artifact it represents a saving of between 1·5 and 2·5 mg./locust/hr. at 25% R.H., 30° C. and between 2·7 and 4·0 mg./locust/hr. at 0% R.H., 30° C. In a dead locust with occluded spiracles this may not represent *active* regulation of water loss, although there may well be a phenomenon associated with cuticle structure which has precisely this result.

The surface of the cuticle of the locust is hygroscopic (Fig. 5), but clearly if adsorption of water vapour occurred at low saturation deficits and the loss of adsorbed water at high saturation deficits it cannot explain the curve (Fig. 7) as it stands. One would expect that if a significant error were involved due to loss or gain of hygroscopic water, then a curve might result, but that loss of rectilinearity would tend to favour an inordinately *high* rate of loss at high saturation deficits. The possibility of error in humidity control was discounted when several replicates using fresh made KOH solutions and fresh P_2O_5 produced the same results.

The fact that high initial rates of water loss occur at low relative humidities does not substantially alter calculations of mean rates of water loss at any particular relative humidity. This can be seen by manipulating water-loss measurements obtained at hourly intervals into groups including the first 3 hr. values and the last 3 hr. values, when the same type of curvilinear relationship is obtained. It appears, therefore, that

the apparent decrease in cuticular permeability with time observed only at the lower range of relative humidities (Fig. 4) can be accounted for by two different phenomena. The initial rapid decrease is an artifact resulting from rapid loss of hygroscopic water adsorbed on to the cuticle. The contribution of permeability change to this high initial water loss is unknown. The secondary decrease (Fig. 4) is of the right order of magnitude to account for the anomalous curvilinear relationship between water loss and saturation deficit. From this evidence, and from the confirmed lower relative permeability at 0% R.H. than at 25% R.H. it is concluded that the relationship between rate of water loss and saturation deficit at 30° C. is curvilinear, falling away at high saturation deficits as depicted in Fig. 7. Thus locusts exposed to low humidities lose less water than might otherwise be expected as cuticle exposed to these low humidities undergoes some sort of permeability change which is reversible when the humidity of the air becomes high once more.

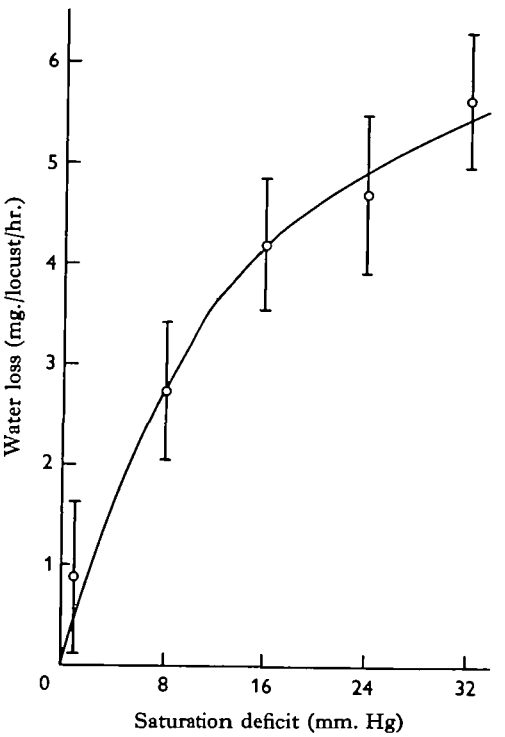

Fig. 7. The relationship between rate of cuticular water loss from a locust of 1·60 g. and saturation deficit at 30° C. The limits indicate ±2 × standard error.

DISCUSSION

It has been shown (Fig. 7) that the permeability of locust cuticle in dry air at 30° C. is 5·63 ± 0·67 mg./locust/hr. using whole animals. This can be converted to 0·71 mg./cm.²/hr., which may be expressed as 0·022 mg./cm²/mm. Hg/hr. for comparison with the permeability figures of other workers (see Bursell, 1964).

The demonstration of a transition temperature in the cuticle of *Locusta* at 46–48° C.

confirms the similarity to *Schistocerca* (Beament, 1959) in this aspect of its water relations. It would appear likely, therefore, that an organized lipid monolayer confers most of the resistance to the outward movement of water in the cuticle of *Locusta*. Although the effect of temperature upon the cuticle permeability of insects has been widely investigated, the effect of relative humidity has not received comparable attention.

The phenomenon of decreasing transpiration with time (from whole animals), shown in this work to occur in *Locusta* under certain conditions, had been previously demonstrated in *Agriotes* and *Aphodius* larvae (Wigglesworth, 1945); in mature *Pieris* pupae, mature *Rhodnius* nymphs and in young *Tenebrio* pupae (Beament, 1959). In all cases the decrease was observed at high saturation deficits. A similar phenomenon has been reported for woodlice by Edney (1951) and by Bursell (1955). It seems possible that the two observed phenomena in the cuticle of *Locusta*—decrease in permeability at high saturation deficits and decrease in rate of water loss with time—could be in some way related. This is a feature of transpiration in some woodlice (Bursell, 1955). There appear to be at least three possible explanations for these features of cuticular water relations, and although scarcely any direct evidence is available it is perhaps worthwhile to discuss their relative merits.

King (1944, 1945) showed that the permeability of keratin membranes to water vapour was higher at 70–80% R.H. than at lower relative humidities. Gluckauf (1944) confirmed this work with keratin and cellulose membranes, and Lovegren & Feuge (1954) have since shown a similar pattern using acetostearin films (di- and triglycerides, waxlike on solidification). The same may well hold true of the cuticle of *Locusta*. An animal in high saturation deficits may experience a quick initial water loss from the cuticle, which will result in shrinkage of the cuticle and decrease in intermicellar pore dimension, reducing the diffusion rate. This effect may be further enhanced by localized build-up of ions, which may further increase contraction if the pH shifts towards the iso-electric point of cuticle proteins (Bursell, 1955). The possibility of dehydration and pH changes affecting the permeability of the lipid epicuticle itself cannot be excluded (Ebeling, 1964).

Wigglesworth (1945) showed that certain detergents and emulsifiers applied to *Rhodnius* nymphs increased the transpiration rate. If the lipid epicuticle is secreted onto the surface of the cuticle in the form of an emulsion (Beament, 1964*b*) it seems likely that the emulsifier will still be present in the wax mixture. Now the emulsifier will become active in the presence of water (e.g. at high R.H.) tending to disorientate the wax molecules and so increasing the permeability of the cuticle to water. It has not, however, been shown that these emulsifiers do exist in insect cuticle, and in the case of *Periplaneta* (Beament, 1955) the wax is secreted on to the surface of the cuticle dissolved in short-chain paraffins and alcohols. In the case of the egg of the tick, *Ornithodorus moubata*, however, the waterproofing wax is rendered dispersable by protein (Lees & Beament, 1948). A similar situation may exist in the egg of the mite *Metatetranychus* (Beament, 1951). Locke (1964) considers that the wax may be transported in lipid-water liquid crystals found in the cuticle of some insects. Dehydration of this lipid-water crystalline system might result in decreased permeability.

Davies & Edney (1952) found that transpiration proceeded at a higher rate from the cuticle of dead than from living spiders and attributed this to 'active secretion' of

water by the epidermal cells in the living animal. Lees (1947) also proposed active secretion of water inward from the cuticle to account for low permeability rates of certain ticks in dry air—an extension of the mechanism of absorption of water vapour from unsaturated air. More recently Winston & Nelson (1965) proposed an active transport mechanism to explain anomalous relationships between water loss and humidity in the mite *Bryobia praetiosa*. Winston (1967) considers that a 'cuticular water pump' exists in the epidermis of *Periplaneta americana* and *Locusta migratoria* (nymphs) which maintains the level of activity of water in the cuticle at a level lower than in the haemolymph. This might, therefore, account for anomalous relationships between cuticle permeability and relative humidity or for active uptake of water, if this were shown to occur. It has, however, been shown that adult *Locusta* do not absorb water vapour or liquid water through the cuticle (Loveridge, 1967). It is felt that the experimental evidence put forward by Winston (1967) to support the theory of a 'cuticular water pump' in *Locusta* is inadequate at present. This does not mean that some water-transport mechanism cannot explain the curvilinear relationship between cuticular transpiration and saturation deficit at 30° C shown by the present work for adult *Locusta* (albeit using freshly killed locusts).

Whatever the mechanism of this phenomenon it does result in substantial reduction in transpiratory water loss through the cuticle at high saturation deficits. If this does occur in the living animal it may be of considerable significance, conserving water reserves at times when reduction in water loss is important.

SUMMARY

1. The rate of water loss from the cuticle of *Locusta* is proportional to weight.

2. The rate of water loss from the cuticle at 30° C., 0% R.H. is $5{\cdot}63 \pm 0{\cdot}67$ mg./locust/hr., giving a permeability of 0·022 mg./cm.^{2}/mm. Hg/hr.

3. The transition temperature at 46–48° C. is similar to that of *Schistocerca* (Beament, 1959) and probably indicates the existence of an oriented lipid monolayer in the epicuticle.

4. At relative humidities of 0–50% the rate of water loss from whole locusts decreases with time. This phenomenon, which does not occur at 75% R.H., is partly due to the loss of adsorbed hydroscopic water during the initial period. A continuing apparent decrease in transpiration is shown to be a true permeability change.

5. The relationship between saturation deficit and rate of water loss at 30° C. is curvilinear, falling away at high saturation deficits. This results in a saving of water amounting to 1·5–2·5 mg./locust/hr. at 25% R.H. and 2·7–4·0 mg./locust/hr. at 0% R.H. and will be biologically significant if not an artifact.

6. The anomalous relationship between saturation deficit and rate of water loss is caused by the permeability change occurring at low R.H. Three theories which may account for these phenomena are discussed.

This work formed part of a thesis submitted for the Ph.D. degree of the University of London. I am indebted to Professor E. Bursell for his supervision and to the Anti-Locust Research Centre, London, for financial support.

REFERENCES

BEAMENT, J. W. L. (1945). The cuticular lipoids of insects. *J. exp. Biol.* **21**, 115–31.

BEAMENT, J. W. L. (1951). The structure and formation of the egg of the fruit-tree red spider mite *Metatetranychus ulmi* Koch. *Ann. appl. Biol.* **38**, 1–24.

BEAMENT, J. W. L. (1955). Wax secretion in the cockroach. *J. exp. Biol.* **32**, 514–38.

BEAMENT, J. W. L. (1958). The effect of temperature on the waterproofing mechanism of an insect. *J. exp. Biol.* **35**, 494–519.

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. (1961*a*). The water relations of insect cuticle. *Biol. Rev.* **36**, 281–320.

BEAMENT, J. W. L. (1961*b*). Electrical properties of orientated lipid on a biological membrane: an electrostatic diffusion barrier and ion pump. *Nature, Lond.* **191**, 217–21.

BEAMENT, J. W. L. (1964*a*). The active transport and passive movement of water in insects. *Adv. Insect Physiol.* **2**, 67–129.

BEAMENT, J. W. L. (1964*b*). The active transport of water: evidence, models and mechanisms. *Symp. Soc. Exp. Biol.* **19**, 273–98.

BURSELL, E. (1955). The transpiration of terrestrial isopods. *J. exp. Biol.* **32**, 238–55.

BURSELL, E. (1964). Environmental aspects: humidity. Chapter 8 (pp. 323–61) in *Physiology of Insecta*, vol. I, ed. M. Rockstein. London and New York: Academic Press.

BUXTON, P. A. (1931). The measurement and control of atmospheric humidity in relation to entomological problems. *Bull. ent. Res.* **22**, 431–47.

CHEFURKA, W. & PEPPER, J. H. (1955). Studies on the cuticle of the grasshopper *Melanoplus bivittatus* (Say.) (Orthoptera: Acrididae). *Can. Ent.* **87**, 145–71.

DAVIES, M. E. & EDNEY, E. B. (1952). The evaporation of water from spiders. *J. exp. Biol.* **29**, 571–82.

EBELING, W. (1964). The permeability of insect cuticle. Chapter 9 (pp. 507–56) in *Physiology of Insecta*, vol. III, ed. M. Rockstein. London & New York. Academic Press.

EDNEY, E. B. (1951). The evaporation of water from woodlice and the millipede *Glomeris*. *J. exp. Biol.* **28**, 91–115.

EDNEY, E. B. (1954). Woodlice and the land habitat. *Biol. Rev.* **29**, 185–219.

GLUCKAUF, E. (1944). Permeability of absorbing substances. *Nature, Lond.* **154**, 831–2.

HUNTER-JONES, P. (1961). *Rearing and Breeding Locusts in the Laboratory*. Anti-Locust Research Centre.

JAKOVLEV, V. & KRÜGER, F. (1953). Vergleichende Untersuchungen zur Physiologie der Transpiration der Orthopteren. *Zool. Jb.* (*Physiol.*) **64**, 391–428.

KING, G. (1944). Permeability of keratin membranes. *Nature, Lond.* **154**, 575–6.

KING, G. (1945). Permeability of keratin to water vapour. *Trans. Faraday Soc.* **41**, 479–87.

KOIDSUMI, K. (1934–5). Experimentelle Studien über die Transpiration und den Wärmehaushalt bei Insekten. *Mem. Fac. Sci. Agric. Taihoku imp. Univ.* **12** (1), 1–179 and (3), 281–337.

KÜHNELT, W. (1928). Über den Bau des Insektenskelettes. *Zool. Jb.* (*Anat.*) **50**, 219–78.

LEES, A. D. (1947). Transpiration and the structure of the epicuticle in ticks. *J. exp. Biol.* **23**, 379–410.

LEES, A. D. & BEAMENT, J. W. L. (1948). An egg-waxing organ in ticks. *Q. Jl microsc. Sci.* **89**, 291–332.

LOCKE, M. (1964). The structure and formation of the integument in insects. Chapter 7 (pp. 379–470) in *Physiology of Insecta*, vol. III, ed. M. Rockstein. London and New York: Academic Press.

LOVEGREN, N. V. & FEUGE, R. O. (1954). Food coatings. Permeability of acetostearin products to water vapour. *J. agric. Fd. Chem.* **2**, 558–63.

LOVERIDGE, J. P. (1967). The water balance of *Locusta*. Ph.D. thesis, University of London.

MALEK, S. R. A. (1958). The appearance and histological structure of the cuticle of the desert locust, *Schistocerca gregaria* (Forskål). *Proc. R. Soc.* (B) **149**, 557–70.

RAMSAY, J. A. (1935). The evaporation of water from the cockroach. *J. exp. Biol.* **12**, 373–83.

UVAROV, B. P. (1948). Recent advances in acridology: anatomy and physiology of Acrididae. *Trans. R. ent. Soc. Lond.* **99**, 1–73.

WIGGLESWORTH, V. B. (1945). Transpiration through the cuticle of insects. *J. exp. Biol.* **21**, 97–114.

WINSTON, P. W. (1967). Cuticular water pump in insects. *Nature, Lond.* **214**, 383–4.

WINSTON, P. W. & BATES, D. H. (1960). Saturated solutions for the control of humidity in biological research. *Ecology* **41**, 232–7.

WINSTON, P. W. & NELSON, V. E. (1965). Regulation of transpiration in the clover mite *Bryobia praetiosa* Koch (Acarina: Tetranychidae). *J. exp. Biol.* **43**, 257–69.