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

II. WATER LOSS THROUGH THE SPIRACLES

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INTRODUCTION

Spiracular control in some insects regulates the amount of water lost from the respiratory surfaces (e.g. Buxton, 1930; Wigglesworth, 1935; Bursell, 1957). Control of spiracular and ventilatory movements has been studied in resting and flying *Schistocerca* by Miller (1960*a*, *b*) and by Miller (1960*c*) and Weis-Fogh (1967). The fact that locusts ventilate the tracheal system (Miller, 1960*a*) introduces a new dimension to problems involving water loss, since water vapour will be lost not only by passive diffusion but also by forced convection. Hamilton (1964) has studied the discontinuous respiration of *Schistocerca*; Buck (1958) indicated (in theory at least) that discontinuous patterns of respiration in insects have their rationale in water conservation. It is with these patterns of ventilation and associated water loss from the tracheal system that this paper is concerned.

MATERIAL AND METHODS

Rearing and breeding conditions and the standard apparatus for relative humidity and temperature control have been described (Loveridge, 1968). Adult male and female *Locusta* aged between 10 and 40 days from the final moult were used since male *Schistocerca* have constant respiratory patterns and live weight during this time (Hamilton, 1964). Locusts were pre-starved at either 0%, 70% or 96% R.H. at 29·5° C. for 24 hr. to empty the gut and minimize the chances of defaecation. Before the experiment animals were exposed to approximately 50% R.H., 29·5° C. for about 15 min., and water loss was measured by weighing to 0·1 mg. before and after an hour's exposure to circulating air of controlled temperature and relative humidity. Careful handling by hind legs and wings prevented 'spitting', but if this or defaecation occurred the result was discarded. These results, therefore, are the sum of water loss through cuticle and spiracles unless otherwise stated.

In experiments where a flow of moist or dry air was required aquarium pumps passed air through concentrated H_2SO_4 and then over columns of $CaCl_2$ and self-indicating silica gel (to give 0 to 5% R.H.) or bubbled air through a Dreschel bottle of water (to give about 90% R.H.). For experiments requiring dry CO_2/air mixtures the required concentration of CO_2 in air was made up in an aspirator and passed through the drying apparatus.

Recordings of dorso-ventral abdominal ventilatory movements were made by a

frontal writing lever connected to the end of the second abdominal sternite recording on a smoked drum. The locust was supported ventral surface uppermost inside a Perspex 'gassing box' (Miller, 1960*a*) through which dry or moist air or CO_2/air mixtures could be passed. A manually operated marker gave a record of the movements of spiracle 2 (if required). During water-loss measurements the rate and pattern of ventilatory movements were assessed using a stopwatch at least four times during the hour's exposure.

RESULTS

The effect of age on transpiration. Loveridge (1968) found that there was no relation between age and cuticular water loss. Within the range of 10–40 days it was found that total transpiration increased linearly with age at two of five relative humidity treatments at a temperature of 30° C. Although not substantiated by the work of Hamilton (1964) it was thought that some loss of spiracular control occurred as the locusts became older. To eliminate possible age effects, the mean age of the sample was standardized to 28–30 days after moult in the case of all treatments in the experiment on the effect of relative humidity on water loss. Locusts 10–40 days old were used in the other experiments.

Table 1. *Correlations between live weight and rate of spiracular (+cuticular) water loss at* 30° C.

R.H. (%)	Regression equation $Y = a+bX$	S.E. of 'a'	No. values	t	P
96 ± 4	$Y = -2{\cdot}3752+1{\cdot}5071X$	0·4279	34	1·5316	0·2–0·1
75 ± 2	$Y = -1{\cdot}4038+4{\cdot}1742X$	0·5972	33	3·7470	< 0·001
50 ± 2	$Y = -3{\cdot}4592+7{\cdot}8705X$	0·4560	129	29·6106	< 0·001
25 ± 2	$Y = 5{\cdot}2309+2{\cdot}5720X$	0·4200	87	2·7467	0·010–0·005
0	$Y = 2{\cdot}0079+5{\cdot}2116X$	0·5079	56	6·0869	< 0·001

The effect of size on water loss. To enable comparisons to be made between samples of different mean size, size correction is necessary. Regressions of water loss (mg./hr.) against live weight (g.) give significant correlations at four of five relative humidities (30° C.) (Table 1). As the regression equations do not extrapolate to the origin, large water-loss samples are corrected to a 'standard locust' of 1·6 g. live weight using the calculated regression, and expressed in mg./locust/hr. The means of small samples are expressed in mg./g./hr. (see Loveridge, 1968).

The effect of activity on water loss. Bursell (1959) has shown that activity in adult *Glossina* increases water loss and abolishes spiracular regulation. Using the standard Perspex box with the false floor tipping about its mid-point, breaking a contact and so giving a recorded activity index, water loss from locusts (starved for 24 hr. at 96% R.H., 29·5° C.) at 50 ± 2% R.H. and 30 ± 1° C. was measured. Regression of water loss (Y, mg./hr.) on live weight (X, g.) in the normal manner (see Table 1) gave the equation

$$Y = -1{\cdot}72+5{\cdot}25X \quad (t = 4{\cdot}352, \text{D.F.} = 27, P < 0{\cdot}001).$$

If water loss (expressed as mg./g./hr.) is plotted against activity (Fig. 1) no apparent correlation can be found, and testing of water loss against live weight *and* activity by multiple regression gave a non-significant relationship. Thus it can be assumed that

there is no increase in water loss with increased activity *at this activity level*, and all the locusts can be assumed to be 'resting'.

The effect of relative humidity on spiracular water loss. Loveridge (1968) showed that during exposure to low humidities cuticular water loss decreased with time. Experiments with live animals (in which cuticular transpiration constitutes less than 50% of total water loss) showed no such phenomenon and so it was considered unnecessary to investigate further any time effects.

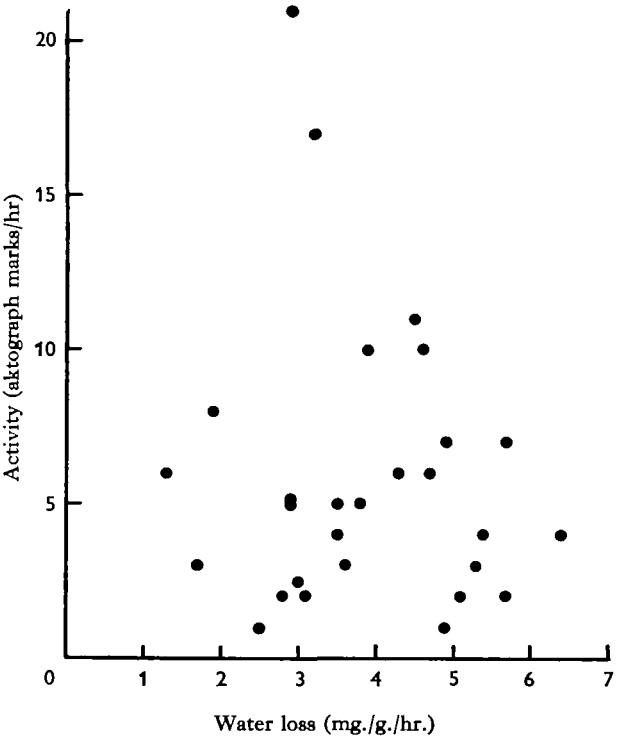

Fig. 1. The relationship between activity and water loss in the 'resting' locust. 50% R.H., 30° C.

To examine the effect of relative humidity on the rate of cuticular plus spiracular water loss, locusts pre-treated for 24 hr. at 96% R.H., 29·5° C. were exposed to humidities of 0, 25, 50, 75 and 96% R.H. at 30° C. From the calculated relationship between size and water loss (Table 1) the rate of water loss of a 'standard locust' at the five relative humidities can be derived (Fig. 2). The relationship between saturation deficit and cuticular plus spiracular water loss at 30° C. is curvilinear, falling away at high saturation deficits. The relationship between cuticular water loss and saturation deficit is known (Loveridge, 1968) so water loss through the spiracles alone can be calculated by simply subtracting mean cuticular water loss from mean spiracular plus cuticular water loss at each relative humidity. The computation of the error estimates of the new relationship was made according to the formula

$$\text{S.E.}_3 = \sqrt{\{(\text{S.E.}_1)^2 + (\text{S.E.}_2)^2\}}.$$

The validity of this procedure is strengthened by the fact that pre-treatments, experimental procedure and apparatus are the same for both sets of figures. The relationship between saturation deficit and spiracular water loss is given in Fig. 3. It is possible to fit a straight line within the fiducial limits of these observations, since the standard errors are relatively large; but the data strongly suggest the existence of curvilinear relationships as drawn (Figs. 2, 3) and it is concluded that there is a trend for propor-

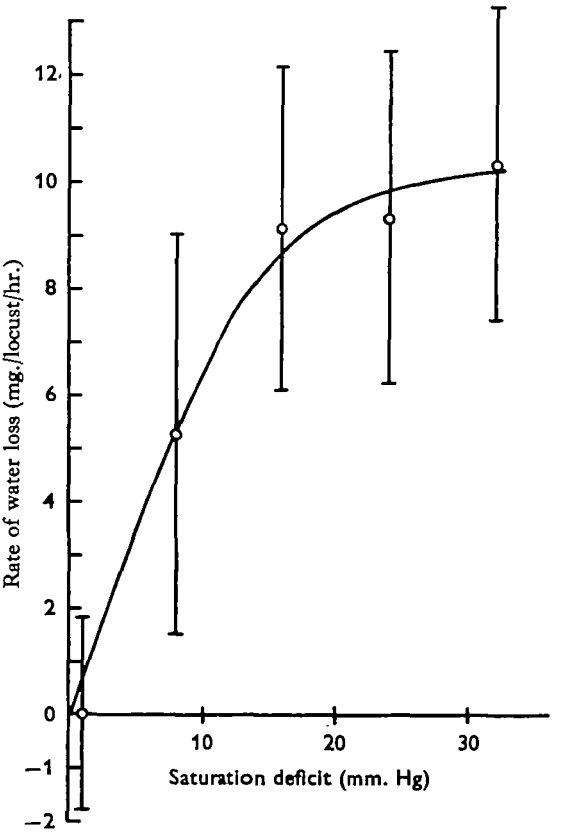

Fig. 2. The relationship between saturation deficit and total transpiration at 30° C. Limits indicate ± 2 × S.E.

tionately less water to be lost through the spiracles at high saturation deficits than in conditions where water loss is less critical. The saving is about 2·5 mg./locust/hr. at 25% R.H., 30° C. and 5·0 mg./locust/hr. at 0% R.H., 30° C., and must represent a useful physiological mechanism to prevent excessive desiccation in dry air.

The effect of carbon dioxide on water loss. The fact that the spiracles of insects can be kept open in air containing low percentages of CO_2 has been utilized by Mellanby (1934), Wigglesworth (1935), Bursell (1957) and others to measure the amount of control the spiracles have over water loss. Hoyle (1960) showed that the spiracles of *Schistocerca gregaria* remained open in 10–15% CO_2, and Miller (1960*b*), working on the same species, showed that in 5% CO_2 expiratory and inspiratory spiracles were

open for 80–90% of the respiratory cycle. During preliminary experiments, however, it was found that CO_2 concentrations of 10% failed to abolish spiracular movement completely, and that ventilatory movements were still strong. In order to clarify further the movements of spiracles and abdominal ventilation in atmospheres containing CO_2, recordings of dorsoventral abdominal ventilatory movements and movements of spiracle 2 were made in CO_2/air mixtures of different concentrations. From the specimen records (Fig. 4) and the graph (Fig. 5) it can be seen that in 30% CO_2 spiracular movements stop abruptly; the valves were observed to be open (Miller, 1960*b*; Hoyle, 1960). Abdominal respiratory movements fall away slowly from a

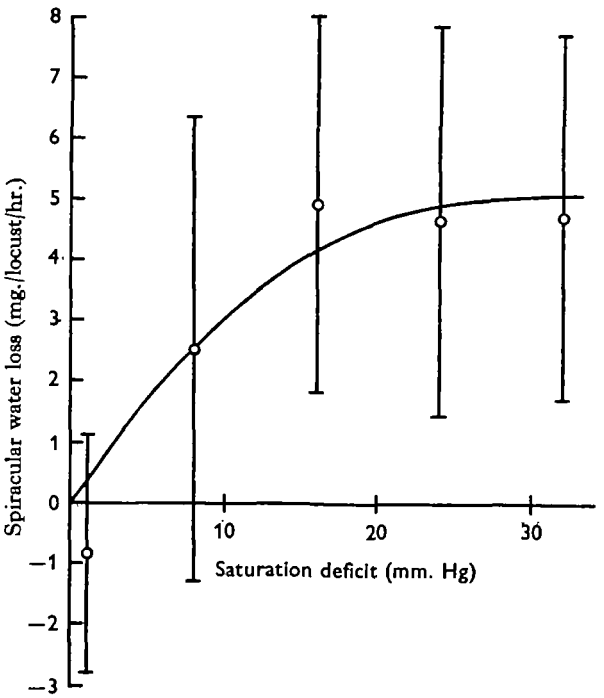

Fig. 3. The relationship between saturation deficit and water loss via the spiracles at 30° C. Limits indicate ±2 × S.E.

maximum of 100/min. in 10% CO_2 to about 60/min. in 70% CO_2, the amplitude of the movements also tending to decrease (Fig. 4). In 75% CO_2 abdominal movements are abolished altogether. Thus the CO_2 concentration required to open the spiracles is about 30%, but ventilatory movements are still present. In 75% CO_2 the spiracles are open and there are no ventilatory movements. The fact that this occurs allows the contributions of ventilation and spiracular opening to water loss to be assessed separately.

This was done in dry air in a chamber modified so that dry CO_2/air mixtures could be passed through it. After the locust had been introduced about 5 l. of the gas mixture were passed through the chamber at 1 l./min. and the taps were shut off. The air inside the chamber was circulated by a magnetically operated fan. Locusts were prestarved at 96% R.H. and samples of similar age were exposed to air, 20% CO_2, 50%

CO_2 and 80% CO_2 at 0% R.H., 30° C. None of the insects showed any sign of ill-effect after a few hours recovery in air. Special care was taken to exclude readings where 'spitting' or defaecation had taken place. The results of this experiment are given in Table 2. In 20% CO_2, where there is ventilation and some spiracular movement (Figs. 4, 5), water loss is just under double the normal rate. In 50% CO_2, where

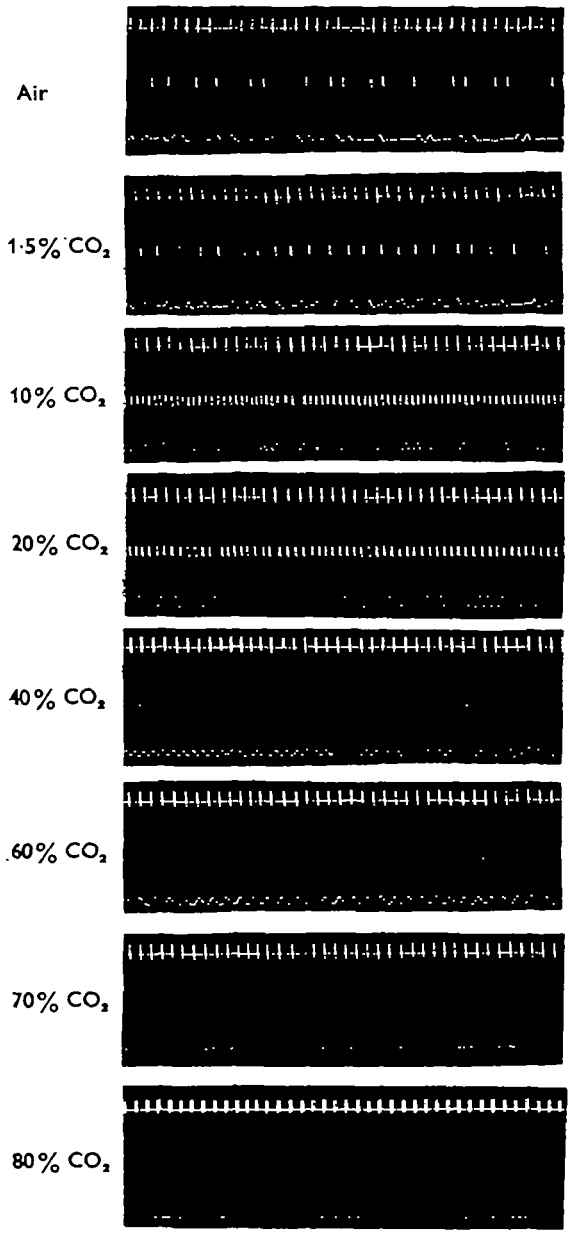

Fig. 4. Specimen records of the respiratory movements of *Locusta* in air and carbon dioxide/air mixtures. Each record consists of time marks (upper trace at one per second), movements of spiracle 2 (centre trace) and dorsoventral abdominal ventilatory movements (bottom trace).

the spiracles are kept open, and ventilation at the rate of about 60 movements/min is still in force, the normal water loss is more than doubled. In 80% CO_2, where all respiratory movements have ceased and the spiracles are open, water loss is only a little more than that in air.

Table 2. *The effect of CO_2 on rates of water loss from locusts exposed to* 0% R.H., 30° *C.*

Concentration of CO_2	Water loss ± 2 × S.E. (mg./g./hr.)	No. of readings
Air	5·4 ± 0·1	—
20% CO_2	10·1 ± 0·6	20
50% CO_2	11·9 ± 1·6	21
80% CO_2	5·8 ± 1·1	21

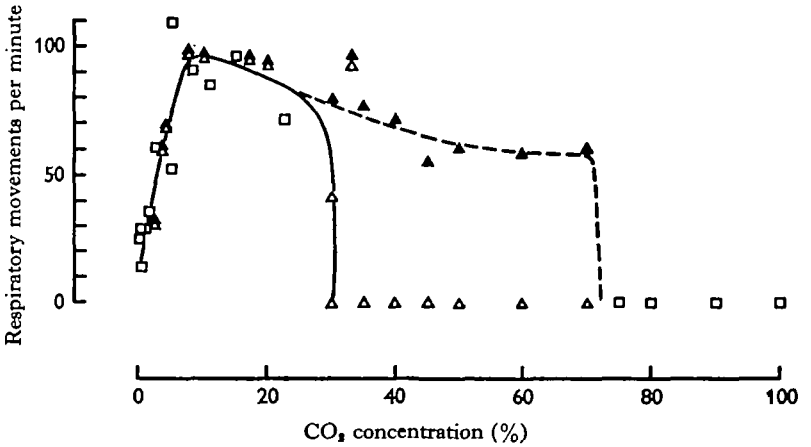

Fig. 5. The rate of respiratory movements of *Locusta* in air and carbon dioxide/air mixtures. △, Spiracular; ▲, abdominal; □, both types of respiratory movement.

The effect of temperature on water loss. Water loss and rates of abdominal ventilatory movements were measured for single locusts exposed to a range of temperatures at 0% R.H. Typical results for two males are given in Fig. 6. Ventilation was discontinuous below 30° C., becoming continuous (dorsoventral abdominal movements) over the range 30–38° C. At 41° C. all four types of ventilatory movements (Miller, 1960*a*) were invoked, and at 46° C. the locust supported itself above the perforated zinc false floor with hindlegs against the wall and fore-legs on the floor. Rapid, deep ventilatory movements of all four types were present for the first 10 min., after which frantic jumping occurred, and the locust was removed after a 15 min. exposure. In Fig. 6 it may be seen that there is a close correlation between rate of ventilation and rate of water loss (expressed in mg./g./mm. Hg/hr. × 10 and so independent of saturation deficit). Between 42° and 45° C. the ventilatory rate increases enormously, and this is accompanied by a similar increase in the rate of water loss. Presumably the hyperventilation ('panting'—Weis-Fogh, 1956) and the increased rate of water loss results in depression of body temperature, but in the absence of body temperature measurements this must be regarded as not proven. The temperature effect, however,

does serve to emphasize the close relationship between ventilation and water loss in *Locusta*.

The effect of pre-treatment on water loss. There are indications that locusts starved for 24 hr. at 96% R.H. lose proportionately less water at low than at high relative humidities (Fig. 3). Locusts with adequate water reserves (such as those starved at

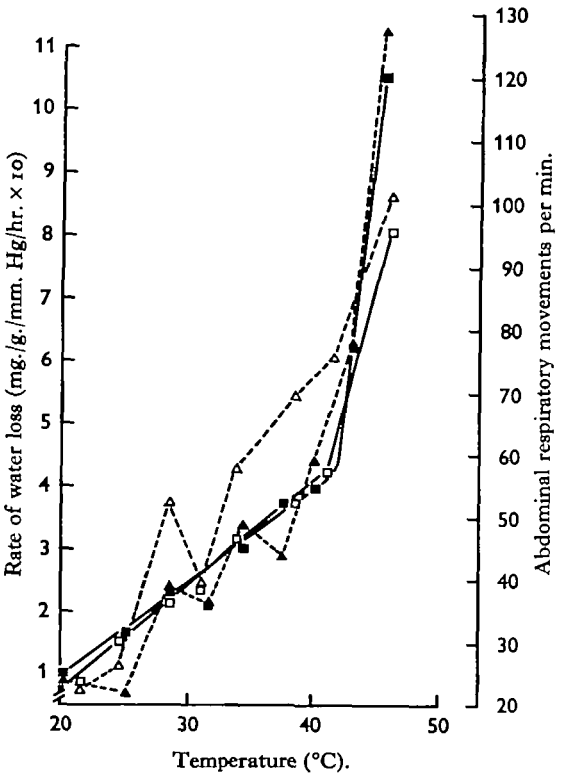

Fig. 6. The effect of temperature on water loss and abdominal ventilatory rate of male *Locusta*. Water loss (■—■) and respiratory rate (▲—▲) of animal 1. Water loss (□—□) and respiratory rate (△—△) of animal 2.

96% R.H.) might not be expected to show the full extent of their powers of water conservation during a 1 hr. exposure to dry air. Thus, for comparison, similar samples of locusts were pre-starved for 24 hr. at low or high relative humidity and water loss was measured in the same apparatus.

In the first experiment samples of male locusts were starved at 96% R.H., about 70% R.H., or 0% R.H. (29·5° C.) for 24 hr. Water loss was measured over 1 hr. exposure to 50% R.H., 30° C. (Table 3). Direct comparison of water loss in the three groups will be valid only if the mean live weights of the samples are the same. In this case, as only males were used, the comparison of rates of water loss is valid as the samples are not significantly different in live weight. Table 3 shows that the samples pre-treated at 70 and 96% R.H. were not significantly different from one another in respect of water loss, but that they were both significantly different from the sample pre-treated at 0% R.H. The extent of water conservation indicated in the group starved

Table 3. *Spiracular (+ cuticular) water loss of male locusts exposed for 1 hr. to 50% R.H., 30° C.*

Sample no.	1	2	3
Pre-conditioned	24 hr. at about 70% R.H., 29·5° C.	24 hr. at 96% R.H., 29·5° C.	24 hr. at 0% R.H., 29·5° C.
No. in sample	21	27	29
Live weight (g.)	1·31 ± 0·07	1·27 ± 0·10	1·30 ± 0·07
Water loss (mg./g./hr.)	4·14 ± 0·78	3·83 ± 0·51	1·37 ± 0·23

Significance of difference in live weight between samples:

1 *vs.* 2	$P > 0·500$	Non-significant
1 *vs.* 3	$P > 0·500$	Non-significant
2 *vs.* 3	$P > 0·500$	Non-significant

Significance of difference in water loss between samples:

1 *vs.* 2	$P = 0·500–0·400$	Non-significant
1 *vs.* 3	$P < 0·001$	Significant
2 *vs.* 3	$P < 0·001$	Significant

Table 4. *The effect of pre-treatment on spiracular (+ cuticular) water loss and abdominal ventilatory rate of locusts exposed to 0% R.H., 30° C. for 1 hr.*

	Locusts starved 24 hr. at 96% R.H.			Locusts starved 24 hr. at 0% R.H.		
	Abdom. resp. movements/min.	H_2O loss (mg./g./hr.)	Live weight (g.)	Abdom. resp. movements/min.	H_2O loss (mg./g./hr.)	Live weight (g.)
	29	4·9	1·36	16	2·7	2·06
	29	3·5	2·70	24	2·7	1·66
	53	5·4	1·62	23	3·6	1·60
	33	4·5	1·16	26	3·9	1·47
	28	5·3	1·50	20	2·3	1·60
	31	6·6	1·62	40	3·7	1·83
	32	4·7	2·99	28	3·6	2·10
	37	5·6	1·63	0	4·2	2·08
	19	4·7	1·35	30	4·9	1·31
	29	3·5	2·69	15	3·9	1·59
	53	4·5	1·61	13	3·6	1·65
	39	5·2	1·77	11	4·1	2·04
	38	6·3	2·90	21	3·0	1·83
	41	4·3	3·13	27	2·6	2·45
	28	4·3	2·36	16	3·2	1·61
	32	7·1	1·36	13	2·9	1·54
	34	5·8	1·52	19	2·8	1·72
	44	4·5	1·75	21	3·5	1·49
	30	6·0	1·59	21	2·0	2·71
	34	5·7	2·88	32	2·5	2·16
	34	7·8	3·11	25	2·5	2·59
	30	4·2	1·39	12	3·1	1·82
	23	6·1	1·35	14	2·8	2·43
	44	3·9	2·34	23	3·9	1·60
	24	7·3	1·51	15	2·8	1·53
Mean ± 2 × S.E.	34 ± 3	5·3 ± 0·5	1·97 ± 0·27	20 ± 3	3·2 ± 0·3	1·86 ± 0·15

Significance of differences between samples in respect of:

(*a*) Live weight $t = 0·712$, D.F. 49 $P = 0·500–0·400$ Non-significant

(*b*) Water loss $t = 7·723$ D.F. 49 $P < 0·001$ Significant

(*c*) Abdominal ventilatory rate $t = 5·985$ D.F. 49 $P < 0·001$ Significant.

at 0% R.H. over the groups starved at high R.H. is in the region of 2·6 mg./g./hr. at 50% R.H., 30° C. Clearly this is a considerable saving of water and it is likely that the conservation mechanism involves the respiratory system.

The second experiment was designed to confirm the differences in water loss between 'dry' pre-treated and 'wet' pre-treated locusts and to elucidate the conservation

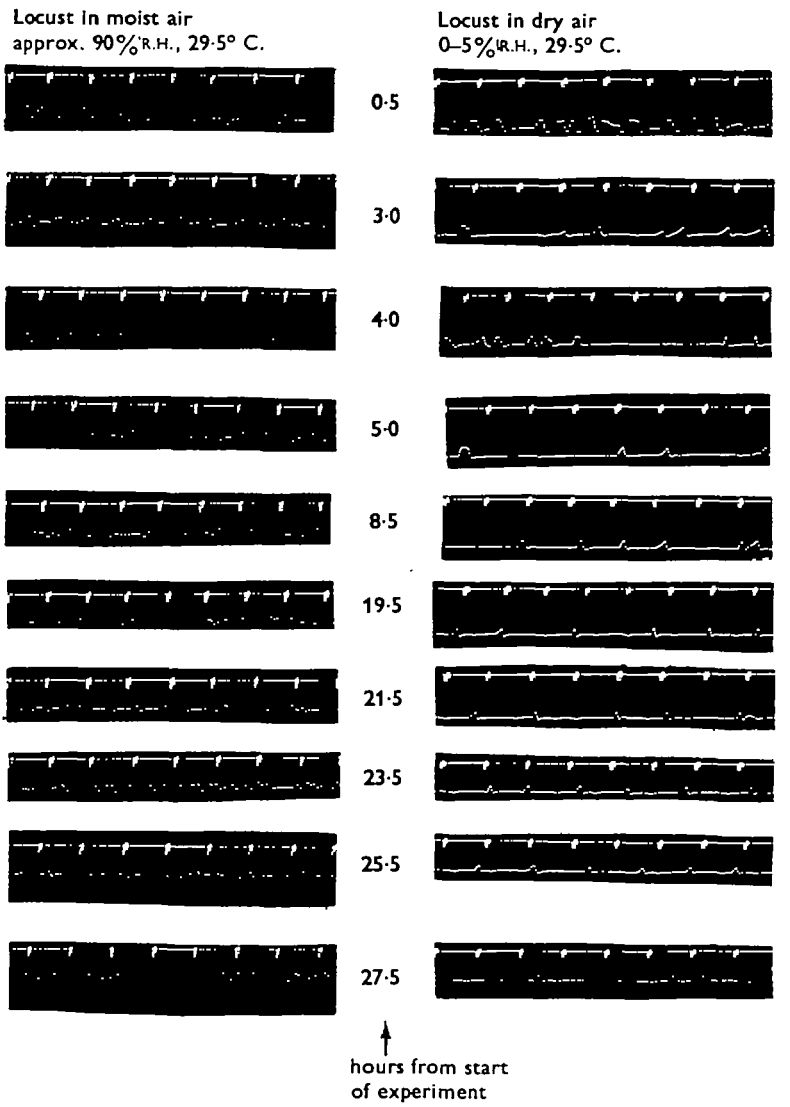

Fig. 7. Dorsoventral abdominal ventilatory movements (expiration upwards, inspiration down) of similar adult male locusts, 27 days after moult. Central figures represent duration of starvation (in hours) in moist air (left) and in dry air (right). Time mark intervals = 5 sec.

mechanism. Two samples of both sexes were starved at 0% R.H. or 96% R.H. and water loss was measured over 1 hr. exposures to 0% R.H., 30° C., during which time the rate of dorsoventral abdominal ventilation was assessed. The results of this experi-

ment are given in full in Table 4. The two samples are similar in mean weight, so direct comparison of water losses and ventilatory rates can be made. The difference between water losses of $5{\cdot}3 \pm 0{\cdot}5$ mg./g./hr. (wet pre-starved) and $3{\cdot}2 \pm 0{\cdot}3$ mg./g./hr. (dry pre-starved) is significant, and so a saving of 2·1 mg./g./hr. at 0% R.H., 30° C. is effected. This represents a 25% conservation of potential water loss. The difference of fourteen abdominal ventilatory movements per minute between 'wet' and 'dry' pre-treated locusts is a reduction of 23% by the dry pre-starved animals. This shows that the locust is capable of reducing its rate of ventilation during desiccation and that this reduction is followed by a commensurate decrease in the rate of water loss.

Ventilation and water conservation. In experiments detailed above it has been shown that the rate of water loss from the tracheal system follows the rate of ventilation quite closely. Reduction in the rate of water loss occurs when locusts are exposed to dry air, and also when locusts have been desiccated. To study the increased ventilatory control under conditions of desiccation, pairs of locusts of the same weight, age, sex and rearing conditions were used. The dorsoventral abdominal respiratory movements of both were recorded at intervals over 1–4 days, the one locust exposed to a flow of dry air, the other exposed to a flow of moist air. Care was taken to arrange the frontal writing levers to give similar recorded movements for a given vertical movement of the locusts' abdominal sternites. Both locusts were starved, but as the metabolic rate of locusts pre-treated in dry or moist air is the same (Loveridge, 1967) starvation will not have substantially different effects on ventilation in the two animals. In effect the only differences between dry and wet treatments are the humidity of the air (which will affect hygroreceptors) and water-loss rates (which will affect water reserves).

From the simultaneous records (Fig. 7) the rate of ventilation, the amplitude of the movements and the incidence of discontinuities can be assessed. The patterns of ventilation, which are similar in the early stages, soon show a marked divergence. Compared with the locust in wet air, the desiccated locust shows reduction in the rate of ventilatory movements and their amplitude, and the incidence of pauses in which no movements are recorded increases. Clearly the amplitude and rate of ventilatory movements determine the volume of air pumped through the tracheal system in a given time. Assuming that the air within the tracheal system of both 'wet' and 'dry' treated insects contains the same amount of water vapour, the locust in dry air will lose considerably more water than the one in wet air, as each inspiratory movement draws unsaturated air into the tracheal system. If the rate of ventilatory flow of air is not curbed, then the locust in dry air will be faced with dangerously high evaporative water loss.

Figure 7 shows that the amplitude of the dorsoventral ventilatory movements of the abdominal sternites fall into two broad classes—large and small. If the contribution of large movements in pumping air is reckoned to be twice that of small movements, then rate and amplitude can be combined and scored. Amplitude is assessed as the vertical extent of the movement only: the broad, flat peaks sometimes obtained between expiration and inspiration do not necessarily increase amplitude—the air within the tracheal system may well be stationary, or at least moving very slowly. The time course of abdominal ventilation in dry and moist air assessed in this way is given in Fig. 8. The locusts in dry air show a rapid decrease in the rate of ventilation, and this low level is maintained over considerable durations. This effect, as well as an increase

in the appearance of discontinuities (which may also reduce water loss (Buck, 1958)), was noticeable in all experiments performed, using females as well as males.

It may be seen that struggling (as seen between 40 and 45 hr. in Fig. 8*a* and 25 and 30 hr. in Fig. 8*c*) substantially increases the rate of ventilation, but that low rates are restored when overt activity ceases. In Fig. 8*a* the ventilatory rate of the locust in wet

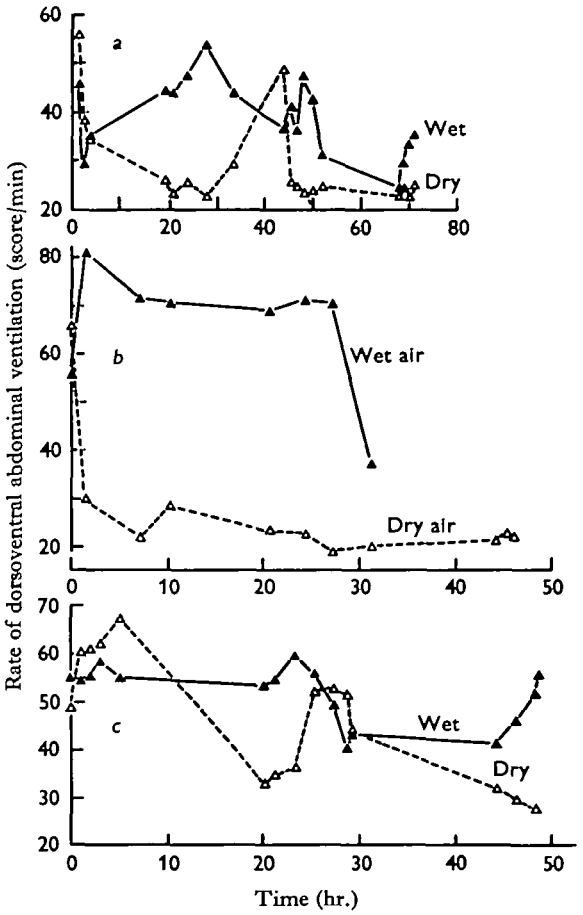

Fig. 8. The effect of starvation in dry (△—△) and in moist (▲—▲) air on the dorsoventral abdominal ventilatory rate of *Locusta* (see text). (*a*) 16-day-old males; (*b*) 20-day-old males; (*c*) 22-day-old males.

air decreased suddenly at about 30 hr., but this was just before it died. The possibility that insects might suffer ill-effects after being restrained would raise objections to the methods employed here (Beament, 1958). Experiments on restrained *Schistocerca* have given no cause to suppose that the same occurs in that species (Dr P. T. Haskell, personal communication). In the present experiments one locust died; in other cases the locusts were released and appeared to have suffered no permanent ill-effects.

The almost immediate reduction of ventilation in dry air may indicate that more rigorous control of water loss is triggered by sensory input from hygroreceptors on the antennae such as those found by Riegert (1960) in *Melanoplus* and by Aziz (1958)

in *Schistocerca*. This might explain why a certain amount of control is exerted in dry air regardless of the state of water reserves, but that the control becomes far more stringent after long exposures to desiccating conditions.

DISCUSSION

Studies on the water balance of various species of insect have shown that the spiracles exercise control over the amount of water lost from the tracheal system (Buxton, 1930; Mellanby, 1934; Wigglesworth, 1935). That spiracular regulation is more stringent under conditions where water is at a premium is evident from the work of Mellanby (1932*a*) on *Tenebrio* larvae, Mellanby (1932*b*) on *Cimex*, Bursell (1957) on *Glossina* adults and Miller (1964*a*) on dragonflies. The work detailed in this paper has shown that, in the locust, water loss is closely dependent upon the rate of ventilation. Normally, the movements of the spiracles are synchronized with the abdominal ventilatory movements, and air flow within the tracheal system is unidirectional (Miller, 1960*a*, *b*). Buck (1962) has suggested that the use of the first two pairs of spiracles for inspiration and the tenth pair for expiration (Miller, 1960*b*) might be a water conservation mechanism. This will be so only if either (*a*) a smaller volume of air is required to pass through the tracheal system to effect a given oxygen uptake than by alternative ventilatory methods, or (*b*) if the air in the main tracheal trunks along which unidirectional air flow occurs is not saturated with water vapour. Directional air flow through the tracheal system is thought to be inefficient for gas exchange (Buck, 1962). That directional flow is commonly found in resting locusts, and tidal air flow involving all the spiracles is found in gravid females, active and flying locusts (Miller, 1960*a*–*c*) supports this view, and makes alternative (*a*) unlikely. Beament (1964) has shown that the air in the tracheoles is almost saturated with water vapour. The fact that the main tracheal trunks are often lined with a lipid intima (Miller, 1964*b*; but see Locke, 1964) might indicate that the air in them need not at all times be saturated with water vapour, although experimental verification of this is clearly necessary. This may particularly be true during bursts of ventilatory activity (Hamilton, 1964), when the first few strokes will clear the tracheal trunks of water vapour, so that successive ventilatory strokes need not expire completely saturated air. Thus, under certain conditions, discontinuous patterns (Buck, 1958) and unidirectional flow of ventilation will combine effectively to reduce water loss. In any event tidal ventilation and a high ventilatory rate lead to high water losses (Table 2). Water loss by simple diffusion from the open spiracles of a non-ventilating locust proceeds at slightly more than the loss from a resting locust with normal respiratory movements (Table 2), so that control of water loss by spiracular regulation alone will be ineffective (at least in the presence of ventilation), whilst control of ventilation has considerable importance as a means of water conservation.

Beament (1964) stated that the air in the tracheoles is at 99% R.H. or more, so that for present purposes it will be assumed that the air in the tracheal system is saturated (although this assumption may not be valid in all circumstances). If the diffusion of water vapour out of the spiracles is ignored, then the rate of water loss through the spiracles at a given ambient humidity can be calculated if the volume of air pumped is known. Thus, with an ambient humidity of 0% and a temperature

of 30° C., 0·030039 mg. of water will be lost for each millilitre of air expired. Weis-Fogh (quoted by Miller, 1964*b*) has found that adult *Schistocerca gregaria* will move 40 l./kg./hr. with the abdominal pumping movements when at rest. When active the maximum abdominal ventilation is 250 l./kg./hr., and the neck and prothoracic movements may contribute 50 l./kg./hr. (Miller, 1960*a*). Thus, the corresponding rates of water loss will be 1·2, 7·5 and 1·5 mg./g./hr. It has been shown (Fig. 3) that the water loss via the spiracles at 30° C., 0% R.H. during normal ventilation is 2·9 mg./g./hr. (4·7 mg./locust/hr.), and that during hyperventilation it may be as high as 6·2–8·0 mg./g./hr. (Table 2). Taking into account age, sex and species differences, the observed results are in reasonably good accord with the calculated figures. McGovran (1931) showed that adult female *Chortophaga viridifasciata* ventilated at 0·222 ± 0·043 ml./g./min. at 28° C, so that in dry air the calculated rate of water loss is 0·40 ± 0·08 mg./g./hr., which is very low in comparison with results obtained with *Locusta*.

The results presented have shown that the ventilation of the tracheal system is the overriding factor in water loss via the spiracles in the locust. The amount of ventilation, its pattern and direction, is under very tight and efficient control. Conservation of water reserves under desiccating conditions is brought about by controlling ventilation in the following ways:

(*a*) Reduction in the volume of air pumped; (i) by decrease in amplitude of ventilatory movements, and (ii) by decrease in the rate of ventilatory movements.

(*b*) Institution of directional air flow.

(*c*) Increase in the discontinuity of ventilatory activity.

The conditions which bring about this regulation are less clearly understood, but it is thought that the combined factors of air humidity and the extent of water reserves will play their part. Control over ventilation is disrupted under the influence of CO_2 gas, after injection of 100 μl. 0·5 M-KCl (Loveridge, 1967) and during exposure to high temperatures, resulting in hyperventilation and increased water loss.

SUMMARY

1. The rate of total transpiratory water loss from *Locusta* is proportional to weight and is not affected by activity within the limits possible in an enclosed box.

2. A trend for proportionately less water to be lost at low humidity than at high humidity probably involves active measures to control water loss from the tracheal system. The saving of water is 5 mg./locust/hr. at 0% R.H., 30° C.

3. Experiments involving exposure of locusts to CO_2 of different concentrations show that little control over water loss is exerted by the spiracles except in so far as they may influence the type of ventilation. Hyperventilation, predominantly of the tidal type, doubles normal water loss.

4. Between 42 and 45° C. the ventilatory rate increases enormously with concomitantly greater water loss.

5. Locusts pre-treated in dry air show a 23% reduction in abdominal ventilatory rate and a 25% reduction in water loss over locusts pre-treated in moist air.

6. Ventilatory movements of locusts under conditions of progressive desiccation show decreased rate and amplitude and an increased incidence of discontinuities, which will conserve water.

7. Ventilation and water loss are closely interdependent. The fact that ventilation can be controlled according to water reserves and the humidity of the air is important in water conservation.

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