Water Budget in Larvae of *Semiadalia undecimnotata* Schn. Studied with Tritiated Water

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The rates of water vapor entry, body water clearance and the net loss of water in larvae of *Semiadalia undecimnotata* (3rd and 4th stage) of different weights were determined from observed changes in the mass of body water and its tritium content for each of several relative humidities (RH) in ambient air.

The water clearance and vapor entry rates as a function of weight increases from emergence to the middle of the stage and then remains constant. The vapor entry rate increases with RH while the clearance rate is independant of RH. The permeability of the cuticle has not the same value in the inward and in the outward direction. The permeability to body water escape is not affected by RH and reaches a maximum at the middle of the stage. The inward permeability does not depend on weight and increases appreciably as RH reaches saturation.

One day of starvation affected these movements of water most in larvae at the middle of their stage.

These results are discussed in relation to some physiological data concerning the cuticular structure and its evolution with age.

The aphid consumption of the coccinella *Semiadalia undecimnotata* in different larval stages has been studied by Ferran and Larroque [1]. These authors have shown that the efficiency of conversion of ingested food into body substance (ECI) varies between and within each developmental stage. They have therefore proposed a method for the measurement of the consumption which is based upon the weight of the larvae [2]. The method is useful for assessing the predation performance of coccinella in the biological control of aphids, but it requires a detailed evaluation of the influences of biotic and abiotic factors on ECI. In this study we examine the water transfer across the cuticle and its dependence upon relative humidity in the absence of food water intake.

The literature provides numerous specific data on the net water loss of body water in dehydrating conditions and some specific data on the physiological absorption of water from the atmosphere [3, 4]. Since 1968 HTO labelled water is used to resolve the rates of body water clearance and vapor entry [5–7]. These principles were utilized in the present investigation of *S. undecimnotata* larvae of third and forth stage of various weights and in nymphs.

Because of reported asymmetry of the cuticle permeability [8], values of permeability are reported here for both the inward and outward movement of water at particular times during the intermolt period, and as a function of relative humidity.

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Materials and Methods

Experimental procedure

Experiments were run at 20 °C. In each 12 to 14 individuals of similar weight were held for 3 h in a 3 l desiccator equipped with a microventilator and containing 50 ml of a saturated salt solution in tritiated water of about 0.1 µCi/µl. Each larva was weighed before exposure to the HTO vapor and again after this exposure. It was then killed with ethylacetate and its radioactivity was measured. The saturated salt solutions and their relative humidities (RH) were: $MgCl_2$ (35%), $Mg(NO_3)$ (54%), NaCl (76%), KNO_3 (92%), and K_2SO_4 (95%).

At a given RH tests were made on each of three sizes of third stage (L 3) larvae (2 to 3 mg *i. e.* L 3 at

emergence, 6 ± 1 mg and 9 ± 1 mg, the maximum weight reached after 2 to 3 days). Forth stage larvae (L4) were studied with 5 sets of larvae weighing respectively 7.5 ± 1 mg (L4 at emergence), 11 ± 1, 20 ± 1, 28.5 ± 1.5 and 34.5 ± 1.5 mg (maximum weight reached after about 4 days).

The influence of fasting on the water balance was studied in L4, of the same weight as above, held without food during one day at 0.76 or 0.92 RH in unlabelled water vapor before exposure to HTO vapor.

Two sets of nymphs (N) weighing 29 mg on the average were also studied at 0.76 and at 0.92 RH.

Measurement of water supply and loss rates

A larva was placed in an airtight tube containing about 100 mg of $CaCl_2$. Ten days or more later the larva was again weighed. The salt was dissolved in 1 ml of water in a counting flask to which 10 ml of liquid scintillator was then added, and the radioactivity was measured in a beta spectrometer. The specific activity S^* was simply the ratio of the radioactivity to the amount of water lost from the body during drying.

Each larva, after extraction of some body water over $CaCl_2$, was dried for 24 h in an oven at 130 °C and again weighed. In control experiment it was determined that the dry weight hardly changed during the test period. Then the difference of the fresh weight at any time from the dry weight is an accurate value of the body water mass.

Wharton and Devine [5] found in the acarina *Lealaps echidmina*, that the tritiated water absorbed across the cuticle is diluted quickly in a single water pool. Another study [9] showed that also in locusts the water turnover fits a one compartment model in which the clearance rate $\dot{W}_T$ is in constant proportion to the pool size Q, $\dot{W}_T = K\,Q$, and the rate of water supply $\dot{W}_S$ is constant in time. In any particular conditions the pool size approaches a steady-state value $Q_\infty = \dot{W}_S/K_T$ were $\dot{W}_S$ and K_T have values characteristic of the conditions. Metabolic water production is one component of the water supply rate $\dot{W}_S$.

$$\dot{W}_S \rightarrow \boxed{} \rightarrow \dot{W}_T = K\,Q.$$

The time dependence of the body water mass during exposure to any particular test condition is:

$$Q = Q_\infty\,(1 - \exp(-K_T\,t)) + Q_0 \exp(-K_T\,t) \quad (1)$$

were Q_0 is the initial pool size.

When the water vapor is labelled with a specific activity S^*_V; the amount of HTO (dq) retained during the time (dt) is the difference between tracer intake ($\dot{W}_S\,S^*_V$) and tracer removal $K_T q$, where q is the amount in the pool at time t.

$$dq/dt = \dot{W}_S\,S^*_V - K_T q. \quad (2)$$

Integration leads to:

$$q = Q_\infty\,S^*_V\,(1 - \exp(-K_T\,t)). \quad (3)$$

Then the value of K_T may be determined from data on Q and S^*_V as

$$K_T = -\ln \frac{Q - q/S^*_V}{Q_0} \Big/ t \quad (4)$$

and

$$\dot{W}_S = K_T \frac{Q - Q_0 \exp(-K_T\,t)}{1 - \exp(-K_T\,t)} \quad (5)$$

and

$$\dot{W}_T = K_T\,Q_0. \quad (6)$$

In water vapor in equilibrium with the labelled solution the specific activity S^*_V is lower than that of the solution S^*_S due to isotopic effects. According to Weston [10] the fractionation factor S^*_V/S^*_S amounts to 0.914 at 25 °C. In our own experiments S^*_V was measured as follows. A known weight of dry $CaCl_2$ is placed in the desiccator together with each set of larvae. Three hours later the $CaCl_2$ is weighed, dissolved in 50 ml of water and an aliquot is tested for radioactivity. The specific activity is calculated from the difference of weights observed. The ratio S^*_V/S^*_S varied from 0.86 to 0.89 in our experiments on *S. undecimnotata* carried out at 20 ± 1 °C.

Estimation of the cuticular permeability

In studies on transpiration, Fick's law is generally used with the assumption that the water vapor diffusion takes place across a homogenous membrane [3] and follows the relationship

$$J_n = P_n\,(C_W - C_V) \quad (7)$$

where J_n is the rate of the net flux in mg cm^{-2} s^{-1}, C_W and C_V are the concentrations inside and outside in mg cm^{-3} and P_n is the permeability in cm s^{-1}. For a tracer Fick's law can be written as follows [11].

$$J_S = P_n\,C_V = \dot{W}_S/S \quad (8)$$

$$J_T = P_n\,C_W = \dot{W}_T/S \quad (9)$$

where J_S is the actual inward flux, J_T the outward flux and S the surface. As stated by some authors quoted by Edney [12] the permeability of the cuticle differs according to the direction of the diffusion, so it is proposed here to substitute respectively the values P_S and P_T to the term P_n in the relationships (8) and (9).

The value of the surface of the cuticle needed for the calculation of the flux is estimated as follows: a larva L3 or L4 is compared to a cylinder whose length l is equal to ten times its radius (r) and the volume at unit density is equal to its weight (P). The ratio α between the surface and the power 2/3 of the volume is then: $\alpha = S/P^{2/3} = 6.94$. If F stands for the vapor pressure at saturation, and f for the partial vapor pressure, if a_V is the activity of vapor and a_W the activity of the vapor in equilibrium with the haemolymph, the number of water molecules in a unit volume is:

$$C_V = f M/R\,T = F\,a_V\,M/R\,T \qquad (10)$$

$$C_W = F\,a_W\,M/R\,T \qquad (11)$$

where R is the gas constant, M the mass of the water molecule and T the temperature in degrees Kelvin. Finally the following relations can be used to determine the permeabilities:

$$\underset{(cm^{-1})}{P_S} = \underset{(mg\,H^{-1})}{\dot{W}_S/S\,C_V} = \underset{(mg\,H^{-1})}{W_S/61.7\,\alpha\,RH}\ \underset{(mg)}{P^{2/3}} \qquad (12)$$

$$\underset{(cm^{-1})}{P_T} = \underset{(mg\,H^{-1})}{W_T/S\,C_W} = \underset{(mg\,H^{-1})}{W_T/61.7\,\alpha\,0.99}\ \underset{(mg)}{P^{2/3}}. \qquad (13)$$

In these formulae the following values have been used:

$R = 62.4 \quad 1 \cdot \text{mmHg} \cdot \text{mol}^{-1} \cdot \text{degree K}^{-1}$
$F = 17.4$ mm Hg
$a_W = 0.99$
$a_V = RH$
$T = 293$ °K
$\alpha = 6.94\ 10^{-2}$ (L3 and L4)
$\alpha = 5.54\ 10^{-2}$ (N)
$P =$ average weight of the set (mg)
$M = 18$ g.

Results

Larvae of third and forth stage normally fed

Fig. 1 shows the rate changes $\dot{W}_S$, $\dot{W}_T$ and $\dot{Q}$ in relation to the weight of L3 and L4 exposed to different relative humidities. In both stages the rates first increase more or less rapidly and become constant at the middle of stage. Fig. 1a demonstrates the influence of relative humidity on $\dot{W}_S$. Thus in L3 for instance $\dot{W}_S$ is six fold higher at 0.95 RH than at 0.35 RH. Inversely the transpiration rate $\dot{W}_T$ is not influenced by RH (Fig. 1b) so that the rate of change of the water mass, $\dot{Q}$, varies with RH in the opposite way from that of $\dot{W}_S$ (Fig. 1c).

The outward permeability P_T (Fig. 2) reaches a maximum at the middle of the stage, which in L3 and L4 respectively is 2 to 4 times the value observed in larvae at emergence. The same figure indicates that P_T is not affected by RH. The inward permeability P_S, on the contrary, is less influenced by weight than by RH. For this reason P_S was plotted in Fig. 3 in relation to the latter variable whose influence is more specially seen at the higher values of RH.

The ratio of permeabilities P_S/P_T (which is also the ratio of the resistances $(1/P_T)/(1/P_S)$ changes with RH (Fig. 4). This ratio does not depend on the

Table I. Water content of starved L4 compared to water content of normally fed L4 of the same initial weight at 0.76 and 0.92 RH. The weight loss induced by starvation is indicated in columns 1 and 1′.

	0.76 (1)	(2)	(3)	0.92 (1′)	(2′)	(3′)
Initial weight [mg]	Weight loss [%]	Water content of larvae starved for one day ±s.d.	Water content of normally fed larvae	Weight loss [%]	Water content of larvae starved for one day ±s.d.	Water content of normally fed larvae
7.5 ± 1	8	0.81 ± 0.01	0.81 ± 0.005	8.2	0.81 ± 0.001	0.81 ± 0.005
11 ± 1	10.7	0.82 ± 0.01	0.81 ± 0.005	13.2	0.82 ± 0.01	0.81 ± 0.01
20 ± 1	14.3	0.78 ± 0.02	0.79 ± 0.02	15.8	0.80 ± 0.02	0.79 ± 0.08
28.5 ± 1.5	13.8	0.80 ± 0.03	0.75 ± 0.02	13.6	0.78 ± 0.03	0.77 ± 0.04
34.5 ± 1.5	8.7	0.76 ± 0.03	0.77 ± 0.02	8.2	0.75 ± 0.03	0.76 ± 0.02

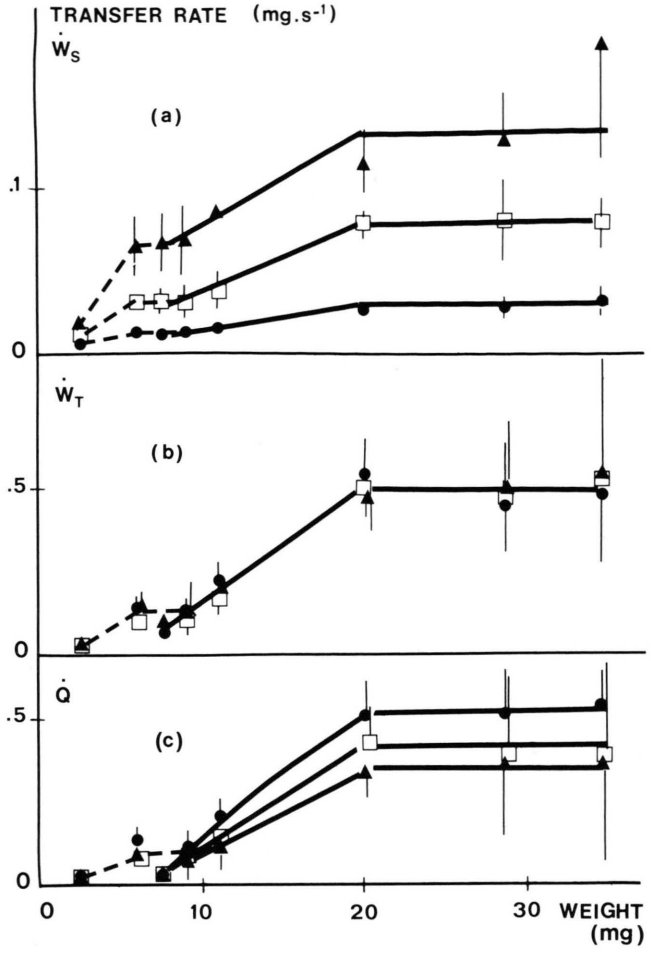

Fig. 1. Changes in sorption rate $\dot{W}_S$, transpiration rate $\dot{W}_T$ and loss rate $\dot{Q}$ as a function of weight in L3 and L4 at three different relative humidities: 0.35 (●), 0.76 (□) and 0.95 (▲). Bars indicate the standard deviation (s. d.).

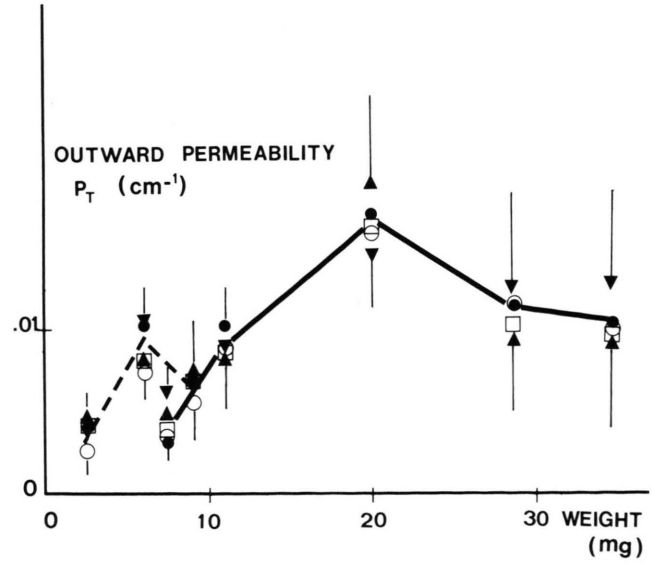

Fig. 2. Changes in outward permeability P_T in relation to weight in L3 and L4 held at 5 different relative humidities: 0.35 (●), 0.54 (□), 0.76 (○), 0.92 (▲) and 0.95 ( ). Bars indicate the s. d. corresponding to the extreme points of a set of a given abscisse.

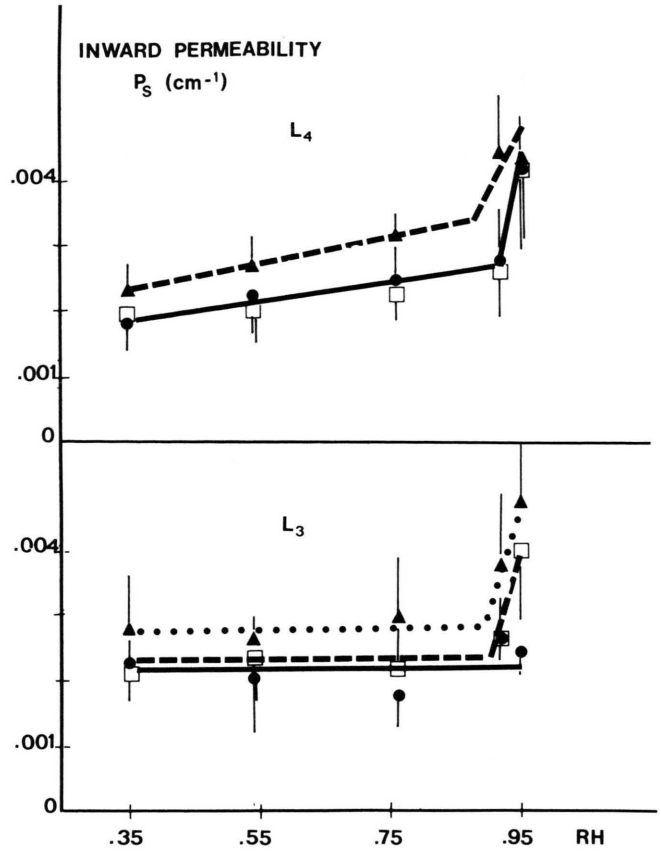

Fig. 3. Influence of relative hymidity on the inward permeability P_S a) in L3 of 2.5 mg (●), 6 mg (▲) and 9 mg (□) and b) in L4 of 7.5 mg (●), 20 mg (▲) and 34.5 mg (□). Bars indicate the s. d.

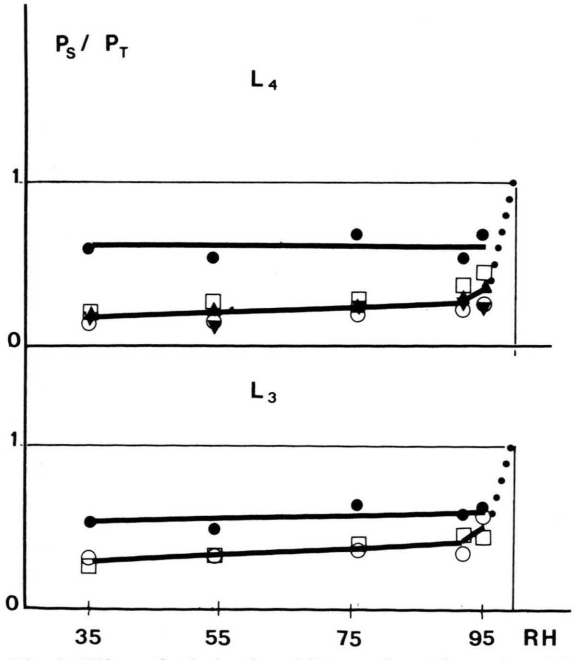

Fig. 4. Effect of relative humidity on the ratio P_S/P_T a) in L3 of 2.5 mg (●), 6 mg (□) and 9 mg (○); b) in L4 of 7.5 mg (●), 11 mg (□), 20 mg (○), 28.5 mg ( ) and 34.5 mg (▲).

calculation of the surface area. In both stage the ratio at emergence (60%) is higher than the one observed in older larvae (30 to 40% in L3, 20 to 30% in L4). In control experiments the weight of larvae placed in a saturated atmosphere remained constant or sometimes increased. In such special conditions we can assume that $a_V = a_W$ and write $\dot{W}_S = \dot{W}_T$. From (12) and (13) we get then:

$$P_S/P_T \cong W_S/W_T \cong 1.$$

The dotted line in Fig. 4 is drawn between the experimental curve and the point for which $P_S/P_T = 1$ in order to show the change of the ratio P_S/P_T in the region 0.95 – 1 RH.

Larvae starved one day

Both in 0.76 RH and in 0.92 RH starved L4 larvae loose between 8 and 15% of their initial weight (Table I). Losses both of dry weight and water occur as one can see from the comparison of the starved

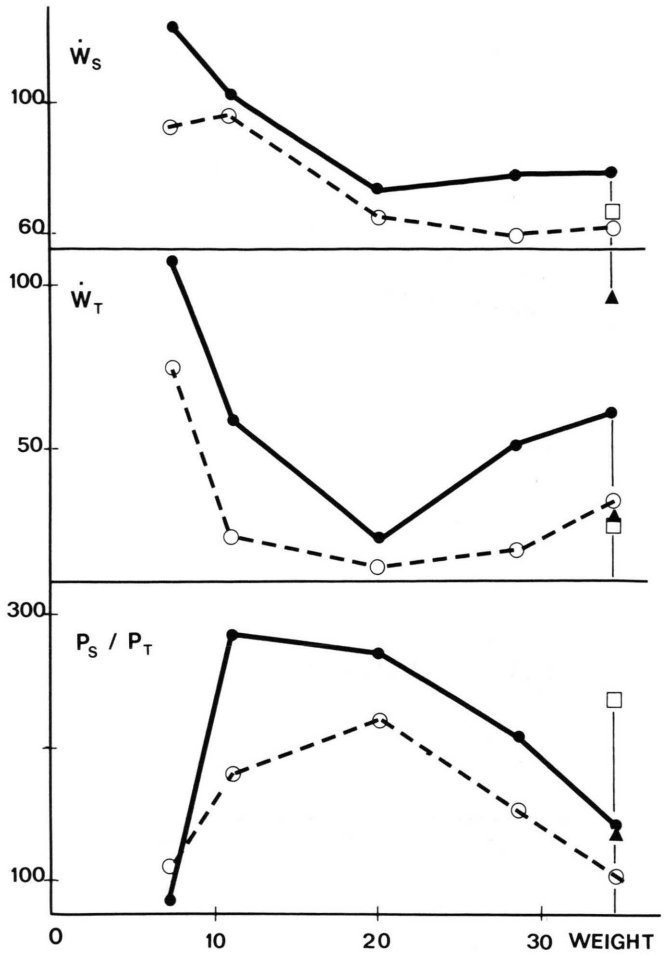

Fig. 5. Changes in the $\dot{W}_S$ and $\dot{W}_T$ rates and in the ratio P_S/P_T expressed as the percentage of the values measured in L4 larvae starved one day and the corresponding values observed in L4 larvae normally fed. As a fuction of weight at 0.76 RH (○) and 0.92 RH (●). Corresponding values for the nymphs at 0.76 RH (□) and 0.92 RH (▲) are plotted on the abscissa 34.5 mg (maximum weight of L4 before pupation).

and the normal L4. The proportion of water in the fresh weight are nearly the same. For both categories of larvae the water content does not depend on RH but decreases slightly with the age.

The rates $\dot{W}_S$, $\dot{W}_T$ and the ratio P_S/P_T, expressed as the percentage of the average value measured in starved L4 and the one measured in the normally fed L4, are plotted versus weight (Fig. 5). Diffusion rates are generally less in starved larvae, especially at 0.76 RH.

Nymphs

The weight of L4 larvae reaches a maximum, then declines for two days, at which time they pupate. The nymphal stage lasts about 6 days. In the present study the nymphs were 3 days old and their weight range from 25 to 34 mg with an average of 29 mg.

The values of $\dot{W}_S$, $\dot{W}_T$ and P_S/P_T expressed as the percentage of the average value measured for the nymphs and the one observed in L4 of 34.5 mg are given in Fig. 5. Values for the nymphs are comparable to values for L4 of 34.5 mg. At 0.92 RH the rates $\dot{W}_S$ and $\dot{W}_T$ are lower in nymphs but the ratio P_S/P_T is similar to the one of starved L4. At 0.76 RH the rates are similar and the ratio is higher in nymphs.

Discussion

The transpiration rate $\dot{W}_T$ depends on three parameters: the cuticular transpiration, the tracheal loss of water and moisture loss with excretion. Control experiments have shown that the loss of weight by excretion amounts to less than 1.2% of the initial weight. Bursell [13] and Edney [14] have reported that the tracheal transpiration is highly affected by physical and metabolic activity. In our experiments the larvae showed little activity and the tracheal transpiration should have been relatively small.

In the study of *Dermacentor variabilis* Knülle and Devine [15] showed that as RH decreases from 100% to 0%, the net water loss rate $\dot{Q}$ increases not because transpiration is higher but because sorption is lower. This is precisely what happens in L3 and L4 which are unable to perform an active sorption contrary to *D. variabilis*. From the same authors the resistance to sorption ($RH/\dot{W}_S$) increases inversely to RH. According to the present study it is also observed in larvae of *S. undecimnotata* that the resistance ($1/P_S$) decreases as RH increases, especially in the higher range of humidity.

It is generally stated that the asymmetry of the cuticular permeability is related to some structural features on which several models have been proposed. According to Locke [8] the filaments observed in the wax canals may be lipid-water liquid crystals which can exist in various phases of which the "middle phase" is hydrophobic and the "complex hexagonal phase" is hydrophilic. If the phase

change is induced by the presence or absence of water in the environment then the change of permeability may be steep in the vicinity of saturation, as is observed for P_S.

The L4 larvae grow regularly from the weight of 7.5 mg to a maximum of about 35 mg four days later [1]. Along with a 4.6 fold increase of weight there will be a 2.8 fold increase of the surface according to our estimations. The observed change of P_S with a maximum in the middle of the stage is likely to be due to the progressive unfolding of the epicuticle [16], and to the cuticle evolution (endocuticle deposition, inner epicuticle formation, cuticle resorption) [8]. Betsch and Vannier [17] have put some light on the connection between histological observations and the loss rate of water in *Allacma fusca* (*collembola*) at different growing stages.

The study of fasting larvae suggests that fasting induces faster cuticular rehandling (normally preceding pupation) which lowers the cuticular permeability and reduces water diffusion. It may finally be stated that the rate of water movement across the cuticle is mainly dependent upon the stage of the development of the cuticle during the intermolt period. The variability of water loss measurements of all the sets of larvae except those at emergence (Fig. 1) is likely to be largely due to the structural dependence of the diffusion permeability and less to the other mechanisms of body water clearance. Because transpiration rates show more variation than sorption rates, one can suppose that the structure dependence is higher for the outward permeability than for the inward permeability.

In a quite different field, the present study leads to the following comments on the water regulation in the environment. Under normal feeding and humidity conditions larvae easily counter any loss of water. Difficulties may well arise in dry conditions and it can be questionned wether larvae are then more active in searching for prey and wether the ECI is modified. From a practical point of view it would be worth knowing wether the performance of larvae can be improved by modifying the ambient relative humidity.

The present study demonstrates the adaptative ability of *S. undecimnotata* larvae that are confronted with a lack of aphids under unfavourable conditions of humidity. Presumably due to modifications in the cuticular structure the water diffusion rates are reduced, especially when their initial value is high and when the relative humidity is low.

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