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WATER BALANCE IN FLESH FLY PUPAE AND WATER VAPOR ABSORPTION ASSOCIATED WITH DIAPAUSE

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Summary

We report the water balance characteristics for diapausing and nondiapausing pupae of the flesh fly *Sarcophaga crassipalpis*. The challenge of maintaining water balance is particularly acute for pupae that spend 9–10 months in diapause, without access to drinking water. While diapausing pupae can tolerate a loss of up to 24.5 % of their total body water content (67.3 %), they have also acquired several other physiological attributes that have enhanced their capacity for maintaining water balance. Net transpiration rates for diapausing pupae (0.008 % h^{-1}) are far lower than rates for nondiapausing pupae (0.023 % h^{-1}). In addition, diapausing pupae can counter water loss with their ability to absorb water vapor from lower humidities (approximate water vapor activity, a_v, 0.58 at 20°C) than can nondiapausing pupae (approximate a_v 0.74 at 20°C). The high critical transition temperature for diapausing pupae (39°C, compared to 30°C for nondiapausing pupae) suggests that epicuticular lipids have been modified to restrict water loss during diapause.

Introduction

One of the most formidable challenges for an insect during diapause, especially a diapause that occurs during the egg or pupal stage, is the maintenance of water balance. In many cases diapause persists for 9–10 months, and during this long period a diapausing egg or pupa has no ability to replenish its water supply by drinking or feeding. This dilemma is further exacerbated by the large surface area:volume ratio characteristic of most insects. Under these circumstances, how is water balance achieved? While it is widely acknowledged as a problem (Lees, 1955; Denlinger, 1986; Tauber *et al.* 1986), few experiments have examined water balance characteristics of diapausing insects. In this paper we investigate water balance in diapausing and nondiapausing pupae of the flesh fly *Sarcophaga crassipalpis* and describe the attributes of diapause that enable the pupa to survive for many months without desiccating.

Water balance is a function of both water loss and water gain (Wharton, 1985).

Key words: diapause, water balance, vapor absorption, critical transition temperature, *Sarcophaga crassipalpis*, *Sarcophaga bullata*.

We determine these rates in diapausing and nondiapausing pupae and demonstrate that diapausing pupae absorb atmospheric water vapor at a much lower water vapor activity, a_v (% RH/100, where RH is relative humidity), than pupae not in diapause. Water vapor absorption allows both types of pupae to recruit water from subsaturated atmospheres to maintain water balance. The effect of temperature on water flux is examined, and our experiments also seek to evaluate the role of the puparium in maintaining water balance.

Materials and methods

Experimental animals

Colonies of *Sarcophaga crassipalpis* Macquart and *Sarcophaga bullata* Parker originating from Urbana, Illinois (40°N), and Lexington, Massachusetts (42°N), respectively, were maintained in the laboratory as described (Denlinger, 1972). To ensure a high incidence of pupal diapause, adults lacking a diapause history were exposed to short daylengths (LD 12 h:12 h) at 25±1°C, and larvae and pupae were reared at a LD 12 h:12 h at 20±0.5°C. Nondiapausing individuals were generated by exposing adults to long daylengths (LD 15 h:9 h) at 25±1°C; larvae and pupae were reared at LD 12 h:12 h, 20±0.5°C. Larvae used in the experiments were in the wandering phase of the third (final) larval instar, 2 days after departure from the food. The day of pupariation was used as a developmental landmark to stage pupae and pharate adults. All adults used in this study were 2- to 4-day-old virgin females that had been deprived of food and water for 24 h to minimize effects of ingestion, excretion, defecation and reproduction on mass changes (Arlian and Eckstrand, 1975).

Experimental conditions

Experiments at 25°C were performed in an environmental room (±1°C), and experiments at other temperatures utilized environmental chambers (±0.5°C). Ambient water vapor activities (a_v) were maintained in sealed desiccators using glycerol-distilled water solutions (Johnson, 1940) or by using anhydrous $CaSO_4$ (Drierite) to generate a_v 0.00. All humidities were verified weekly with a Taylor hygrometer (a_v±0.03) (Thomas Scientific, Philadelphia).

Within the desiccators, pupae were placed on a steel mesh grid held on a porcelain plate. Individual adult female flies and third-instar wandering larvae were placed in separate 1.5 cm^3 polypropylene Eppendorf tubes perforated by 12–1 mm diameter holes, as described by Lighton and Feener (1989), and weighed individually. No change in mass was observed for empty tubes during the experiment. No excretory material was observed in the tubes and no excretory material was deposited in the tubes by the stages of flies used in these experiments. All components of the system were predesiccated at a_v 0.00 for 24 h before use.

Body water pools, dehydration tolerance and net transpiration rate

To determine body mass, individuals were weighed on an electrobalance (Cahn

25, Ventron Co.). Initial mass at day 0 was recorded as wet mass, and dry mass was determined after drying the fly over anhydrous $CaSO_4$ at 50°C to constant mass. Water mass (m) was defined as the difference between wet mass and dry mass. The quantity of water in the insect was expressed as the ratio of water mass (m) to total wet mass×100, or as water as a percentage of total mass (Wharton, 1985).

Dehydration tolerance for diapausing pupae was calculated by placing groups of pupae at 30°C over anhydrous $CaSO_4$. Subgroups were removed each day and weighed. The percentage change in body mass was determined as the ratio of the difference between the mass at time 0 and at any given time t to the original mass×100, or as the percentage change in body mass from the original wet mass. Diapause was terminated in the pupae by exposing them to a closed system of hexane vapor for 2 h (Denlinger *et al.* 1980), and pupae were held at LD 15 h:9 h, 25°C until eclosion. The limit of dehydration tolerance was defined as the minimum amount of water loss that prevented 50 % eclosion.

To calculate net transpiration (integumental and respiratory water loss), the insects were weighed, placed at a_v 0.00 for a specified time, and reweighed. The ratio of the water mass at time t (m_t) to original mass at time 0 (m_0) represents water lost over the time interval with respect to initial water mass. In the case of transpiration into dry air, water content at any time t (m_t) is equal to water mass at time 0 (m_0)×e^{-k_t}, where k is the percentage of water lost in unit time and t is time elapsed between m_0 and m_t (Wharton, 1985):

$$m_t = m_0 e^{-k_t} \tag{1}$$

or $\ln(m_t/m_0) = -k_t$. The observed change in mass of water results from the difference between the rate of movement of water from insect to air ($\dot{m}_T$) and the rate of movement of water from air to insect ($\dot{m}_S$). This difference is the rate of change of water mass ($\dot{m}$) (Wharton, 1985):

$$\dot{m} = \dot{m}_S - \dot{m}_T . \tag{2}$$

If $\dot{m}_S < \dot{m}_T$, then $\dot{m} < 0$ and water mass decreases per unit time. If $\dot{m}_S > \dot{m}_T$, then $\dot{m} > 0$ and water mass increases in time. When $\dot{m}_S = \dot{m}_T$, $\dot{m} = 0$, and the pupa is in water balance. At a_v 0.00, $\dot{m}_S$ is zero and thus $\dot{m}_T$ equals water moving out or $\dot{m}$, and transpiration proceeds so that a constant amount (k) of water mass (m) is lost in unit time (equation 1). The instantaneous rate of loss at any time t is equal to the product $-k_t$. Water loss obeys kinetics governing first-order rate laws in which loss is dependent on a single component and is expressed in % h^{-1}. The first-order rate constant (% h^{-1}) is equal to the negative slope of an exponential regression between $\ln(m_t/m_0)$ and elapsed time (Wharton and Devine, 1968; Devine and Wharton, 1973). An insect's water balance is evaluated by determining net water loss in addition to its competing flow, net absorption (Wharton, 1985).

Uptake kinetics, critical transition temperature

Rates of uptake were determined as described by Machin (1984). Briefly, net mass changes (mg) at hydrating vapor activities were corrected for cuticular losses

($mg\,h^{-1}$) at vapor activities to which water was lost. The slope of the regression through the points of water vapor exchange ($mg\,h^{-1}$) on a_v describes the rate of uptake ($mg\,h^{-1}\,\Delta a_v^{-1}$). Pupae were synchronized physiologically by predesiccation at a_v 0.00 and 20°C for 24 h. Uptake rates were determined 24 h after exposure to the experimental a_v.

The critical transition temperature (CTT) is the temperature at which epicuticular lipids undergo a phase change resulting in dramatic water loss (Edney, 1977). The CTT was estimated for predesiccated (a_v 0.52, 20°C for 24 h) nondiapausing and diapausing pupae. Rates of net water loss ($\ln k$) were determined at various temperatures, as previously described, and plotted as a function of the reciprocal of absolute temperature (T^{-1}). The slope of the plot of $\ln k$ against T^{-1} is equal to $-E_a R^{-1}$ (Seethaler *et al.* 1979; Toolson, 1980),

$$\ln k = -E_a R^{-1} T^{-1} + \ln A \tag{3}$$

$$E_a = -\{(t_i \ln k_i - t_i n^{-1}) \times [t^2_i - (t_i)^2 n^{-1}]^{-1}\} R\,, \tag{4}$$

where E_a is the energy of activation, R is the gas constant and A is the frequency factor; t represents temperature over range i with respect to the loss rate (k) of the amount (n) of water. A simultaneous change in E_a with absolute temperature (T^{-1}) designates a new temperature range (i), and the CTT is defined at the intercept of the slopes describing the two activation energies (Wharton, 1985). A new temperature range was selected when the correlation coefficient describing the relationship between net transpiration ($\ln k$) and temperature ($K^{-1} \times 10^3$) was less than 0.95.

All water balance variables were compared using analysis of variance (ANOVA) and Sokal and Rohlf's (1981) test for equality of slopes of several regressions.

Results

Water pool

Changes in the total exchangeable water pool through development for nondiapausing and diapausing flies are shown in Fig. 1. Throughout development there is a general decrease in body water mass (m) between stadia (ANOVA, $P<0.05$). When the total body water mass is expressed as a percentage adjusted to the original mass, no significant difference is observed between pupae and adult flies; larval values, however, remain significantly different from those of pupae and adults (ANOVA, $P<0.05$). Throughout development, the total available water pool is not significantly different between nondiapausing and diapausing flies. For nondiapausing and diapausing pupae, dry mass is positively correlated to body water mass ($r>0.89$) and significantly different from zero ($F>519.27$, d.f.=399, $P<0.0001$).

Dehydration tolerance limit

Preweighed diapausing pupae (6 weeks in diapause at 20°C) were transferred to a_v 0.00 at 30°C. The dehydration tolerance limit was defined as the point where

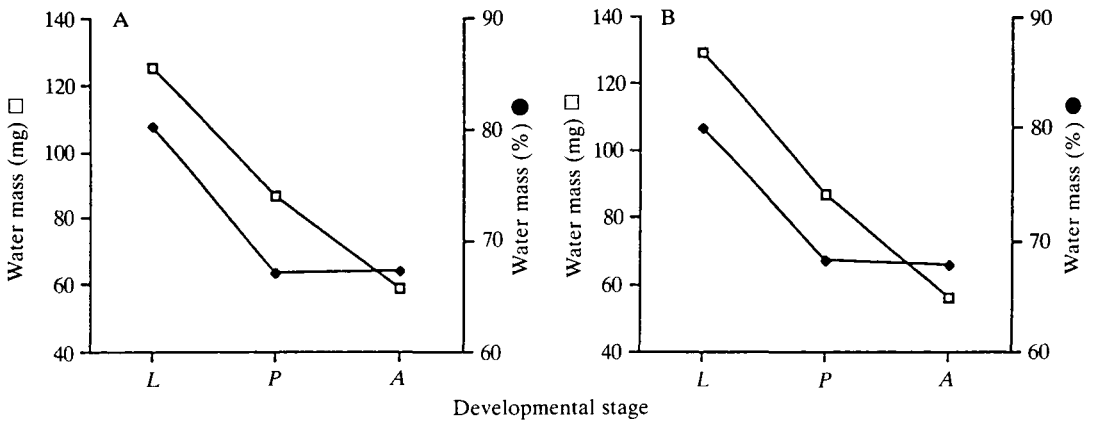

Fig. 1. Total body water pool expressed in mg (□) or as a percentage (●) for nondiapausing (A) and diapausing (B) groups of flesh flies (*Sarcophaga crassipalpis*) at different stages of development. *L*, third-instar wandering larvae, 2 days after leaving food (N=100); *P*, day of pupation (N=200); *A*, adult virgin females, 2–4 days after eclosion (N=50). Data points represent the mean of N individuals. Vertical error bars lie within the confines of the symbols used on the graph.

50 % of the subgroup (each N=30) failed to eclose. This point was reached when pupae lost 24.5±2.5 % of their original body mass (initial wet mass range= 128.5–133.1 mg).

Net transpiration rates

In an atmosphere where the water content is zero, water lost from the pupa is described by a first-order kinetic relationship of exponential decay (equation 1). Nondiapausing pupae held at a_v 0.00 and 20°C lost water at a rate of 0.023 % h^{-1}, which is significantly faster than diapausing cohorts, which lost water at a rate of 0.008 % h^{-1} (F=5.447, d.f.=89, P<0.01, Fig. 2). When held at a_v 0.00 and 20°C, third-instar larvae not destined for pupal diapause lost water at a rate of 0.43 % h^{-1}. Adult females that emerged from nondiapausing pupae exhibited a water loss rate of 1.10 % h^{-1}. Transpiration rates for third-instar larvae and adult females were not significantly different between the nondiapause and diapause groups. All flies were synchronized physiologically by predesiccation at a_v 0.52 and 20°C for 24 h so that mass changes equalled the mass of water lost (Wharton, 1985).

Water retention at hydrating vapor activities

Percentage change in mass was documented for 14 days at 20°C for diapausing pupae (predesiccated a_v 0.52 and 20°C for 24 h) in hydrating (a_v 0.85) and dehydrating (a_v 0.33) atmospheres (Fig. 3B) to determine how long pupae could retain absorbed water and to examine the possibility that infradian O_2 consumption cycles (Denlinger *et al.* 1984) might alter water balance physiology during diapause. Examination of nondiapausing pupae under the same humidity regimes

demonstrated that water loss increased with decreasing vapor activity and that no water was gained in nondiapausing pupae at a_v 0.85 (Fig. 3A). After being held at a_v 0.85 for 24 h, diapausing pupae gained 0.6±0.1 % while nondiapausing pupae lost 2.0±0.1 % of their original mass. At a_v 0.33, diapausing pupae lost 0.3±0.1 % of their original mass while nondiapausing cohorts lost 3.2±0.1 % within 24 h. For

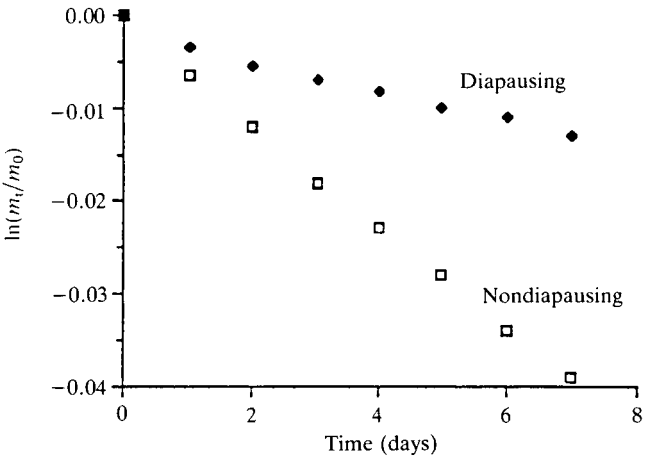

Fig. 2. Net transpiration at 20°C from nondiapausing (□) and diapausing (◆) *Sarcophaga crassipalpis* pupae (predesiccated at a_v 0.52, 20°C, 24 h) into dry air (a_v 0.00). Semi-logarithmic plot of water mass ratio (m_t/m_0; mass at time t, m_t, divided by original mass at time 0, m_0) *versus* time describes transpiration rate ($-k_t$) as the negative slope (mg day^{-1}) of exponential decay (equation 1). Values are the means for 45 pupae. Vertical error bars lie within the confines of the symbol used on the graph (±S.D. ≤0.005).

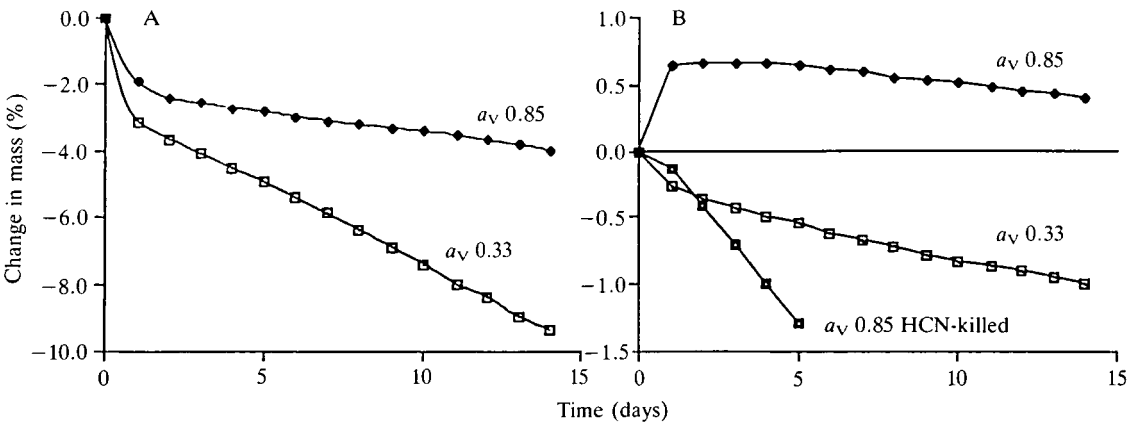

Fig. 3. Percentage change in mass in response to long-term exposure to hydrating (a_v 0.85) and dehydrating (a_v 0.33) atmospheres at 20°C for nondiapausing (A) and diapausing (B) *Sarcophaga crassipalpis* pupae (predesiccated at a_v 0.52, 20°C, 24 h). Mass change was also monitored in a group of diapausing pupae killed with HCN. Data points represent the mean of 45 pupae. Vertical error bars lie within the confines of the symbol used on the graph (±S.D. ≤0.2).

diapausing pupae held at a hydrating a_v (a_v 0.85) the initial water vapor absorbed was maintained above 0 % mass change and extended over the 14 day period. The percentage change in mass observed for both types of pupae in different humidities demonstrates that total body water loss is reduced in diapausing pupae. The smooth rate of mass change in diapausing pupae suggests that there is no connection between water flux and the infradian cycles of O_2 consumption that have been observed during pupal diapause.

Mechanism of water gain

To test the possibility that water gain at hydrating vapor activities might be due to production of metabolic water, a group (N=90) of diapausing pupae was held in a hydrating atmosphere (a_v 0.85), then dehydrated for 12 h (a_v 0.33). A subset of the group (N=45) was dried to constant mass, while the remaining pupae were rehydrated (a_v 0.85) for 12 h. Following rehydration, the group was dried to constant mass, and dry masses were compared (Knülle, 1967). If metabolism contributes to water gain, oxidation of fatty acids should decrease residual dry mass. Residual dry masses of diapausing pupae were not significantly different following hydration–dehydration (mean dry mass 35.66±2.18 mg) and hydration–dehydration–rehydration (mean dry mass 34.95±1.72 mg) a_v regimes, indicating that the water gain in hydrating atmospheres cannot be attributed to the production of metabolic water. A subgroup of HCN-killed diapausing pupae (N=45) held in a hydrating atmosphere (a_v 0.85, 20°C) lost 1.4±0.1 % of its original mass over a period of 5 days (Fig. 3B). Control pupae maintained a net gain of 0.6±0.2 % over the elapsed time under the same conditions. The water loss observed when HCN-killed diapausing pupae (predesiccated at a_v 0.52 and 20°C for 24 h) were held at a hydrating vapor activity suggests that they rely on oxygen consumption for water absorption and thus the possibility of an active process or that water loss (Fig. 1) is under spiracular control.

Role of the puparium

Blocking different regions (anterior, middle and posterior third) of the puparium of diapausing pupae (each N=45) with wax did not alter the ability to gain water at a_v 0.85 and 20°C. Gains did not differ significantly from those of control pupae (net gain of 0.62±0.15 %). The only significant blockage occurred when the entire puparium was covered with wax (net gain of 0.03±0.05 %) (F=5.407, d.f.=89, P<0.01). This suggests that the entire puparium is sufficiently porous to permit water entry.

To examine the role of the puparium in restricting water loss, head capsules were removed from a subgroup (N=45) of 6-week-old diapausing pupae (predesiccated at a_v 0.52 and 20°C for 24 h) that were placed at a_v 0.60 and 20°C. Open puparia (head capsules removed) lost water at a rate of 3.0×10^{-4} % h^{-1}, which was significantly greater than the loss (2.2×10^{-4} % h^{-1}) observed with intact puparia held under the same conditions (F=5.431, d.f.=89, P<0.01).

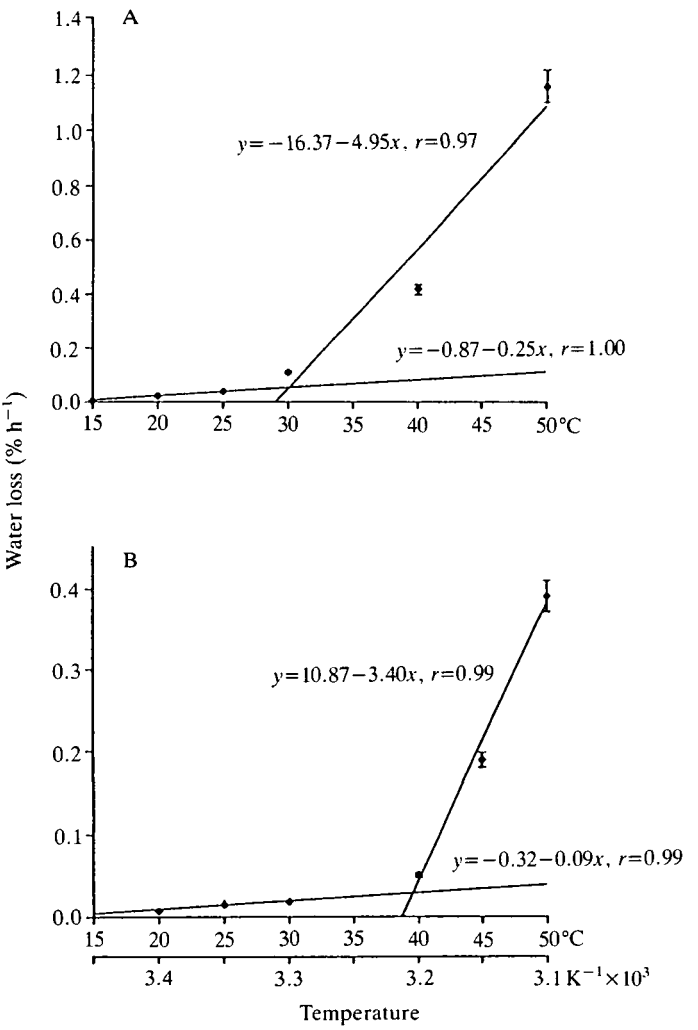

Fig. 4. Arrhenius plot of ln$k$ against $K^{-1}\times10^3$ for nondiapausing (A) and diapausing (B) *Sarcophaga crassipalpis* pupae (predesiccated a_v 0.52, 20°C, 24 h). The slope of the lines gives the mean activation energy for each temperature range. The intersection of the two lines is the critical transition temperature. Data points represent the mean of 45 pupae; vertical error bars signify 95% confidence limits.

Critical transition temperature

The critical transition temperature (CTT) for nondiapausing pupae was 30.2±2.1°C (N=45), and in diapausing cohorts the CTT was 39.1±1.4°C (N=45) (Fig. 4). Differences between nondiapausing and diapausing pupae were significant (F=4.369, d.f.=89, P<0.025). Activation energies, which are used in the CTT calculation, were 1.05±0.59 kJ mol^{-1} (N=45) for nondiapausing pupae in the low temperature range and 20.69±0.46 kJ mol^{-1} (N=45) at higher temperatures. These activation energies differed significantly from those of diapausing pupae

activation energies of $0.38\pm0.08\,kJ\,mol^{-1}$ and $14.2\pm0.79\,kJ\,mol^{-1}$, respectively ($F_{<CTT}=3.679$, d.f.=89, $P<0.05$; $F_{>CTT}=3.243$, d.f.=89, $P<0.05$). CTT values for nondiapausing and diapausing pupae killed with HCN were nearly identical to values observed in viable pupae (29.8±1.6°C and 40.2±2.3°C, respectively).

Uptake kinetics

Rates of water uptake, determined from the slope of a regression of water exchange ($mg\,h^{-1}$) on a_v (Machin, 1984), were estimated at different temperatures for pupae predesiccated at a_v 0.00 and 20°C for 24 h. At 20°C, nondiapausing pupae exhibited a net uptake rate of $3.49\pm0.09\,mg\,h^{-1}\,\Delta a_v^{-1}$, which differed significantly from that of diapausing pupae at the same temperature ($1.76\pm0.13\,mg\,h^{-1}\,\Delta a_v^{-1}$) ($F=3.549$, d.f.=89, $P<0.05$, Fig. 5). At 25°C, nondiapausing pupae exhibited an uptake rate of $8.44\pm0.07\,mg\,h^{-1}\,\Delta a_v^{-1}$, which differed from that of diapausing pupae ($5.32\pm0.17\,mg\,h^{-1}\,\Delta a_v^{-1}$) ($F=4.427$, d.f.=89, $P<0.025$). At 30°C, the estimated CTT for nondiapausing pupae, the rate of uptake declined to $6.67\pm0.18\,mg\,h^{-1}\,\Delta a_v^{-1}$, while diapausing pupae had an uptake rate of $7.40\pm0.09\,mg\,h^{-1}\,\Delta a_v^{-1}$ ($F=3.147$, d.f.=89, $P<0.05$). At 42°C, a temperature slightly above the estimated CTT for diapausing pupae, the rate of uptake declined to $6.50\pm0.08\,mg\,h^{-1}\,\Delta a_v^{-1}$. Below the CTT, the rate of uptake increased with temperature; once within the range of the CTT, however, the rate of uptake declined dramatically. With respect to temperature, rates of uptake between nondiapausing and diapausing groups were significant at $P<0.01$.

A vertical line drawn from the intercept of the regression line describing the rate

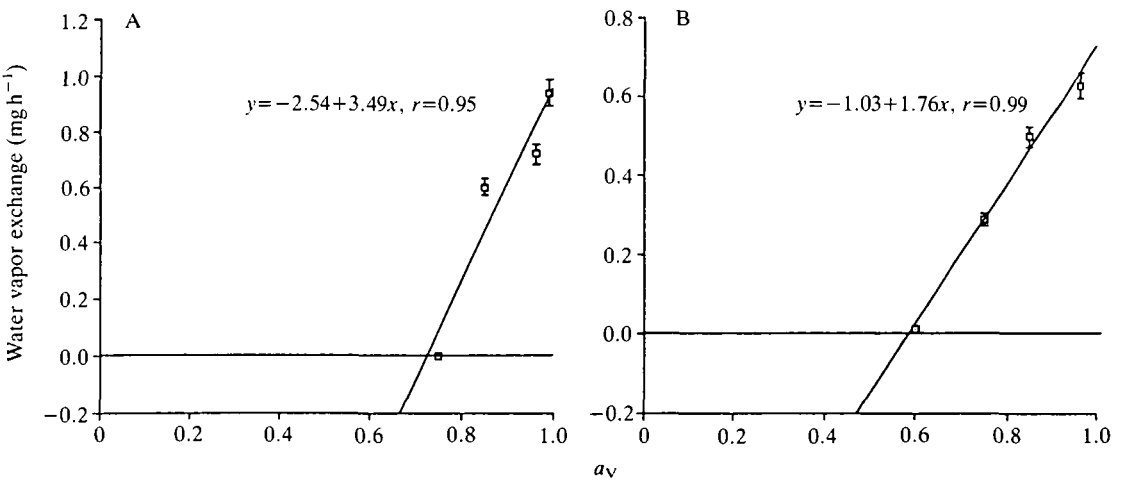

Fig. 5. Rates of water vapor exchange for nondiapausing (A) and diapausing (B) *Sarcophaga crassipalpis* pupae (predesiccated at a_v 0.00, 20°C for 24 h). The slope of the line at hydrating values of a_v indicates the mean rate of uptake ($mg\,h^{-1}\,\Delta a_v^{-1}$). Data points represent the mean of 45 pupae; vertical error bars signify 95 % confidence limits.

of uptake and zero water vapor exchange to the x axis (Fig. 5) defines the minimum a_v at which water can be absorbed under these conditions. This calculation demonstrates that diapausing pupae can absorb water vapor from lower vapor activities (approx. a_v 0.58) than nondiapausing pupae (approx. a_v 0.74) at 20°C. For both types of pupae, those predesiccated at a_v 0.52 and 20°C for 24 h exhibited different changes in mass at a_v 0.85 (Fig. 3) from those predesiccated at a_v 0.00 (Fig. 5). Pretreatment at a_v 0.00 removes a greater percentage of body water than pretreatment at a_v 0.52 (because $\dot{m}_S=0$, equation 2); thus, rehydration occurs at a higher rate after exposure to a lower a_v. Both pretreatments, however, demonstrate the capacity of diapausing pupae to absorb water vapor from lower vapor activities than nondiapausing pupae.

Comparative observations in Sarcophaga bullata

Water balance variables were also examined for a more northern population of a closely related species, *S. bullata*, under the same set of conditions as described for *S. crassipalpis*. The total water pool for nondiapausing and diapausing *S. bullata* pupae (66.8 %) was not significantly different from the available water in *S. crassipalpis* pupae. As in *S. crassipalpis*, net water loss rates for nondiapausing pupae of *S. bullata* (0.018 % h^{-1}) differed significantly from those in diapause (0.006 % h^{-1}) (F=5.281, d.f.=89, P<0.01). For both types of pupae, an interspecific difference exists between the two sarcophagid species (F_{ND}=5.141, d.f.=89, P<0.01; F_D=5.267, d.f.=89, P<0.01). Though water loss rates for nondiapausing and diapausing pupae vary interspecifically, the rate ratio of nondiapause:diapause remains constant (approx. 3.0). The water vapor absorption and CTT values for both nondiapausing and diapausing pupae of *S. bullata* lie within the respective ranges seen in *S. crassipalpis*. Activation energies from which the CTT is derived are significantly lower for the more northern species (F=3.067, d.f.=89, P<0.05). Activation energies for nondiapausing pupae were 0.59±0.25 kJ mol^{-1} below the CTT and 14.84±0.50 kJ mol^{-1} at higher temperatures. Diapausing pupae had energies of 0.305±0.017 kJ mol^{-1} at low temperatures and 9.49±0.38 kJ mol^{-1} above the CTT (F=4.891, d.f.=89, P<0.025).

Discussion

This study demonstrates that diapause has a profound effect on several aspects of water balance. In diapausing flesh fly pupae, net transpiration rates are far lower than in nondiapausing pupae, water vapor absorption occurs at lower humidities, and a high critical transition temperature associated with diapausing pupae suggests the presence of an epicuticular barrier that is unique to diapause.

Water content of insects can range from 45 to 90 % (Rapoport and Tschapek, 1967), but for most insects mean body water content is about 70 % (Edney, 1977). The percentage body water in flesh fly pupae (67.3 %) is thus close to this reported mean value, as is that of the tsetse fly pupae (71 %) (Bursell, 1958). Some insects

and even small mites can lose more than 50% of their water masses and still survive (Wharton, 1985), but others tolerate only about a 20% loss (Arlian and Veselica, 1979). Across a broad size range of *Glossina* species, Bursell (1958) reports that tsetse fly pupae can withstand a loss of about 28% of their body water. The dehydration tolerance limit is slightly less (24%) for diapausing pupae of *S. crassipalpis*.

The low net transpiration rate observed in diapausing flesh fly pupae ($0.008\ \% \ h^{-1}$) makes a major contribution to the conservation of the body water pool for the long duration of diapause. For flesh fly pupae not in diapause, the rate of water loss ($0.023\ \% \ h^{-1}$) approximates that of tsetse fly pupae (*Glossina morsitans*) at a comparable developmental stage (Bursell, 1958). In tsetse fly pupae, Bursell (1958) found that the puparium confers resistance to desiccation. This is also observed in diapausing flesh fly pupae.

Throughout the 9–10 months of diapause, a pupa is vulnerable to the loss of a large percentage of its body water. To counter this loss, pupae can absorb water against the water activity, a_w (0.99), of their hemolymph. In tsetse fly, a species that lacks the capacity for diapause, Jack (1939) and Bursell (1958) observed no uptake of water by pupae held in saturated air beyond that which could be accounted for by the hygroscopic properties of the puparium. Some water gain in flesh fly pupae may be attributed to the hygroscopic properties of the puparium, as proposed by Bursell (1958), but the vapor activity at which water absorption occurs in both diapausing and nondiapausing pupae is below vapor saturation and presents a dramatic case of water movement against a large atmospheric gradient.

Water vapor absorption at subsaturated atmospheres is often assumed to be an active process because it ceases at death (Edney, 1977). Killed diapausing flesh fly pupae lost water when exposed to a vapor activity that hydrated viable pupae; however, killed pupae are not definitive controls for active uptake because spiracular closing mechanisms will be inoperable, so that much of the water may be lost from respiratory surfaces. It is likely that water gain may be due to passive chemisorption and physical adsorption of water vapor, because predesiccated pupae increase in mass only by a small amount when transferred to a higher a_v, the uptake is not maintained and a new equilibrium water content is approached after 1–2 days. The amount of water gained by adsorption decreases with temperature (Glasstone and Lewis, 1960), and the rates of water gain we observe increase with temperature (until the CTT), implying that water is absorbed and contributes to sorption ($\dot{m}_S$, equation 2). HCN-killed diapausing pupae show no evidence of first-day passive absorption that could account for the water gain (even though quite small) observed in living pupae held at the same humidity. Furthermore, no significant (passive) absorption was observed in pupae transferred from dry air to humidities below 'hydrating' humidities. Thus, these data indicate that passive absorption cannot completely account for the total water gained from subsaturated air, but the methods we have used in these experiments are not fully adequate to distinguish between active and passive processes.

Water vapor absorption is usually restricted to a specific body region (Noble-

Nesbitt, 1970; Machin, 1975; O'Donnell, 1977; Rudolph and Knülle, 1982). To identify the site of absorption, different body regions can be coated with molten wax that, once solidified, forms an obstruction to vapor uptake. Only when puparia of diapausing pupae are completely covered with wax do they lose their ability to absorb water. This implies that the puparium, the inert third-instar exocuticle that encapsulates the pupa, is permeable to water over its entire surface. Whether there is a specific site of uptake on the pupa has not been determined.

Metabolic water is available to insects as a by-product of oxidative catabolism primarily of carbohydrates and fats (Wharton, 1985). Fat constitutes the main energy reserve of flesh fly pupae (Adedokun and Denlinger, 1985) and tsetse fly pupae (Buxton and Lewis, 1934). Bursell (1958) reported that the fat content of pupae held at 0 % RH is not statistically different from that of pupae held at 98 % RH and concluded that metabolic water did not increase to compensate for water loss at lower humidities. The same conclusion can be drawn for flesh fly pupae following one cycle of dehydration–rehydration.

The critical transition temperature (CTT) is the point above which transpiration rates dramatically increase with temperature, and CTTs in insects range from 30 to 60 °C (Edney, 1977; Lighton and Feener, 1989). The temperature-dependency function of water loss fits a Boltzmann temperature function. An Arrhenius equation predicts the proportion of water molecules permitted to escape from the cuticle as necessary energies approach a given temperature (Williams and Williams, 1967). A change in cuticular permeability denotes a new activation energy ($kJ\,mol^{-1}$) indicated by a change in the slope of the Arrhenius plot (Needham and Teel, 1986). Though the basis for the CTT remains controversial (Edney, 1977; Toolson, 1978; Seethaler *et al.* 1979; Gilbey, 1980; Monteith and Campbell, 1980; Machin, 1980), most workers agree that the CTT denotes an important transition in the epicuticular lipids, an important barrier to transpirational water loss. The observation that the CTT of diapausing pupae is 9 °C higher than that in nondiapausing pupae implies that pupae in diapause require a higher temperature to induce a phase change in these epicuticular lipids. This study suggests that the high CTTs for diapausing flesh fly pupae and their low rates of water loss may be related to increases in quantity or qualitative changes in epicuticular lipids, as demonstrated in other diapausing pupae, the tobacco hornworm *Manduca sexta* (Bell *et al.* 1975) and the Bertha armyworm *Mamestra configurata* (Hegdekar, 1979). Our recent experiments have verified that such changes are indeed associated with the diapause of *Sarcophaga* (J. A. Yoder and D. L. Denlinger, unpublished observations). The two- to threefold increase in cuticular hydrocarbons we observe in association with diapause is likely to require a higher temperature to elicit a phase transition, i.e. the CTT should be higher. According to reaction energetics, a large E_a, as shown in this study, corresponds to low collision frequencies of water molecules, thereby partitioning more water into the gaseous phase and increasing the ease of water escape. As shown by these data, at higher CTT values (implying a quantitative or qualitative change in

epicuticular lipids) activation energies (describing the frequency of water molecule collisions) are suppressed and lead to a lower water loss rate.

During diapause, flesh fly pupae exhibit infradian cycles of oxygen consumption (Denlinger *et al.* 1984), yet the rates of water absorption and loss that we observed in this study occur at a fairly constant rate for many days. It is thus unlikely that the pupa's cycles of respiration and metabolism are affecting water flux.

The water balance properties we have observed in *S. crassipalpis* are likely to be shared by other related species. Our results with *S. bullata* were quite similar to our observations with *S. crassipalpis*. Again, diapausing pupae absorb water at lower vapor activities and have higher CTTs than nondiapausing pupae. The rate of water loss in *S. bullata* is lower than in *S. crassipalpis*, and lower activation energies in *S. bullata* correspond to lower transpiration rates into dry air. This may indicate that *S. bullata* is better adapted to surviving in a dry environment, but insufficient distribution data are available to verify this.

We dedicate this paper to the memory of George W. Wharton, a valued colleague who inspired our interest in arthropod water balance. We thank Drs K. H. Joplin, D. C. Smith, M. D. Sigal and anonymous reviewers for their helpful comments. This research was supported in part by USDA-CRGO grant no. 88–37153–3473.

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