The Journal of Experimental Biology 203, 783–789 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JEB2373

EXTREME RESISTANCE TO DESICCATION IN OVERWINTERING LARVAE OF THE GALL FLY *EUROSTA SOLIDAGINIS* (DIPTERA, TEPHRITIDAE)

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Accepted 22 November 1999; published on WWW 26 January 2000

Summary

During winter, larvae of the goldenrod gall fly *Eurosta solidaginis* are exposed for extended periods to severe low ambient temperatures and low humidities within plant galls. The resistance of these larvae to desiccation at various temperatures and humidities, the transition (critical) temperature, and the effects of treatment with organic solvents on the larval rates of water loss and on changes in osmolality during desiccation were examined. The water loss rates of the flesh fly *Sarcophaga crassipalpis* under desiccating conditions were also measured.

The water permeability of the cuticle of *E. solidaginis* larvae was very low (0.038 $\mu g\,h^{-1}\,cm^{-2}\,Pa^{-1}$ at 20 °C and 4 % relative humidity) compared with that of larvae of other species. The value for *E. solidaginis* is equivalent to that of the very drought-resistant larvae of the tenebrionid beetle *Tenebrio molitor* (0.038 $\mu g\,h^{-1}\,cm^{-2}\,Pa^{-1}$ at 30 °C). In contrast, the permeability of larvae of the flesh fly *Sarcophaga crassipalpis* at 20 °C and 4 % relative humidity was 0.331 $\mu g\,h^{-1}\,cm^{-2}\,Pa^{-1}$.

The thermal dependence of the cuticular permeability increased with temperature by approximately 0.0010 $\mu g\,h^{-1}\,cm^{-2}\,Pa^{-1}\,°C^{-1}$ in the interval between 4 and 40 °C. At the transition temperature of 40 °C, the thermal dependence of the permeability increased abruptly to 0.0400 $\mu g\,h^{-1}\,cm^{-2}\,Pa^{-1}\,°C^{-1}$. Larvae treated with hexane and acetone remained remarkably resistant to water loss. However, treatment with chloroform:methanol increased the water loss rate approximately 25-fold.

During desiccation at 4 °C and 4 % relative humidity for 21 days, *E. solidaginis* larvae showed a mass loss of 18.5±4.4 % (mean ± S.E.M., N=6). Animals dried under the same conditions over the same period showed a haemolymph osmolality of 851±75 mosmol kg^{-1} (N=4). Larvae freshly removed from the galls showed a haemolymph osmolality of 918±67 mosmol kg^{-1} (N=3). A higher osmolality in the dried compared with the fresh larvae would have been expected. The present observation suggests that important ions in the haemolymph may have been excreted or rendered osmotically inactive during desiccation.

Key words: desiccation, cold-hardiness, insect, water balance, winter, *Eurosta solidaginis*, *Sarcophaga crassipalpis*.

Introduction

The emergence of arthropods as the most successful group of land animals required solutions to problems associated with water balance. Their high surface area to body volume ratio posed particular challenges to life in desiccating habitats. Comparative physiologists have often turned to arthropods living in deserts to study extreme adaptations for maintaining water balance. These investigations examined the reduction of transpiratory losses *via* cuticular and respiratory pathways as well as osmoregulatory and excretory mechanisms for water conservation (for reviews, see Edney, 1977; Hadley, 1994). However, alpine and polar arthropods or species overwintering in temperate regions have received little attention despite the fact that they must also sometimes endure severely desiccating conditions.

The goldenrod gall fly *Eurosta solidaginis* (Diptera, Tephritidae) is widely distributed in North America, ranging from Texas into southern Canada (Lee et al., 1995). Third-instar larvae of this species overwinter within spherical stem galls on several species of goldenrod (*Solidago* spp.). Although the above-ground portion of their host plant senescences and dies in late summer or early autumn each year, the stem and gall often remain upright and extend above the snowpack throughout the winter. This species has been studied extensively with regard to its freezing tolerance (for references, see Storey and Storey, 1988; Baust and Nishino, 1991; Lee et al., 1995). Larval freezing is promoted by the susceptibility of its cuticle to inoculative freezing by external ice and by the ice-nucleating activity of calcium phosphate crystals within the Malpighian tubules (Layne et al., 1990; Mugnano et al., 1996). Survival of freezing to temperatures as low as −55 °C is promoted by various responses including the synthesis of a multi-component system of low-molecular-mass

cryoprotectants (glycerol, sorbitol and trehalose), that collectively may reach levels exceeding 1 mol l^{-1}, and increases in the unsaturation of membrane fatty acids (Storey and Storey, 1992; Bennett et al., 1997).

Previous work on water balance in this species established that the body water content remains relatively constant throughout the winter. In a Texas population of this species, Rojas et al. (1986) reported that between November and January the larval water content remained constant between 60 and 64 % even though the water content of plant gall tissues decreased from 65 to 20 %. Furthermore, the synthesis and accumulation of the cryoprotectant glycerol within the larvae was strongly correlated with the decrease in the water content of the gall, suggesting that plant senescence triggers glycerol production. In an Ohio population, two separate studies found that the water content of larvae remained relatively constant between 59 and 63 % during the winter (Lee et al., 1995; Bennett and Lee, 1997). Layne and Medwith (1997) reported similar results for a population from Pennsylvania.

Recent work by Layne (1991, 1993) and Layne and Medwith (1997) has demonstrated the extreme variability of temperatures and moisture conditions experienced by larvae during the winter. On a daily basis, gall temperatures often vary by 20–30 °C and reach absolute temperatures in excess of 35 °C during September and October in western New York (Layne, 1991). Furthermore, the water content of gall tissues varied markedly from 10 % to more than 60 % during the winter and generally matched precipitation patterns (Layne, 1993). Fluctuations in the water content of the gall tissues significantly affects the susceptibility of the larvae to inoculative freezing by external ice that forms in the plant tissues and thus influences the temperature at which the larvae freeze and the number of freeze–thaw cycles they will experience seasonally (Layne et al., 1990; Layne, 1993).

Consequently, overwintering larvae are exposed for months to variable and low gall water contents and sometimes to severe low ambient temperatures within plant galls that offer little protection against these environmental extremes. Despite these extreme conditions, larvae maintain a relatively constant water content. Since surprisingly little is known concerning the water relations of this species, we examined the resistance of larvae to desiccation at different temperatures and humidities, the transition (critical) temperature above which transpiration rates increase rapidly and the effects of treatment with various solvents on larval rates of water loss.

Materials and methods

Galls containing *Eurosta solidaginis* (Fitch) larvae were collected in February 1996 and kept at −22 °C until used. Larvae were removed from the galls and weighed before and after desiccation using a Mettler (AG 245) balance. Desiccation was performed over Drierite (W. A. Hammond Drierite C., Ohio, USA) with a measured relative humidity (RH) of 4 % and over saturated solutions of NaCl (75 % RH). The larvae were kept in ELISA plastic wells in desiccators or in glass test tubes fitted with netting over Drierite during exposure to the various conditions.

The surface area of the larvae was calculated from the initial mass of the animals using Meeh's formula:

$$S = kW^{0.667} \quad (1)$$

(Wigglesworth, 1945; Hadley, 1994), where S is the calculated surface area (mm^2), k ($mm^2\,mg^{-1}$) is a species-specific constant and W is the mass (mg) of the animal. A value of 2.56 $mm^2\,mg^{-1}$ was calculated for k by measuring the surface area of *E. solidaginis* larvae (N=5) of known mass. The surface area was obtained by cutting open the body of the larva and expressing the contents before measuring the surface area on millimetre square graph paper.

The vapour pressure deficit ΔP between the surrounding air and the haemolymph of the animals was calculated using the equation:

$$\Delta P = [55.556/(55.556 + O) - \mathrm{RH}/100]P_w^*, \quad (2)$$

where P_w^* is the standard vapour pressure of pure water at the given temperature (Lundheim and Zachariassen, 1993) and O is the osmolality of the larvae. All calculations using this formula were corrected for P_w^* (Weast, 1986). The ΔP values were: 4 °C, 4 % RH, 767.8 Pa; 4 °C, 75 % RH, 190.6 Pa; 20 °C, 4 % RH, 2206 Pa; 20 °C, 75 % RH, 546.5 Pa. The following values were all based upon a relative humidity of 4 %: 30 °C, 4003 Pa; 37 °C, 5920 Pa; 40 °C, 6960 Pa; 45 °C, 9043 Pa; 50 °C, 11.64 kPa.

Osmolality was determined by drawing haemolymph into a capillary from animals freshly removed from the galls and from animals kept at 4 °C at 4 % RH for 21 days. Samples (10 µl) were measured using a Wescor 5500 vapour pressure osmometer. The osmolality of fresh larvae was 918±66 mosmol kg^{-1} (mean ± S.E.M., N=3).

To determine the water loss rate at various times during exposure, living larvae (N=4–6) were exposed to 4 % and 75 % RH at 4 ° and 20 °C, and the mass loss was measured at intervals during this period. To investigate whether water loss rate was under physiological control, larvae (N=6 in each treatment) were killed by exposing them to cyanide for 5 h at room temperature. They were then exposed to 20 °C and 4 % RH. The onset of pupariation was identified by a decrease in larval activity coupled with an initial smoothing of the cuticle and later by cuticular darkening.

The critical or transition temperature was determined by exposing larvae (N=6) to temperatures of 4, 20, 30, 37, 40 and 45 °C for 24 h and to 50 °C for 7 h over Drierite. The reason for exposing the larvae to 50 °C for only 7 h was that water loss was so fast that exposure for 24 h at this temperature would have given an excessively low rate of loss because of the total water loss from the larvae. The effects of solvents on water loss rate were investigated by gently washing larvae (N=7) for 5 or 15 min in hexane, methanol/chloroform (1:2) or acetone. Larvae were lightly blotted with filter paper, weighed, placed over Drierite at 20 °C for 24 h and the mass loss determined.

Larvae of *Sarcophaga crassipalpis* (Diptera,

Sarcophagidae) were reared on raw liver and used when they entered the wandering phase (Lee and Denlinger, 1985). Water loss rate was determined by weighing the larvae (N=8) and exposing them to 20 °C and 4 % RH for up to 11 h. The larvae were then weighed again, and the water loss rate was calculated. The surface area was calculated using Meeh's formula as for *E. solidaginis* but using a k value of 5.044 $mm^2 mg^{-1}$ obtained by measuring the surface area of *S. crassipalpis* larvae of known mass (N=8).

Values are presented as means ± S.E.M. (N).

Results

The mean mass of the *E. solidaginis* larvae was 54.9±1.5 mg (N=24). The body water content was 64.7±4.5 % (N=24), a value consistent with previous reports for the Ohio population of this species (Lee et al., 1995; Bennett and Lee, 1997). The osmolality of larvae freshly removed from the galls was 918±67 mosmol kg^{-1}. Surprisingly, this value was significantly greater than for larvae that had been desiccated for 21 days at 4 °C and 4 % RH (851±75 mosmol kg^{-1}, N=4; Student's t-test, P=0.05). Animals dried under the same conditions over the same period showed a mass loss of 18.5±4.4 % (N=6).

The water loss rates of larvae held at 20 ° and 4 °C at 4 % and 75 % RH are shown in Table 1. At 4 °C and 75 % RH, the rate of water loss was 25.2 μg $cm^{-2} h^{-1}$, whereas at 20 °C and 4 % RH the rate was 84.6 μg $cm^{-2} h^{-1}$, these two treatments being the extremes and the only ones showing significant differences in their rates of water loss. Dead animals held at 20 °C showed approximately the same water loss rates as living animals held under the same conditions, suggesting that respiratory water loss was minimal. The water loss rate for *S. crassipalpis* larvae was substantially higher (730 μg $cm^{-2} h^{-1}$) than that for *E. solidaginis* larvae. The mean mass of the *S. crassipalpis* larvae was 174.9±3.7 mg (N=8) with a body water content of 73.2±0.3 % (N=8).

The permeability of *S. crassipalpis* larvae was higher than that of *E. solidaginis* larvae (Table 2). As was the case with water loss rates, the values of water permeability were similar for living and dead *E. solidaginis* larvae held under similar conditions, i.e. 20 °C, 4 % RH, 0.038 μg $cm^{-2} h^{-1} Pa^{-1}$ (living) and 20 °C, 4 % RH, 0.030 μg $cm^{-2} h^{-1} Pa^{-1}$ (dead). *Eurosta solidaginis* larvae held at 4 °C and 4 % RH had a cuticular permeability of 0.087 μg $cm^{-2} h^{-1} Pa^{-1}$, whereas larvae held at 20 °C and 4 % RH had a cuticular permeability of 0.038 μg $cm^{-2} h^{-1} Pa^{-1}$. One might have expected that, at higher temperatures (i.e. 20 °C), the cuticular permeability would be higher because of the greater saturation deficit ΔP=767.8 Pa at 4 °C, 4 % RH *versus* ΔP=2206 Pa at 20 °C, 75 % RH. Previous workers have attributed this apparent anomaly to dividing the water loss term by the saturation deficit (Appel et al., 1986; Hadley, 1994).

Cuticular permeability increased as temperature increased (Fig. 1). In the temperature range from 4 °C to approximately 40 °C, water permeability increased only slightly with temperature, at approximately 0.0010 μg $cm^{-2} h^{-1} Pa^{-1} °C^{-1}$. The transition temperature at which water permeability

Table 1. *Water loss rates of living and dead third-instar larvae of* Eurosta solidaginis *at 4 °C and 20 °C and 4 % and 75 % relative humidity after 90–120 h of exposure*

		Water loss rate (μg $cm^{-2} h^{-1}$)	
	Treatment	Living	Dead
20 °C	4 % RH	84.6±10.1*	65.6±4.5
	75 % RH	54.3±12.6	73.4±23.9
4 °C	4 % RH	67.0±11.1	–
	75 % RH	25.2±10.2*	–

Values are means ± S.E.M. (N=4–6).

*Indicates the only mean values that were significantly different from each (Kruskal–Wallis nonparametric ANOVA test followed by Dunn's multiple-comparisons test, P<0.04).

RH, relative humidity.

Table 2. *Permeability of third-instar larvae of* Eurosta solidaginis, *wandering larvae of* Sarcophaga crassipalpis *and values from the literature for selected species*

Species	Permeability (μg $h^{-1} cm^{-2} Pa^{-1}$)	Stage	Conditions	Habitat	Remarks	Reference
Eurosta solidaginis	0.087	Larva	4 °C, 4 % RH	Mesic to xeric	Living	Present study
Eurosta solidaginis	0.083	Larva	4 °C, 75 % RH	Mesic to xeric	Living	Present study
Eurosta solidaginis	0.038	Larva	20 °C, 4 % RH	Mesic to xeric	Living	Present study
Eurosta solidaginis	0.077	Larva	20 °C, 75 % RH	Mesic to xeric	Living	Present study
Eurosta solidaginis	0.030	Larva	20 °C, 4 % RH	Mesic to xeric	Dead	Present study
Eurosta solidaginis	0.134	Larva	20 °C, 75 % RH	Mesic to xeric	Dead	Present study
Sarcophaga crassipalpis	0.331	Larva	20 °C, 4 % RH	Mesic	Living	Present study
Eleodes armata	0.129			Xeric		Ahearn and Hadley (1969)
Onymacris plana	0.011	Adult	27 °C, 5 % RH	Xeric		Edney (1971)
Onymacris laeviceps	0.026	Adult	27 °C, 5 % RH	Xeric		Edney (1971)
Tenebrio molitor	0.038	Larva	30 °C	Xeric	Dead	Mead-Briggs (1956)
Calliphora erythrocephala	0.383	Larva	30 °C	Mesic	Dead	Mead-Briggs (1956)

RH, relative humidity.

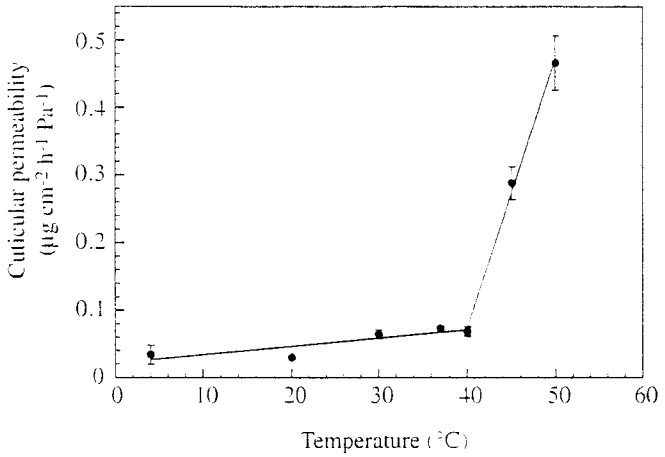

Fig. 1. Cuticular permeability of third-instar larvae of *Eurosta solidaginis versus* temperature. The transition (critical) temperature was 40 °C. The regression line for temperature points below the transition temperature is: $y=0.0013x+0.0218$ ($r^2=0.77$, $P=0.051$); the regression line for temperature points above the transition temperature is: $y=0.0398x-1.5178$ ($r^2=1.0$, $P=0.038$). Values are means ± S.E.M., $N=6$).

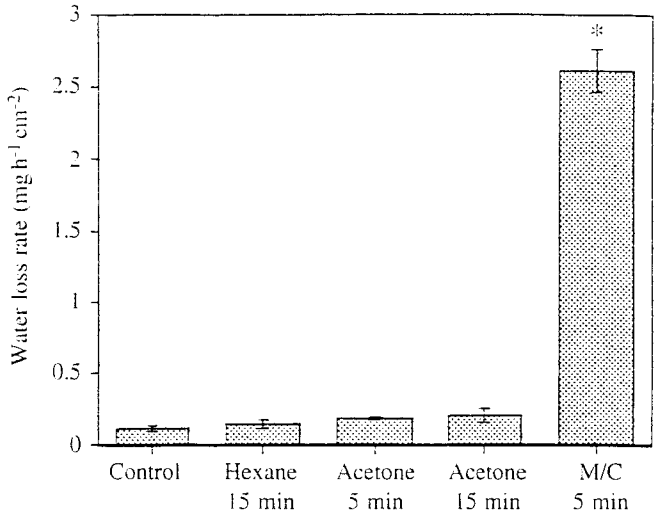

Fig. 2. The effects of hexane, acetone and methanol/chloroform (M/C) (1:2) on water loss rate of third-instar larvae of *Eurosta solidaginis*. The asterisk denotes that the value that is significantly different from the control (Kruskal–Wallis nonparametric analysis of variance, KW=19.820, $P=0.0005$, followed by Dunn's multiple-comparisons test, $P<0.001$). Values are means ± S.E.M., $N=7$).

increased abruptly was at 40 °C, at which the water permeability was 0.0705 μg cm^{-2} h^{-1} Pa^{-1}. Between 40 and 50 °C, the thermal dependence of the water permeability increased markedly to 0.0400 μg cm^{-2} h^{-1} Pa^{-1} °C^{-1}.

To determine the effects of organic solvents on the water loss rate, larvae were treated with three solvent systems (Fig. 2). Treatment with neither acetone nor hexane significantly increased the water loss rate. The only solvent that changed the water loss rate significantly compared with the control value was methanol/chloroform, which caused an increase in the water loss rate of approximately 25-fold.

As larvae began to pupariate, the rate of mass loss greatly increased, as illustrated for four individuals held at 20 °C and 75 % RH over a 22 day period (Fig. 3). Pupariation was evident approximately 8 days after the experiment began when the larval cuticle began to sclerotize and darken, and larvae assumed a coarctic shape. However, pupariation begins internally before it is evident externally. This process apparently began approximately 5 days after the experiment began when the rate of mass loss increased. The mean rates of mass loss were approximately 0.75±0.13 % day^{-1} for the first 3–7 days, 3.46±0.77 % day^{-1} for the next 3–4 days and 0.37±0.07 % day^{-1} for the last 12–14 days ($N=4$) (Fig. 3).

Discussion

Overwintering larvae of *E. solidaginis* exhibited remarkably

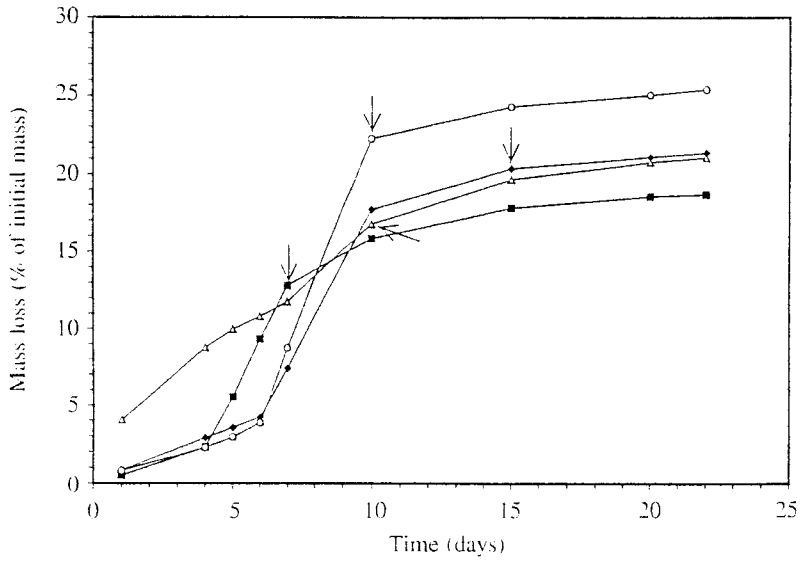

Fig. 3. Mass loss (% of initial mass) *versus* time of four third-instar larvae of *Eurosta solidaginis* before and during pupation while held at 20 °C and 75 % relative humidity. The time at which pupation was first observed for each individual is indicated by an arrow. Different symbols represent the four individuals.

Table 3. *Water loss rates of third-instar larvae of* Eurosta solidaginis *and* Sarcophaga crassipalpis *and values from the literature for selected species*

Species/Stage	Water loss rate ($\mu g\,cm^{-2}\,h^{-1}$)	Conditions	Reference
Eurosta solidaginis Larva	85	20 °C, 4 % RH	Present study
Eurosta solidaginis Larva	240	30 °C, 4 % RH	Present study
Sarcophaga crassipalpis Wandering larva	730	20 °C, 4 % RH	Present study
Tenebrio molitor Beetle larva	240	30 °C, 0 % RH	Mead-Briggs (1956)
Reticulitermes spp Termite nymphs of five species	740–2260	34 °C, 0 % RH	Collins and Richards (1963)
Agriotes sp. Beetle larva in soil	17200	30 °C, 0 % RH	Wigglesworth (1945)
Periplaneta americana Cockroach adult	470	20 °C, 0 % RH	Mead-Briggs (1956)
Onymacris plana Desert beetle adult	41	27 °C, 5 % RH	Edney (1971)

RH, relative humidity.

low rates of water loss comparable with the lowest rates reported for species living in deserts or highly xeric environments (Tables 2, 3). Our search of the literature revealed only a few reports of water loss rates for larval insects. When we tested wandering larvae of the flesh fly *S. crassipalpis*, their rate of loss was almost nine times greater than that of *E. solidaginis* larvae (Table 3). Of particular note is the fact that the reported rate ($240\,\mu g\,cm^{-2}\,h^{-1}$) of water loss at 30 °C from *T. molitor* beetle larvae, which have a much more heavily sclerotized cuticle, was the same as we determined for the soft-bodied larvae of *E. solidaginis* at this temperature (Table 3). Furthermore, the rate of water loss ($85\,\mu g\,cm^{-2}\,h^{-1}$) for the gall fly larva at 20 °C was only slightly greater than that of the desert beetle *Onymacris plana*, even though this heavily sclerotized adult beetle was reported by Hadley (1994) to have the lowest reported rate of water loss for any insect. The permeability of $0.038\,\mu g\,h^{-1}\,cm^{-2}\,Pa^{-1}$ for gall fly larvae ranks it among the most xeric-adapted species (Table 2; Hadley, 1994).

The fact that the rates of water loss for dead *versus* living larvae were not significantly different, when determined under comparable conditions, indicates that respiratory water losses appear to be a relatively minor factor for this species, at least at lower temperatures (Table 1). Another factor contributing to this could be that polyol accumulation may have reduced the rate of oxidative metabolism substantially with a subsequent and proportional reduction in the rate of transpiratory water loss. Furthermore, the fact that cuticular permeabilities (corrected for vapour pressure deficit) were largely independent of temperature over the range 0–35 °C (Fig. 1) suggests that even large fluctuations in diurnal temperatures of the gall would not markedly affect larval transpiration. Even though we found very low rates of water loss in larvae that had been removed from their plant galls, in nature it is likely that rates of water loss would be even more moderated by the surrounding gall tissues whose moisture levels are reported to vary between 10 and in excess of 60 % during the winter (Layne, 1993).

The fact that the haemolymph osmolality of larvae desiccated for 21 days was lower ($851\,mosmol\,kg^{-1}$) and not very different from that of fresh larvae ($918\,mosmol\,kg^{-1}$) during a loss of approximately 19 % of their initial body mass indicates either that some organic solutes are metabolized or that *E. solidaginis* larvae control the ion content of their haemolymph, possibly by excreting or rendering important ions osmotically inactive, perhaps after redistribution to the intracellular compartment, during desiccation (see Hyatt and Marshall, 1985; Bjerke and Zachariassen, 1997). The calculated change in osmolality of an ideal solution desiccated to the same extent would have been an increase of $382\,mosmol\,kg^{-1}$.

The transition temperature is identified by a dramatic increase in the cuticular permeability, which presumably reflects a loss of cuticular waterproofing by epicuticular lipids (Hadley, 1994). For terrestrial arthropods, this value is often at 40–60 °C or higher, with the highest values often reported for desert species that also have very low rates of water loss. In contrast, the transition temperature of 40 °C for *E. solidaginis* larvae is relatively low. Layne (1991) reported that the maximum temperature experienced by overwintering larvae in the field was 31.6 °C. He also noted that larvae survived 24 h of exposure to 35–36 °C, but did not survive for 24 h at 45.5 °C. Consequently, overwintering larvae would not be expected to encounter environmental temperatures above their transition temperature.

An increase in water loss rates in connection with moulting

has been observed in various species of insect. Wigglesworth and Gillett (1936) reported that, in *Rhodnius prolixus*, water loss rate more than doubled some hours after moulting. Twenty four hours after the beginning of moulting in the beetle *Tenebrio molitor*, Wigglesworth (1948) observed that the water loss rate increased four- to sixfold, the fastest transpiration occurring in the early hours after shedding the skin. Edney (1957) argued that this change in cuticular permeability is because the old exo- and endocuticle are digested before the skin is cast, so that only the thin epicuticle is left. Gilby and Rumbo (1980) observed a major water loss 20–22 h after puparium formation in the sheep blow fly *Lucilia cuprina*. This increase in water loss was attributed to casting of the posterior larval tracheae and to mechanical expulsion of liquid through the exposed spiracles. After this expulsion, the water loss rate fell to a very low value because of the creation of airspaces between the pupal cuticle and the outer membranes (Gilby and Rumbo, 1980). In contrast to Gilby and Rumbo (1980), we found that the rate of water loss began to increase before external signs of pupariation were evident, indicating that changes in water permeability occurred as a result of changes in the cuticle.

The main water barrier in the insect cuticle is considered to be the epicuticular lipids (Hadley, 1989). A number of studies have shown that the removal of these lipids by abrasion or by chemical treatment significantly increases the cuticular water permeability (Wigglesworth, 1945). Hadley (1989) showed that rubbing the cuticle of the cricket *Acheta domesticus* with hexane increased the water permeability by 11-fold. Hadley and Quinlan (1989) also found that a similar treatment of the abdominal cuticle of the black widow spider *Latrodectus hesperus* with chloroform:methanol (2:1) increased its water permeability by up to 190-fold. Our results show that gently washing *E. solidaginis* larvae with chloroform:methanol (2:1) had a similar, although somewhat smaller, effect on water loss rate. Gently washing *E. solidaginis* larvae with hexane and acetone had very little effect on the water loss rate (Fig. 2). Since hexane alone had only a minor effect on the water loss rate, the results suggest that polar lipids may play an important role in waterproofing the cuticle of *E. solidaginis* larvae.

Within their galls, the overwintering larvae are challenged not only by low winter temperatures but also by diurnal variations in temperature that may span more than 30 °C (Layne, 1991), causing them to undergo multiple cycles of freezing and thawing. The water content of the gall also varies with precipitation. Since the larvae apparently do not have access to free water to drink nor do they feed during the winter, they must rely on mechanisms that conserve water during their extended overwintering period of 6–8 months. Lundheim and Zachariassen (1993) demonstrated, in accordance with physical laws, that frozen insects would not be expected to be under desiccation stress since the unfrozen portion of their body fluids is in vapour pressure equilibrium with the surrounding ice. However, during periods of desiccating conditions, when inoculative freezing is not likely to occur and temperatures are above the supercooling point of their body fluids, the extreme resistance to desiccation of *E. solidaginis* larvae may be critical for water conservation.

The resistance of *E. solidaginis* to water loss, revealed by the present study, suggests that conditions within the gall are highly desiccating and that the cuticle plays a major role in water conservation. This conclusion is supported by the exceptionally low rates of water loss, which are among the lowest reported for any insect. This result is a particularly surprising discovery in a relatively soft-bodied larval dipteran.

The results of this study also raise interesting evolutionary scenarios and questions concerning linkages between adaptations to survive desiccation and those enhancing cold-hardiness (for a discussion, see Ring and Danks, 1994). Did the development of desiccation resistance promote the evolution of freeze tolerance in this species? The larval cuticle is both susceptible to inoculative freezing by external ice (Layne et al., 1990) and highly resistant to water loss. What is the explanation for this paradox?

Jackie Litzgus is thanked for assistance with statistics. This research was supported by the Danish Science Research Council (H.R.) and NSF IBN-9728573 (R.E.L).

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