Plant senescence cues entry into diapause in the gall fly *Eurosta solidaginis*: resulting metabolic depression is critical for water conservation

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Summary

Mechanisms and possible cues for seasonal increases in desiccation resistance in larvae of the goldenrod gall fly *Eurosta solidaginis*, were examined before and after natural and premature plant senescence, or after being removed from their gall and placed in either 100, 95 or 75% relative humidity (RH). Rates of water loss were 8.6-fold lower, averaging 0.7 ± 0.2 μg mm^{-2} h^{-1}, in larvae from senescent gall tissue and after all RH treatments than in control larvae from pre-senescent plants. Enhanced desiccation resistance occurred quickly, within 3 days of removal from their gall. Contrary to most previous reports, a large majority of the increased desiccation resistance (~85%) was due to reduced respiratory transpiration with the remainder being the result of a lowered cuticular permeability. Rates of cuticular water loss were reduced by the presence of a vapor pressure gradient between the larval hemolymph and environmental water vapor and were probably due to increases in cuticular lipids and/or production of the cryoprotectant glycerol. Metabolic rate was reduced by over fourfold, averaging 0.07 ± 0.01 μl CO$_2$ g^{-1} h^{-1}, in larvae from senescent gall tissue and all RH treatments compared to larvae from pre-senescent plants. The magnitude of the reduction in metabolic rates indicated that these larvae had entered diapause. In addition, larvae entered diapause in response to removal from, or degeneration of, the gall tissue they feed, on rather than seasonal changes in temperature or photoperiod. The low metabolic rates of the diapausing larvae probably allowed them to dramatically reduce their respiratory transpiration and total rate of water loss compared with non-diapausing controls. Thus, diapause, with its associated lowered metabolic rate, may be essential for conserving water in overwintering temperate insects, which may be dormant for six or more months of the year.

Key words: desiccation resistance, diapause, *Eurosta solidaginis*, cuticular permeability, respiratory transpiration

Introduction

Insects are intrinsically at risk of desiccation because of their small size and high surface area to volume ratio. Nevertheless, the risk of desiccation can vary greatly throughout an insect's life cycle. For example, temperate and polar insects are potentially at a much higher risk of desiccation in the winter because cold air can contain little water vapor, and overwintering insects are typically dormant and unable to imbibe free water. The seasonal contrast in desiccation stress for galling insects can be even greater. Temperate galling insects usually do not experience desiccation stress in the summer while the active plant tissue they inhabit is fully hydrated (Williams et al., 2004). However, galls can quickly desiccate as the plants senesce and the gall tissues dry in the autumn. In addition, dried gall tissue provides little buffering against environmental extremes and insects remaining in their galls experience the full desiccating conditions of winter (Layne, 1993; Layne and Medwith, 1997).

To survive the desiccating conditions of winter, galling insects such as cynipid wasps (Williams et al., 2002) and the

goldenrod gall fly *Eurosta solidaginis* (Ramløv and Lee, 2000), have extremely low rates of water loss. These soft-bodied, immature insects have rates of water loss that are similar to heavily sclerotized, adult desert beetles (see Edney, 1977 and references therein; in Hadley, 1994a). To attain such high levels of desiccation resistance, overwintering goldenrod gall fly larvae seasonally reduce their rates of water loss from late summer to mid-winter. In particular, *E. solidaginis* reduce their rate of water loss more than sixfold, within a two-week period in early autumn (Williams et al., 2004). However, the mechanisms by which *E. solidaginis* reduce their rate of water loss or the cues triggering the enhanced desiccation resistance during this period are unknown.

The goldenrod gall fly (Diptera: Tephritidae) ranges throughout much of central and eastern North America, from Texas to southern Canada. In late spring or early summer, females oviposit in terminal buds (future stems) of goldenrod plants (*Solidago* spp.) (Uhler, 1951). Larvae feed and grow within the moist gall tissue throughout the summer and early

autumn. In southwest Ohio goldenrod plants and gall tissues senesce and rapidly dry in late September and early October (Irwin et al., 2001; Williams et al., 2004). In addition to reducing their rate of water loss, larvae undergo other physiological changes as their gall tissue senesces. For instance, larvae produce the cryoprotectant glycerol in response to plant senescence (Baust and Lee, 1982; Rojas et al., 1986), suggesting this low molecular mass polyol may be important in preventing dehydration. Larvae also enter diapause during this period (Irwin et al., 2001). Diapause is defined as a genetically determined state of low metabolic activity, suppressed development and heightened resistance to environmental extremes, which generally begins before, and lasts longer than, the adverse conditions (Tauber et al., 1986; Danks, 1987).

Overwintering and dormant insects lose water to the environment primarily through cuticular and respiratory transpiration (Edney, 1977; Hadley, 1994a; Danks, 2000). During the fall and winter, larvae of *E. solidaginis* reduce their rates of cuticular water loss by producing epicuticular lipids, which increase by 40-fold from summer to mid-winter (Nelson and Lee, 2004), and possibly by producing cryoprotectants (Williams et al., 2004). These larvae may also reduce their rates of loss by lowering respiratory transpiration, as their metabolism decreases when entering diapause. However, reductions in metabolic rate for inactive insects frequently have a relatively minor effect on desiccation resistance (for reviews, see Chown, 2002; Chown and Nicolson, 2004). Regardless, larvae of *E. solidaginis* probably reduce both avenues of water loss as they increase their desiccation resistance in early autumn as gall tissue senesces, but the relative contributions of decreased cuticular and respiratory transpiration are unknown.

The purpose of this study was to determine whether plant senescence and diapause induction were closely associated with the seasonal reduction in rates of larval water loss that occurs in early October as documented previously (Williams et al., 2004). Specifically, we measured the cuticular and estimated respiratory contributions to total organismal water loss as gall tissue naturally senesced. We also determined whether mild desiccation stress and premature plant senescence would trigger enhanced larval desiccation resistance. We measured total rates of water loss, rates of cuticular water loss, carbon dioxide production (as an estimate of metabolic rate and diapause induction), body water content, hemolymph osmolality and glycerol content of larvae collected from the field just prior to, and after, natural plant senescence. To determine if the field-collected larvae were taken from desiccating and senescent plant tissue, we measured the water content and water activity of gall tissues.

Materials and methods
Insect collection

Fresh galls containing *Eurosta solidaginis* Fitch were collected before (1 October 2003) and after (20 October 2003) the larval rates of water loss decrease markedly (Williams et al., 2004). To induce premature drying of the gall tissue, goldenrod plants (*Solidago altissima)* were cut at ground level and tied upright to a stake on 17 September 2003. The cut plants remained in the field until being brought into the laboratory and analyzed on 1 October 2003 and were termed the 'stem cutting' treatment. All galls were collected at the Ecology Research Center near Oxford, Ohio, USA and tested within 12 h of being harvested. To standardize for size, only larvae weighing between 48 and 58 mg were used in all experimental groups except the stem cutting treatment. Because larvae grow rapidly prior to gall senescence (Bennett and Lee, 1997; Layne and Medwith, 1997) and goldenrod plants in the stem cutting treatment senesced prematurely, larval mass in this group were much lower, averaging 41.6±1.5 mg (mean ± S.E.M.).

Gall measurements

Hydric conditions of the gall tissue were assessed by measuring the total water content and water vapor potential of the gall tissue immediately surrounding the larvae. Gall water content was determined by weighing 10 galls that had contained larvae to ±0.1 mg using a Mettler Toledo AG245 balance (Columbus, OH, USA), before and after drying in an oven at 65°C until they reached a constant mass. Water vapor potential of the gall tissue was determined by the psychrometric vapor pressure depression technique described by Hølmstrup and Westh (1994). Immediately after opening an occupied gall, 10–20 mg of gall tissue surrounding the larval chamber was transferred to a Wescor C-52 sample chamber (Logan, Utah, USA) and allowed to equilibrate for 30 min. Water potential was then determined with a Wescor HR 33T Dewpoint Microvoltmeter (Logan, Utah, USA) operated in the dewpoint mode. Measurements were taken on 10 randomly selected galls collected on 1 October. Galls in the stem cutting treatment and those collected on 20 October were too dry to accurately measure water activity.

Measurements of desiccation resistance

Resistance to desiccation was determined by measuring rate of water loss, in $\mu g \ mm^{-2} \ h^{-1}$; body water content, as a ratio of wet mass to dry mass; and rate of cuticular water loss. To determine rate of organismal water loss, which includes both respiratory and cuticular components, larvae (N=10) were weighed to ±0.01 mg to obtain a fresh mass. Larvae were then re-weighed after being desiccated non-convectively over Drierite (W. A. Hammond Drierite Co., Ohio, USA) at 4% relative humidity (RH) and 20°C until they lost 5–10% of their fresh mass. Cuticular surface area was estimated from initial wet mass using an equation determined by Williams et al. (2004): $y=0.912x+4.204$, $r^2=0.804$, where y = surface area in mm^2 and x = mass in mg. Body water content was determined by placing the desiccated larvae in an oven at 65°C until a constant dry mass was obtained.

Rates of cuticular water loss ($\mu g \ mm^{-2} \ h^{-1}$) were measured to determine the relative contributions of respiratory and cuticular components of overall organismal water loss.

Cuticular water loss was assessed by weighing larvae (*N*=10) before and after exposure to 4% RH at 20°C as described above, however, prior to testing, the spiracles of each larva were topically blocked with a small amount of Thomas Scientific Lubriseal stopcock grease (Swedesboro, New Jersey, USA), to eliminate respiratory water loss. Stopcock grease was carefully applied with a 10 ml syringe to ensure that only the spiracles were covered with the stopcock grease. In addition, larvae were examined after the experiment to ensure that the grease did not spread to other areas of the cuticle. This procedure is extremely effective at blocking the four larval spiracles of *E. solidaginis*, as CO_2 emission is completely eliminated after application of only a small amount of stopcock grease to the spiracles (data not shown).

Hemolymph osmolality and cryoprotectant concentration

Hemolymph osmolality (*N*=10) was determined by drawing 7-10 μl of hemolymph into a capillary tube through a small incision in the larval cuticle. The hemolymph was then analyzed in a Wescor Vapro 5520 Hemolymph Osmometer (Logan, Utah, USA). To measure glycerol concentration, larvae (*N*=7) were frozen at –80°C until whole body measurements were performed by enzymatic assay (Sigma Chemical Co., St Louis, Missouri, USA, no. 337) as described by Hølmstrup et al. (1999).

Measurement of metabolism

To determine larval diapause status, we assessed metabolic rate by measuring CO_2 emission. Larvae (*N*=7 per treatment) were weighed and individually placed into ~14 ml^3 glass respirometry chamber kept within a temperature-controlled bath held at 20°C. Carbon dioxide was measured using a flow through (50 ml min^{-1}) respirometer (TR-3 model, Sable Systems, Las Vegas, Nevada, USA). Room air was used for respirometry measures after it was drawn through a column of ascarite (Thomas Scientific, Swedesboro, New Jersey, USA) to remove CO_2 and drierite to reduce water content of the air. Larvae were equilibrated to the chamber for 1 h prior to analysis. Metabolic rate data were converted into units of μl CO_2 emitted per mg fresh mass per hour using DATACAN software (Sable Systems). We did not measure larval activity during metabolic rate determinations because CO_2 production was relatively stable after the 1 h acclimation period, and larvae, regardless of diapause status, rarely moved, unless prodded, after being removed from the gall.

Effect of mild and moderate desiccation stress on larval desiccation resistance

A second group of larvae were collected between September 28 and October 3, prior to the dramatic seasonal decrease in rates of water loss, and were used to determine the effect of mild and moderate desiccation stress on desiccation resistance. Immediately after collection, larvae were removed from their galls and placed in desiccators over either double distilled water to produce a RH of 100%, a saturated solution of sodium sulfate producing a RH of 95% or a saturated

solution of sodium chloride producing a RH of 75%. Each desiccator was placed in an incubator maintained at 20°C. After 3, 6 or 10 days exposure to these conditions, larvae were removed and assessed for water loss rate, body water content, cuticular permeability, hemolymph osmolality, glycerol concentration and metabolic rate using the methods described above.

Statistical analysis

After data were determined to be normally distributed with homogeneous variances, a one-way analysis of variance followed by a Bonferroni multiple comparisons (Statview 5.0, SAS Institute Inc., Cary, North Carolina, USA) was used to determine differences in means between larvae collected on 1 and 20 Oct., the stem cutting treatment, and all relative humidity treatments. To increase statistical power when examining the larval treatments exposed to various relatively humidities, a one-way ANOVA followed by a Bonferroni multiple comparisons was used to identify differences in RH treatments on a given day. A significance level of α=0.05 was used for all tests.

Results

Gall measurements

Hydric parameters of the gall tissue changed dramatically in post-senescent goldenrod plants. Gall water content was more than fourfold lower in the stem cutting group and galls collected on 20 October than those examined on 1 October, which averaged 1.97±0.01 mg water mg dry mass^{-1} (Table 1). Hydric changes in the gall tissue were also evident as water potential of the tissue surrounding the larvae was high on 1 October (–12.8±0.7 bars) but was so low in the stem cutting group and those collected on 20 October that no measurement could be made (Table 1). Taken together, and compared with previous reports, the high water content and water activity of the 1 October control group indicate these galls were pre-senescent, while the low values for the stem cutting and 20 October galls were fully senesced (Williams et al., 2004).

Table 1. *Gall water content and water activity of goldenrod galls containing* E. solidaginis *larvae (*N=10*) collected during October 2003*

Collection date	Gall water content (mg water mg^{-1} dry mass)	Gall water activity (kPa)
1 Oct.	1.97±0.07[a]	1280±70
1 Oct. (stem cutting)	0.30±0.04[b]	–
20 Oct.	0.46±0.12[b]	–

In the stem cutting group, plants had their stems cut two weeks prior to the 1 Oct. collection. Galls were too dry in the stem cutting and 20 Oct. collections to accurately measure gall water activity. Means (± S.E.M.) for gall water content not sharing a letter are significantly different (*P*<0.05).
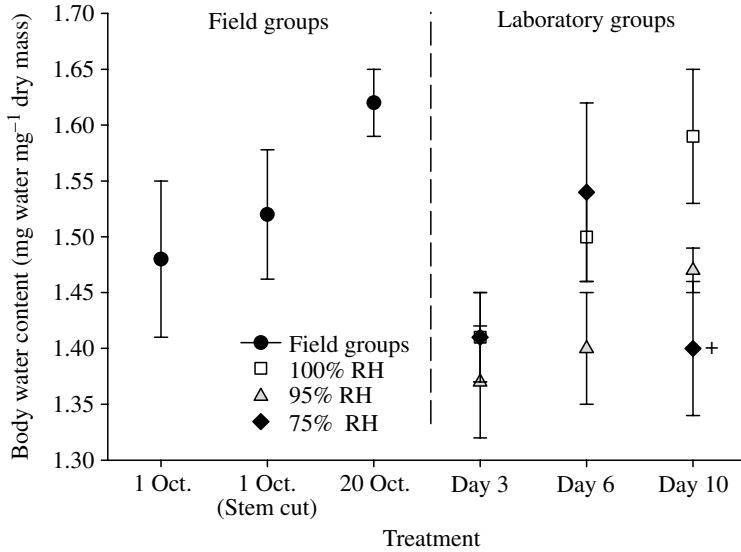
Fig. 1. Body water content (N=10) of *Eurosta solidaginis* larvae analyzed immediately after collection from the field or after 3, 6 or 10 days exposure to various relative humidities (RH). On a given day, laboratory group data with a '+' are significantly different from the 100% treatment using a one-way ANOVA and Bonferroni multiple comparisons test.

Measures of desiccation resistance

Although the larvae were exposed to desiccating conditions after gall senescence, they were able to maintain their hydration levels, as the body water content did not differ among the 1 October, stem cutting and 20 October groups (Fig. 1). In addition, body water content did not differ in the laboratory RH treated larvae and 1 October controls (Fig. 1). Values ranged between 1.62±0.03 and 1.37±0.05 mg water mg dry mass^{-1} (58–62% of fresh mass).

Rates of larval water loss were dramatically (P<0.05) lower for larvae in the stem cutting group and those collected on 20 October (averaging 0.7± 0.2 μg mm^{-2} h^{-1}) compared to larvae collected on 1 October (6.0±1.8 μg mm^{-2} h^{-1}; Fig. 2A). After just 3 days of exposure to 75% RH, larvae had rates of water loss (1.3±0.1 μg mm^{-2} h^{-1}) that were statistically similar to those in the stem cutting and 20 October groups (Fig. 2A). Furthermore, humidity may influence *E. solidaginis* to increase desiccation resistance as 6-days exposure to 100% RH and 95% RH were required before rates of larval water loss were significantly lower that those collected and analyzed on 1 October (Fig. 2A). In addition, larvae exposed to 75% RH for 10 days had significantly lower rates of water loss (0.8±0.1 μg mm^{-2} h^{-1}; P<0.05) than those in the 100% RH, 10 day treatment (2.1±0.5 μg mm^{-2} h^{-1}).

To estimate the cuticular transpiration component of organismal water loss, we measured rates of larval water loss after topically blocking their spiracles. Rates of cuticular water loss were significantly lower for larvae analyzed on 20 October (0.4±0.1 μg mm^{-2} h^{-1}) compared with those in the 1 October group (1.1±0.4 μg mm^{-2} h^{-1}; P<0.05) and there was a trend
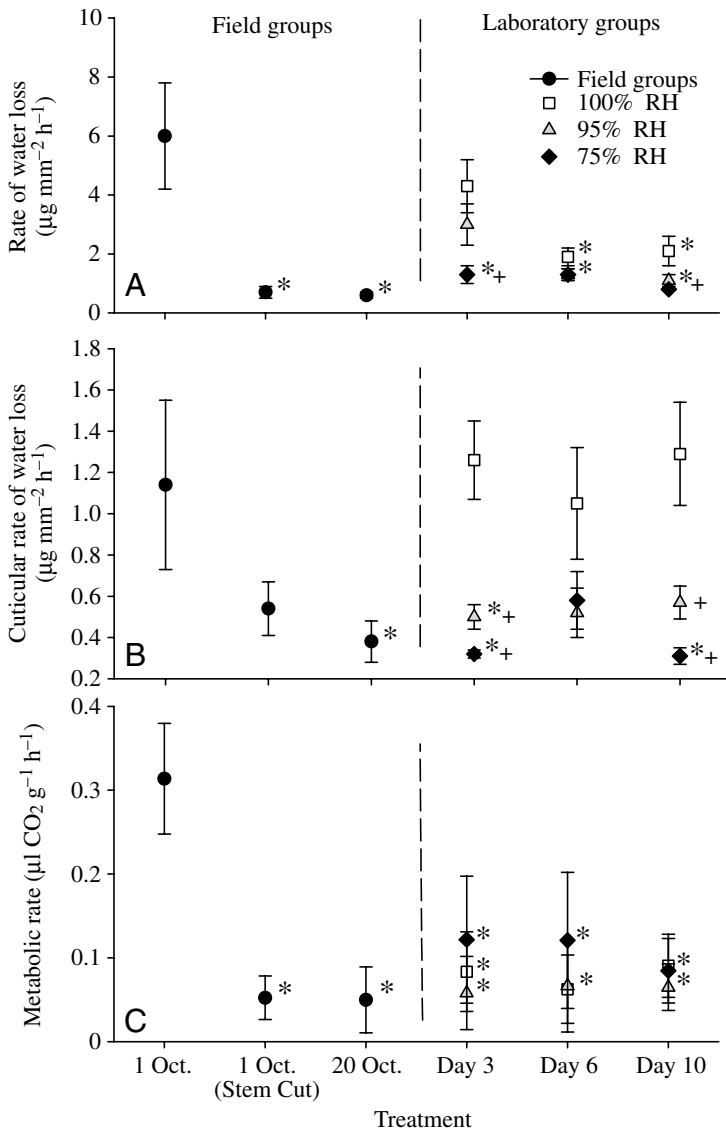

Fig. 2. (A) Rates of total water loss (N=10), (B) rates of cuticular water loss (N=10) and (C) rates of metabolism (N=7) of *Eurosta solidaginis* larvae analyzed immediately after collection from the field or after 3, 6 or 10 days exposure to various relative humidities. Rates of cuticular water loss were measured on larvae after their spiracles were topically blocked with stopcock grease. Values are means ± S.E.M. An asterisk indicates a significant difference from the 1 Oct. data point using a one-way ANOVA and Bonferroni multiple comparisons test. On a given day, laboratory group data with a '+' are significantly different from the 100% treatment, using a one-way ANOVA and Bonferroni multiple comparisons test.

toward lowered rates of cuticular water loss for the stem cutting group (Fig. 2B). Larvae exposed to 75% and 95% RH for only 3 days had rates of cuticular water loss averaging 0.4 ± 0.1 μg mm^{-2} h^{-1}, which were significantly lower than for larvae analyzed on 1 October ($P<0.05$). In contrast, larvae in high humidity treatments did not lower their cuticular transpiration; larvae exposed to 100% RH for 3, 6 and 10 days had the same rates of cuticular water loss as those examined on 1 October (Fig. 2B). In addition, rates of cuticular water loss were significantly higher in larvae exposed to 100% RH than in those in the 95% and 75% RH treatments for 3 and 10 days (Fig. 2B). It is important to note that the above measure of cuticular permeability may also include water loss through the mouth and anus, however, these avenues of water loss are probably minimal, as *E. solidaginins* larvae are not actively feeding or producing frass during this period.

Measures of metabolism

Metabolic rate was significantly lower ($P<0.05$) for larvae analyzed on 20 October (post-plant senescence) and in the stem cutting group (premature plant senescence), averaging 0.05 ± 0.01 μl CO_2 g^{-1} h^{-1} compared with larvae from the 1 October group (pre-senescent goldenrod; 0.31 ± 0.06 μl CO_2 g^{-1} h^{-1}; Fig. 2C). Interestingly, metabolic rate lowered rapidly once the larvae were removed from their gall; all larvae in the 100%, 95% and 75% RH treatments had similar metabolic rates (0.09 ± 0.05 μl CO_2 g^{-1} h^{-1}) to those in the stem cutting and 20 October group just 3 days after being removed from the gall. No larvae, in any experimental group, demonstrated discontinuous gas exchange.

Hemolymph osmolality and cryoprotectant production

Hemolymph osmolality increased by an average of 105 mOsm kg^{-1} in the stem cutting and 20 October treatments, compared with the 1 October collection, which averaged 534 ± 14 mOsm kg^{-1} (Fig. 3A). Larvae exposed to 100%, 95% and 75% RH for 6 and 10 days had significantly higher hemolymph osmolalities, averaging 673 ± 27 mOsm kg^{-1}, than those in the 1 October collection ($P<0.05$). Mildly desiccating conditions did not appear to influence hemolymph osmolality as solute concentrations did not differ between the 100% RH and 95 or 75% RH treatments during the 3, 6 or 10 days of exposure (Fig. 3A).

Changes in hemolymph osmolality were largely due to increases in glycerol content. Glycerol concentrations increased by over 3.4-fold in larvae collected on 20 October compared with the 1 October group, which averaged 47 ± 5 mOsm kg^{-1} (Fig. 3B). The effect of relative humidity on glycerol concentration is less clear. Although there was no difference between RH treatments on day 3 and day 10, the larvae in the 75% RH treatment had higher glycerol levels than the 1 October group on day 6 and day 10 of the study. Considering all field and laboratory groups together, glycerol production constituted approximately 61% of the overall
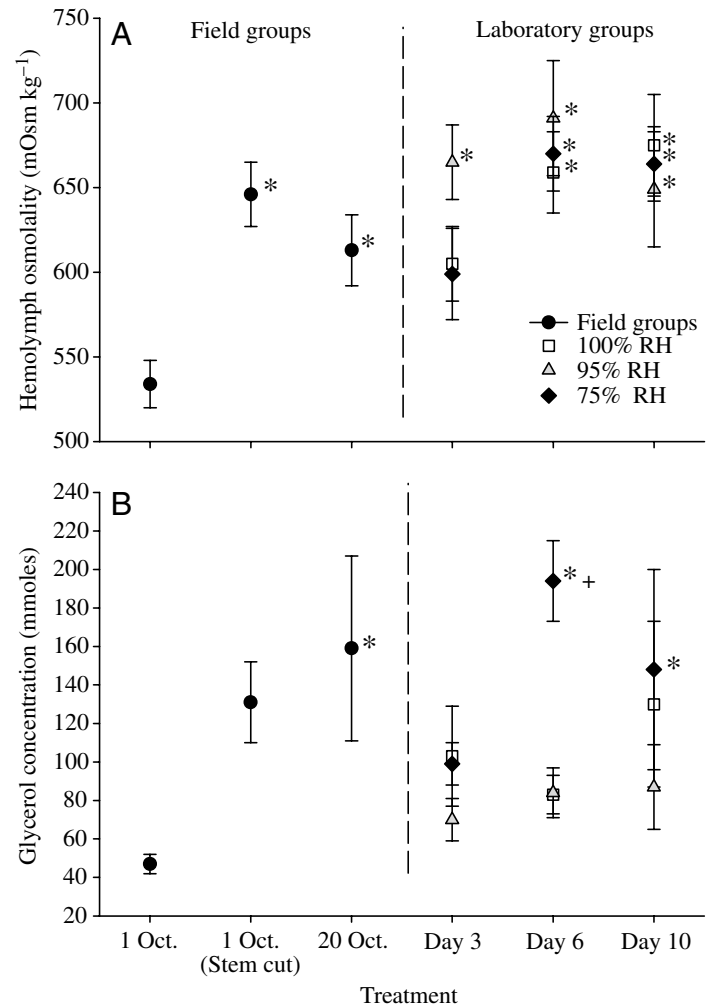

Fig. 3. (A) Hemolymph osmolality ($N=10$), and (B) glycerol concentration ($N=7$) of *Eurosta solidaginis* larvae analyzed immediately after collection from the field or after 3, 6 or 10 days exposure to various relative humidities. Values are means ± S.E.M. An asterisk indicates a significant difference from the 1 Oct. data point using a one-way ANOVA and Bonferroni multiple comparisons test. On a given day, laboratory group data with a '+' are significantly different from the 100% treatment using a one-way ANOVA and Bonferroni multiple comparisons test.

increase in hemolymph osmolality compared to the 1 Oct. control group (Fig. 3A).

Discussion

Rapid increase in desiccation resistance

Since stem galls often project above the snowpack, larvae of *E. solidaginis* may experience severely desiccating conditions during winter. To survive six months or longer in their desiccated galls, larvae must reduce their rates of water loss from the relatively high levels in early autumn to mid-winter (Ramløv and Lee, 2000; Williams et al., 2004). Larvae from prematurely and naturally senesced plants had

significantly reduced rates of water loss compared to the 1 October control group. The reduced rates of water loss for larvae from senesced plant tissue (stem cutting and 20 October treatments) occurred quickly, within a few weeks, and were similar to rates measured from mid-winter-acclimated larvae (Williams et al., 2004; Fig. 2A). In fact, mid-winter rates of water loss were probably attained within days after the plant tissue senesced because it only took 3 days for larvae in the 75% RH treatment and 6 days for larvae in the 95% and 100% RH treatments to reduce their rates of water loss to near mid-winter levels. Regardless of the speed at which these larvae reduced their rates of water loss, enhanced desiccation resistance of these larvae can be attributed to a combination of decreases in cuticular and respiratory transpiration.

Enhanced cuticular resistance plays a minor role in rapid increase in desiccation resistance

It is generally thought that increased desiccation resistance in arthropods is primarily due to a reduction in cuticular water loss (Hadley, 1994a; Hadley, 1994b). However, reduced cuticular transpiration contributed only a small component to lowering the rates of total water loss in this study (Fig. 4). Three groups of larvae significantly reduced both their rates of total water loss and rates of cuticular water loss compared to the 1 October control (20 October field group, 75% RH day 3, and 75% RH day 10 groups; Fig. 2A,B). Larvae in these groups averaged a decrease in their rate of total water loss of 5.1 μg mm^{-2} h^{-1}, but reduced their rate of cuticular transpiration by only 0.8 μg mm^{-2} h^{-1} (Fig. 4). Thus, reductions in rate of cuticular water loss only accounted for a small portion (15%) of the overall decrease in rate of water
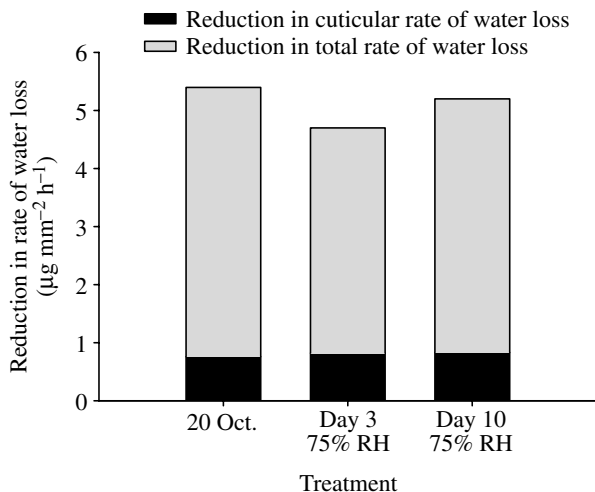

Fig. 4. Reductions in the total rate and cuticular rate of larval water loss between the non-diapausing 1 Oct. control and diapausing 20 Oct., 75% RH day 3, and 75% relative humidity (RH) day 10 experimental groups. These three experimental groups were chosen for this comparison because they had significantly reduced rates of both total water loss and cuticular water loss compared with the 1 Oct. control.

loss in these groups. Consequently, because the decrease in the rate of water loss for *E. solidaginis* larvae at this time in their life cycle is only due to changes in cuticular and respiratory transpiration, the majority (~85%) of the overall reduction in rate of water loss was the result of a reduction in respiratory transpiration (Fig. 4).

Mechanisms of reduced cuticular water loss

Reductions in cuticular water loss were probably due to increases in cuticular lipids. The integument of an insect is the primary barrier to cuticular water loss (Hadley, 1994a; Gibbs, 1998) and many insects at risk of dehydration enhance their desiccation resistance by increasing epicuticular lipids when entering a dormant stage or diapause (s.f. *Manduca sexta*, Bell et al., 1975; *Sarcophaga crassipalpis*, Yoder et al., 1992; *Mamestra configurata*, Hegdekar, 1979). *Eurosta solidaginis* larva also increases the amount of its epicuticular lipids by 40-fold over several months, from late summer to mid-winter (Nelson and Lee, 2004). Thus, even though the abrupt decrease in rates of water loss for *E. solidaginis* occurred within days, increased hydrocarbons probably reduced rates of cuticular water loss of the larvae exposed to desiccating conditions.

Less clear is the effect that cryoprotectant accumulation may have had on reducing cuticular water loss. Cryoprotectants are so-called because they enhance insect cold tolerance. Recently, several authors have suggested that cryoprotectants may be beneficial for water conservation through colligative action (Ring and Danks, 1994; Block, 1996; Bayley and Hølmstrup, 1999) or by binding water at the cuticular basement membrane (see discussion in Williams et al., 2004). Synthesis of the cryoprotectant glycerol accounted for most (~60%) of the overall increase in hemolymph osmolality in both field and laboratory groups (Fig. 3A,B). The observed increases in larval glycerol concentration confirmed earlier reports that *E. solidaginis* produces this cryoprotectant in response to gall tissue senescence (Baust and Lee, 1982; Rojas et al., 1986), suggesting that glycerol may have a protective role when entering a dormant state or desiccating environment. However, it is unclear if glycerol production lowered rates of cuticular water loss. For instance, larvae in the 75% RH treatments had lower rates of cuticular water loss than larvae in the 100% RH treatments on days 3 and 10 of the study (Fig. 2B) and larvae in the 75% RH treatments were also the only laboratory groups to have an increased glycerol concentration when compared to the 1 October control group (Fig. 3B). By contrast, larvae in the 95% RH treatment did not follow this trend. These larvae had lower rates of cuticular water loss than the 100% RH treatment (Fig. 2B) but did not have increased glycerol levels compared with control values (Fig. 3B). Thus, it appears unlikely that glycerol production was a primary factor in reducing rates of cuticular water loss during this study.

Reductions in cuticular water loss were triggered by environmental moisture, or more specifically, the presence of a water potential deficit between the larvae's hemolymph and the environment. A water potential deficit of only 1700 kPa (100 kPa=1 bar) between the hemolymph of the collembolan

Folsomia candida and its environment induce a marked increase in its drought tolerance (Sjurnsen et al., 2001). On 1 October, larvae were in a potentially hydrating environment as their gall tissues were quite moist and had a higher water activity than their hemolymph (plant tissue averaged 1280 kPa; Table 1; larval hemolymph averaged 1300 kPa as calculated from osmolality measures). Larvae placed in the 100% RH treatment continued to experience a potentially hydrating environment and did not reduce their rate of cuticular water loss (Fig. 2B). In contrast, larvae placed in the 95% and 76% RH treatments were subjected to desiccating conditions with an average water potential deficit between their hemolymph and environmental water vapor of 5820 and 39 210 kPa, respectively. In addition, larvae in the stem cutting and 20 October groups also had similarly reduced rates of cuticular water loss after being subjected to water potential deficits in their galls (Fig. 2B; Table 1). Consequently, mild desiccation stress cued larvae to reduce their rate of cuticular water loss and did so in as little as 3 days.

Plant senescence triggers entry into larval diapause

Low metabolic rates for various field- and laboratory-treated larvae indicate that they had entered diapause. Larvae from southwest Ohio reduce their metabolic rate by more than 75% as they enter diapause and maintain a metabolic rate of ~0.1 μl CO_2 g^{-1} h^{-1} at 20°C throughout diapause (Irwin et al., 2001). Carbon dioxide production of larvae taken from post-senescent galls or larvae exposed to various RH treatments decreased by an average of 75% (ranging between 61% and 84%) compared to the 1 October control group, and averaged 0.07 μl CO_2 g^{-1} h^{-1}. Taken together, the reduction and resulting level of CO_2 production indicate that these larvae were in diapause. The induction of diapause occurred rapidly for larvae placed in the RH treatments as they entered the dormant state within 3 days of being removed from the gall.

Plant senescence triggered *E. solidaginis* larvae to enter diapause. A variety of cues induce insects to enter diapause, including food availability or quality, moisture, oxygen and pH. However, the most common cues for temperate insects are related to temperature and/or photoperiod (Tauber et al., 1986; Danks, 1987). Irwin et al. (2001) suggested that a combination of low temperature, such as experiencing an initial frost, photoperiod, or host plant senescence induced larvae to enter diapause. Yet in our study, photoperiod and temperature did not appear to influence diapause induction. For example, all larvae in the various RH treatments entered diapause even though they were held at a relatively high constant temperature (20°C) after collection (Fig. 2C). In addition, field groups experienced the same temperatures and photoperiods prior to testing; however, larvae in the stem cutting group had entered diapause by 1 October, whereas larvae from the control group had not (Fig. 2C). However, the fact that larvae in the stem cutting group, taken from dried gall tissue, entered diapause by 1 October suggests that plant senescence induced these larvae to enter the dormant state. As gall tissues senesce the environment that the larva inhabits changes in two distinct ways. The nutritive gall layer on which the larvae feed deteriorates, eliminating their only food source (Uhler, 1951). In addition, the larvae are subjected to a desiccating environment for the first time (Williams et al., 2004). Moisture is an important cue for many insects to enter diapause (see references in Tauber et al., 1998), yet moisture, or more specifically a desiccating environment, does not appear to influence diapause induction for *E. solidaginis*. All larvae removed from their galls and placed in various relative humidities entered diapause regardless of whether they were in a non-desiccating environment (100% RH treatment) or a desiccating environment (95% and 75% RH treatments; Fig. 2C). However, larvae in all the RH treatments were removed from their food and water source, suggesting that food and water availability or quality is the primary cue that triggers *E. solidaginis* to enter diapause.

Importance of diapause in the water balance of dormant insects

Larvae of *E. solidaginis* probably reduce their respiratory transpiration by lowering their metabolic rate as they enter diapause. It is well established that elevated metabolic rate, due to activities such as flight, is directly related to increased respiratory water loss and, consequently, total water loss (Nicolson and Louw, 1982; Lehmann, 2001). However, for most inactive insects, respiratory water loss constitutes a minor portion of their total water loss, 20% or less (Chown, 2002). Therefore, most studies have found that reductions in basal metabolic rate have little effect on maintaining water balance for these insects (Quinlan and Hadley, 1993; Hadley, 1994b; Djawden and Bradley, 1997; Rourke, 2000). In spite of that, others contend that reductions in basal metabolic rate can be important in limiting insect water loss (Zachariassen, 1996; Davis et al., 2000; Addo-Bediako et al., 2001). Reductions in metabolism may have a greater impact on water conservation for xeric-adapted insects, in which respiratory transpiration constitutes the majority of their total water loss, such as the beetle *Phrynocolus petrosus*, which loses 69% of its water through respiration (Zachariassen, 1991). For the non-diapausing 1 October control larvae, respiratory water loss was 4.9 μg mm^{-2} h^{-1} (estimated by subtracting the rate of cuticular water loss, Fig. 2B, from the rate of total water loss, Fig. 2A), or 80% of the total water loss. However, transpiratory water loss was dramatically reduced, to only 0.6 μg mm^{-2} h^{-1}, for larvae in diapause (Fig. 4). These larvae also reduced their metabolic rate by 4.2-fold over the same testing periods. Therefore, a substantially lowered metabolism due to diapause would reduce the need for gas exchange and would allow the larvae to reduce the rates of respiratory and total water loss by regulating their spiracular openings (Gibbs et al., 2003).

Temperate insects benefit from diapause in a variety of ways. Insects in the state of diapause are often more resistant to adverse conditions. Diapause also synchronizes spring emergence and prevents premature development, which would be fatal, during unseasonably warm periods in late winter (Tauber et al., 1986; Danks, 1987). The reduced metabolic rate

of diapausing insects also conserves stored energy needed for spring development and reproduction (Danks, 1987; Irwin and Lee, 2003; Williams et al., 2003). However, few authors consider desiccation resistance as an important function of diapause for temperate and polar insects. Our data suggest that a lowered metabolic rate, due to diapause induction, is extremely important in conserving body water. For instance, if the non-diapausing 1 October control larvae maintained the respiratory transpiration rate of 4.9 μg mm^{-2} h^{-1} (Fig. 2A minus Fig. 2B), we estimate it would only take them 26 days to loose 10% of their body water to the desiccating conditions used in this experiment through respiration transpiration. In contrast, diapausing larvae had an average transpiration rate of 0.6 μg mm^{-2} h^{-1} and it would take these larvae 214 days to loose 10% of their body water through respiratory transpiration. Since many temperate insects remain in diapause for 6 months or more, a dramatically reduced rate of respiratory water loss, through a lowered metabolic rate, would have a profound impact on their overwintering water balance.

In summary, the large seasonal reduction in rate of water loss for *E. solidaginis* larvae is cued by the senescing of their gall tissue and is primarily due to reduced respiratory transpiration as the larvae enter diapause.

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