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Cold winter microenvironments conserve energy and improve overwintering survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis*

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We studied the influence of two overwintering microenvironments on survival and potential fecundity of goldenrod gall flies, *Eurosta solidaginis* (Fitch) (Diptera, Tephritidae). These freeze-tolerant larvae overwinter above the snow on standing goldenrod stems (elevated) or below the snow on broken stems (ground-level). When covered by snow, the ground-level larvae were well insulated and thus protected from the lowest temperatures of the winter, but, because they were warmer, they consumed more energy than their elevated counterparts. The ground-level group also experienced greater warming from the soil during sunny spring days, and their galls were less prone to drying than their elevated counterparts. By winter's end the ground-level larvae exhibited significantly lower rates of emergence (83.5% vs 93.0%) and reduced potential fecundity (274 ± 11 eggs/female vs 336 ± 17 eggs/female). Models of seasonal energy use indicate that these differences were due to higher metabolic rates in the ground-level microenvironment due to insulation by snow and warming from the soil, which reduced the energy available for morphological development and egg production in the spring. We conclude that colder winter microenvironments can have a strong positive effect on overwintering ectotherms, particularly those that rely on energy stores accumulated during the autumn to produce eggs in spring. The enhanced reproductive output of insects overwintering in colder microenvironments may be a selective force promoting the evolution of increased cold-hardiness.

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Many northern insects hibernate in sites that provide little protection from the harsh conditions of winter (Danks 1991). The model for this study, larvae of the goldenrod gall fly (*Eurosta solidaginis* (Fitch)), overwinters in galls on the stem of goldenrod (*Solidago* spp.). The galls offer little insulation so the larvae within them experience large diel fluctuations in temperature (up to 20°C), the extreme cold of winter, and large fluctuations in relative humidity depending on recent precipitation (Layne 1991, 1993). Given the stressors in such an environment, it would seem that such sites would be a poor place to overwinter. However, laboratory studies have demonstrated that colder winters promote energy conservation (Pullin and Bale 1989a), thus enhancing survival (Pullin and Bale 1989b, Irwin and Lee 2000) and spring fecundity (Irwin and Lee 2000). We wanted to test further the hypothesis that cold is advantageous by examining whether colder microclimates in nature have similar positive effects on larval energy conservation and, thus, adult fecundity.

The cold tolerance of our model species, the goldenrod gall fly, has been studied extensively. This species is exceptionally cold tolerant, surviving freezing to -50°C (Lee 1991), and it is also extremely resistant to desiccation (Ramløv and Lee 2000). These adaptations allow larvae to survive in the poorly insulated galls in harsh

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temperature climates as far north as the Canadian prairie provinces (Uhler 1951). In addition to tolerating freezing, this species can also manipulate the temperature at which its tissues freeze (the supercooling point) by the production of endogenous ice nucleators (Mugnano et al. 1996) and, thus, to a limited extent, controls the amount of time it spends frozen.

Most goldenrod gall fly larvae overwinter on standing, goldenrod stems. However, plant stems are often broken by wind, snow, deer, etc. Thus, a portion of each population overwinters above the snow on standing stems (elevated), while others lying on or near the ground are covered by snow (ground-level). Because snow is an excellent insulator (Geiger 1966, Corbet 1972), we hypothesized that larvae spending the winter in a subnivean environment (i.e. under the snow) would consume more of their energy reserves and thus produce fewer eggs as adults. In contrast, those larvae in galls above the snow would be exposed to lower air temperatures, freeze more often, and thus have lower metabolic rates and higher reproductive output than their subnivean counterparts.

This study measured the thermal and hydric conditions of the elevated and ground-level microenvironments to which the galls were exposed during winter and the influence of these conditions on the survival and potential fecundity of gall flies. Also, using recent measurements of the relationship between temperature and metabolic rate, we modeled energy consumption for both habitats, thus allowing us to visualize when, and under what conditions, one habitat was energetically advantageous over the other.

Methods

Larvae were collected on November 26, 1998, from old-field habitats at Miami University's Ecology Research Center (39° 31′ 57″ N, 84° 43′ 23″ W) and stored at 4°C until the following day. On November 27, the galls were split into two groups of approximately 600 galls each. Both groups were spread between 2 layers of loose plastic mesh mounted in a wooden frame. The frames were placed less than 1 m apart in a field of *Solidago*: one elevated on posts 1.2 m above ground and the other only 10 cm above ground. These two locations simulated the approximate height of galls that are on upright stems throughout the winter ("elevated") or on broken stems lying near the ground ("ground-level"). Alongside the galls we mounted Hobo H8 Pro dataloggers (Onset Computer Corp.) that recorded temperature and relative humidity every 30 min throughout the winter. Overlying the entire structure was a loose covering of 2-cm square mesh to deter mammalian and avian predators. Snow that accumulated on the elevated platform was removed twice daily.

Samples of ~ 100 galls were taken from each treatment on January 21, February 3, and April 20 for measurements of larval mass and water content, and gall tissue water content. Each larva and its corresponding gall were weighed ± 0.1 mg, dried to constant mass at 60°C, then reweighed. On April 20, 50 of the remaining galls were split open and the proportion of apparently healthy pupae in each group recorded. Also, another 115 apparently healthy pupae from each group were placed into individual wells of cell tissue plates and moved to an incubator at 20°C. One well in each plate was filled with water to maintain high humidity for the pupae. The pupae were checked daily and the date of adult emergence was recorded. Once emerged, the adult flies were given 5 d before being frozen in a microcentrifuge tube at -80°C. (Females develop a full complement of eggs within 5 d, even without mating; Uhler 1951.) The adults were subsequently weighed (± 0.1 mg, Mettler-Toledo microbalance) and measured (head width and wing length; ± 0.01mm, Linkham VTO video analysis system). Females were dissected and all of the eggs in each ovary counted under a dissecting microscope ($40\times$) as described previously by Irwin and Lee (2000). Because egg size in this species is constant and independent of female body size and fecundity (Irwin and Lee 2000), we did not measure egg size in this study.

Finally, we used our previous measurements of the relationship between metabolic rate and temperature for diapausing and nondiapausing larvae (Irwin et al. 2001, Irwin and Lee 2002) to estimate energy use for the two treatment groups. Such a model allowed us to (1) illustrate the conditions under which the greatest differences in energy expenditure were likely to take place, and (2) estimate the difference in the total energy used by the two groups. A second-order polynomial fit was applied to actual temperature/metabolic rate data over the range of -15 up to 20°C (diapausing larvae) or up to 15°C (nondiapausing larvae) (Fig. 1). The models fit the data very well ($r^2 = 0.9946$ and 0.9939 for diapause and nondiapause larvae, respectively) and increased nearly linearly up to 30°C (close to the highest temperature recorded in nature) as was observed in Layne and Eyck's (1996) study of *E. solidaginis* metabolic rates. For the first part of the winter, we used the metabolic rates estimated for diapausing larvae. We switched to the non-diapause model at noon on Feb. 18, the approximate date when *E. solidaginis* breaks diapause at this geographic location (Irwin et al. 2001). Because temperature was recorded every 30 min., we estimated the total CO_2 produced for each 30 min. interval through the winter. The sum of all 30 min. intervals was used to estimate the total energy expenditures from November 27 to April 20 (the actual dates during which the larvae were exposed to the field treatments). The original estimates were made in ml CO_2 produced per gram of fresh mass per hour and

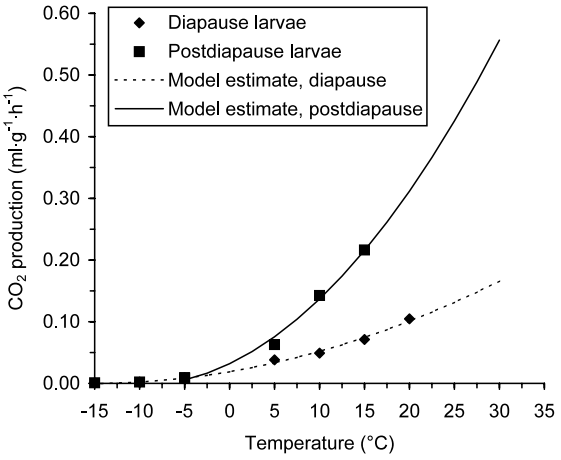

Fig. 1. Summary of metabolic rates (measured using CO_2 production) for subzero (Irwin and Lee 2002) and above zero temperatures (Irwin et al. 2001). These data were used to estimate energy consumption based on environmental temperatures.

were converted to estimated weight loss (mg per gram of animal mass) by assuming a respiratory quotient of 0.924 (Irwin and Lee, unpubl. data). This is similar to the RQ of 0.89 obtained for *E. solidaginis* by Stinner and Abrahamson (1979) and the RQ of 1.0 calculated for other dipteran larvae (Berrigan and Lighton 1993). We assumed that only lipids and carbohydrates were used as energy sources. With an RQ of 0.924, 26.2% of the energy produced is via the lipid pathway (similar to the rough estimate of 17% made by Layne and Medwith 1997) and the remaining via the catabolism of carbohydrates. We also assumed that 2 liters of O_2 were consumed per gram of lipid and 0.84 liters of O_2 were consumed per gram of carbohydrate (Schmidt-Nielsen 1990).

Results

Although the larvae in elevated and ground-level microenvironments were in close proximity, there were significant differences in the physical conditions that they experienced (Figs. 2 and 3). Only early in the experiment (November) did the ground-level group have an advantage: the ground-level group was cooler than the elevated, but only during the night (Fig. 2B). At this time of year, the soil lacked an insulative layer of snow and had cooled considerably. Thus, during the night, when insolation was no longer playing a role, heat was lost from the galls of the ground-level group into the nearby soil (Corbet 1972). In contrast, the elevated group experienced less cooling because of their greater distance from the soil. The advantage experienced by the ground-level larvae was short-lived, occurring only during this short period in the autumn.

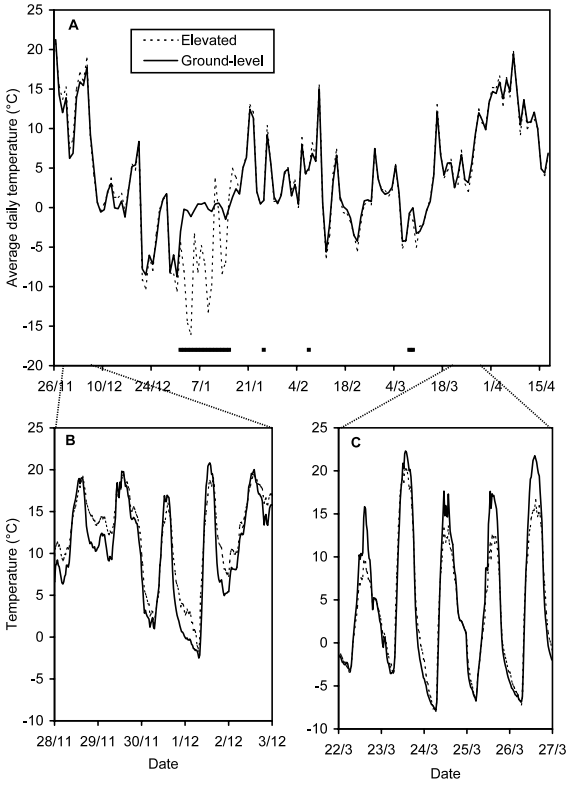

Fig. 2. Average daily temperature experienced by the ground-level and elevated groups during the experiment (A). Insets are actual (not average) microenvironmental temperatures measured half-hourly for ground-level and elevated larvae: B represents a period in early winter and C is during the spring warming. Dark bars along lower portion of A indicate periods of snow cover.

The greatest temperature differences between the treatment groups were observed during periods of snow cover. Snow is an excellent insulator (Geiger 1966) and during periods of snow cover, the ground-level group

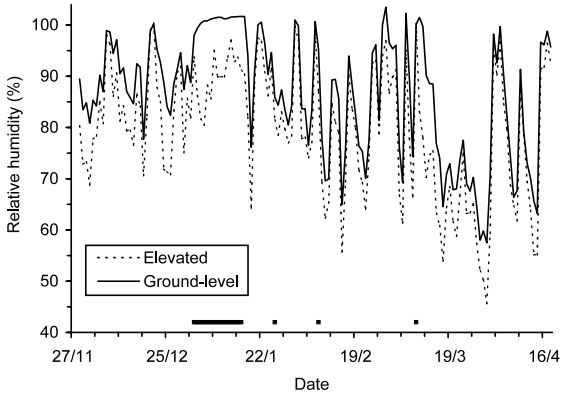

Fig. 3. Average daily relative humidity experienced by the elevated and ground-level groups during the experiment. Dark bars along lower portion of graph indicate periods of snow cover.

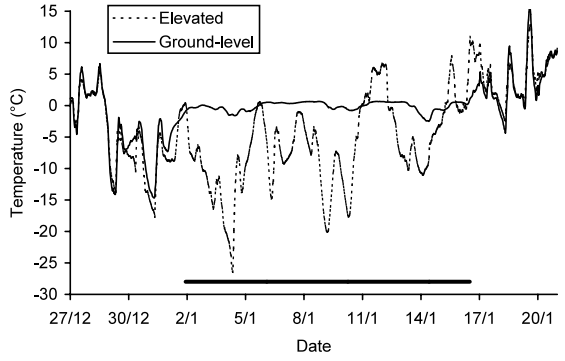

Fig. 4. Half-hourly microenvironmental temperatures experienced by elevated and ground-level larvae during the longest period of snow cover. The dark bar along the bottom of the graph indicates the actual period of snow cover.

rarely parted from 0°C, whereas the elevated larvae were frequently exposed to subzero temperatures (Fig. 4). As a result, the minimum winter temperature experienced by the ground-level group was -14.7°C. Without the insulative layer of snow, the elevated larvae experienced a minimum temperature of -26.5°C. Because they were so much cooler, the elevated larvae expended less energy during this period (Fig. 5). The advantage in energy savings was significant despite the very low metabolic rates in this temperature range (near or below 0°C).

There were also temperature differences between the treatment groups after the snow had melted. As the day-length increased in March and April, the ground-level group was as much as 5°C warmer when exposed to direct sunshine (Fig. 2C). Vegetation near the ground reduces windspeed and the consequent convective heat losses (Corbet 1972), and the vegetation also re-radiates energy gained from the sun (Geiger 1966). These two factors increased heat gain and retention of the ground-level galls so that they were typically warmer than those of the elevated group during periods of sunshine. The ground-level galls were typically less than 5°C warmer than the elevated galls, but this difference resulted in a great disadvantage for the ground-level larvae.

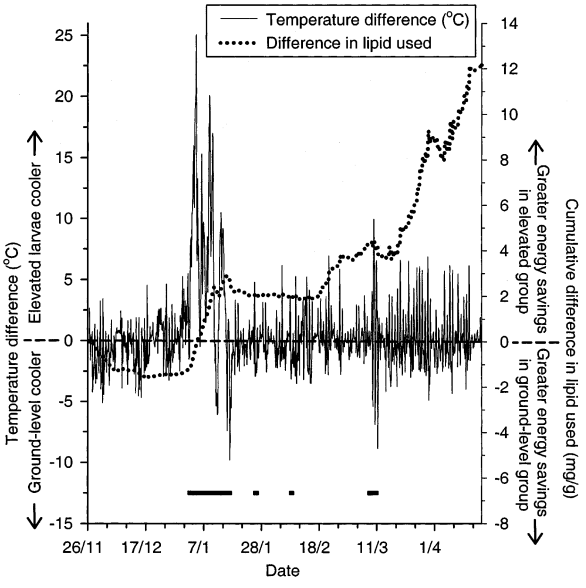

Fig. 5. Differences between the ground-level and elevated groups in microenvironmental temperature (half-hourly) and the estimate of total lipid and carbohydrates consumed. Dark bars along lower portion of graph indicate periods of snow cover.

Over the entire experimental period the average temperature experienced by the ground-level larvae was 3.96 ± 0.10°C (mean $\pm$ SEM), significantly higher than the 3.33 ± 0.10°C experienced by the elevated group (two-tailed, unpaired t-test: $t = 4.46$, $p < 0.0001$). If we assume that larvae supercooled to -10°C and, after freezing, remained frozen until the melting point of -2°C (Lee and Lewis 1985) was reached, then larvae in the ground-level group were frozen fewer times during this winter (10 times versus 13 times in the elevated group) and were frozen for less time (170 h versus 391 h) than those in the elevated group.

Our estimates of energy utilization during the winter were based on the known relationship between metabolic rate and temperature and were thus related to the temperature differences outlined above. Fig. 5 illustrates the difference in estimated lipid consumption during the winter. In late November and early December there was a slight advantage to the ground-level group in terms of energy conservation. However, there was a marked shift toward an advantage in energy savings for the elevated larvae during periods of snow cover. The ground-level group never recovered from this shift. In fact, as spring approached, higher day-time temperatures caused the ground-level larvae to consume even more energy than their elevated counterparts, thus improving the advantage of the elevated larvae. By the end of the experiment the elevated group had catabolized an estimated 12.7 mg g^{-1} of animal mass less than the ground-level group, a difference of 4.5% over the entire winter. Our model of energy consumption predicted reductions in body mass of 26.8% and 28.1% in the elevated and ground-level groups, respectively (Fig. 6). Actual losses of dry weight were 26.4% and 29.4% for the elevated and ground-level larvae, respectively, but this difference was not statistically significant (Fig. 6).

The elevated larvae had higher survival and fecundity than the ground-level group, but did not differ in body size. The groups did not significantly differ in the number of galls producing apparently healthy pupae at the end of the field exposure (44% healthy in the ground-level group and 52% in the elevated

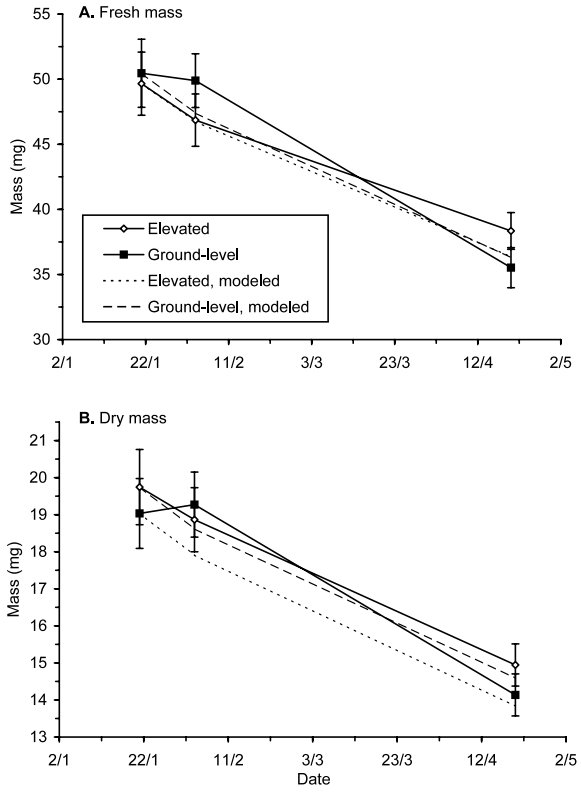

Fig. 6. Seasonal reductions in larval fresh mass (A) and dry mass (B). Dotted lines indicate weight loss estimated by the model. Both fresh and dry mass fell significantly over time (fresh: $F = 24.24$, $p < 0.001$; dry: $F = 21.77$, $p < 0.001$) but there were no significant differences between the treatment groups (fresh mass: $F = 0.04$, $p = 0.842$; dry mass: $F = 0.30$, $p = 0.583$).

group; Table 1). However, of the 115 pupae from each group moved into conditions that would allow development, 93% of the elevated group successfully emerged as adults whereas significantly fewer (83.5%) of the ground-level group reached adulthood, and the flies of the ground-level group emerged slightly later (Table 1). Despite these differences, measures of size (head width, wing length, and adult body weight) did not differ significantly between the groups (Table 1), nor did larval or pupal body mass (fresh or dry mass) (Fig. 6). Despite the lack of a difference in body size, potential fecundity (number of eggs per female) differed significantly with females from the ground-level group producing 18.5% fewer eggs (Table 2).

What accounts for this difference in potential fecundity? In our previous study of temperature effects on potential fecundity (Irwin and Lee 2000), we demonstrated that differences in body size alone accounted for the observed differences in potential fecundity (i.e., the warmest group had lower potential fecundity only because it produced small females that produced fewer eggs). This was not the case in the more natural experiment presented here. Adult body mass (log-transformed) did have a significant effect on potential fecundity (ANCOVA $F = 11.65$, $p = 0.001$) but when adult body mass was included as a factor in the analysis of covariance, the treatment effect was still significant ($F = 9.46$, $p = 0.004$) (Table 2, Fig. 7). Therefore, although adult body mass had a significant effect on potential fecundity, adult body mass does not explain the differences due to the treatment.

Temperature was not the only parameter that could have affected the larvae. Relative humidity was typically higher for the ground-level group and, while covered by snow, the ground-level galls were in a constant 100% relative humidity environment (Fig. 3). Thus, the ground-level galls tended to remain relatively moist, reaching a plateau of approximately 58% water content. In contrast, the elevated galls stabilized at approximately 25% water (Fig. 8). Regardless of differences in the gall environment, larval water content was tightly regulated between 61.0 and 63.5% and, at the experiment's end, did not differ significantly between the treatment groups (Fig. 8).

Discussion

Our experiment demonstrated that even slightly colder natural microenvironmental conditions provide a substantial benefit in terms of energy conservation and thus higher potential fecundity. Energy savings in the elevated environment can be attributed to (1) lack of insulation by snow during the coldest days of the year and (2) a superior ability of the elevated galls to disperse heat gained during insolation in the spring.

Studies of insect freeze tolerance have historically considered only the adverse effects of freezing (but see discussion by Danks 1996, Irwin and Lee 2000). Also, there is a paucity of studies that consider natural microclimatic conditions and studies that include long exposures to freezing (Sømme 1999). Although our previous work (Irwin and Lee 2000) demonstrated that freezing at -22°C for several months increased mortality of *E. solidaginis* larvae, the greater exposure to cold in the exposed group in this study did not have an adverse effect. The elevated group was frozen for more of the winter, went through more freeze/thaw cycles, and experienced colder minimum temperatures than the ground-level group. Regardless, the survival and potential fecundity was superior to that of the ground-level larvae. Thus, it seems that freezing in nature, at least for the conditions seen during this study, is not particularly stressful to *E. solidaginis* larvae hibernating in a supranivean location. This species is well adapted to freezing, and easily survives the freezing conditions of winter in southwestern Ohio. This being the case, larvae overwintering above the snow can use the cooler environment experienced on a standing goldenrod gall stem

Table 1. Survival and emergence rates, time to emergence, and morphometric measurements of male and female *Eurosta solidaginis* in the elevated and ground-level groups.

Measure[1]	Elevated	Ground-level	Treatment effect	
			Test statistic	*p*
Overall				
Galls with pupae (n = 50)	26	22	Fisher's exact	0.24
Failed to emerge (n = 115)	8	19	Fisher's exact	0.02
Days to emerge (n = 115-failures)	8.93 ± 0.11	9.27 ± 0.20	$U' = 338$	0.01
Males				
Sample size[2]	33	15		
Adult weight	15.6 ± 0.6	16.6 ± 1.0	$t = 0.97$	0.17
Head width	2.28 ± 0.03	2.20 ± 0.04	$t = 0.62$	0.27
Wing length	6.34 ± 0.23	6.64 ± 0.15	$t = 1.08$	0.15
Females				
Sample size[2]	27	18		
Adult weight	22.1 ± 0.8	20.2 ± 1.3	$t = 0.62$	0.27
Head width	2.25 ± 0.03	2.29 ± 0.04	$t = 1.26$	0.11
Wing length	6.66 ± 0.08	6.31 ± 0.29	$U' = 132$	0.44

[1] Linear measurements are in mm, weight in mg, and both are presented as mean ± SEM.
[2] Sample sizes on morphometric measures may be lower than indicated because damaged animals (e.g. wings torn) were excluded from the analysis.

Table 2. Total number of eggs per female *Eurosta solidaginis* following overwintering in elevated or ground-level sites. Data are present both with and without correction for body mass using analysis of covariance.

Measure	Elevated	Ground-level	Treatment effect	
	($n = 27$)	($n = 18$)	Test statistic	*p*
Mean no. eggs/female	336 ± 17	274 ± 11	$U' = 338$	0.014
Mean no. eggs/female corrected for log-transformed body mass (SAS, PROC GLM)	335 ± 13	275 ± 15	$F = 9.46$	0.004

[1] Values presented are mean ± SEM.
[2] Values presented are least-square mean ± SEM.

to conserve energy. This contrasts with other freeze-tolerant species that require insulation by snow for protection against cold (e.g. *Celatoblatta qinquemaculata*; Sinclair 2001).

Although the greatest absolute differences in temperature took place during periods with snow cover, the spring days drove the energetic advantage of the elevated larvae well beyond that gained during snow cover. This difference in energy consumption was amplified by (1) the high metabolic rates at these temperatures (15 to 20°C during the day) and (2) the higher metabolic rates in these larvae because they had broken diapause. Post-diapause larvae had a Q_{10} of approximately 3 in the 10 to 20°C temperature range (Fig. 1). Therefore, ground-level larvae that were 5°C warmer than their elevated counterparts had metabolic rates 1.5 times higher. The temperature differences during the spring accounted for 10.4 mg g^{-1} animal mass of the total 12.4 mg g^{-1} difference between the groups, thereby exceeding the advantages gained during periods of snow cover (Fig. 5).

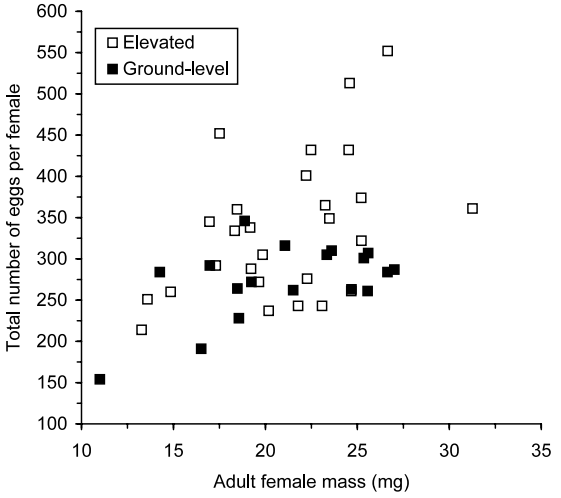

Fig. 7. Total number of eggs produced per female. Body size significantly affected the number of eggs produced ($F = 11.65$, $p = 0.001$), but the treatment effect was still significant ($F = 9.46$, $p = 0.004$) even after adjustment for body size by ANCOVA.

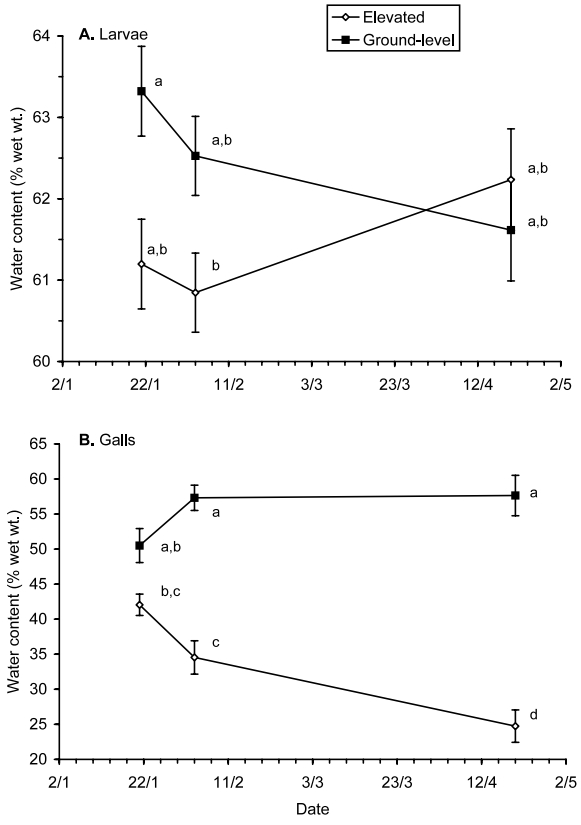

Fig. 8. Seasonal changes in larval (A) and gall (B) water content (percent fresh mass). Larval water content was affected by treatment ($F = 4.78$, $p = 0.03$) but the date of collection did not have a significant effect. Gall water content was significantly affected by both date ($F = 3.87$, $p = 0.02$) and treatment ($F = 132.65$, $p < 0.001$), as well as the interaction between the two ($F = 15.54$, $p < 0.001$). Means sharing a letter were not significantly different.

The differences in energy consumption between the treatment groups were not severe enough to cause changes in body size. None of the body size measures were adequate to demonstrate that the ground-level larvae had been in a more stressful environment. This is consistent with our previous study (Irwin and Lee 2000) where, despite very large differences in laboratory overwintering temperature regimes (12, 0, -22°C) and large changes in potential fecundity, adult size did not differ significantly. As in our previous study, it is apparent that more subtle measures of health and reproductive fitness are required to identify the influence of microenvironment on *E. solidaginis*.

Although there were no significant differences in body size between the treatment groups, there was an 18.5% reduction in potential fecundity in the ground-level group compared to the elevated. This can not be attributed to even subtle differences in body size between the groups (which may have been undetectable when comparing means) because, even after adjustment for the influence of body mass using analysis of covariance, the flies of the ground-level group were still estimated to have produced 17.9% fewer eggs. There was also other evidence that the ground-level larvae were under more stressful conditions. Mortality (measured as the number of pupae failing to emerge) was higher in the ground-level group, and they eclosed (emerged as adults) significantly later than their elevated counterparts, suggesting that they were weaker in some way.

Temperature had a strong effect on the energy consumption of ground-level and elevated larvae but it was not the only microenvironmental factor that may have played a role in this experiment (Fig. 3). The gall environment in the ground-level group was more moist because of wetting of the galls by rain and snow, the high vapor pressure that is typical close to the ground (Geiger 1966), and because they were not exposed to the dry winds present during the coldest part of the year (Corbet 1972). More experiments are required to improve our understanding of the influence of humidity on *E. solidaginis* health and fecundity.

The higher potential fecundity of the elevated larvae suggests a mechanism for the selection of enhanced cold-hardiness in this species. Other species of *Eurosta* (e.g. *E. comma*, *E. cibrata*) hibernate underground in galls formed on the plant rhizome (Novak and Foote 1980, Ming 1989). Given the great reproductive advantage gained from overwintering above the snow (i.e. on the stem or crown rather than the rhizome), there would be strong selection for the development of cold-hardiness to allow overwintering in a more exposed environment. By this mechanism, one can envision evolution toward stem- and crown-galling by improvements in the cold-hardiness of rhizome-galling species. More work on the evolution of *Eurosta* is required to test this scenario (Ming 1989).

Our data support the hypothesis that the development of enhanced cold-hardiness allows overwintering in a colder environment which, in turn, substantially reduces energy consumption during winter and enhances reproductive fitness. We observed that an increase in average winter temperature of only 0.6°C reduced fecundity by 18%. This leads us to wonder how *E. solidaginis* and other ectotherms will respond to global warming with predicted increases in average winter temperatures as great as 5°C over the next century (Hengeveld 2000). To fully understand the influence of climate change on ectotherms, we must consider not only the severe stress of intense cold (or lack thereof), but also subtle responses (e.g. fecundity) to seemingly minor changes in local conditions.

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