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Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae)

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Abstract

We tested the hypothesis that mild winter temperatures are detrimental to the survival and reproductive potential of insects. We measured survival, body size, and potential fecundity of a freeze tolerant insect, the goldenrod gall fly (*Eurosta solidaginis*), after overwintering in the laboratory for ~3 mo. frozen at -22°C , unfrozen at 0°C , or unfrozen at 12°C . Larvae held at 12°C suffered high mortality (70%) and relatively low potential fecundity as adults (mean \pm SEM=199 \pm 11 eggs/female), while those held at 0°C had both low mortality (11%) and high potential fecundity (256 \pm 15 eggs/female). Freezing (-22°C) increased mortality (30% overall) but did not significantly reduce fecundity (245 \pm 13 eggs/female). Egg length and width were constant regardless of treatment group or female body size. Analysis of covariance indicated that reduced fecundity in the 12°C group was related to reduced larval body weight following treatment. Patterns of larval weight loss in the experimental treatments were generally correlated with previous reports of latitudinal trends in weight loss through the winter. We conclude that mild winter temperatures may be detrimental to some overwintering insects, particularly species that do not feed following winter diapause. Low temperature and even freezing are beneficial, allowing conservation of energy reserves to maintain high survival and potential fecundity. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Overwintering conditions strongly affect the survival and fitness of northern insects. Since the pioneering work of R.W. Salt (see review in Ring and Riegert, 1991), the field of insect cold-hardiness has largely focused on low temperature tolerance and the physiological responses to cold. Some insect species tolerate high subzero temperatures but do not survive ice formation and many succumb to chilling injury before ice forms in the tissues (Lee, 1991). A few species are freeze tolerant, surviving the formation of substantial quantities of ice within the tissues.

More recently, some studies of insect cold tolerance have addressed more subtle sublethal effects of cold injury, including those that may reduce fitness (Bale,

1987). Chilling injury in *Sarcophaga crassipalpis*, for example, reduces the capacity of flies to emerge from the puparium (Yocum et al., 1994), weakens the proboscis reflex in response to food, and reduces nerve conduction velocity (Kelty et al., 1996). Turnock and coworkers have shown that exposure of dipteran and lepidopteran larvae to subzero temperatures above the supercooling point reduces post-diapause developmental rate (Turnock et al., 1985; Turnock and Bodnaryk, 1991). These studies have typically focused on chill-sensitive species and on the role of low temperature as a physiological stressor.

In contrast to previous work, our study sought to examine the positive ecological and evolutionary consequences of freezing in the freeze tolerant *Eurosta solidaginis*. The goldenrod gall fly is found through eastern North America from Texas to southern Ontario, west across the plains, and north to Alberta (Uhler, 1951). Throughout its range, third instar larvae overwinter in galls on the stems of senescent goldenrod plants. Because the gall provides little insulation and closely

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tracks air temperature, larvae experience daily temperature fluctuations as great as 20°C as well as extremely low winter temperatures (Layne, 1993). The senescent plant tissues are also subject to drying and wetting according to levels of precipitation (Layne and Medwith, 1997). To survive these conditions, this species is highly resistant to desiccation (Ramløvs and Lee, unpubl. data), reduces its supercooling ability to ensure freezing of its tissues (Baust and Lee, 1981; Mugnano et al., 1996), and has a high degree of freeze tolerance (surviving temperatures below -50°C; Lee, 1991). Physiological adaptations for freeze tolerance have been studied extensively and include the synthesis and accumulation of high concentrations of glycerol, sorbitol, and trehalose (reviews: Storey and Storey, 1988; Baust and Nishino, 1991).

Several aspects of the goldenrod gall fly's life history make this species a useful model for studying the effects of overwintering temperature. After spending the winter in the gall, the third instar larvae pupariate and emerge as non-feeding adults with vestigial mouthparts (Uhler, 1951). Females (including virgins) fully develop their eggs within 5 d of eclosion and, in nature, lay these eggs on the young leaves near the apical meristems of goldenrod plants, then die ~10 d later (Uhler, 1951; Abrahamson and Weis, 1997). Therefore, the metabolic reserves available to females to survive the winter, pupate, mate, and produce and lay eggs are procured during the previous summer/early fall by the third instar larva as it feeds on plant tissues. Without nutrient acquisition by adults complicating our analysis, we can directly examine the effect of overwintering temperature on larval and adult body size and potential fecundity.

Because freeze tolerant species often choose overwintering sites where freezing temperatures are common, we predicted that low overwintering temperatures and freezing may be beneficial in terms of reducing winter mortality and enhancing adult fecundity. In contrast, mild winters may be detrimental because larvae consume more energy through the winter (Danks, 1987) and thus reduce their reproductive output as adults—either through reductions in egg number and/or egg size.

2. Materials and methods

Galls were collected in Butler Co., OH, on 22 January 1996, and were stored at -22°C until use on 6 March. Each larva was removed from its gall, weighed (± 0.1 mg), then replaced in its original position in the gall. The galls were resealed with pins and placed into one of three temperature treatments: 12, 0, or -22°C ($n=44$, 44, and 43, respectively). We stored the larvae in desiccators over a supersaturated glucose solution to maintain relative humidity at ~80%. Relative humidity was measured in each treatment group with a HoboRH datalogger (Onset, Inc.).

On 28 May, all the galls were moved to 12°C for 24 h to allow frozen larvae to thaw, then moved to room temperature (23°C) to allow development to continue. Five days after eclosion (a sufficient time for females to fully develop their eggs; Uhler, 1951) the flies were placed in 8 ml vials, then killed by freezing and stored at -22°C. Both males and females were weighed for total body weight (± 0.1 mg), then measured for total length, head width, and wing length (± 0.01 mm) under 7 \times magnification. To determine potential fecundity, females were dissected, the total number of eggs in both ovaries counted, and the length and width (± 0.0001 mm) of a random sample of 10 eggs from each ovary measured under 100 \times magnification. All eggs within the ovaries were mature and were similar in size both within a female and between females. Measurements were made on an Olympus dissecting microscope with a Linkam VTO 220 video analysis system. Sample sizes were variable through this portion of the experiments as a result of differential mortality in the treatment groups.

We measured change in body water content at various stages throughout the experiment. First, a sample of five larvae was made from the initial group on 6 March. These larvae were weighed fresh, dried to constant weight at 60°C, then weighed (± 0.1 mg) to allow calculation of water content. Similar samples were made from each treatment group on 28 May following the temperature treatments ($n=5$ per treatment group). Finally, we calculated water content of all adult males 5 d after eclosion ($n=7-16$ per treatment group).

Analyses included nonparametric ANOVA (Kruskal-Wallis test) followed by Dunn's multiple comparison to identify the effect of treatment on fecundity, body size (total length, head width, wing length), and egg length and width. Analysis of covariance was used to correct for female size and allow us to determine whether fecundity was affected directly by treatment or only through effects on female size. Binomial comparison for multiple proportions with Tukey multiple comparisons (Zar, 1984) were used to describe differences in mortality between the treatment groups. A significance level of $\alpha=0.05$ was used for all tests and values presented in the text are mean \pm SEM.

3. Results

Simulated overwintering at 12°C adversely affected survival of *E. solidaginis* (Fig. 1). Of 44 larvae held at 12°C, 10 died before pupariation and an additional 13 died as pupae. In contrast, only one larva died in the 0°C group (initial $n=44$) and six in the -22°C treatments (initial $n=43$). In the 0°C group another four individuals died during pupariation, and seven in the -22°C group died during pupation. In the 12°C group, four failed to emerge completely from the puparium, and another three

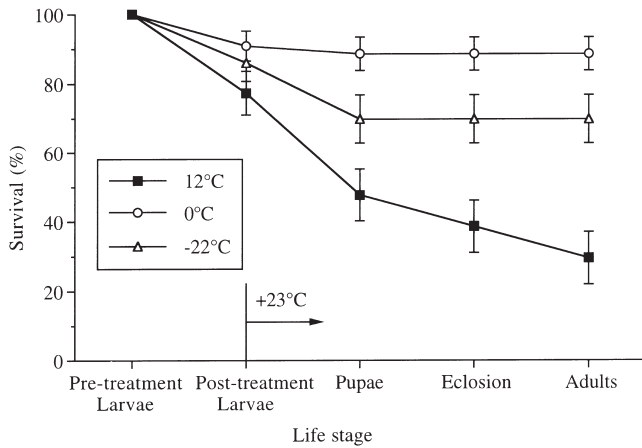


Fig. 1. Survival of *Eurosta solidaginis* overwintered at 12, 0, and -22°C at different life stages. Total mortality was significantly higher after being held at 12°C ($P < 0.01$) or -22°C ($P < 0.01$) than at 0°C . Error bars indicate binomial errors calculated as in Zar (1984).

died before 5 d as an adult. In contrast, the two colder treatment groups exhibited no mortality beyond the pupal stage. Total mortality was significantly different between all the treatment groups ($P < 0.01$ in all pairwise comparisons).

A portion of the high temperature group broke diapause during their 12°C exposure: 12 of 16 pupariated before being moved to room temperature. Larvae of the other two groups all pupariated after 1–2 d at 23°C . Of these, the -22°C group took significantly longer (Kruskal–Wallis, $P < 0.001$) to eclose (18.5 ± 0.6 d) than those in the 0°C group (17.5 ± 0.1 d). Because we knew the time to eclosion for only four individuals of the 12°C group (the others having pupariated before being moved to room temperature), this group could not be included in the Kruskal–Wallis multiple comparisons test, but their mean time to eclosion was 19.0 ± 0.8 d.

The high temperature treatment also resulted in great losses of larval body weight in both males and females (Fig. 2) during the treatment period. At 12°C males lost 30.3% and females lost 31.0% of their initial body weight. In contrast, those at 0 and -22°C did not lose a significant amount of weight during treatment. The higher losses in the 12°C group were not due to water loss, as these dropped only ~4% (Fig. 3) while ~30% of body weight was lost. Once the flies reached adulthood, no significant differences in weight or water content in either sex were present among the treatment groups (Figs. 2 and 3). Linear morphological indicators of size (head width, wing length, total body length) were not significantly different between the treatment groups with the exception of wing length in females where the 12°C group was significantly smaller than the -22°C group (Table 1).

Potential fecundity was strongly affected by the 12°C treatment where total egg production was reduced by more than 20% over the 0°C group (Table 2). The num-

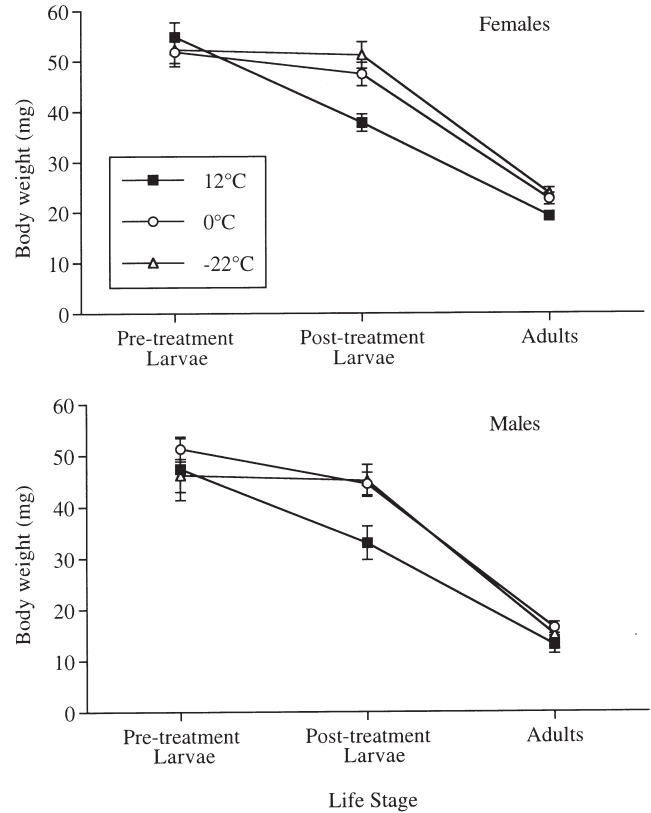


Fig. 2. Reductions in female and male body weight before the overwintering treatment (pre-treatment), immediately following treatment (post-treatment), and after 5 d as an adult.

ber of eggs in the -22°C group was not statistically different from the 0°C group nor the 12°C group. Female post-treatment larval weight strongly influenced the number of eggs produced (Fig. 4), at least in the 0 and -22°C treatment groups where sample sizes were large enough to allow detection of this trend. An analysis of covariance was used to standardize egg production for differences in post-treatment larval weight. In this analysis, weight was a strong effect ($F = 79.4$, $P < 0.0001$) and, once standardized for weight, fecundity was no longer significantly different between treatment groups ($F = 1.4$, $P = 0.25$). Therefore, overwintering at 12°C reduced potential fecundity because it reduced post-treatment larval weight, not through direct effects on egg production. Interestingly, the reduced body weight of the 12°C group did not extend into the adult stage as adult weight and most morphological measures showed no differences between treatment groups. Also, regardless of post-treatment larval weight, adult size, number of eggs produced, or treatment group, egg length and width remained quite constant (Table 2).

4. Discussion

Mild overwintering temperatures reduced survival and, through effects on larval weight, potential fecundity

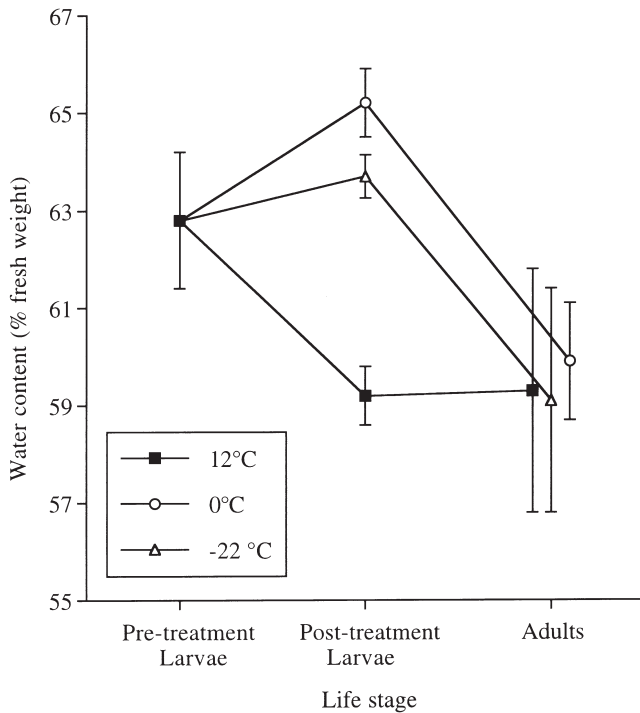


Fig. 3. Water content of individuals before the overwintering treatment (pre-treatment), immediately following treatment (post-treatment), and after 5 d as an adult. Sex was not determined for pre-treatment and post-treatment larvae; adults were males.

in *E. solidaginis*. Adult flies in the 12°C treatment group were consistently (although not always statistically) smaller in all linear morphological measures and body weight. This may be attributed to the higher metabolic rate associated with this relatively high overwintering temperature: metabolic rate more than doubles from 0

to 12°C (Layne and Eyck, 1996; Irwin and Lee, unpubl. data). In contrast, those larvae at low temperature (0°C) or frozen (−22°C) would have had low metabolic rates (Lee et al., 1995; Layne and Eyck, 1996; Irwin and Lee, unpubl. data) and thus conserved energy stores.

The nutrient reserves of the larvae are used during metamorphosis to produce adult tissues (Wigglesworth, 1972). Individuals held at 12°C, with high metabolic rates and no ability to replenish nutrient reserves after entering diapause or during the adult stage, had lower nutrient reserves for egg production than those at 0 and −22°C. These flies produced 22% fewer eggs than those at 0°C, and 18% fewer than those held frozen at −22°C (although this difference was not statistically significant; Table 2). Because female size is highly correlated with fecundity in the Diptera (Berrigan, 1991), we used an analysis of covariance to remove the effect of body weight (specifically, post-treatment larval weight) on potential fecundity. This analysis indicated that high temperature did not directly reduce fecundity. Rather, the reductions in fecundity were explained by the influence of high temperature during treatment on post-treatment larval weight: those females that were smaller in the larval stage produced fewer eggs (Fig. 4).

Although mild temperatures decreased potential fecundity, egg size was not affected. In fact, eggs from all treatment groups were very similar in both length and width (Table 2). Thus, although an egg size/egg number trade-off exists for some other Diptera (e.g. Drosophilidae; Berrigan, 1991), this was not the case in *E. solidaginis*. Two other tephritids also have fixed egg size across female size classes (Krainacker et al., 1989). Female *E. solidaginis* lay their eggs in the young leaves near the

Table 1
Morphometric differences between the treatment groups in females and males^a

Morphometric measure	12°C group	0°C group	−22°C group	H	P
<i>Males</i>					
Sample size	7	16	15		
Post-treatment larval weight	33.0±3.3 ^b	44.5±2.2 ^{b,c}	45.1±3.1 ^c	7.53	0.023
Adult weight	13.0±1.7	16.3±1.1	15.0±1.4	2.35	0.309
Body length	6.70±0.25	7.30±0.13	7.11±0.20	3.42	0.181
Head width	2.26±0.08	2.28±0.04	2.30±0.05	0.66	0.718
Wing length	6.20±0.29	6.57±0.08	6.56±0.18	2.91	0.234
<i>Females</i>					
Sample size	7	23	15		
Post-treatment larval weight	37.8±1.7 ^b	47.4±2.3 ^{b,c}	51.2±2.6 ^c	7.61	0.022
Adult weight	19.2±0.9	22.6±1.2	23.7±1.2	5.39	0.067
Body length	8.13±0.20	8.40±0.11	8.46±0.15	2.23	0.328
Head width	2.19±0.04	2.26±0.04	2.33±0.04	3.89	0.143
Wing length	6.09±0.11 ^b	6.51±0.10 ^{b,c}	6.72±0.13 ^c	8.14	0.017

^a Linear measurements are in mm, weight in mg, and both are presented as mean ± SEM. In the case of a significant Kruskal–Wallis test, means were compared using Dunn's test at $P < 0.05$. Where Dunn's tests were performed, means within a row not sharing a letter are significantly different.

Table 2

Mean (\pm SEM) total number of eggs per female, mean egg length, and mean egg width for female *Eurosta solidaginis* after overwintering at 12, 0, and -22°C ^a

Measure	12°C group (n=7)	0°C group (n=23)	-22°C group (n=15)	H	P
Mean no. eggs/female	199 \pm 11 ^b	256 \pm 15 ^c	245 \pm 13 ^{b,c}	6.19	0.045
Mean egg length	894 \pm 11	893 \pm 5	890 \pm 6	0.48	0.786
Mean egg width	262 \pm 5	260 \pm 3	253 \pm 3	3.85	0.146

^a Egg length and width are in μm . In the case of a significant Kruskal–Wallis test, means were compared using Dunn's test at $P < 0.05$. Where Dunn's tests were performed, means within a row not sharing a letter are significantly different.

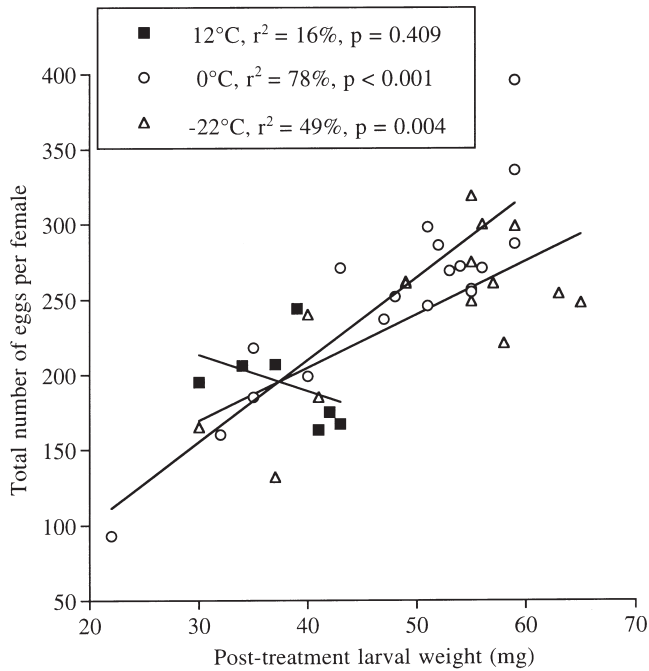


Fig. 4. Potential fecundity in the three overwintering treatment groups (12, 0, and -22°C) by female post-treatment larval weight. Lines are least-square regressions for each of the treatment groups.

host plant's apical meristem. Upon hatching, the larvae migrate several millimeters from the oviposition site to the apical meristem of the host plant (Uhler, 1951). Perhaps this specific microhabitat of early larval life restricts the optimal size of eggs and/or newly hatched larvae in *E. solidaginis*, thus preventing an egg size/fecundity trade-off. Although egg size is constant, we did not measure egg quality (e.g. egg weight, lipid content). Reductions in egg quality might reduce egg hatchability and larval survival (see Wigglesworth, 1972).

Larvae overwintering in nature show latitudinal gradients in weight loss, presumably due to thermal conditions, similar to the degree of weight loss in our experimental groups. During the treatment period, our larvae at 0 and -22°C did not lose significant weight, but those at 12°C lost ca. 30% of their initial body weight. As in our 0 and -22°C treatments, larvae overwintering in Ontario, near the northern edge of this species' range,

lost only 7.5% of their body weight between September and April (not a statistically significant loss; Storey and Storey, 1986). In contrast, larvae overwintering farther south in western Pennsylvania lost 20–22% from September to March (Layne and Medwith, 1997) and Texas larvae lost 50% during their short winter period (November to January) (Rojas et al., 1986). These field data support our observation that overwintering at higher temperatures results in greater weight loss during the winter. Potential fecundity was not measured in those studies. We suggest that future work should examine southern populations of *E. solidaginis* for physiological adaptations that might improve fecundity where winter temperatures are mild.

Our measure of reproductive output was potential fecundity, the number of eggs in the ovary. Abrahamson and Weis (1997) and Uhler (1951) both reported that female *E. solidaginis* oviposit only a small portion of their available eggs ($\sim 10\%$). However, these experiments were performed in greenhouses at high population density. It is likely that a greater proportion of the total egg production is laid under natural conditions (Uhler, 1951). Mild winters, and the resulting low energy reserves, may also reduce the fitness of females by adversely affecting their ability to disperse, find males, copulate, and oviposit. Similar effects could reduce fitness of males as well.

Although mortality was higher in the -22°C group than the 0°C group, flies in the -22°C group did not have reduced fecundity. Thus, in terms of energy consumption and, ultimately, egg production, freezing produces the same effects as being unfrozen at 0°C . The metabolic rate of larvae at 0°C and -22°C is likely very low ($< 50 \mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$; Layne and Eyck, 1996) as would be expected for temperatures in this range (Irwin and Lee, unpubl. data; Salt, 1958; Asahina, 1966). The physiological stress associated with freezing apparently did not directly affect fecundity. However, freezing is not without costs as mortality was 20% higher in the -22°C flies than those at 0°C .

Our freezing treatment of 3 mo. at -22°C is far more severe than would be experienced by this population of *E. solidaginis* in nature (Layne, 1993). Despite this, the high potential fecundity in the frozen group is in sharp

contrast with studies on freeze intolerant species exposed to subzero temperatures. Exposure of *Musca domestica* to -7°C without previous cold-hardening reduced fecundity by $>50\%$ (Coulson and Bale, 1992). Unlike this chill-sensitive, freeze intolerant dipteran, the freeze tolerant *E. solidaginis* did not exhibit reduced fecundity following exposure to subzero temperatures.

Overwintering at high temperatures reduced both fecundity and survival. We combined these estimates to determine the average number of eggs produced per initial female in each treatment group ($n=43-44$). A population overwintering at 12°C would produce an average of 59 eggs per initial female while those at 0°C would produce 227 eggs and those frozen at -22°C would produce 171 eggs per initial female. Thus, although our severe freezing treatment had some adverse effects on average potential fecundity, a simulated mild winter was much more detrimental. This leads us to predict that during mild winters or when larvae overwinter in relatively warm microclimates (e.g. fallen stems insulated by snow are generally warmer than those above the snowpack) goldenrod gall flies will experience reduced survival and fecundity. Also, we hypothesize that *E. solidaginis* inhabiting regions where winters are typically warm may exhibit low Q_{10} values during the winter to conserve their energy reserves.

In contrast to the traditional perspective on freezing injury, our data show that low temperature during winter is important to maintain high fecundity. Low temperature is particularly important to reduce energy conservation in *E. solidaginis* and other species that do not feed following winter diapause (Tauber et al., 1986) because post-winter energy reserves are required for egg production, dispersal, mating activities, and oviposition.

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