Deleterious Effects of Mild Simulated Overwintering Temperatures on Survival and Potential Fecundity of Rose-Galling Diplolepis Wasps (Hymenoptera: Cynipidae)

JASON B. WILLIAMSI*, JOSEPH D. SHORTHOUSE2, AND RICHARD E. LEE, JR.1
1Department of Zoology, Miami University, Oxford, Ohio 45056
2Department of Biology, Laurentian University, Sudbury, Ontario P3E 2C6, Canada

ABSTRACT Prepupae of the rose galling Diplolepis spinosa from areas with relatively cold winters in southern Canada, and Diplolepis variabilis from a milder locale in western Canada, were used to test the hypothesis that mild winter temperatures are detrimental to the survival and potential fecundity of insects. Prepupae of D. spinosa held within or removed from their galls were exposed to simulated overwintering temperatures (−22, 0, 5, or 10°C) for approximately four months before measuring their survival, body size, and potential fecundity. Similar studies were conducted using prepupae of D. variabilis that were removed from their gall and subjected to 0°C or 10°C treatments. Diplolepis spinosa, with or without their galls, averaged 66% more mortality at 10°C than at 0°C. Female D. spinosa that survived the 10°C treatment had 32% fewer eggs than those held at 0°C. In contrast, there was no difference in survival or numbers of eggs between D. variabilis held at 0°C and 10°C. Body size of adult females and size of eggs did not differ among temperature treatments for either species. We conclude that mild overwintering temperatures may be detrimental for insects by raising their metabolism, and consequently reducing energetic reserves needed for development to the adult stage and subsequent production of eggs the following spring.


INTRODUCTION

Overwintering insects in northern regions must endure severe cold and desiccation stress to survive. Because of this, many studies have examined physiological adaptations that promote insect survival in these extreme conditions (see reviews in Lee and Denlinger, '91; Leather et al., '95; Danks, '96). However, potential consequences of mild overwintering temperatures on insects have received little attention.

Many overwintering insects feed only as larvae, and do so for only a few weeks or months prior to winter. These insects have limited metabolic resources which must be allocated for building up and maintaining protection against the cold, for use in overwintering metabolism, and for metamorphosis in the spring. Insects that do not feed as adults must also use these limited energy reserves for reproductive activities and egg production.

Most insects that overwinter enter diapause to conserve metabolic reserves. Diapause is a genetically determined state of suppressed development induced by environmental factors in which the duration of the suppressed development lasts longer than the adverse conditions (Tauber et al., '86; Danks, '87). However, even in diapausing insects, metabolic rate is directly dependent on temperature (Irwin and Lee, 2000). Consequently, unusually warm, mild winters may deplete metabolic reserves needed for adult development and reproduction in the spring. The current study examines the effects of elevated winter temperatures on two species of cynipid gall wasps, Diplolepis spinosa (Ashmead) and D. variabilis (Bassett). Ranging throughout much of southern

*Correspondence to: Jason B. Williams, Department of Zoology, Miami University, Oxford, OH 45056. E-mail: william37@mumichio.edu
Received 28 October 2002, Accepted 18 February 2003
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/jez.a.10264
and central Canada, *D. spinosa* produces spherical, multichambered galls on the stems of *Rosa blanda* Ait. in eastern Canada, *R. woodsii* Lindl. in the prairies and *R. nutkana* Presl. in southern British Columbia (Shorthouse, '88, '93). In contrast, *D. variabilis* is only found in the Okanagan Valley of southern British Columbia and induces multichambered leaf galls which remain on the host plant, *R. woodsii*, throughout the winter. All *Diplolepis* species overwinter within their galls as freezing intolerant prepupae (Somme, '64; Rickards and Shorthouse, '89).

Senescent gall tissue offers little protection from winter stresses. Ring ('81) and Ring and Tesar ('83) found the freeze-intolerant dipteran, *Rhabdophaga* sp. collected in the arctic supercooled to -61.6°C while the leaf galling hymenopteran, *Diastrophus kineauidii* from the relatively mild climate near Victoria British Columbia supercooled to only -33.0°C. Members of the *Diplolepis* genus are freeze-intolerant and supercool extensively (-31 to -40°C) depending on the winter microclimate they inhabit (Somme, '64; Rickards and Shorthouse, '89; Williams et al., 2002) and are highly resistant to desiccation (Williams et al., 2002).

Several life history traits make *D. spinosa* and *D. variabilis* useful subjects for temperature-related studies of overwintering. Both species spend up to eight months in their senescent, dry galls as prepupae before they pupate, emerge, and exit their galls as non-feeding adults the following spring (Shorthouse, '93). Females of most species are considered parthenogenetic (Shorthouse, '93) and exit from their galls with most of their eggs fully developed (Schröder, '67). *Diplolepis* females lay their eggs within one to seven days of exiting their galls at either the base of leaf buds or on developing leaflets in unforced buds (Shorthouse, '98). Larvae initiate gall formation and then feed on its tissues until the gall senesces and dries in late summer. Consequently, all metabolic reserves for winter survival, development to adulthood, dispersal, mating activities, and egg production the following spring are acquired as larvae during the previous summer.

Although these two species overwinter for similar lengths of time, they can experience very different temperature regimes during the winter. Prepupae of *D. spinosa* normally experience lower winter temperatures than *D. variabilis*. Consequently, we hypothesize that mild overwintering temperatures will reduce survival and/or fecundity of *D. spinosa* more than *D. variabilis*. In this study, we measured prepupal mass and water content, prepupal survival, size of adult females, survivorship of adults and potential fecundity in terms of the number and size of eggs for both species undergoing simulated overwintering at different temperatures.

**MATERIALS AND METHODS**

Galls containing *D. spinosa* were collected from two sites: Sudbury, Ontario and Medicine Hat, Alberta. Galls of *D. variabilis* were collected near Kelowna, British Columbia as they matured in October 1999. Temperature data, taken from archives of Environment Canada's climate normals from 1971 to 2000, were recorded near the collecting sites at Sudbury (46°37'N, 80°48'W), Medicine Hat (50°01'N, 110°43'W), and Kelowna weather stations (49°57'N, 119°22'W).

Galls were held at 15°C until all *D. spinosa* collected from Medicine Hat and *D. variabilis* prepupae were removed from the galls and weighed (±0.01 µg, Mettler Toledo UMT-2 balance). These prepupae, which are termed "extracted," were placed in 96-well microplates. Extracted *D. spinosa* were separated into 10, 5, and 0°C treatment groups (n:75 per group) while extracted *D. variabilis* prepupae were placed into 10, and 0°C treatment groups (n:55 per group). Extracted *D. spinosa* and extracted *D. variabilis* prepupae were also placed into a -22°C treatment. However, because of condensation from improper handling these individuals presumably froze inoculatively and were not included in this paper. Intact galls containing *D. spinosa* collected from Sudbury were placed into 10, 5, and 0°C treatment groups which contained 18 to 21” galls of approximately 80 g wet mass. We stored the extracted prepupae and intact galls over supersaturated NaCl solutions to maintain a relative humidity of ~75%. Prepupae of *D. spinosa* left to emerge from their galls are termed "galled *D. spinosa*.”

After 120 days of exposure to their assigned treatment temperature, extracted prepupae were weighed, placed in 0.5 ml microcentrifuge tubes, and placed at room temperature (~23°C) to allow continued development. Survival was assessed every 12 hr and recorded at each developmental stage. Death of non-adults was determined when tissue degradation was apparent and/or develop-
ment had not progressed after 40 days exposure to 23°C, death of adults was determined when they did not respond to repeated tactile stimuli. To ensure full egg development, extracted females were held for six days after pupation before being killed by freezing and stored at −80°C. The intact galls containing D. spinosa were also transferred to room temperature after 120 days of exposure. Females, having exited their galls, were held for six days before being stored at −80°C. After three consecutive days without wasps exiting, the intact galls were transferred to −80°C for later dissection.

Head width and wing length were determined for ten randomly selected females from each treatment group of extracted D. spinosa and D. variabilis, and galled D. spinosa. To measure potential fecundity, the randomly selected females were dissected, the total number of eggs in both ovaries was counted, and the length and width (±0.1 μm) of a random sample of 10 eggs from each female were measured under 100× magnification. Measurements were taken using an Olympus dissecting microscope with a Linkam VTO 220 video analysis system.

Total body water content of extracted D. spinosa and D. variabilis prepupae was measured prior to and after the 120 days of exposure to the treatment temperatures. Initially, five live individuals from each species were weighed, dried to a constant weight at 65°C and re-weighed to determine water content. Similar measurements were taken (n=5 per treatment group) for each species within 24 hours after their removal from the temperature treatments.

Analyses included one-way ANOVA followed by Fishers PLSD test (Sokal and Rohlf, '95) to identify differences between treatment groups for larval body water content, adult body size (head width and wing length), number of eggs, egg length and width, and number of days exposed to room temperature prior to pupation and eclosion for extracted D. spinosa and D. variabilis after the treatment regimen was complete. A paired t-test was used to examine differences in mass between pre and post-treatment prepupae (Samuels and Witmer, '99). Analysis of covariance was used to determine if fecundity was directly influenced by the temperature treatment by adjusting for the effects of female body size. Differences between treatment groups for mortality and the proportion of wasps which exited their gall were evaluated using a one-way ANOVA model with proportions transformed via an angular transformation (Zar, '84). Tukey's multiple comparison procedure was then used to determine which treatment groups differed. Significance was determined at alpha =0.05.

RESULTS

Survival was adversely affected at the highest simulated overwintering temperature (10°C) for both extracted and galled D. spinosa, but not for extracted D. variabilis (Figs. 1 and 2). Of the 65 extracted D. spinosa prepupae held at 10°C, 10 died before pupation, five died as pupae, five failed to emerge from their pupal case, and an additional 34 died within 24 h after emerging as adults. In contrast, only 13 individuals in the 5°C group and 10 in the 0°C group failed to become adults. Total mortality was significantly different between the

![Graph](attachment:image.png)

Fig. 1. Survival of extracted Diplolepis spinosa held at simulated overwintering temperatures of 10, 5, or 0°C and Diplolepis variabilis held at 10 or 0°C for 120 days before transfer to room temperature. The asterisk indicates total survival was significantly lower (p<0.01) for D. spinosa in the 10°C group compared to the two lower temperature treatments.
Fig. 2. Effect of simulated overwintering temperature on the proportion of *Diplolepis spinosa* that were able to emerge naturally from their galls. Emergence percentages not sharing a letter were significantly different. Error bars (±SE) are less than the size of the symbol.

10°C treatment and the two lower temperature treatment groups (p < 0.01 in both pair-wise comparisons). In addition, galled *D. spinosa* held at 10°C had an emergence rate of only 32.3% compared to the three lower temperature treatments, which averaged greater than 87% (Fig. 2). In contrast, total mortality was not significantly different (p = 0.16) between extracted prepupae of *D. variabilis* held at the 0 and 10°C.

Male and female *D. spinosa* in the -22°C treatment group took much longer to exit their galls (20.9 ± 0.4 d for males, and 25.7 ± 0.4 d for females) than individuals held at the three higher temperature treatments, which averaged 10.2 ± 0.04 d for males and 11.0 ± 0.05 d for females (Fig. 3). In all four temperature treatments, males exited their galls before females by an average of 1.9 d. Similarly, extracted *D. spinosa* males that were able to eclose did so 1.1 d sooner than did females, while *D. variabilis* males eclosed 2.9 d before females in all treatment groups (Table 1). Extracted male and female *D. spinosa* had similar rates of eclosion within the three temperature treatments; however, the slight differences among the males (0.7 d) in the three treatment temperatures, as well as the females (0.9 d), were significantly different. Female *D. variabilis* held at 10°C took significantly longer (3.5 d) to pupate and 3.8 d longer to eclose than females in the 0°C treatment group (Table 1).

Prepupae undergoing simulated overwintering at higher temperatures lost more weight (Table 2) and showed slight increases in body water content compared to ones held at lower temperatures (Table 3). All temperature treatments of extracted *D. spinosa* and *D. variabilis* exhibited significant weight losses (p ≤ 0.001 for each group); however the greatest losses, ranging between 9.6 and 16.9%, were caused by the 10°C treatment. In contrast to weight, body water content was higher for most post-treatment groups of extracted prepupae compared to pre-treatment levels (Table 3). All three temperature treatments of extracted *D. spinosa* and the 0°C treatment of *D. variabilis* had significantly higher body water contents, ranging from 2.8 to 4.9% higher. However, no change in water content was observed in *D. variabilis* prepupae subjected to 10°C. Size of adult wasps (head width and wing length) was not significantly different among treatment groups for extracted females of *D. spinosa*, and *D. variabilis*, or galled females of *D. spinosa* (Table 4).

Fig. 3. Cumulative percent of daily emergence for galled *Diplolepis spinosa* held for 120 days at 10, 0, 5 or -22°C before transfer to room temperature (23°C).
Potential fecundity, measured in numbers of eggs produced, was strongly reduced by higher simulated overwintering temperatures for galled (p<0.001) and extracted \textit{D. spinosa} (p<0.001) (Fig. 4). The mean 337±13 eggs produced by extracted \textit{D. spinosa} in the 0°C group was significantly higher, averaging 29% more, than the number produced by females in the 5°C and 10°C groups. The number of eggs from galled \textit{D. spinosa} females in the -22°C and 0°C groups, 493±19 and 524±12 respectively, did not differ, but were significantly more than the 5°C and 10°C groups, which averaged between 79 and 174 fewer eggs. In contrast, \textit{D. variabilis} females held at 0°C and 10°C produced similar numbers of eggs. Even though numbers of eggs differed between treatment groups for \textit{D. spinosa}, egg length and width remained constant for both species (Table 4).
TABLE 4. Wing length, head width, egg length, and egg width (mean ± SEM) for female Diplolepis spinosa and Diplolepis variabilis after simulated overwintering at - 22, 0, 4, and 10°C

<table>
<thead>
<tr>
<th>Measure</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-22°C</td>
</tr>
<tr>
<td>Galled D. spinosa</td>
<td></td>
</tr>
<tr>
<td>Wing length</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Head width</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Egg length</td>
<td>367 ± 3</td>
</tr>
<tr>
<td>Egg width</td>
<td>54.0 ± 0.1</td>
</tr>
<tr>
<td>Extracted D. spinosa</td>
<td></td>
</tr>
<tr>
<td>Wing length</td>
<td></td>
</tr>
<tr>
<td>Head width</td>
<td></td>
</tr>
<tr>
<td>Egg length</td>
<td></td>
</tr>
<tr>
<td>Egg width</td>
<td></td>
</tr>
<tr>
<td>Extracted D. variabilis</td>
<td></td>
</tr>
<tr>
<td>Wing length</td>
<td></td>
</tr>
<tr>
<td>Head width</td>
<td></td>
</tr>
<tr>
<td>Egg length</td>
<td></td>
</tr>
<tr>
<td>Egg width</td>
<td></td>
</tr>
</tbody>
</table>

1Body measurements are in mm, while egg measurements are in μm.

Fecundity is highly correlated with size of females in other species of insects (Berrigan, '91), so an analysis of covariance was used to determine if the variation in egg production for D. spinosa was the result of differences in body size or temperature treatment. Using head width as an indicator of size, the interaction between temperature and adult size was not significant (p=0.22 for extracted and p=0.49 for galled females of D. spinosa). Similarly, other measures of size, such as wing length for galled and extracted D. spinosa, and weight of post-treatment prepupae for extracted females, did not have a significant interaction with temperature and were not used as a covariate. When body size was standardized, egg number was still significantly different between temperature treatments (p<0.001 for both extracted and galled D. spinosa). Thus, differences in egg production were dependent on the simulated overwintering temperature, not female size.

**DISCUSSION**

Immature stages of insects with depleted energy reserves may not have the energy needed to undergo metamorphosis and develop adult tissue (Wigglesworth, '72). Alternatively, adults that develop from malnourished larvae may have reduced fecundity compared to ones with more metabolic reserves. Galled and extracted D. spinosa exposed to mild overwintering temperatu-
OVERWINTERING TEMPERATURES ON DIPLOLEPIS WASPS

Extracted D. variabilis maximum averages ranging between -0.2°C in January to 15.4°C in April. These temperatures are substantially warmer than average temperatures taken from weather stations near D. spinosa collecting sites, -6.4°C at Sudbury and -3.3°C at Medicine Hat with daily maxima averaging -8.4 and -4.5°C respectively in January and 8.5 and 13.7°C in April. A relatively warm winter climate would apply strong selective pressure on D. variabilis to develop better mechanisms for energy conservation, such as lowering their metabolic rates. Therefore, in contrast to D. spinosa, the difference in metabolic rate between the treatment temperatures of 0°C and 10°C for prepupae of D. variabilis may have been negligible, resulting in minimal differences in their post-treatment metabolic reserves and consequently similar survival rates, adult size, and egg number.

Further evidence that D. variabilis prepupae are adapted to overwinter in a milder climate is the fact that they required an additional 6.5 days, or 39°Cdays, longer to pupate than did D. spinosa (Table 1). Post diapause morphogenesis will commence when conditions such as temperature, food availability, and moisture are suitable for development (Danks, '87). Prepupae of Diplolepis are unusual in that they do not feed as adults and overwinter encased in chambers of their galls with walls composed of dried, thick-walled sclerenchyma tissues (Shorthouse, '93). As a result, moisture, as well as food availability, are unlikely cues for initiation of post diapause morphogenesis (Rickards and Shorthouse, '89), therefore, the most probable cue is thermal. Since overwintering D. variabilis inhabit a milder environment, they may encounter unusually warm temperatures more frequently than D. spinosa. Thus, prepupae of D. variabilis may require increased exposure to warmer spring-like conditions to begin post diapause morphogenesis, which functions to avoid premature adult development and ensure that they emerge during suitable environmental conditions.

Differences in developmental rates between extracted D. spinosa and D. variabilis were evident. However, males of both species eclosed prior to females. By emerging first, males would be able to find females as soon as they leave the gall and have a better chance to inseminate more females, as has been found for other species of insects (Wiklund, '95). The fact that a considerable proportion of the adults of both species were male (all treatments of galled D. spinosa, extracted D. spinosa, and extracted D. variabilis

to exit their galls than wasps at warmer treatment groups (Fig. 3). Assuming the rate of post diapause development was the same for each treatment group, individuals in the -22°C were at a suboptimal temperature that slowed diapause development compared to those at milder temperatures. Irwin et al. (2001) found that larvae of the gall fly Eurosta solidaginis continued diapause development even when frozen at -22°C; however, they did so at a slower rate than ones that were unfrozen at 0°C.

The observed differences in survival and fecundity between D. spinosa and D. variabilis may be due to the different climates these species inhabit. Daily mean temperatures from the Kelowna weather station, near the D. variabilis collection site, averaged only 0.9°C between November and April from 1971 to 2000 with daily
averaged 51.3, 40.6, and 37.0% males respectively) suggests that a large part of the populations of these species are reproducing sexually. However, Stille and Davring ('80) showed that males of Diplolepis roseae (L.) are reproductively inactive and suggested that the genus exhibits “obligate homzygous automicictic deuteroiky” which is defined as a strictly parthenogenetic system where homozygous females as well as males are produce through meiotic activity. However, males are rare and reproductively inactive.

We hypothesized that milder overwintering temperatures would reduce potential fecundity for both D. spinosa and D. variabilis. Milder temperatures did lower the number of eggs produced by D. spinosa, though egg size remained constant for both species regardless of the temperature treatment. Even though egg number to egg size trade-offs have been reported for other Hymenoptera (e.g. Chalcidoidea; Berrigan, ‘91) this phenomenon was not evident in D. spinosa. Females of Diplolepis deposit their eggs in highly restricted areas on either unfolded leaflets or at the base of apical meristems of leaf buds (Shorthouse, ’93, ’98). Even before completely exiting the egg shell, larvae begin to feed on plant tissue, thus stimulating gall formation (Shorthouse, ’93; Brooks and Shorthouse, ’98). Such specific microhabitats for deposition of eggs as well as the initial larval development may prevent an egg size to egg number trade-off by placing optimal limits on egg size for these species, as was suggested for the gall Eurosta solidagninis (Irwin and Lee, 2000). Although egg size was constant, quality of eggs (total weight or lipid content), which can influence hatching and initial larval development (Wigglesworth, ’72), was not measured.

The results from this study support the hypothesis that relatively low overwintering temperatures promote conservation of metabolic reserves even in dormant insects. For insects that do not feed as adults, such as D. spinosa, energy reserves that remain after winter must be used for adult tissue development as well as egg production. However, the designation of a high or low overwintering temperature is relative to an organism’s climate. Diplolepis variabilis, which overwinters in a comparably warmer climate than D. spinosa, was not affected by the experimental temperature treatments used in this study. We predict, consequently, that higher experimental temperatures, perhaps in the range of 15–20°C, would negatively affect survival and fecundity in this species. In summary, mild overwintering temperatures may increase metabolism, deplete metabolic reserves, and have a deleterious effect on insect survival and potential fecundity.

ACKNOWLEDGMENTS

We thank Dr. R.G. Lalonde for collecting galls of Diplolepis variabilis and helping to locate weather data for southern British Columbia, Marilyn R. Shorthouse for help with collecting galls of D. spinosa, and Dr. Jason Irwin for help with counting eggs.

LITERATURE CITED


