

Winter Biology**Freeze Tolerance****in the Goldenrod Gall Fly**

Birds migrate. Bears hibernate. Turtles and frogs retreat to the bottom of lakes. Most animals must avoid harsh winter conditions; few can survive freezing. Larvae of the goldenrod gall fly (*Eurosta solidaginis*), can survive freezing to -40°C or below. The study of survival at low temperature is called cryobiology. This article provides an introduction to the winter biology of this widely distributed and unusual species, and suggests classroom activities that illuminate principles of cryobiology through insect overwintering.

A variety of opportunities for educational activities are found in the complex, yet easy-to-manipulate, trophic relationships between goldenrod plants, insects that induce gall formation, and the natural enemies of these gallmakers. Gall collection, measurement, and observation (exit holes, larval response, temperature, etc.) can help students develop scientific process skills including observation, classification, measurement, inference, prediction, control of experimental variables, and material manipulation (Peard, 1994). Galls can also be studied to learn about insect ovipositing behavior and plant responses to three types of gallmakers—each with its own distinct gall type (Newell, 1994). Likewise, classroom activities can focus on the collection and study of galls to discover principles of ecology and insect life cycles (Kahn, 1997).

LUKE H. SANDRO is a biology teacher at Springboro High School in Springboro, OH; e-mail: lsandro@springboro.k12.oh.us. RICHARD E. LEE, JR. is a Professor in the Department of Zoology, Miami University, Oxford, OH; e-mail: leere@muohio.edu.

LUKE H. SANDRO RICHARD E. LEE, JR.

One aspect of goldenrod gallmakers that has received little attention in the science education literature is the winter biology of these unusual insects. In autumn, the overwintering larva enters a state of dormancy, called diapause, and gradually acquires the capacity to survive freezing to temperatures of -40°C and below (Baust & Lee, 1981). In contrast, a beetle larva and two parasitic wasps that also overwinter in goldenrod galls are intolerant of freezing and must avoid internal ice formation.

Life Cycle

Only a single generation of the goldenrod gall fly occurs each year, with more than 11 months of the insect's life spent inside the gall. Adults emerge in late spring. Mating occurs on the apex of the plant where the male waits to attract females soon after they emerge from the gall (Abrahamson & Weis, 1997). After extensive inspection of the goldenrod plant for a suitable site, the female deposits her eggs (usually singly) into the leaves surrounding a bud. Each female deposits about 20-25 eggs in her life (at least under laboratory conditions).

Eggs hatch within 5-8 days, and the larvae immediately tunnel inward to the meristematic bud tissue where a chamber is created as the larvae feed on the plant matter found there. The presence of the larvae induce the plant to form a spherical stem gall, approximately 15-30 mm in diameter. The precise mechanism of induction remains a mystery, but it clearly involves complex control of the developmental processes within the plant (Abrahamson & Weis, 1997).



Gall tissue is the only food for the growing larvae, and the adults are nonfeeding. Within the gall, the larvae undergo two molts. The first (from first to second instar) usually occurs in mid-July, and the second (from second to third and final instar) in mid-August. In September, the third instar larva excavates an exit tunnel that extends up to the epidermis of the gall. The following spring, the adult fly will use this tunnel to emerge from the gall.

Insect Overwintering: Two Strategies for Survival

In temperate climates, both active and dormant insects are sometimes exposed to subzero temperatures, increasing the chance that their body fluids could freeze. In response to this threat, insects have evolved two primary strategies: freeze-avoidance and freeze-tolerance (Lee, 1989).

Freeze-Avoiding Insects

Most insects cannot survive freezing of body fluids; consequently, they must avoid severe cold by moving to warm microhabitats and/or physiologically enhancing their capacity to avoid freezing. The freezing point may be defined as the temperature below which existing ice crystals will grow and equals the melting point. For an insect, the temperature of crystallization (T_c) is the temperature at which spontaneous ice nucleation occurs in body water and the ice lattice begins to grow. Since many insects have an extensive capacity to supercool (i.e., remain liquid at temperatures below the freezing point of their body fluids), the T_c may be many degrees below the freezing point. In the laboratory, an insect's T_c is measured by placing a thermocouple on the body to detect the exothermic heat of crystallization that is released as body water freezes (visible as the "spike" in Figure 1).

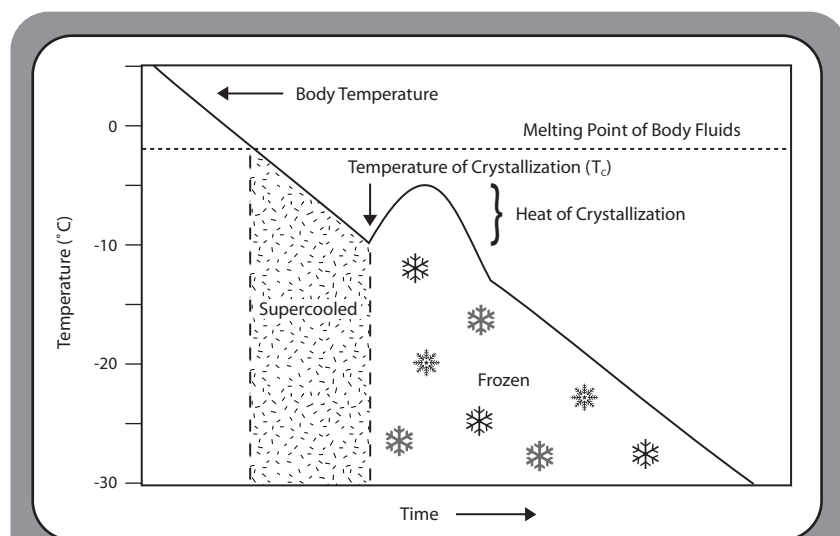


Figure 1.

Body temperature of an insect during cooling and freezing. Insect freezing at the temperature of crystallization (T_c) is indicated by the initial detection of the heat of crystallization (adapted from Lee 1989).

An interesting aspect of supercooling is that as the volume of liquid decreases, its capacity to supercool increases—this is one reason that insects, being essentially small bags of fluid, often supercool extensively. In addition, during the autumn, freeze-avoiding insects often prepare for winter by decreasing their T_c even further through the production of cryoprotectants, such as glycerol, sorbitol, and trehalose—which act as “antifreeze” — and through the elimination of potential ice nucleating agents, such as grains of soil, certain crystals, and even bacteria that inhibit supercooling (Lee & Costanzo, 1998).

Freeze-Tolerant Insects

Insects that survive freezing must overcome two major problems. First, ice may cause mechanical damage due to intracellular freezing or due to the formation of ice crystals between layers of tissues/organs that forces the layers apart. The second is cellular dehydration. When ice forms extracellularly, water molecules are gradually taken out of solution to join the growing ice lattice; this process is termed freeze concentration. Solute concentration is increased in the remaining extracellular water, thus creating an osmotic gradient which causes increasing amounts of water to leave the cell. If excessive, this dehydration causes lethal injury.

Comparatively few insects survive the freezing of their body water. These species avoid freezing injury, in part, by increasing their T_c through the synthesis of ice-nucleating agents that function to inhibit supercooling. Since freezing occurs more gradually at higher temperatures, the insect gains more time to physiologically adjust to ice formation. Freeze-tolerant insects also synthesize

high levels of the cryoprotectants glycerol, sorbitol, and trehalose. These compounds act in a number of ways. As mentioned, they function as “antifreeze” by colligatively depressing the freezing point and decreasing the total amount of ice formed. For example, one mole of cryoprotectants decreases the freezing point of a solution (or an insect’s blood) by 1.86°C. Cryoprotectants may also stabilize proteins and cell membranes to prevent injury during freezing and thawing. Glycerol and sorbitol appear to change the shape of the ice crystals, effectively “blunting” them and making freezing less damaging. Finally, penetrating cryoprotectants, such as glycerol, enter cells and raise the osmotic pressure, thus reducing the amount of cellular dehydration caused by the freeze concentration (Davidson & Lee, 1998).

Energy Conservation

While subzero temperatures can present problems for insects, they are beneficial

in some ways. During the winter most insects enter a state of hibernation or dormancy, referred to as diapause in insects, that is marked by decreased metabolism, slowed or arrested development, and increased tolerance of environmental extremes (Danks, 1987; Tauber et al., 1986). The primary function of diapause is to conserve energy reserves. Low winter temperatures promote conservation by further depressing metabolism and energy use. Indeed, it appears that some overwintering insects, especially ones that feed only as larvae, *depend* upon subzero temperatures to help them conserve enough energy to complete their life cycle. The gall fly in particular shows a high mortality and low fecundity when exposed to mild winter temperatures (Irwin & Lee, 2000).

Eurosta solidaginis: A Case Study in Freeze-Tolerance

The overwintering larva of the goldenrod gall fly is a well-studied example of a freeze-tolerant species. Like similar insects, this species only acquires freeze-tolerance as it cold-hardens for winter. During autumn, the larva prepares itself for overwintering in a number of ways. First, the insect stops feeding. Its metabolic rate drops significantly and remains consistently low throughout the winter. This is an indication that the gall fly has entered diapause (Irwin et al., 2001). In addition, the larva undergoes the aforementioned elevation of its T_c from -15°C to -9°C , ensuring that ice formation will occur at a more survivable higher temperature (Figure 2).

The T_c of the larva is elevated in two ways. One is inoculative freezing, or freezing induced by contact with already-formed ice crystals on the integument of the larva. In autumn, plant galls with a high water content freeze at approximately -4.5°C (Layne et al., 1990). As ice forms in the plant's tissues, it initiates freezing of the larva at higher temperatures than an isolated larva would freeze. Thus, in early autumn a larva within moist, green galls freezes inoculatively at relatively high temperatures, while in late autumn a larva within brown, dry galls must rely on another mechanism to elevate its T_c . In this case, the larva uses an internal ice nucleator to increase its T_c —but in an unusual way. While many freeze-tolerant insects contain ice-nucleating proteins in their hemolymph (Lee & Costanzo, 1998), the gall fly larva relies on large calcium phosphate crystals within its Malpighian tubules, or primitive kidneys, to catalyze freezing (Mugnano et al., 1996). During this period of cold-hardening, the larva also synthesizes the cryoprotectants glycerol and sorbitol. Finally, the gall fly larva alters the lipid composition of its cell membranes, making them more resistant to freezing damage.

Another problem faced by overwintering insects is how to synchronize the various physiological changes that enhance cold-tolerance with the arrival of winter. In the gall fly larva, two phe-

nological “triggers” have been identified (Figure 2). First, drying of the plant tissue as the goldenrod plant senesces in late summer triggers the production of the cryoprotectant glycerol. A few weeks later, when temperatures fall below 5°C , sorbitol is synthesized from the larva's glyco- gen stores. Thus, the larva monitors two environmental cues: the moisture level of the gall tissue and the environmental temperature. This two-step process decreases the chance that it will be “fooled” into premature or late cold-hardening.

Gall Ecology: Interactions with Parasitoids, Predators & Competitors

Though the gall larva is our primary focus here, it is important to note that the goldenrod gall and its inhabitants comprise a complex, tritrophic ecological system. While feeding on gall tissue, the fly larva sometimes becomes a meal for various animals. The most easily identified predators in the field are the black-capped chickadee, which leaves a messy, irregular hole in the gall, and the downy woodpecker, which leaves a neater, cone-shaped hole.

In addition to bird predation, there are three opportunistic insects that commonly overwinter in the goldenrod gall. Two species of wasps are direct predators of the gall fly, which they consume before overwintering in the gall. One species of beetle is not a direct predator but competes with the gall fly larva for the food resources within the gall (Abrahamson & Weis, 1997).

The overwintering strategy of these insects differs from that of the gall fly larva. These species avoid freezing by markedly lowering their T_c —in some cases to temperatures as low as -40°C (Baust et al., 1979). In this way, the

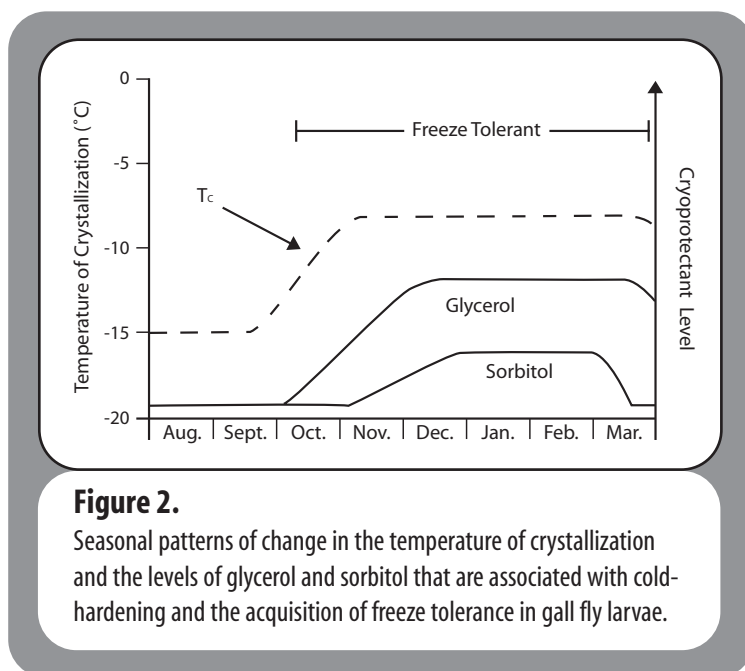


Figure 2.

Seasonal patterns of change in the temperature of crystallization and the levels of glycerol and sorbitol that are associated with cold-hardening and the acquisition of freeze tolerance in gall fly larvae.

three competitors of the gall fly larva enhance their capacity to supercool, surviving extremely low temperatures without freezing internally.

Winter Biology Activities Using the Goldenrod Gall

Perhaps the most attractive aspect of gall activities for teachers and students is that the field season for gall cryobiology begins with the first snow! This is a unique opportunity to get students outside during the winter months, which otherwise offer limited opportunities for fieldwork.

Science teachers of all grade levels can use the goldenrod gall to help students discover and understand various biological principles. Although we will focus mainly on activities appropriate for the secondary biology curriculum, these activities may be adapted for use at any level of instruction.

Collecting, Observing, Identifying

The goldenrod gall provides a wealth of opportunities to develop such process skills as those found in collecting, observing, measuring, inferring, hypothesizing, and designing and performing experiments (Peard, 1994). Galls can be collected systematically at any time of the year and will have different observable characteristics during each season.

Examining gall contents provides a perfect opportunity for students to use a simple dichotomous key to identify the gall fly and its competitors. In addition to excellent pictures and video on the biology of the goldenrod and the gallmakers, Dr. Warren G. Abrahamson provides such a key at <http://www.facstaff.bucknell.edu/abrahmsn/solidago/main.html>.

Freeze-Tolerant vs. Freeze-Avoiding Insects

Since it is only freeze-tolerant for a few months of the year, the gall fly larva can be used to demonstrate both freeze-tolerance and freeze-susceptibility. A household freezer (typically set at approximately -20°C) is perfect for keeping galls at winter-like temperatures until they are examined. Some larvae will revive upon thawing, even after several months of storage in a freezer, while others will not. A simple, impressive way to demonstrate this freeze tolerance in the classroom is to drop a frozen larva on an aluminum pie pan. The distinctive “tink” sound made by the larva will convince students that it is indeed frozen—however, within several minutes, winter-collected larvae will thaw, respond to touch, and crawl.

Students could investigate the seasonal acquisition of freeze-tolerance by collecting galls periodically between September and May and testing whether the larvae can survive freezing. The larva can only survive freezing after physiologically cold-hardening by increasing glycerol and sorbitol levels and T_c . Larvae collected in late autumn will

survive for extended periods in a classroom freezer, while those collected too early will not.

Testing for Diapause Development

The gall fly larva can only survive the winter if it is allowed to enter diapause. Diapause development requires an extended period of chilling, without which the insect cannot complete its life cycle (Irwin et al., 2001). This investigation requires one collection trip after temperatures have dropped well below freezing, e.g. late December or early January. The galls should be stored immediately in a freezer. Every two weeks, a portion of the galls can be removed, thawed, and kept in an aquarium or otherwise contained. Adults should emerge successfully after a long enough period of freezing.

Sample Gall Activity

The following is a sample gall activity, including guidelines for collection, classroom use (including a sample data table), and questions for fall and winter.

Goals

- The learner will gain an understanding of field biology collection, observation, and identification techniques.
- The learner will understand the trophic interactions between gall inhabitants.
- The learner will understand the overwintering strategies of the goldenrod gall fly.

Objectives

- The learner will be able to use a dichotomous key to identify specimens.
- The learner will be able to organize collected data into a table.
- The learner will be able to infer relationships between gall height/diameter/location and gall fly survival.
- The learner will be able to define freeze-tolerance, freeze-susceptibility, cold-hardening, and diapause.

I. Gall Field Collection Guidelines

Note: It is reasonable to expect each student to collect 30 galls.

Materials

- tape measure
- black permanent marker

Instructions

1. Measure the height of each gall from the ground.
2. Write this height **directly on each gall**.
3. If a gall is 10 ft. or closer to edge of field, write E on the gall.

- If it is more than 10 ft. from edge of field, write M (for middle) on the gall.
- Break goldenrod stem directly above and below gall to collect.
- While collecting, decide whether your galls were dispersed **randomly** throughout the field, or if you noticed that they were **grouped** in some way, i.e. concentrated in clumps. Write this down.
- Place galls in your freezer at home until the next day.
- Bring to class before 1st period so they can be put in the freezer before they thaw.
- Galls will be kept frozen for the next two weeks.

II. Gall In-Class Activity Guidelines

Materials

galls gall identification key
 utility knife data sheet
 ruler

Instructions

- Read the writing on each gall before opening it. Mark height and location on the chart.
- Measure the diameter of each gall before opening it. Mark this on the chart.
- Important:** Hold the blade retracting tab on your utility knife firmly in place (so it doesn't slide back in).
- Insert the tip of the blade longitudinally (parallel with the stem) as far as it will go.
- Gently rotate your wrist so that the blade twists and cracks the gall open along the grain. Watch an instructor do this before you try it. If it's done right, very little stress is put on the blade and it won't break.
- Use the key to figure out what's in your gall. Check the correct box on the chart.

III. Data

Gall #	Date	Height (cm)	Location (M or E)	Diameter (cm)	CHECK ONLY ONE OF LAST 5 BOXES				
					Gall fly	Wasp	Beetle	Bird	Nothing
1.									
2.									
3.									
4.									
5.									

What is the:

Total number of galls with **gall fly larvae**? _____
 Avg. height of these galls? _____
 Avg. diameter of these galls? _____
 Number of these galls in middle of field? _____
 Number of these galls on edge of field? _____

Total number of galls with **bird holes**? _____
 Avg. height of these galls? _____
 Avg. diameter of these galls? _____
 Number of these galls in middle of field? _____
 Number of these galls on edge of field? _____

Total number of galls with **wasp or beetle parasites**? _____
 Avg. height of these galls? _____
 Avg. diameter of these galls? _____
 Number of these galls in middle of field? _____
 Number of these galls on edge of field? _____

IV. Data Analysis/Discussion

All-Season Collection Questions

From the previous data, what conclusions can you draw about the relationship between:

- Gall height and gall fly survival?
- Gall diameter and gall fly survival?
- Gall location (edge or middle) and gall fly survival? Might the results be different depending on whether the edge is close to wooded areas vs. agricultural fields?
- Is bird predation more likely at specific gall locations (e.g. higher on the stem)? At specific locations in the field? Describe your findings.
- What is the most successful competitor or predator of the gall fly?
- If galls were found in a clumped distribution, explain why.
- How are exit holes indicative of what is inside the gall?
- Do the different types of larvae react differently to stimuli?

Fall Collection Questions

1. What percentage of the gall fly larvae survived freezing?
2. Did larvae from green (i.e., wet) galls survive differently than those from brown (dry) galls? Why might this be?
3. It is known that gall fly larvae survive sub-zero temperatures in the winter. Based on the survival rate of these galls, what conclusion can you draw about freeze-tolerance in gall flies?
4. What possible factors could contribute to this freeze-tolerance?

Winter Collection Questions

1. After freezing, what percentage of gall fly larvae were alive when thawed?
2. Why did these gall fly larvae survive freezing better than the ones collected in the fall?
3. Explain why galls were kept in the freezer until we opened them.
4. How might freezing be beneficial to these larvae?
5. When would be the best time to thaw these galls if we wanted the adults to emerge and complete their life cycle?
6. How long must larvae be frozen so they can successfully emerge later?
7. How long a freezing period is too long?
8. What is the effect of holding larvae at 5°C instead of -20°C?
9. What might be the consequences of an especially mild winter?
10. How does average gall size and weight change during the year?

Exploring Cryobiology –The Role of Cryoprotectants

When exploring the cold-hardiness of specific organisms, it is useful to introduce students to the concept of cryoprotective solutes and their function in preventing freeze damage. A classroom experiment, described by Davidson and Lee (1998), testing the effects of solutes like sugar and salt (both of which can function as cryoprotectants) on freezing damage to beets or potatoes is an appropriate introduction to these concepts. Beets in particular are an excellent choice for measuring freeze damage to living cells since beet cells, when ruptured, leak red pigment. Possible areas of investigation include:

- How does varying the concentration of solute affect freeze damage?
- Is salt or sugar a better cryoprotectant for beets? Why might this be?
- How does freezing damage cells?
- Why might losing water be a good thing for cells that are about to freeze?

Microrespirometry & Energy Conservation

The effect of low temperatures on the metabolism of insects can be effectively explored in the classroom using a microrespirometer (Lee, 1995). An accurate, practical, and inexpensive device can be made from a plastic syringe with a length of micropipet glued into its tip, using potassium hydroxide to remove the CO₂ produced from respiration. Students could place larvae in several of these microrespirometers then place the microrespirometers in water baths at different temperatures (ice water vs. room temperature, for example). An empty microrespirometer should also be run to control for possible changes in temperature and barometric pressure. By measuring the effect of temperature on the rate of oxygen consumption, students will discover that low temperature helps conserve metabolic energy reserves. Questions to investigate:

- How does temperature affect the respiration rate of larvae?
- How is carbon dioxide emission related to oxygen consumption?
- How might low temperatures enhance insect survival in winter?

Conclusions

Hands-on investigations of insect overwintering can be used to demonstrate many new and exciting concepts in the classroom. Exploration of the habitat, predators, and life cycle of the freeze tolerant gall fly sets the stage for introducing aspects of cryobiology and winter survival. As a result, students will enhance skills ranging from observation to prediction to experimentation. Exciting discoveries await students who investigate these “frozen maggots!”

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