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Diapause development in frozen larvae of the goldenrod gall fly, *Eurosta solidaginis* fitch (diptera: tephritidae)

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**Abstract** Seasonal changes in metabolic rate and the potential for morphological development demonstrated that third-instar larvae of the goldenrod gall fly, *Eurosta solidaginis* Fitch, exhibit a distinct winter diapause. Metabolic rate (CO₂ production) was significantly lower from 15 October to 9 February than in early autumn (9 September) and spring (1 March) samples. The induction of diapause coincided with the development of cold-hardening, maximum larval mass, and gall senescence, but our experiments did not identify specific cues triggering diapause induction. We examined the influence of exposure to 0 °C and −20 °C on diapause development. Diapause development in larvae stored at 0 °C occurred at approximately the same rate as in nature. Until 15 December the larvae were in the refractory phase of diapause (incapable of morphological development, even at permissive temperatures), but afterward moved to the activated phase within which diapause intensity decreased until termination in February. Diapause development occurred in larvae collected during the winter and stored at −20 °C for periods of 1 week to 3 months. Diapause intensity decreased in frozen larvae through the winter but at a slower rate than in larvae stored at 0 °C.

**Key words** Diapause · Freezing · Cold · Goldenrod gall fly · Metabolic rate

**Abbreviation** JH juvenile hormone

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**Introduction**

Diapause is a stage of arrested morphological development, common in insects during periods of adverse environmental conditions. Tropical insects typically enter diapause during the dry season, while many temperate insects do so during winter. Diapause involves a suite of neuroendocrine and other physiological changes manifested in a reduced metabolic rate and the cessation of morphological development (Tauber et al. 1986). A variety of cues signals entry into and termination of the diapause stage, and these cues may act individually or in combination depending on the species of insect and local conditions. The most common cues are temperature and photoperiod, but others include food availability or quality, moisture, oxygen, pH, and salinity (Danks 1987). A diapause stage allows insects to conserve energy during periods of unfavorable environmental conditions, and synchronizes reproduction both within a population and with environmental conditions that are optimal for reproduction (Mansingh 1971).

Diapause is a dynamic process which varies in intensity and “develops” so that the cues (and often the magnitude of the cues) necessary to terminate diapause change over time (Tauber et al. 1986; Danks 1987). The terminology used to describe the types and phases of diapause have taken many forms in the literature. Some authors have created new terms with operational definitions to describe biological responses resulting from experimental manipulation of animals in diapause (Tauber et al. 1986; Danks 1987). For simplicity, we use the general terminology proposed by Mansingh (1971) which is applicable to the responses of insects and other invertebrates both in the laboratory and in the field.

First, the general phrase “diapause development” refers to the physiological changes associated with progression through diapause. Diapause is preceded by a “preparatory phase” during which energy reserves are accumulated. The entry of an insect into diapause is referred to as “diapause induction.” Early in diapause,
insects are typically in the “refractory phase” when they cannot terminate diapause and continue morphological development, even if exposed to optimal environmental conditions. Later in diapause, however, insects gradually move into the “activated phase” during which an insect is able to terminate diapause and continue development if environmental conditions are favorable. “Diapause intensity” changes throughout the phases mentioned above; it is typically measured as the duration of permissive conditions required for the insect to terminate diapause.

This study focuses on the winter diapause of the goldenrod gall fly, *Eurosta solidaginis* Fitch. This species, which ranges from Texas to southern Canada, forms galls on the stems of goldenrod plants. In the early summer (late May and early June in New York) newly emerged *E. solidaginis* mate and the short-lived female adults (9–10 days) lay eggs in the terminal buds of young *Solidago* spp. (Uhler 1951). Because the window of opportunity to mate and lay eggs is quite narrow, it is important that reproductive activity be synchronized with other members of the species and with the emergence of the host plant. Larvae feed and grow within the galls throughout the summer and into the fall. In late September the host plant begins to senesce and, about this time, larvae excavate an exit tunnel to the surface of the gall, then retreat to the center. The larvae overwinter as freeze-tolerant third-instar larvae, and do not pupariate and eclose until spring when temperatures increase and new host plants are available (Uhler 1951).

Overwintering third-instar larvae of *E. solidaginis* experience extreme variation in temperature and hydration state of the gall tissues. The galls are generally above the snowpack and the gall itself provides little insulation to the larva within. In fact, gall temperature closely follows ambient air temperature and therefore overwintering larvae may experience daily fluctuations as great as 29 °C and the extreme cold of winter (Layne 1991, 1993). Correspondingly, these larvae are extremely cold-tolerant (Salt 1959; Morrissey and Baust 1976; Lee et al. 1995) and highly resistant to desiccation (Ramløv and Lee 2000).

Previous reports have suggested the existence of diapause in overwintering *E. solidaginis* larvae, but none of these studies sought evidence for diapause by systematically measuring both metabolic rate and developmental potential (key indicators of diapause) through the entire autumn-to-spring transition. The first suggestion of diapause was made by Uhler (1951), who found that larvae collected prior to January 13 did not develop and emerge as adults when moved into a greenhouse. However, 18% of a sample collected on January 20, following 4 days of subzero temperatures, did develop to adulthood. Similarly, Bennett and Lee (1997) observed that only a small proportion of *E. solidaginis* larvae collected in November or December pupariated after placement at 23 °C following 24 h of freezing to either −10 °C or −20 °C. In contrast, almost 100% of the larvae collected in late January developed to the adult stage after the same treatments. Also, January larvae demonstrated little change in oxygen consumption after 5 days at 15 °C, whereas the metabolic rates of March larvae increased significantly (Lee et al. 1995). The best evidence comes from measurements of oxygen consumption on seasonal (summer, autumn, and winter) samples of larval *E. solidaginis* (Layne and Eyck 1996). Summer larvae exhibited high metabolic rates and a high Q10 compared to the autumn and winter larvae. However, large changes in larval size and local weather during collecting prevented Layne and Eyck (1996) from attributing these patterns to diapause.

The study we present here critically tested for the existence of diapause in overwintering *E. solidaginis* using measures of metabolic rate and developmental potential and sought to describe the phenology of diapause development in this species. Because of this species’ extreme cold tolerance, we also examined the effects of freezing on diapause development. To achieve our goals, we have provided a seasonal characterization of metabolic rate in this species, both throughout the winter and during diapause termination. We have used morphological development: (1) as an indicator of diapause development (including progression through the stages of diapause and changes in diapause intensity) through the fall and winter, and (2) to explore the effects of freezing on diapause development. Our results are considered within the framework of seasonal changes in the gall microenvironment and the physiological condition of the larvae.

### Materials and methods

Seasonal changes in metabolic rate were measured in third-inst ar goldenrod gall fly larvae collected from Miami University’s Ecology Research Center at 2–3 week intervals throughout the fall and winter of 1997–1998. Larvae were individually placed in glass respirometry chambers and the chambers were submerged in water inside a double-walled beaker. A refrigerated alcohol bath (Neslab, RTE-8DD) was used to control the temperature of coolant flowing through the beaker wall, thus allowing precise control of larval body temperature. CO2 production of the larvae was measured using flow-through (40 ml min⁻¹) respirometry (TR-3 system, Sable Systems) followed by analysis with DATACAN software (Sable Systems). CO2 production was measured at 5 °C intervals from 10 °C to 20 °C with 1 h equilibration at each temperature before measurements were made. Larvae were weighed (±0.01 mg) following the experiments to allow expression of metabolic rates as microliters CO2 produced per gram fresh weight per hour. Larvae were then dried to constant mass and weighed to allow calculation of water content. The plant galls were also weighed, dried, and weighed again for calculation of water content.

The same protocol and apparatus described above were used to measure metabolic rates for the diapause termination experiment except that metabolic rate was only measured at 23 °C. (Preliminary experiments demonstrated that 23 °C was above the developmental threshold for larvae collected in late winter.) We used elevation of metabolic rate as an indicator that a larva had broken diapause and was continuing development. Because several days were required for January larvae to break diapause, each point on the graph represents a different group of eight larvae. In contrast, because measurements were made over only a period of hours, the February sample represents a single group of eight larvae measured repeatedly.