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Winter Predation of Diapausing Cocoons of Slug Caterpillars (Lepidoptera: Limacodidae)

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ABSTRACT Predators exert strong top-down pressure on herbivorous insects, but research on how predators affect herbivore fitness often focuses on the more active juvenile and adult life stages while ignoring the pupal or cocoon life stage. Few studies have investigated predation of lepidopteran pupae or cocoons and even fewer have investigated species that are not forest pests. Here we present a study on overwinter survival for two moth species in the family Limacodidae, a group of polyphagous species found in deciduous forests. We placed cocoons of the saddleback caterpillar, *Acharia stimulea* (Clemens), and the spiny oak-slug caterpillar, *Euclea delphinii* (Boisduval), in the field under saplings of six different tree species and monitored predation and survival. This is the first study to examine predation rate among different host plants within a site. We found that cocoon predation was fairly high and differed significantly between limacodid species (29% for *A. stimulea* vs. 22% for *E. delphinii*). Predation rate did not differ among the six host plant species that we tested and also did not vary annually. Through phenotypic selection analyses, we found that cocoon mass affected both the likelihood of predation and overwinter survival; larger cocoons were less likely to be depredated and more likely to successfully emerge the following year. Overall our results indicate that cocoon predation is an important source of mortality for these two limacodid species and that there may be positive selection for greater cocoon mass for both limacodid species.

KEY WORDS cocoon predation, diapause, Limacodidae, phenotypic selection analysis, pupal predation

Natural enemies often exert strong top-down pressure on populations of herbivorous insects and thus can be important agents of natural selection (Hairston et al. 1960, Strong et al. 1984, Bernays and Graham 1988, Schoonhoven et al. 1998, Lill 2001, Murphy 2004, Singer and Stireman 2005). Many studies have investigated how predators negatively affect herbivorous insect populations, but the vast majority of these studies examine predation on the feeding stages (Strong et al. 1984, Schoonhoven et al. 1998). One life stage that is often overlooked when studying predation of holometabolous insects is the pupal or prepupal cocoon stage. These non-feeding stages typically enter a rather prolonged quiescent or diapause state that is considered a life history adaptation for insects that inhabit seasonal environments (Tauber et al. 1986). These diapausing insects may serve as an important food source for a variety of vertebrate and invertebrate predators during times of food scarcity; however, field studies of predation rates on pupae and cocoons are relatively sparse and to-date have largely focused on the bionomics of outbreaking forest pests.

Research on pupal predation has focused primarily on three species, all of which are forest pests and known to outbreak: autumnal moth (*Epirrita autumnata*), winter moth (*Operophtera brumata*), and gypsy moth (*Lymantria dispar*). Experiments have shown that up to 100% of winter moth pupae may disappear in the field, and these disappearances have generally been attributed to predation (Varley and Gradwell 1960, Frank 1967b, a). Pupal predators can be an important driver of winter moth population dynamics (Hunter et al. 1997); although predators are unlikely to suppress an ongoing outbreak (Hunter et al. 1991, Tanhuanpaa et al. 1999, Heisswolf et al. 2009), pupal predators may be able to prevent outbreaks from occurring and their high abundance in certain forest types may explain why these forests are rarely subject to winter moth outbreaks (Raymond et al. 2002). Studies of winter and autumnal moths have investigated how density-dependence (Tanhuanpaa et al. 1999, Heisswolf et al. 2009), geographic region (Tanhuanpaa et al. 1999), habitat type (Raymond et al. 2002, Riihimaki et al. 2005), and altitude (Hansen et al. 2009) affect pupal predation risk (Table 1). Gypsy moth studies have investigated how rodent density (Hastings et al. 2002, Connors et al. 2005, Goodwin et al. 2005), pupal density (Schauber et al. 2004), geo-

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Table 1. Compilation of pupal (or cocoon) predation rates from studies of other Lepidoptera gathered from the literature. Percentages represent the range of mean values reported from different treatments or experiments in each of the studies

Family	Species	% Predation for pupae		Study findings	Source
		Low	High		
Geometridae	<i>O. brumata</i> (winter moth)	6	89	Predation by shrews uniformly high along an altitudinal gradient	(Hansen et al. 2009)
		20	100	Generalist invertebrate predators respond positively to increasing pupal density	(Heisswolf et al. 2009)
		10	43	Pupal predators more abundant in oak habitats than spruce or moorland habitats	(Raymond et al. 2002)
	<i>E. autumnata</i> (autumnal moth)	10	90	Predation by shrews uniformly high along an altitudinal gradient	(Hansen et al. 2009)
		0	35	Generalist invertebrate predators respond positively to increasing pupal density	(Heisswolf et al. 2009)
			60	Pupal predation does not differ between birch monoculture forests vs two-species mixture forests	(Riihimaki et al. 2005)
	40	70	Pupal predation is density independent in outbreak area and positively density dependent outside outbreak area	(Tanhuanpaa et al. 1999)	
Lymantriidae	<i>L. dispar</i> (gypsy moth)	13	92	Rodent activity positively correlated with pupal predation rate	(Connors et al. 2005)
			25	Pupal predation did not differ between thinned vs. uncut forest stands	(Grushecky et al. 1998)
		56	99	Rodent density positively correlated with pupal predation rate and rodent density varies among geographic areas	(Hastings et al. 2002)
		31	96	Pupal predation varies among forest types and is highest in oak stands, where small mammals are most abundant	(Liebhold et al. 1998)
			81	Pupal predation is higher in urban and xeric oak forests than mesic oak forests	(Liebhold et al. 2005)
		20	80	Pupal predation rate positively correlated with pupal density	(Schauber et al. 2004)
Lasiocampidae	<i>G. postica</i>	2	7	Pupal defense traits did not explain differences in predation rate between these two species; the authors suggest that pupal cocoon structure may be important in determining predation rate	(Veldtman et al. 2007)
	<i>G. rufobrunnea</i>	7	79		

If no range was given in the study, only a single value is reported under 'High'.

graphic region (Hastings et al. 2002), forest thinning (Grushecky et al. 1998), and forest type (Liebhold et al. 1998, 2005) affect pupal predation risk (Table 1). It remains unclear, however, whether the results from these studies of forest pest species extend to nonpest species as well (but see Veldtman et al. 2007; Table 1).

Studies of the bottom-up effects of host plant quality on insect fitness focus on larval or adult life stages and typically measure survival on a variety of different host plant species (e.g., Strong et al. 1984, Thompson 1988, Jaenike 1990, Bernays and Chapman 1994, Schoonhoven et al. 1998, Murphy 2007, Tilmon 2008, Gripenberg et al. 2010). This experimental approach, however, has rarely been applied to the pupal life stage. Previous studies on pupal predation of winter, autumnal, or gypsy moths have measured predation rates within homogenous forests types, such as birch or oak (Varley and Gradwell 1960, Frank 1967a, b, Tanhuanpaa et al. 1999, Hansen et al. 2009, Heisswolf et al. 2009), or have compared predation rates between forest types (Raymond et al. 2002, Liebhold et al. 2005), between different types of managed forests (Grushecky et al. 1998, Liebhold et al. 1998, Riihimaki et al. 2005), or between geographic regions (Tanhuanpaa et al. 1999, Hastings et al. 2002). To our knowl-

edge, no study has investigated how pupal or cocoon survival may vary in association with different tree species within a single forest.

The goal of our research was to investigate overwinter survival of cocoons in the microlepidopteran family Limacodidae. In eastern North America, limacodid larvae feed during late summer and early autumn in deciduous forests (Covell 1984). Their common name, slug caterpillars, derives from their unusual locomotory habit, characterized by a high degree of ventral contact with the substrate and the use of abdominal "sucker" appendages in movement (Epstein 1995). The larvae are highly polyphagous, feeding on trees and shrubs in well over a dozen plant families (Epstein 1988, Wagner 2005, Lill et al. 2006, Lill 2008, Murphy et al. in review). Although limacodid species are polyphagous, individual larvae are functionally monophagous as they are restricted to feeding upon the host plant on which they hatched; there are significant differences in host quality among potential host species and this variation in host quality leads to significant differences in larval size and cocoon mass depending on which host plant the larva feeds upon (Lill and Murphy, unpublished data). A previous study of leaf-tying microlepidopterans (*Psi-*



Fig. 1. Slug caterpillars included in this study and their cocoons. (A) *A. stimulea* larva that is heavily defended with stinging spines. (B) *E. delphinii* larva that is also defended with stinging spines, but less so than *A. stimulea*. (C) An

locorsis quercicella; Lill 2001) found a strong link between intraspecific variation in host plant quality, which declined over the season, and the mass of diapausing pupae; pupal mass was a consistent target of positive directional selection because of the fitness benefits of diapausing at a larger size, which increased overwintering survival. Presumably, larger pupae may have larger metabolic reserves that could enhance overwintering survival (Lill 2001). Alternatively, for species that produce cocoons, larger cocoon mass could offer some protection against pupal predators if larger cocoons were also more difficult to penetrate. In the case of the generalist Limacodidae examined in this study, the diets of larvae feeding on different *host plant species* are perhaps even more likely to produce wide variation in cocoon mass. Therefore, we used a similar approach as Lill (2001), phenotypic selection analysis, to explore the relationship between this trait and multiple measures of herbivore fitness.

Limacodids, are perhaps best known for their unusual morphologies; limacodid larvae are often intricately colored and possess various types of protuberances on their dorsal surfaces, including stinging spines that are an effective defense against a variety of generalist predators (Murphy et al. 2010). Nonetheless, predators and parasitoids remain important sources of mortality for limacodid larvae in both temperate and tropical ecosystems (Godfray et al. 1987, Murphy et al. 2010). Late-instar larvae spin cocoons and then diapause as prepupae within their cocoons through the winter before pupating and emerging the following summer. Larvae commonly remain in this diapausing stage for 8 mo or more (Lill and Murphy, unpublished data), during which they are potentially exposed to predation and parasitism from a wide variety of litter-dwelling natural enemies. However, both the sources and levels of mortality during this life history stage are currently unknown.

We studied the ecological factors that influence overwinter survival of two species of slug caterpillars (Lepidoptera: Limacodidae): the saddleback caterpillar, *Acharia stimulea* (Clemens), and the spiny oak-slug caterpillar, *Euclea delphinii* (Boisduval) (Fig. 1). Through field experiments conducted over 2 yr, we addressed a series of five research objectives: (1) Quantify levels of predation and parasitism for overwintering *A. stimulea* and *E. delphinii* cocoons. (2) For *E. delphinii*, the species for which we have consecutive years of data, test if percent mortality because of cocoon predation varies annually. (3) Investigate whether predation rates differ between *A. stimulea* and *E. delphinii*; *A. stimulea* is more heavily spined in the larval stage than *E. delphinii*, but whether *A. stimulea* is also relatively more defended against predation in the cocoon stage is unknown. (4) Investigate whether predation differs in the litter under different

intact *E. delphinii* cocoon. (D) A depredated *E. delphinii* cocoon. The predator bisected the cocoon and ate most of the prepupa within (located in the photo directly above the pencil tip).

tree species. (5) Characterize the relationship between cocoon mass and overwintering survival for each study species to determine if cocoon mass is the target of phenotypic selection.

Materials and Methods

Study System. In temperate regions, including the Washington, DC, metropolitan area, *A. stimulea* and *E. delphinii* are reported to be univoltine. Adults emerge from cocoons in June and July to mate and larvae can be found in the field from August through October (Wagner 2005, Murphy et al. in review); they are considered late-season herbivores. Late-instar larvae spin cocoons and then diapause as prepupae through the winter within their cocoons; pupation and adult emergence occurs the following year. Limacodid larvae spin their cocoons in the litter beneath their host plant, either between leaves or by cementing the cocoon to twigs or exposed stems close to the ground (Lill and Murphy, unpublished data). How they get there is not well understood; we have observed late-instar limacodid larvae moving down tree trunks in the late season presumably to spin their cocoons in the litter.

Both *A. stimulea* and *E. delphinii* are endemic to deciduous forests in the eastern United States and are highly polyphagous as larvae, with host ranges that include well over a dozen trees and shrubs throughout their range (Epstein 1988, Wagner 2005, Lill 2008, Murphy et al. in press). While their diets are broad taxonomically, they have been shown to strongly prefer smooth-leaved species over species with pubescent leaves (Lill et al. 2006). As larvae, both species are brightly colored and possess stinging setae (commonly referred to as spines) for all or a portion of their larval development (Dyar 1899); these stinging spines protect the larvae from a variety of generalist predators (Murphy et al. 2010). When limacodid larvae, including the two examined in this study, spin their cocoons, they incorporate the spines into their cocoons (Epstein 1996), which causes the cocoons to be irritating to humans when handled (Lill and Murphy, unpublished data), and suggests that the chemical irritants retain their activity during this period. Although the spines are known to function as a defense for the larvae, whether the cocoons are similarly defended against predators has never been investigated.

Field Experiment. To determine the extent to which predation is a significant source of mortality for limacodid cocoons and whether predation rates differed among host plants, between moth species or between years, we placed lab-reared *A. stimulea* and *E. delphinii* cocoons in the field and recorded their fate. During the first winter (2007–2008) we tested only *E. delphinii* cocoons and in the second winter (2008–2009) we used both *A. stimulea* and *E. delphinii* cocoons in our field experiment. We conducted our experiments at Little Bennett Regional Park (Clarksburg, MD), a 15 km² park in northern Montgomery County mostly covered in second growth oak-hickory-beech forest. The park is located in the Piedmont

region of Maryland and our study site is on a wooded upland slope containing mostly channery silt loam soils (U.S. Department of Agriculture, Natural Resources Conservation Service data).

All of the limacodid larvae used in our experiments were from our laboratory colonies; we maintain genetic diversity within our colonies by adding new individuals every year through extensive sampling for adults and/or larvae from field sites in the greater metropolitan area of Washington, DC. In the late fall, after larvae from our colonies had spun cocoons and entered diapause, we weighed the cocoons individually on a microbalance (Mettler Toledo XS205DU, Columbus, OH) and then glued them to 8 × 2 cm aluminum tree tags (Forestry Suppliers, Inc., Jackson, MS) with Elmer's glue. The cocoons were glued to one end of the tree tag, a unique number was etched into the center of the tag and then a hole was punched through the other end so that the tag could be secured to the ground in the field with an 8 cm aluminum nail.

In 2007, we marked five understory saplings of each of six common, co-occurring host plants, for a total of 30 trees. The species we used were black cherry (*Prunus serotina*; Rosaceae), American beech (*Fagus grandifolia*; Fagaceae), blackgum (*Nyssa sylvatica*; Nyssaceae), pignut hickory (*Carya glabra*; Juglandaceae), northern red oak (*Quercus rubra*; Fagaceae), and white oak (*Quercus alba*; Fagaceae). Each of these six tree species serves as a host for a variety of caterpillars in the family Limacodidae, including the two study species. This set of host plants was also the one used in lab-rearing the larvae used in this experiment, but we randomized larval host plant identity in placing out sentinel cocoons (i.e., cocoons were randomly assigned to host plants without regard to their larval host plant affiliation).

Under each tree, we placed two *E. delphinii* cocoons, each ≈0.3 m from the tree's trunk; we deployed a total of 60 sentinel cocoons for this experiment (two cocoons per tree, five trees of six species). We chose a haphazard direction for the first cocoon (N, S, E, or W of the tree) and then placed the second cocoon on the opposite side of the tree (180° from the first cocoon). We temporarily moved the litter aside, secured the aluminum tag to the ground with the nail, and then covered the cocoon and tag with the displaced litter (litter layer ≈10–20 cm). We put all of the cocoons in the field on 7 November 2007. In mid-March and again in late April of 2008, we relocated each surviving cocoon with the aid of a metal detector (National Geographic, Wild Planet Entertainment Inc., San Francisco, CA). Over the course of the winter, several of the cocoons became either completely or partly exposed to the elements through natural processes that removed the leaf litter from underneath the trees (e.g., runoff from snowmelt or heavy rainstorms); trees that were located on hillsides were more likely to lose their leaf litter and cocoons tended to become exposed whereas trees that were located in hollows gained litter and these cocoons were sometimes covered in litter layers >40 cm. Each time we censused the cocoons in the field we noted whether they were

present or missing; if present, we noted whether they were exposed or covered in litter and also whether they were intact or showed evidence of predation, such as tooth marks or holes chewed through the cocoon wall (see Fig. 1). All surviving cocoons were returned to the lab for adult rearing following the second census. In the lab, we placed recovered cocoons into individual 0.5 liters deli containers (Fabrikal, Kalamazoo, MI), provisioned them with a moistened filter paper disc (7.5-cm diameter; VWR, West Chester, PA), periodically misted them with water to avoid desiccation and recorded the sex of all emerging moths.

In 2008 we expanded the experiment to include both *A. stimulea* and *E. delphinii* cocoons. *A. stimulea* larvae are more heavily spined than *E. delphinii* larvae and predators tend to avoid them to a greater degree in lab trials (Murphy et al. 2010). Thus, we wanted to test whether predators would also avoid *A. stimulea* cocoons more than *E. delphinii* cocoons in the wild. Similar to the experiment in 2007, we marked trees from the same six plant species. For *A. stimulea* we marked eight understory saplings of each of the six plant species, for a total of 48 trees. There were again two cocoons placed under each tree (same methods as in 2007) for a total of 96 cocoons. For *E. delphinii* we marked six trees of each of the six plant species, for a total of 36 trees and 72 cocoons. The variation in sample sizes among these experiments reflects the availability of lab-reared cocoons of each species. We placed all of the cocoons in the field on 10 November 2008. The following spring we censused the cocoons in mid-March and late April and then returned the cocoons to the laboratory to assess their final fates, again recording the sex of all emerging moths.

Data Analysis. To address whether predation is an important source of mortality for limacodid cocoons (Objective 1), we calculated the proportion of cocoons that were either: (1) known victims of predation (tooth marks or holes chewed through the cocoon wall), or (2) possible victims of predation (the tags were recovered, but the cocoons were missing from the field). None of the cocoons were parasitized. To test if cocoon predation for *E. delphinii* differed between years (Objective 2), we used a contingency table with two categorical variables: year (2007–2008 or 2008–2009) and predation (yes or no). For this analysis, we used only the cocoons for which we could positively determine that predation had occurred (i.e., we did not include missing cocoons that were likely victims of predation). Because there was no difference in predation of *E. delphinii* cocoons during the two winters, we combined the *E. delphinii* data from both years in subsequent analyses. To test if predation rates differ between *A. stimulea* and *E. delphinii* cocoons (Objective 3), and if cocoon predation differs among host plants (Objective 4), we performed an ordinal logistic regression with predation (yes/no) as the dependent variable and limacodid species, tree species, cocoon mass, and exposure status of the cocoons (i.e., cocoons that were visible as we approached the study tree were considered ‘exposed’ and cocoons that were

still covered by litter were considered ‘not exposed’) as independent predictor variables. Finally, we used a contingency table analysis to test whether exposure differed among the six tree species.

To examine the importance of body size (measured as cocoon mass) on both rates of predation and overwintering survival (Objective 5), we performed phenotypic selection analyses on each cohort of deployed cocoons. Phenotypic selection analysis examines the relationship between relative fitness and phenotypic traits of interest, in this case cocoon mass, which varied considerably within species (*Acharia stimulea*: 185–601 mg; *Euclea delphinii*: 30–297 mg). For each of three cohorts (*A. stimulea*, 2008–2009; *E. delphinii*, 2007–2008 and 2008–2009), we measured two bouts of mortality selection (episodes *sensu* Arnold and Wade 1984a): (1) the period from deploying the cocoons to collecting them back the following spring (field survival); and (2) the entire period from deploying the cocoons until pupation and adult emergence (field + lab survival). The first selection episode assessed the importance of cocoon mass on predation and disappearance (we considered missing larvae as predated here) whereas the second selection episode assessed the importance of cocoon mass on all possible sources of overwintering mortality. We combined the data for all host plants in these analyses because field predation did not vary significantly among host plants for either caterpillar species (see Results). For each episode, phenotypic selection was measured following the methods outlined in Lande and Arnold (1983) and Arnold and Wade (1984a, b) and following Kalisz (1986) and Lill (2001). Specifically, we measured directional selection (β) during the k th episode by regressing individual relative fitness (ω_k) on the phenotypic trait (z) using the model:

$$\omega_k = \beta z + \text{error}$$

All directional selection gradients were standardized by multiplying the linear regression coefficient by the standard deviation of the trait mean. Because mortality selection involves binary fitness measures (0 or 1), these selection gradients are approximations (Kalisz 1986). By comparing the magnitude of phenotypic selection between these two episodes (the first of which is nested within the second), we aimed to distinguish between the effects of biotic factors (predation) and abiotic factors (e.g., climate, exposure) on overwintering survival of cocoons that naturally varied in initial size. We completed all statistical analyses with either JMP v. 6.0.3 or SAS v. 9.1 (SAS Institute Inc., Cary, NC).

Results

Objective 1: Quantify Levels of Predation and Parasitism for Limacodid Cocoons. We found that predators killed 29% of *A. stimulea* cocoons and 22% of *E. delphinii* cocoons (Figs. 1 and 2). An additional $\approx 30\%$ of cocoons of both species were missing in the field and may have also been killed by predators. Many of the depredated cocoons that we did find were ripped

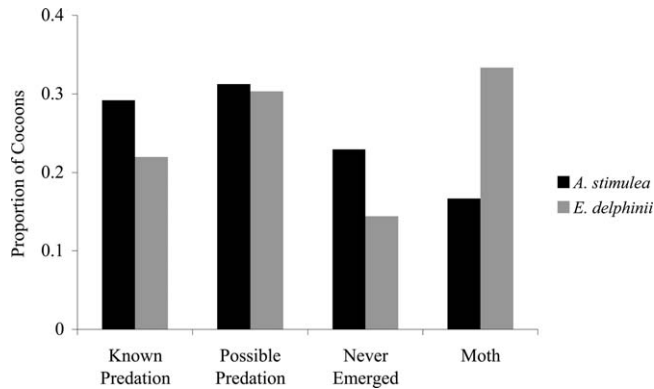


Fig. 2. Proportion of *A. stimulea* and *E. delphinii* cocoons in our field experiments that were either: (1) known victims of predation, (2) possible victims of predation, (3) recovered from the field but never emerged, or (4) recovered from the field and successfully emerged as an adult moth. The data presented is for the single winter that *A. stimulea* cocoons were placed in the field (2008–2009, $n = 96$) and for both winters that *E. delphinii* cocoons were placed in the field (2007–2008, $n = 60$ and 2008–2009, $n = 72$).

from their aluminum tag and the partially eaten cocoon was found nearby after extensive searching; the missing cocoons may have also been removed from the aluminum tags, but simply never found. Thus, the 29% predation rate of *A. stimulea* cocoons is conservative, but predation may account for up to 60% of cocoon mortality in the field. Similarly, 22% mortality is a conservative estimate for predation of *E. delphinii* cocoons, but could be as high as 52% if we consider instances of possible predation. Although we do not know the identity of the predators, tooth marks on the aluminum tags as well as on the cocoons suggest that microtine rodents are likely culprits. We also note here that we reared no parasitoids from these exposed cocoons, suggesting that larvae and pupae inside of cocoons are not subject to high levels of parasitism at our study site. We routinely rear tachinid flies from overwintering pupae (*Austrophorocera* sp.), as well as a few trigonalid hyperparasitoids (*Orthogonalys pulchella*) (Murphy et al. 2009), but these parasitoids attack the larvae before cocoon formation. Because we used lab-reared cocoons in this experiment, there was no possibility that larvae were parasitized before cocoon formation.

Objective 2: Test if Cocoon Predation for *E. delphinii* Varies Annually. Considering only known instances of predation, we found no significant differences in the percentage of *E. delphinii* cocoons attacked by predators during the two winters during which we conducted this study. Over the 2007–2008 winter, 20% of the *E. delphinii* cocoons were depredated and over the 2008–2009 winter, 25% of the *E. delphinii* cocoons were depredated, but these percentages did not differ significantly ($\chi^2 = 0.47$; $df = 1$; $P = 0.5$). Thus, overwinter predation upon limacodid cocoons appears to be fairly consistent over this 2 yr period, at least for *E. delphinii*. These two winters (2007–2008 and 2008–2009) were similar to each other in terms of average monthly temperature and both were slightly warmer than the long-term mean (mean temperature for 2007–2008 = 2.8°C, mean tempera-

ture for 2008–2009 = 1.8°C. The long-term mean winter temperature is 1.2°C).

Objective 3: Test if Cocoon Predation Rates Differ Between *A. stimulea* and *E. delphinii*. *A. stimulea* cocoons suffered significantly greater predation than *E. delphinii* cocoons (Fig. 2; Likelihood ratio $\chi^2 = 3.8$, $df = 1$, $P = 0.05$). For *A. stimulea*, we found that 29% of the cocoons were killed by predators, but only 22% of *E. delphinii* cocoons were killed by predators.

Objective 4: Test if Cocoon Predation Rates Differ Among Host Plants. We found that predation levels did not differ beneath the different tree species (Fig. 3; Likelihood ratio $\chi^2 = 5.9$, $df = 5$, $P = 0.3$), but instead depended heavily on whether the cocoons were exposed or covered by litter (Likelihood ratio $\chi^2 = 11.1$, $df = 1$, $P = 0.0009$). Although predation of cocoons did not differ among tree species, the presence or absence of litter beneath the trees did differ among tree species ($\chi^2 = 15.4$, $df = 5$, $P = 0.009$). This host plant effect was driven primarily by high amounts of litter under American beech (where only 13% of cocoons were exposed) relative to the other five species, which had more variable litter layers (37–47% of cocoons were exposed).

Objective 5: Characterize the Relationship Between Cocoon Mass and Overwintering Survival for Each Study Species to Determine if Cocoon Mass is the Target of Phenotypic Selection. On average, *A. stimulea* cocoons had twice the mass of *E. delphinii* cocoons (mean = 289 vs. 144 mg, respectively, in the combined experiments). We found significant positive selection on cocoon mass following the field episode of survival for both *A. stimulea* and *E. delphinii* cocoons during the 2008–2009 winter; in other words, predation was significantly greater on smaller cocoons within each species than larger cocoons (Table 2; Fig. 4). Although selection for larger *E. delphinii* cocoons during the 2007–08 winter was not significant ($P = 0.11$; Table 2), the selection gradient is still positive and thus the trend is the same as the following winter. When comparing the entire overwintering period

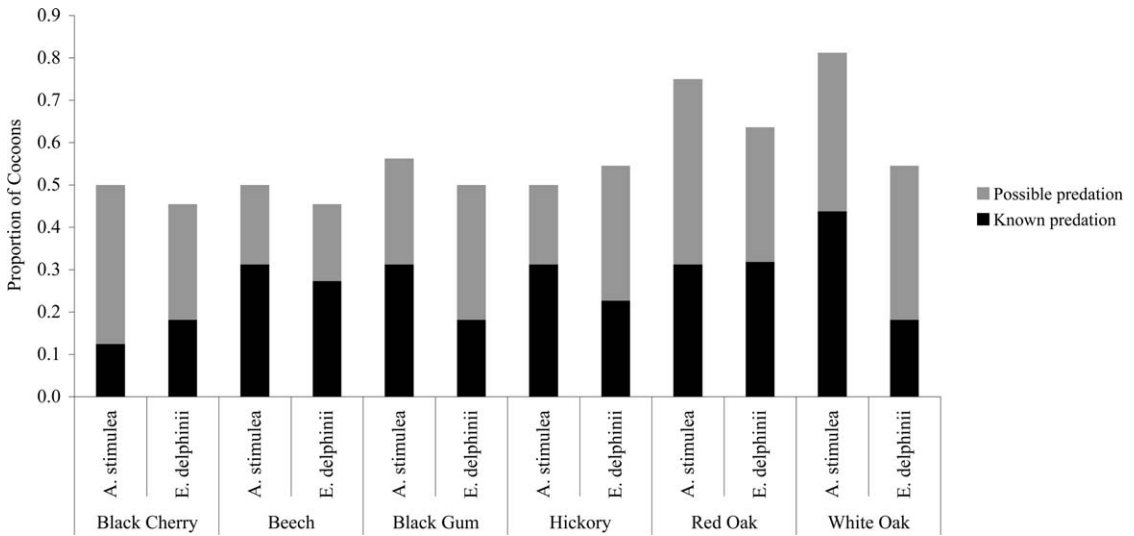


Fig. 3. Proportion of *A. stimulea* and *E. delphinii* cocoons in our field experiments that were known victims of predation (black portion of the bars) and possible victims of predation (gray portion of the bars) for each of the six host plants we studied. The data presented are for the single winter that *A. stimulea* cocoons were placed in the field (2008–2009, $n = 96$) and for both winters that *E. delphinii* cocoons were placed in the field (2007–2008, $n = 60$ and 2008–2009, $n = 72$).

(i.e., the mean cocoon mass of the individuals that survived to adult emergence), we detected significant positive selection on cocoon mass in all three episodes (Fig. 5; Table 2).

Discussion

We found that predation was an important source of mortality for limacodid cocoons, with predators killing at least 29 and 22% of experimental cocoons of *A. stimulea* and *E. delphinii*, respectively. For the species that we tested in two consecutive years, *E. delphinii*, predation rates appeared to be consistent over time, but the two winters during which we conducted this study were similar to each other and were relatively mild; predation rates may vary during winters that are more severe. If we compare our results to the relatively few other studies that have investigated pupal predation in the field, we see that our measures for predation pressure for limacodid cocoons are comparable to predation rates reported in the literature (Table 1). Three of the species listed in Table 1 (winter moth, autumnal moth, and gypsy moth) are capable of causing serious damage to trees during population

outbreaks and for all of these species reported pupal predation rates can be extremely high (90–100%). Our measures of cocoon predation, while still substantial, are not nearly so high. We suggest that the chemical irritants from the stinging spines of larval limacodids that are incorporated into their cocoons may provide some measure of protection for the prepupal larvae within these cocoons. One other study has also investigated cocoon predation on species that are putatively defended as cocoons. Larvae in the genus *Gonometa* are similar to limacodids in that they also have urticating setae that they incorporate into their cocoons (Veldtman et al. 2007) and although predation rate is highly variable for the two *Gonometa* species (Table 1), it is generally lower than for the nondefended moth species (winter moth, autumnal moth, and gypsy moth). Other species, such as the flea beetle *Longitarsus melanocephalus* and the Buckeye moth (*Junonia coenia*), sequester chemical compounds as pupae (Bowers and Collinge 1992, Bowers and Stamp 1997), which protects them from predators and pathogens in lab trials (Baden and Dobler 2009).

The chemical defenses of cocoons may explain broad patterns of pupal predation among families, but

Table 2. Results of phenotypic selection analyses on cocoon mass

Selection episode	Insect species	Year	N	l_x	β (SE)	P
Field	<i>E. delphinii</i>	2007–2008	60	0.62	+0.16 (0.10)	0.11
Field + Lab	<i>E. delphinii</i>	2007–2008	60	0.42	+0.30 (0.15)	0.05
Field	<i>E. delphinii</i>	2008–2009	72	0.36	+0.35 (0.15)	0.03
Field + Lab	<i>E. delphinii</i>	2008–2009	72	0.26	+0.40 (0.20)	0.043
Field	<i>A. stimulea</i>	2008–2009	96	0.40	+0.27 (0.13)	0.03
Field + Lab	<i>A. stimulea</i>	2008–2009	96	0.17	+0.49 (0.23)	0.031

Selection gradients (β) are standardized and bold values indicate that the gradient was significant ($P < 0.05$). N is the sample size at the beginning of the exp, l_x is the survival over the episode.

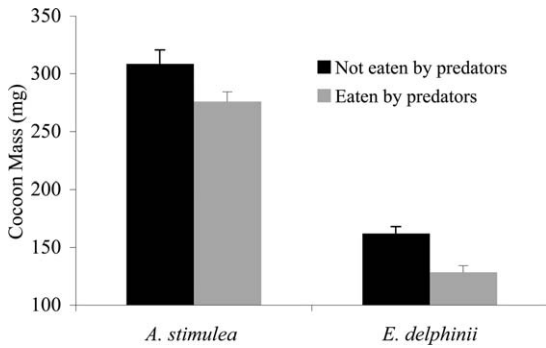


Fig. 4. Mean cocoon mass (mg) for *A. stimulea* ($n = 96$) and *E. delphinii* ($N = 132$) cocoons in our field experiment that were either eaten by predators (gray bars; *A. stimulea* $N = 58$, *E. delphinii* $N = 69$) or not eaten by predators (black bars). All cocoons were weighed before being placed in the field, and for *E. delphinii* the data includes the cocoons studied during both winters. The 'eaten by predators' category includes both cocoons that were known victims of predation and those that disappeared in the field. The 'not eaten by predators' category includes all cocoons that were recovered intact during the final census for each year, including both cocoons that emerged as moths and those that had died sometime during the winter.

it does not explain the difference in predation rate between *A. stimulea* and *E. delphinii*. We had predicted a priori that *A. stimulea* cocoons would be relatively more protected from predators than *E. delphinii* cocoons because *A. stimulea* larvae are more heavily defended with stinging spines than are *E. delphinii* larvae and generalist predators have been shown to distinguish between these two species as larvae (Murphy et al. 2010). However, despite having cocoons that are highly irritating when handled by humans, *A. stimulea* cocoons were more susceptible to predation than *E. delphinii* cocoons (29 vs. 22%, respectively). Future research should investigate this difference in predation rate further.

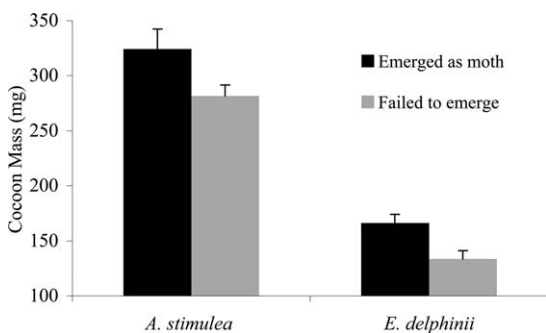


Fig. 5. Mean cocoon mass (milligram) for *A. stimulea* ($n = 96$) and *E. delphinii* ($N = 132$) cocoons in our field experiment that either successfully emerged as adult moths (black bars; *A. stimulea* $N = 16$, *E. delphinii* $N = 44$) or died during the winter, either by predation or exposure (gray bars). All cocoons were weighed before being placed in the field, and for *E. delphinii* the data includes the cocoons studied during both winters.

We did not find any direct host plant effects on cocoon survival; cocoons were equally likely to be depredated in association with any of the six tree species that we studied. Cocoon predation is dissociated from the host plant to some degree because the cocoons are found in the litter beneath the host plants and although host plant identity did not affect predation rate, the presence or absence of litter above a cocoon did affect predation rate; cocoons that were exposed were more likely to be depredated than cocoons covered by litter. The amount of litter differed significantly among tree species, a pattern driven primarily by American beech, which has a thicker litter layer than any other tree species in our study; however, litter depth is also strongly affected by other nearby plants in the forest (including canopy trees), which may partially explain why we found a significant effect of litter but not a significant effect of host plant per se on the level of cocoon predation. In this case, host plant identity does not affect predation rates directly, but rather indirectly through the presence or absence of litter.

An additional argument for the indirect importance of host plant on cocoon survival is the effect of host plant on cocoon mass. In another study, we have found that larvae reared on these same six host plant species differ significantly in growth rate, cocoon mass, and lifetime fitness with some species (e.g., cherry) serving as high quality hosts while others (e.g., red oak) are poor quality hosts (Lill and Murphy, unpublished data). As a consequence, larvae reared on high quality hosts attain a greater cocoon mass than larvae reared on low quality hosts. Because we randomized large and small cocoons under each of the tree species in our experiment to test for a direct effect of host plant on cocoon survival independent of cocoon mass, we were not able to detect potential indirect effects of host plant species on cocoon survival via differences in cocoon mass. Our phenotypic selection results show that larger cocoons are more likely to survive the winter and emerge the following spring. Had we matched larval and cocoon 'hosts' (i.e., placed cocoons of larvae reared on cherry under cherry trees in the field exposure experiment), we would likely have detected an effect of host plant on overwintering survival. Future studies matching larval and cocoon 'hosts' are needed to tease apart potential direct and indirect effects of host plant species on overwintering mortality.

Cocoon mass was found to be an important predictor of overwintering survival, both for predation risk as well as surviving winter conditions to adult emergence. We detected positive directional selection on cocoon mass for two of the three episodes of field survival, which suggests that the predator community discriminated based on prey size and preferred to attack smaller cocoons for both limacodid species; this may be correlated with differences in the thickness of the cocoon walls if bigger cocoons tend to have thicker walls. Greater cocoon mass was also selected for when considering the entirety of the overwintering period; larger cocoons of both species consistently had higher

probabilities of surviving to adult emergence, which was reflected in the significant, positive selection gradients for all three episodes (Table 2). Previous studies have found similar patterns of positive directional selection on pupal mass during overwintering periods (e.g., McGregor 1996, Lill 2001). Individuals that enter diapause at a larger size may have a greater store of fat reserves that increase survival by providing them with a valuable energy reserve, particularly during the metabolically challenging warmer periods of late fall and early spring (Tauber et al. 1986). Our data are consistent with this interpretation and suggest that differential effects of host plant species on body size in these polyphagous insects may extend well beyond the most commonly studied larval stage.

Our research on overwinter survival of limacodid cocoons demonstrates that although this life stage is often ignored by ecological studies of herbivorous insects, the selective pressures exerted by both biotic and abiotic forces can be important. Notably, we found that both predators and the harsh winter environment selected for larger cocoons in both limacodid species. Additional experiments are needed to study the specific predators involved and whether different predator taxa (e.g., vertebrate vs. invertebrate) may be selecting for different cocoon traits. Although there is a great deal of research on diapause and the environmental cues that trigger it (Tauber et al. 1986), very little seems to be known about the ecology of diapausing cocoons in the field and their interactions with predators. Future comparative studies that estimate predation for a wide array of lepidopteran lineages are needed to better understand how variable pupal and cocoon predation rates are in nature.

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