

## ORIGINAL ARTICLE

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# The effects of warm and cold periods on resource depletion and emergence synchrony in diapausing *Hypena opulenta*: Implications for biological control of invasive swallow-worts in North America

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**Abstract**

Temperature plays an important role in winter diapause of temperate insects. Its effects can cause problems for biological control programs, both for the establishment of insects in novel climates and for the mass rearing of insects in the laboratory. *Hypena opulenta* (Christoph) (Lepidoptera: Erebidae), a biological control agent for invasive swallow-wort species in North America, has been observed to enter diapause in mid-summer in its introduced range. Additionally, *H. opulenta* has proved complicated to mass-rear, in part because individuals sometimes enter diapause unexpectedly, meaning that diapausing pupae may need to be stored over winter for varying lengths of time. We conducted pupal storage experiments to determine the effects of high pre-winter temperatures, like those experienced by early diapausing individuals in the field, on lipid store depletion. We conducted a second experiment to compare the effects of cold treatments of different lengths (1, 3, and 6 months), during diapause, on pupal mortality, development times, and the synchrony of adult emergence. Exposure to warm periods ranging from 25 to 75 days during early diapause did not affect pupal weight, adult emergence rates, or lipid store depletion in *H. opulenta*. Conversely, the length of cold exposure (between 1 and 6 months) during diapause clearly affected moth mortality, and the timing and consistency of adult emergence. Longer cold periods (3 and 6 months) resulted in earlier, more synchronous adult emergence, and lower diapause mortality, than shorter cold periods (1 month). Our results indicate that in terms of energy depletion, early diapause will not affect the fitness of emerging adults, which is important for the viability of univoltine populations in the southernmost part of the introduced range. Additionally, our results will assist with the *H. opulenta* mass rearing program, indicating the timing required to synchronize adult emergence.

**KEYWORDS**

Apocynaceae, biological control, diapause, Erebidae, *Hypena opulenta*, insect rearing, Lepidoptera, lipid store, resource depletion, swallow-worts, *Vincetoxicum nigrum*, *Vincetoxicum rossicum*

**INTRODUCTION**

Diapause is generally an adaptation for surviving adverse periods, such as cold winter. Low temperatures play an important role in winter diapause of temperate insects

for several reasons. First, low temperatures can be a cue that triggers the onset of diapause, although many insects, particularly in temperate climates, enter diapause in response to short day-lengths below a critical threshold (Gill et al., 2017). Second, low temperatures reduce insect

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metabolic rates allowing them to conserve resources while in diapause. High temperatures can result in the faster depletion of fat reserves needed for post-diapause morphogenesis and adult fitness (Irwin & Lee, 2003). Third, temperatures below a certain threshold prevent the resumption of post-diapause morphogenesis and help to synchronize insect life-cycles (Lehmann et al., 2017). Finally, cold periods contrast rising temperatures in spring, providing insects with clearer seasonal cues for diapause termination (Hodek & Hodkova, 1988). For many temperate insects, however, the environmental conditions that contribute to the termination of diapause are more complex and less well understood than those that stimulate diapause initiation. In some cases, the experience of cold periods for a certain time are a requirement for diapause termination, or at least hasten it (Yaginuma et al., 1990; Denlinger, 2002). In other insects, temperature appears to play little or no role in diapause maintenance and termination (Hogan, 1960; de Wilde, 1969; Chippendale & Reddy, 1973).

When biocontrol agents are introduced to new locations, novel environmental conditions such as temperature and photoperiod can result in the early or late onset of diapause. This can prove a barrier to establishment and can hamper the success of biological control (e.g., Bean et al., 2007).

*Hypena opulenta* (Christoph) (Lepidoptera: Erebididae) is a defoliating moth native to eastern Europe, Central Asia, and the northern part of western Asia (Fibiger et al., 2010). This insect has recently been released as a classical biocontrol agent in Canada and the USA for the control of two invasive weeds, pale swallow-wort, *Vincetoxicum rossicum* (Kleopow) Barbar., and black swallow-wort, *Vincetoxicum nigrum* (L.) Moench (Apocynaceae). Female *H. opulenta* lay eggs predominantly on the underside of leaves, and the resulting larvae defoliate the plant, developing through five instars. The moths overwinter as pupae in the soil, but diapause is facultative and thus multiple generations are possible in a single season (Hazlehurst et al., 2012). A joint petition for the release of *H. opulenta* in Canada and the USA was submitted in November 2011, and was approved for Canada in 2013 and for the USA in 2017. The first releases were conducted in the same years. In Canada, in total 52402 *H. opulenta* have been released across 26 sites in Ontario (Jones et al., 2023). Although the insect has been established at early release sites since 2014 (Bourchier et al., 2019), population densities remain lower than would be required to negatively affect swallow-wort fitness. It is possible that the slow growth of *H. opulenta* populations at North American release sites is caused in part by a mismatch between environmental conditions and the insect's diapause response.

Experimental cage releases, as well as growth chamber experiments, have determined that most *H. opulenta* enter diapause when larvae are exposed to photoperiods below 15 h 35 min. Shorter summer day-lengths in the introduced range, compared to Donetsk, Ukraine, where the moths were originally collected, will likely result in some *H. opulenta* populations entering diapause early,

after a single generation (Jones et al., 2020). The early onset of diapause may be problematic for the biocontrol program in several ways, most of which have been studied experimentally. First, univoltine populations will take longer to reach the densities required to affect the target weed (Bourchier et al., 2019). Second, the absence of active *H. opulenta* larvae later in the season will allow invasive swallow-worts to recover and compensate for early season damage (Milbrath et al., 2016). Third, early diapausing *H. opulenta* will be exposed, in their most vulnerable stage, to predators that become increasingly active later in the summer (Jones et al., 2022). One consequence of early diapause that has yet to be studied in *H. opulenta* is the metabolic costs associated with undergoing diapause through periods of relative warmth, such as at the beginning of an early diapause.

We conducted two laboratory experiments designed to aid our understanding of diapause in *H. opulenta*. The first experiment explored the effects of extended warm periods during early diapause on lipid store depletion. The second examined the effects of cold period length on diapause termination and synchrony of adult emergence. We discuss the results in terms of how understanding these elements of *H. opulenta* diapause can inform the biocontrol program, both in terms of field releases and monitoring, and in terms of laboratory storage and mass rearing of the moths.

## MATERIALS AND METHODS

### Insect production

All insects used in this study were from laboratory-reared colonies maintained at Agriculture and Agri-Food Canada in Lethbridge, AB, and at the University of Toronto, ON. Both of these laboratory populations originate from insects collected in 2006 and 2012 from Donetsk, Ukraine, by CABI-Switzerland (Weed & Casagrande, 2010). Moth rearing during our experiments took place in large growth chambers set at 22 °C and L12:D12 h photoperiod. Day-lengths shorter than 15.5 h are known to stimulate diapause in *H. opulenta* (Jones et al., 2020). Emerging adult moths (up to eight males and eight females) were placed into plastic oviposition cages (55 × 40 × 22 cm) containing cut *V. rossicum* stems. Plant material was replaced every 2–3 days to ensure that the moths had sufficient space to lay their eggs, and that emerging larvae were not overcrowded. Once larvae had reached the second instar, they were transferred individually into small plastic cups so that access to food was controlled among individuals. Each plastic cup (30 mL) contained a disk of moistened tissue paper in the base, and 2–3 leaves of *V. rossicum*. All of the *V. rossicum* foliage used for insect rearing was collected from a field site in Uxbridge, ON, Canada (44.088681, –79.106804). Cups were cleaned every 2 days to remove frass, and leaves were replaced as necessary until pupation. Once the

pupal case was hardened, as indicated by a dark brown color, pupae were placed individually on moistened cotton wool, in the same plastic cups, and retained at 22 °C for a period of 2 weeks to monitor for adult emergence. Because emergence of non-diapausing pupae typically occurs within 14 days (Weed & Casagrande, 2010), non-emerged pupae that remained after this period were assumed to be in diapause.

### Experiment 1: Effects of extended warm periods on lipid store depletion

We conducted a pupal storage experiment to determine the effects of exposure to high temperatures, during diapause, on resource depletion in emerging adult moths. We produced 90 diapausing pupae, as described above. All individuals used in the experiment pupated between 20 August and 30 September, 2019. Pupae were then sexed and weighed using an analytical balance (readability 0.0001 g), and males and females were divided evenly between three treatments: controls (short warm period), medium warm period, and long warm period. Pupae in these three treatments were held at 25 °C for 25, 50, and 75 days, respectively, before being placed in cold storage. The 25-day warm period (treatment 1) was designed to mimic conditions experienced by second-generation moths pupating towards the end of summer. The 50- and 75-day warm periods (treatments 2 and 3) were designed to mimic the longer warm periods experienced by moths pupating early after a single generation. For winter cold storage, all pupae were transferred into small plastic cups (10 mL) containing a piece of moist cotton wool. Cups containing experimental pupae were placed in closed cardboard boxes to exclude light, and held at 10 °C for 1 week before being moved to a 5 °C fridge for the winter. Pupae from all three treatments were held in cold storage for approximately the same length of time (ca. 5 months) prior to lipid extraction. For each treatment, all individuals were removed from cold storage at the same time.

Pupae were weighed, as above, immediately after being removed from cold storage to assess any change in weight that had occurred during the winter. Lipid stores for each individual were determined by calculating the loss in dry weight of emerged adults after Soxhlet lipid extractions (Atkins, 1969). Pupae were removed from cold storage and placed back in a growth chamber at 25 °C, 75% r.h., and L16:D8 h photoperiod. Pupae were monitored daily for emergence, and emerged adults were placed immediately in the freezer at –18 °C. After 20 days, the percentage of pupae that had emerged within each treatment was calculated, and any non-eclosed pupae was removed from the experiment. Emerged adults were removed from the freezer and their wings were removed using a scalpel. Wings were removed because

these fragile body parts would disintegrate during the lipid extraction process, leading to misleading results. The wingless adults were placed in a drying oven at 50 °C for 72 h, before being weighed. Dried moths were then placed individually into 1-mL pipette tips (7 cm length) plugged at both ends with glass wool. Lipid extraction was conducted by Soxhlet extraction using iso-octane over a period of 6 h. Following extraction, moths were dried, as above, and reweighed. The lipid content of each moth was calculated as the difference between initial dry weight and the extracted dry weight. Lipid content was then expressed as a percentage of the initial dry weight for each moth.

### Experiment 2: Effects of cold treatment duration on diapause termination

We conducted a pupal storage experiment to determine the effects of cold treatment length on diapause termination, mortality, and synchrony of adult emergence. Diapausing pupae ( $n=580$ ) were generated as above and sexed. Pupae were then transferred to smaller plastic cups (10 mL) with moist cotton wool as in experiment 1. All pupae were transferred to a growth chamber at 10 °C and L12:D12 h photoperiod for a period of 1 month, before being transferred to a fridge at 4 °C. Pupae were then divided among three treatments based on the length of cold storage, 1 month (90 females and 78 males), 3 months (98 females and 106 males), and 6 months (108 females and 100 males). After their assigned periods of cold storage, pupae were removed from the fridge, transferred to larger plastic cups (35 mL) to allow space for unobstructed adult emergence, and placed at room temperature (21–23 °C) and L16:D8 h photoperiod. Cups were inspected daily for moth emergence and the cotton was checked once a week, exchanged when mold developed and re-moistened if necessary. After 300 days at room temperature, pupae that had not eclosed and were unresponsive to being touched with forceps were assumed to have died.

### Statistical analysis

For experiment 1, the change in pupal weight over the course of diapause was analyzed using a repeated measures ANOVA. The effect of storage treatment and sex (male/female) on adult moth emergence was compared using a generalized linear model with a binary logistic response. The effects of the three high-temperature exposure treatments, sex, and their interaction, on adult moth lipid stores (proportion of total dry weight) were compared using a generalized linear model. All statistical analyses for experiment 1 were conducted using IBM-SPSS v.28.0 (IBM, Armonk, NY, USA).

For experiment 2, two-sample tests for equality of proportions with continuity correction based on  $\chi^2$  tests were

used to compare (1) proportions of females vs. males dying after cold storage for each cold storage duration separately, and (2) proportions of females vs. males not emerging after >300 days at room temperature. Distribution curves of moth development times (time to emergence after removal from cold storage) were compared between 3- and 6-month cold-storage treatments using the non-parametric Kolmogorov–Smirnov test after residuals could not be normalized using development rate as the response variable. Analyses for experiment 2 were conducted using R 4.1.2 (R Core Team 2019).

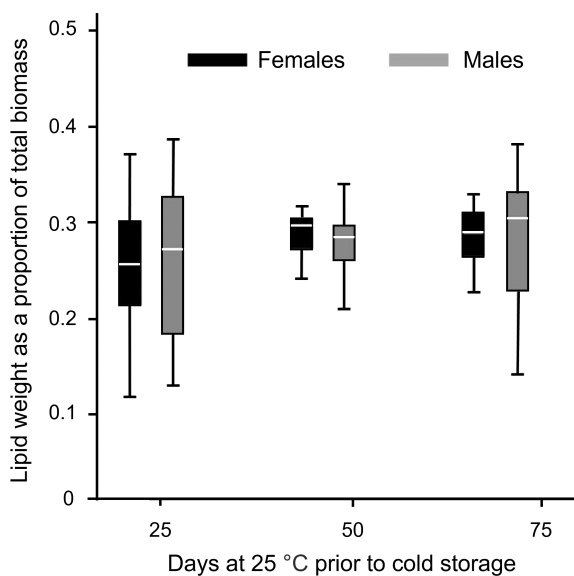
## RESULTS

### Experiment 1: Effects of extended warm periods on lipid store depletion

Pupal weights did not change significantly between pre- and post-diapause measurements ( $F_{2,89}=0.008$ ,  $P=0.93$ ;  $n=90$ ) and no interaction was observed between storage treatments and changes in pupal weights ( $F_{1,89}=0.039$ ,  $P=0.96$ ).

The overall emergence rate of adult moths after the storage treatments was relatively high (76.7%). Neither storage treatment ( $\chi^2=3.004$ , d.f. = 2,  $P=0.22$ ;  $n=90$ ) nor sex ( $\chi^2=0.571$ , d.f. = 1,  $P=0.45$ ) had an effect on the proportion of pupae that successfully eclosed and there was no interaction between the two factors, storage treatment and sex ( $\chi^2=0.884$ , d.f. = 2,  $P=0.64$ ).

Of the 90 pupae placed in cold storage, 69 emerged as adults and were used for lipid extractions. Neither



**FIGURE 1** The effect of warm periods (at 25 °C) of 25, 50, and 75 days prior to cold-storage on post-diapause lipid stores in *Hypena opulenta* adults, expressed as lipid weight proportional to total biomass. Box plots represent the first and third quartiles of the data (bottom and top boxes), the median value (the line in between), and the data range (the whiskers).

storage treatment ( $\chi^2=3.999$ , d.f. = 2,  $P=0.14$ ;  $n=69$ ) nor sex ( $\chi^2<0.001$ , d.f. = 1,  $P>0.99$ ) had an effect on the lipid content of emerging *H. opulenta* adults. No interaction was observed between storage treatment and sex ( $\chi^2=0.174$ , d.f. = 2,  $P=0.92$ ) (Figure 1).

### Experiment 2: Effects of cold treatment duration on diapause termination

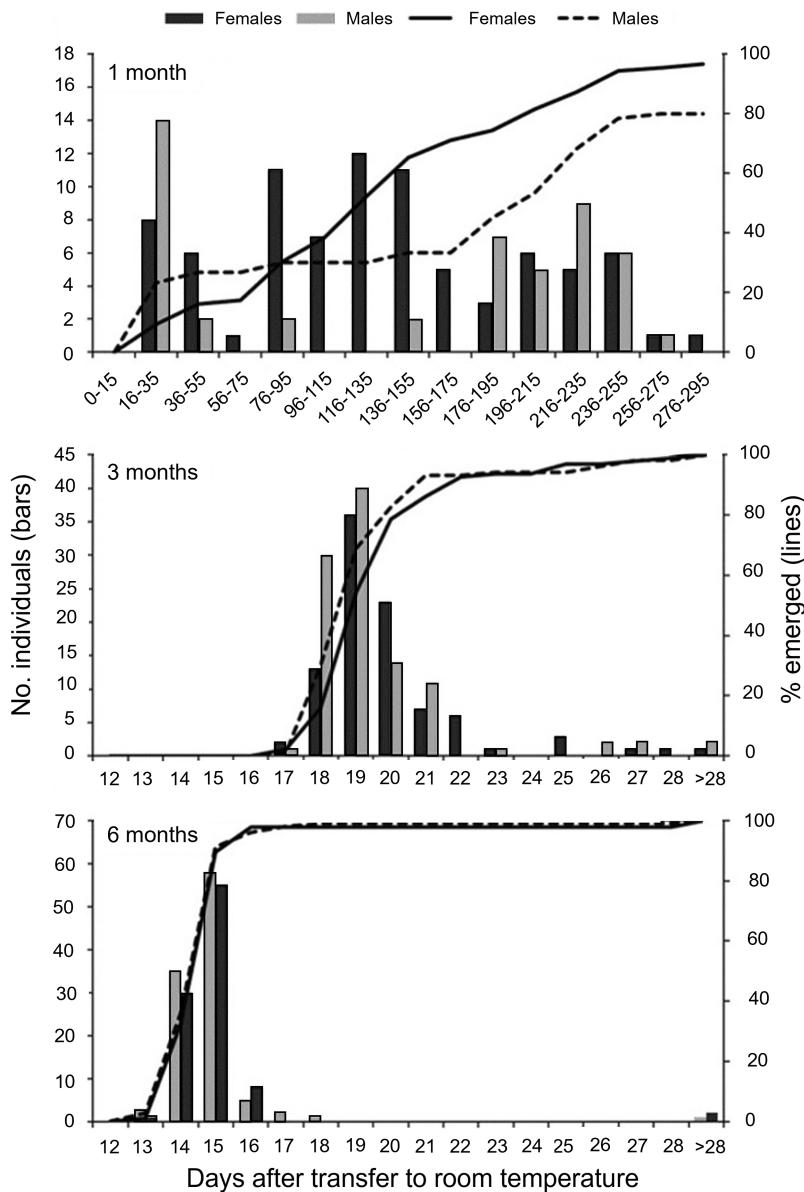
We exposed diapausing *H. opulenta* pupae to cold treatments of 1, 3, and 6 months to examine their effects on moth mortality, as well as the timing and synchrony of adult emergence. After 1 month of cold storage, the timing of adult emergence was scattered, starting 16 days after transfer to room temperature (21–23 °C). The cumulative emergence of females was linear, whereas male emergence was more random, with two main waves of emergence occurring 16–35 and 176–255 days after transfer (Figure 2). Pupal mortality was higher in males than in females (23.1 vs. 4.4%;  $\chi^2=11.16$ , d.f. = 1,  $P=0.0008$ ) (Figure 3).

When pupae were transferred to room temperature after 3 months of cold storage, moths started emerging after 17 days, with a peak of emergence at 19 days (Figure 2). For both males and females, cumulative emergence produced a sigmoid curve (Figure 2), with a few individuals emerging at 17 days, >50% emerging at  $\leq 19$  days, and 94% emerging at  $\leq 23$  days. Thereafter, only a few more moths emerged until day 28 and only three individuals took longer than that (31, 33, and 38 days). No significant difference was found in mortality between males (2.8%) and females (4.1%; Fisher's exact test:  $P=0.71$ ) (Figure 3).

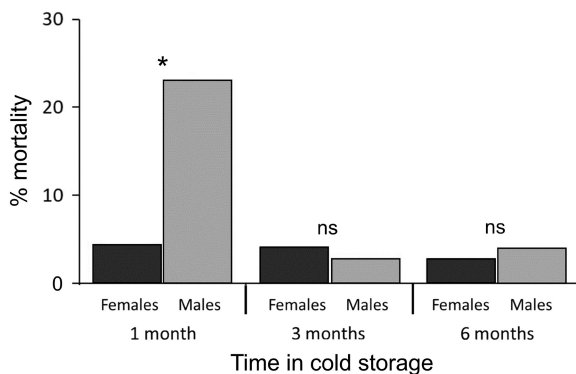
After 6 months of cold storage, adult emergence was even more synchronized, starting 13 days after transfer to room temperature, and with a pronounced peak at 15 days (Figure 2). The cumulative emergence curve was also sigmoid but much steeper than for the 3-month cold storage treatment. The cumulative emergence curves differed between 3 and 6 months (Kolmogorov–Smirnov test:  $D=0.480$ ,  $P<0.001$ ;  $n=398$ ). After 6 months of cold storage, >90% of all males and females had emerged after 15 days, and 99% at 18 days (Figure 2). Only three individuals emerged many days later (75, 76, and 124 days). There was no significant difference between male (4.0%) and female (2.8%) mortality of pupae (Fisher's exact test:  $P=0.71$ ) (Figure 3).

## DISCUSSION

Exposure to warm periods ranging from 25 to 75 days during early diapause did not affect pupal weight, adult emergence rates, or lipid store depletion in *H. opulenta*. Conversely, the length of cold exposure (between 1 and 6 months) during diapause clearly affected moth mortality, and the timing and consistency of adult emergence. Longer cold periods (3 and 6 months) resulted in earlier, more



**FIGURE 2** Emergence of adult *Hypena opulenta* moths after 1, 3, and 6 months of cold-storage at 4 °C. Bars represent the numbers of adults emerging during each time period, lines represent the cumulative emergence of adult moths (note the different scales between the panels).



**FIGURE 3** Mortality (%) of *Hypena opulenta* pupae after diapause in cold storage for 1, 3, and 6 months at 4 °C. The asterisk indicates a significant difference between males and females (two-sample test for equality of proportions with continuity correction based on  $\chi^2$  tests:  $P < 0.05$ ; ns,  $P > 0.05$ ).

synchronous adult emergence, and lower diapause mortality, when compared to shorter cold periods (1 month).

We exposed diapausing *H. opulenta* pupae to high temperatures for extended periods, prior to winter cold storage, to mimic the conditions experienced by individuals that enter diapause in summer at North American release sites. Our results showed that even the longest high-temperature treatments (75 days) had no effect on pupal weight loss during diapause, the rate of successful eclosion, or lipid reserves as a proportion of adult weight. These results are somewhat surprising because high winter temperatures have been shown to reduce pupal weight and adult fitness in other overwintering insects (Irwin & Lee, 2003; Sgolastra et al., 2011). For example, the rice stem borer, *Chilo suppressalis* Walker, enters winter diapause as late-instar larvae in response to short day-lengths. Exposure to higher temperatures during the winter results in lower pupal weights, reduced overwintering survival, and decreased fecundity in the resulting adults

(Xiao et al., 2017). Our observation that high temperatures during diapause do not affect *H. opulenta* lipid stores is particularly surprising, as most insects consume mainly lipids during the diapause period (Sinclair & Marshall, 2018), and high winter temperatures have been shown to increase the rate of resource depletion in other overwintering insects. Irwin & Lee (2003), for example, compared goldenrod gall flies overwintering in the relatively warm under-snow layer, with those overwintering in exposed stems. Larvae in the snow layer consumed significantly more lipids than those in upright stems. In addition, larval mortality in the snow layer was higher, and adult fecundity in those that emerged was reduced by around 18%. Our results suggest that diapausing *H. opulenta* pupae can withstand extended warm periods without any significant increase in pupal mortality, or depletion of lipid reserves. These results are positive for the biocontrol program as many of the activities and traits that confer fitness in adult insects, such as egg production and flight capacity, are fueled by lipid stores that are retained at the end of winter (Sinclair & Marshall, 2018). The ability to tolerate extended warm periods during diapause may facilitate a bet-hedging strategy, whereby voltinism varies within *H. opulenta* populations in order to mitigate against unfavorable late summer conditions such as drought. Such a strategy is supported by the observation that a proportion of *H. opulenta* appear to enter diapause early, even in optimal conditions (Weed & Casagrande, 2010; Jones et al., 2020).

Our work indicates that *H. opulenta* are resilient to extended high temperatures during early diapause. Studying the relationship between diapause temperatures and resource depletion in the laboratory allowed us to apply controlled temperature treatments and produce clear results. Two issues could, however, be addressed to ensure that our results translate to field conditions. First, fluctuations in temperature can cause accelerated resource consumption, and exacerbate the effects of increased mean temperatures (Hodek & Hodkova, 1988; Zhao & Wang, 2021). Although our experimental pupae suffered no measurable resource depletion, fluctuating temperatures that are common under field conditions could potentially change this. Second, high temperatures early in diapause can increase the temperature sensitivity of insects and reduce their ability to survive cold winters (Zhao & Wang, 2021). Indeed, outdoor overwintering experiments using laboratory-reared *H. opulenta* pupae found that mortality due to cold stress was quite high (ca. 30% in the colder of two winters) (Jones et al., 2022). Finally, resource depletion in response to warm diapause conditions can take the form of accelerated consumption of carbohydrates and proteins, rather than lipids (Zhao & Wang, 2021). In the fall webworm, *Hyphantria cunea* Drury, for example, higher fall temperatures result in lower pupal size and weight, but their lipid stores are not affected (Zhao & Wang, 2021). Although we did not measure carbohydrate and protein consumption during this study, the fact that *H. opulenta* pupae showed no significant weight loss over the course of the experiment suggests that depletion of these resources was minimal.

We exposed diapausing *H. opulenta* pupae to cold periods of varying lengths (1, 3, and 6 months) to explore the role of cold temperatures on diapause termination and adult emergence. Diapausing *H. opulenta* exposed to longer cold treatments exhibited earlier and more synchronized adult emergence. The observed relationship between cold periods and adult synchronization is not surprising, as similar patterns have been seen in several other Lepidoptera that undergo winter diapause as pupae (Chen et al., 2014; Stalhandske et al., 2015). However, even among Lepidoptera, responses to different lengths of cold periods vary markedly. In the peach twig borer, *Anarsia lineatella* (Zeller), for example, chilling treatments of 4 °C for a period of 30 days are enough to elicit synchronized reactivation of diapausing larvae (Damos & Savopoulou-Soultani, 2010). In contrast, in the fall webworm, *H. cunea*, exposure to 5 °C for a period of 90–120 days is needed for faster post-diapause development, lower mortality, and more synchronized adult emergence, when compared to a shorter or no cold period (Chen et al., 2014).

After the shorter cold exposure periods, especially after only 1 month, emergence of *H. opulenta* was scattered, with some individuals taking a very long time to emerge. Asynchronous adult emergence following abnormal temperature cues (such as overly short winters) may be a bet-hedging strategy employed by insects in unpredictable climates (Bradford & Roff, 1993; Ragland et al., 2009). For biocontrol agents, this can be a desirable trait, as it helps them to establish in a greater variety of environments and climatic conditions (e.g., Borowiec et al., 2018). For *H. opulenta*, we observed marginally earlier adult emergence after 6 months of cold storage, compared with 3 months. Indeed, after 6 months of cold storage, emergence began 15 days after transfer to room temperature, which represents a similar development time to non-diapausing *H. opulenta* pupae (Hazlehurst et al., 2012). These results suggest the onset of a post-diapause quiescence stage at some point between 3 and 6 months of cold storage.

In addition to asynchronous adult emergence, the shortest cold treatment (1 month) resulted in significantly higher moth mortality, particularly for males. The reasons for this increased male mortality are not clear, given that we observed similar post-diapause fat reserves in male and female moths. One possible explanation is that, in protandrous insects, males are heavily penalized for late emergence through lost mating opportunities (Iwasa et al. 1983; Parker & Courtney, 1983). The need to be ready to mate early means that diapausing males must allocate resources both to winter survival and sperm production, whereas females (in income breeders at least) can focus solely on winter survival (Wiklund et al., 1996). This hypothesis is supported by the fact that *H. opulenta* is known to continue to develop eggs after adult emergence (Seehausen et al., 2019).

Photoperiod was not manipulated as a variable during our diapause termination experiment. The effects of photoperiod on diapause termination in *H. opulenta* could be a subject for future investigation; however, for several

Lepidoptera species tested, photoperiod appears to have little influence (Tobin et al., 2002; Poitou et al., 2020). Indeed, rearing programs for *H. opulenta* across the USA and Canada have employed various light regimes with no reported effects of diapause termination (L Tewksbury, pers. comm.). In the box tree moth, *Cydalima perspectalis* Walker, an invasive species in Europe and North America, resumption of larval development after a sufficiently long cold period (Nacambo et al., 2014) appears to be mediated entirely by temperature (Poitou et al., 2020). Resuming development in response to direct temperature cues, rather than indirect photoperiodic cues, is likely beneficial for invasive species and biocontrol agents alike, allowing them to undergo synchronous and effective diapause in a wide range of climates across their introduced range.

*Hypena opulenta* pupae did not suffer lipid resource depletion as a result of high temperatures at the beginning of diapause. Extended cold treatments during diapause were, however, required to promote timely and synchronous adult emergence. These results are informative for the *H. opulenta* mass rearing program, indicating that diapausing pupae can be stored at 25 °C for up to 75 days, and remain in diapause for up to 200 days, without suffering from reduced viability or significant resource depletion. Rearing efficiency can be maximized by storing diapausing pupae at 5 °C for 6 months, but cold storage can safely be reduced to 3 months if the rearing season needs to be extended. Our results also address key obstacles for establishment that have been identified for *H. opulenta* in North American release sites, such as the effects of early diapause on resource depletion, and the impacts of longer, colder winters (Jones et al., 2022). The effects of early diapause in *H. opulenta* on winter survival and adult fitness in regards to energy depletion may not be as detrimental as expected. Additionally, long, cold winters in the introduced range are likely to promote synchronous adult emergence, despite regional differences in photoperiod. These findings offer hope for the viability of univoltine populations of the moth in the southernmost part of its introduced range.

## AUTHOR CONTRIBUTIONS

**Ian Jones:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (lead); writing – review and editing (equal). **M. Lukas Seehausen:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); validation (equal); writing – original draft (supporting); writing – review and editing (supporting). **Sandy M. Smith:** Funding acquisition (equal); project administration (equal); resources (equal); supervision (equal); writing – original draft (supporting); writing – review and editing (equal). **Robert Bouchier:** Conceptualization (equal); funding acquisition (equal); project administration (equal); resources (equal); supervision (equal); writing – original draft (supporting); writing – review and editing (equal).

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## CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest to disclose.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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