Graphical Abstract

Impact of the defoliating moth *Hypena opulenta* on invasive swallow-worts (*Vincetoxicum* species) under different light environments

Lindsey R. Milbrath* and Jeromy Biazzo

United States Department of Agriculture, Agricultural Research Service, Robert W. Holley

Center for Agriculture and Health, 538 Tower Road, Ithaca, NY 14853-2901, USA

*Corresponding author email: Lindsey.Milbrath@ars.usda.gov, fax: 1-607-255-1132, phone: 1-607-254-7268

Abstract

Black and pale swallow-wort (*Vincetoxicum nigrum* and *V. rossicum*, Apocynaceae) are twining vines from Europe that have become invasive in eastern North America. *Hypena opulenta* (Lepidoptera: Erebidae), a defoliating moth from the Ukraine, has recently been released in Canada and a request for release in the United States is under review. Its potential multivoltinism, and hence repeated defoliation of swallow-worts, combined with low-light levels in an understory environment may be promising for controlling forest infestations of swallow-worts. We conducted a greenhouse study of the impact of larval defoliation on seedlings and mature (flowering) plants of black and pale swallow-wort at different frequencies (once or twice) and degrees of defoliation (50 or 100%-seedlings; three or six larvae per stem-mature plants). Defoliation occurred under two forest light-regimes: low-light conditions typical of deeply-shaded forest habitats and higher light levels representative of a more open canopy or forest edge. Plants grown under higher light conditions were generally larger and produced many more seeds than heavily-shaded plants. One round of complete defoliation by *H. opulenta*, but not necessarily two, generally reduced biomass and seed production for both species and life stages, except for root mass of mature plants. No mature plants died and little seedling mortality occurred apart from black swallow-wort that had been completely defoliated twice under low light. Pale and black swallow-wort seedlings and mature plants appear to be tolerant of moderate defoliation damage. Complete defoliation repeated within and across years may be needed for suppression of forest infestations of swallow-wort.

Keywords: *Cynanchum rossicum*; *Cynanchum louiseae*; swallow-wort; dog-strangling vine; agent impact; weed biological control

1. Introduction

Documentation of a candidate biological control agent's potential impact, or efficacy, on a target weed continues to be encouraged (McClay and Balciunas, 2005; Morin et al., 2009; Müller-Schärer and Schaffner, 2008; Raghu et al., 2006). One common approach is to quantify an agent's impact on individual plant performance to help inform the prioritization of agents for further study or to estimate plant impacts post-release (e.g., Balciunas and Smith, 2006; Gerber et al., 2007; Reddy and Mehelis, 2015). Impact data should ideally be collected from all life stages of the plant that are attacked by an agent. Such data cannot reliably be used to predict effects on plant populations (Müller-Schärer and Schaffner, 2008). However, per capita impact data of candidate agents can be incorporated in demographic models to prospectively assess potential effects on plant population growth (e.g., Davis et al., 2006; Raghu et al., 2006).

Plants can reduce the negative effects of insect or pathogen damage by altering their growth form, phenology, or allocation of resources (Strauss and Agrawal, 1999; Stowe et al., 2000). However, seedlings are often less tolerant of herbivory than mature plants (Strauss and Agrawal, 1999; Colpetzer et al., 2004). Also, the effect of herbivore damage can be enhanced by the addition of other environmental stresses (Berg et al., 2015; Harris, 1981). For example, defoliation can have a more severe effect on shaded plants relative to unshaded plants or on species that may be less shade tolerant (Baraza et al., 2004; Blundell and Peart, 2001; Lentz and Cipollini, 1998; Norghauer et al., 2008; Rogers and Siemann, 2003). Assessing the impact of candidate agents under a variety of environmental conditions and on different life stages of the target weed can aid our understanding of potential outcomes of weed biological control programs (Berg et al., 2015; Lockett et al., 2012).

Black swallow-wort (*Vincetoxicum nigrum* (L.) Moench) and pale swallow-wort (*V. rossicum* (Kleopow) Barbar.) (Apocynaceae-subfamily Asclepiadoideae) are herbaceous, long-lived perennial twining vines that were introduced into North America from Europe in the mid-to late-1800s (Monachino, 1957; Moore, 1959; Pringle, 1973; Sheeley and Raynal, 1996). Over the past 30-40 years, they have increased in abundance in various natural and managed habitats in the northeastern United States and southeastern Canada. The largest infestations occur in New York State, the southern part of New England, and Ontario, with additional U.S. records west to Nebraska and Kansas (DiTommaso et al., 2005; USDA-NRCS, 2015). The one recorded infestation in California apparently has died out in recent years (Sanders, 1996; A. Sanders, personal communication). Both species have been declared noxious weeds, banned or prohibited invasive species in Connecticut, Massachusetts, New Hampshire, New York, and Vermont (NYS-DEC, 2015; USDA-NRCS, 2015). Pale swallow-wort is reported to reduce grassland bird and old-field arthropod densities (DiTommaso et al., 2005; Ernst and Cappuccino, 2005), and both species pose a risk to threatened and endangered species and to the globally rare alvar (limestone barrens) ecosystems in the Lower Great Lakes region (DiTommaso et al., 2005; Lawlor, 2000; Tewksbury et al., 2002).

Pale and black swallow-wort can establish and grow under a wide range of soil pH, disturbance regimes, and light levels (Averill et al., 2010, 2011; Hotchkiss et al., 2008; Magidow et al., 2013; Smith et al., 2006). Both species generally produce a similar number of stems and seeds (Averill et al., 2011). Although both species are found in open field to forest understories, black swallow-wort has not been observed as frequently in forest environments in North America for unknown reasons, especially heavily-shaded understories (Averill et al., 2011; Milbrath, 2008). Light levels in forests infested with swallow-wort typically range from 2-30%

of ambient light in forest interiors and up to 34% of ambient light at forest edges (Averill, 2009; Milbrath, 2008).

Mechanical control of swallow-wort appears ineffective although it may be of value in reducing the spread of the wind-dispersed seeds if properly timed (Averill et al., 2008; McKague and Cappuccino, 2005; Milbrath et al., 2016). Herbicidal control is currently the only effective control tactic available, but it can be costly, impractical for large acreage infestations such as in forests, and prohibited on certain properties. Also, currently registered herbicides have a broad-spectrum of activity and are therefore of potentially limited use in natural areas (Averill et al., 2008; Cain and Irvine, 2011; DiTommaso et al., 2013; Lawlor and Raynal, 2002; Mervosh and Gumbart, 2015). A biological control program has been in development for long-term suppression, and several insect and pathogen candidate agents have been assessed to varying degrees (Berner et al., 2011; Dolgovskaya et al., 2014; Gibson et al., 2014; Hazlehurst et al., 2012; Leroux, 2014; Maguire et al., 2011; Weed and Casagrande, 2010, 2011; Weed et al., 2011a, b).

The defoliating moth *Hypena opulenta* (Christoph) (Lepidoptera: Erebidae, formerly Noctuidae) was found to be specific to European swallow-worts in host range tests (Hazlehurst et al., 2012), and it was released in Ontario, Canada in 2013 (Young and Weed, 2014). A release petition for the U.S. is still under review (R. Casagrande, personal communication). *Hypena opulenta* was originally collected in a forested habitat in the Ukraine (Weed and Casagrande, 2010). It overwinters in the pupal stage, and mating and oviposition commence in the spring. Larvae have five instars and spin cocoons in leaves that have been tied together or in the soil (Weed and Casagrande, 2010). The moth is expected to produce at least two generations per summer (Weed and Casagrande, 2010).

An artificial defoliation study of swallow-worts by Milbrath (2008) indicated that one or two rounds of 100% defoliation under low light conditions could be particularly damaging, even fatal, to both seedlings and mature plants. Simulated herbivory studies can provide initial insights into plant responses and agent selection, but they may over- or underestimate some plant responses (Schat and Blossey, 2005; Schooler et al., 2006; Ziganira and Olckers, 2012). Follow-up studies with agents are thus needed (Raghu and Dhileepan, 2005).

A previous impact study by Weed and Casagrande (2010) with *H. opulenta* indicated that a single infestation of four-eight larvae per mature plant could substantially reduce biomass and seed production for pale swallow-wort but not black swallow-wort. However, different plant sizes (e.g., stem number) between the two swallow-wort species were noted as a potential confounding variable in their study. Infesting plants on a per stem, rather than per plant, basis could help standardize defoliation damage, and thus a plant's response to herbivory, across plants of differing stem number. Furthermore, as noted above, the addition of an abiotic stress, such as low light levels, might enhance the impact of a defoliating agent like *H. opulenta*, particularly on seedlings. The effect of repeated defoliation within a growing season by an herbivore on swallow-wort also has not been examined, although it is expected to occur because *H. opulenta* is likely bivoltine.

Our objective was to document the impact of *H. opulenta* on swallow-worts under a previously unstudied range of stressful factors. We evaluated in a quarantine greenhouse the effect of larval density or percentage defoliation, including repeated defoliation, on seedlings and mature flowering plants of the two swallow-wort species grown under two lower light conditions representative of forest infestations. Specifically, we were interested in the effect of defoliation under low-light conditions typical of deeply-shaded forest habitats and higher light levels

representative of a more open forest canopy or forest edge. We hypothesized that defoliation would negatively affect seedlings to a greater degree relative to mature plants, greater frequencies of defoliation would be increasingly damaging to swallow-wort plants, and increased shading would enhance the effect of defoliation.

2. Materials and methods

2.1. Insect culture

Pupae of *H. opulenta* were obtained in 2012 from a colony maintained at the University of Rhode Island (R. Casagrande and E. Tewksbury). The Rhode Island colony originated from larvae and pupae collected from pale swallow-wort and *V. scandens* (Sommier et Levier) Pobed. in 2006 at two forested locations near Donetsk, Ukraine (N 47° 34.497' E 37° 46.168'; N 47° 48.681' E 38° 32.738') (Weed and Casagrande, 2010). The Rhode Island colony initially had been reared on black swallow-wort, pale swallow-wort, and the related *V. hirundinaria* Medik., although in later years only black swallow-wort was used. We mass reared one generation of moths into pupal diapause on pale and black swallow-wort, and overwintered the pupae at 5°C and a 10:14 hr (L:D) photoperiod before use in the impact study. Insect performance is comparable on pale and black swallow-wort (Weed et al., 2011b).

Pupae were transferred to a chamber set at 14:10 hr (L:D) and 25:20°C for adult emergence, mating, oviposition, and egg hatch. Two sets of adult moths were reared out, approximately 6 wk apart, to supply larvae for two different rounds of defoliation (c. 100 adults of each sex in the first set and c. 70 adults of each sex for the second set). Upon emergence, groups of four moths (two male, two female) were each held for mating in 14.5 x 10 x 6.5 cm ventilated, plastic cages containing honey-water and one to two swallow-wort leaves placed in a moist foam block. After

4-5 d, 10 mating pairs each were transferred into 30 x 30 x 75 cm mesh and plastic-sided cages with honey-water and a potted swallow-wort plant for oviposition. Leaves with eggs were collected every other day and placed into 14.5 x 2 cm petri dishes lined with moist filter paper. Fresh leaves were placed into the dishes just prior to hatch, and neonates were collected for infesting plants.

2.2. Impact on mature plants

Rootstocks of pale swallow-wort were collected from the field in fall 2011 from Robert G. Wehle State Park, Jefferson County, New York, USA (N43°52', W76°15'). The rootstocks were stored at 4°C until transplant the following spring. Black swallow-wort rootstocks collected in fall 2011 were harmed by an unidentified fungus during cold storage, so new rootstocks were collected in May 2012 from Bear Mountain State Park, Rockland County, New York, USA (N41°18', W73°58') for immediate transplant. Individual rootstocks were planted into individual 1.9-L pots containing a soilless mix (Metro-Mix 560, Sun Gro Horticulture Distribution Inc., Bellevue, Washington, USA) and 15-9-12 N-P-K slow-release fertilizer (Osmocote Plus, Scotts-Sierra Horticultural Products Co., Marysville, Ohio, USA). Plants were grown outdoors for one summer to reestablish their root systems. Upon natural senescence all potted plants were placed in cold storage at 4°C for c. 6 months until the start of the experiment.

Overwintered plants were transferred in April to a quarantine greenhouse at the Sarkaria Arthropod Research Laboratory, Cornell University, Ithaca, New York, USA. Plants were grown under a 25:20°C thermophase (actual range 19-32°C) and 67% relative humidity (range 38-88%) with lights set for a 14:10 hr (L:D) photoperiod. The photoperiod was planned to naturally change over the course of the experiment (April to August), with natural daylengths increasing to

and then decreasing from a maximum photophase of 15 hr 18 min (sunrise to sunset). Pots were watered as needed. Plants were allowed to grow without shade for 18 d to approximate the open canopy conditions that swallow-wort plants experience in the spring. Plants were then randomly assigned to experimental treatments of shading (see below), the number of stems were counted, and they received 5 g of a slow-release fertilizer. Stem number did not include immature stems which were 1 cm or less in length and were not actively growing. Plants were either vegetative or initiating flower bud development. Additional plants (non-experimental but treated similarly) were harvested and divided into above-ground tissue (stems and leaves) and root fractions. These were dried at 70°C for 7 d and weighed. Black swallow-wort had 1.5 ± 0.7 (range 1-4) stems per plant ($n=60$, mean $\pm$ SD). The above-ground dry mass was 0.93 ± 0.25 g and the root dry mass was 4.61 ± 1.29 g ($n=13$). In contrast, pale swallow-wort had 6.5 ± 2.1 (range 3-12, $n=60$) stems per plant. The above-ground dry mass for pale swallow-wort was 6.95 ± 1.64 g and the root dry mass was 18.64 ± 5.41 g ($n=18$). Preliminary analyses were conducted to determine if we could use stem number as a covariate to standardize the unplanned size difference in adult plants between the two species. Because there was very little overlap in stem number between the two species, i.e., the covariate was not independent of the factor of interest (species), we analyzed species separately.

For each of the two swallow-wort species, the experimental design was a two-way factorial treatment structure in a split plot design. Light environment (high light forest or low light forest) served as the whole plot treatment and was arranged as six pairs (blocks) of the two light environments in a randomized complete block design. Blocks were used to account for potential light and temperature gradients in the greenhouse as well as different infestation dates. The subplot treatment consisted of a one-way treatment structure in a randomized complete block

design, with five combinations of larval density and defoliation frequency (zero larvae per stem [non-defoliated control], three larvae per stem infested once or twice, and six larvae per stem infested once or twice). All subplot treatments were represented by one potted plant in each of 12 blocks for a total of 60 experimental units for each species.

Groups of potted plants (blocks) were placed inside 0.91 x 0.83 x 1.08 m shade cages constructed of PVC pipe and surrounded by either 40% or 80% shade cloth (Greenhouse Megastore, <http://www.greenhousemegastore.com/>). Additional shade cloth was added to the tops of the low light cages beginning late June to maintain the desired light levels. Depending on the date on which measurements were taken, light levels on a sunny day inside the shade cages varied from 90.9 ± 3.9 to $126.7 \pm 8.7 \mu\text{mol s}^{-1} \text{m}^{-2}$ (mean $\pm$ SE, $n=6$) for the simulated high light forest treatment and from 13.3 ± 0.7 to $35.8 \pm 4.5 \mu\text{mol s}^{-1} \text{m}^{-2}$ ($n=6$) for the low light forest treatment. Light levels in high light forest understories and some forest edge environments typically range from 120-289 $\mu\text{mol s}^{-1} \text{m}^{-2}$ or 9-34% of ambient light. Light levels in low light, or deeply-shaded, forest understories typically range from 13-30 $\mu\text{mol s}^{-1} \text{m}^{-2}$ or 1.0-2.5% of ambient light (Averill, 2009; Milbrath, 2008). Greenhouse temperature and humidity records indicated that the plants in the low- and high-light environments experienced similar conditions during the course of the experiment.

Because plants varied in the number of stems, and hence leaf biomass available to defoliating larvae, we adjusted the number of larvae per plant according to the pretreatment number of stems a given plant possessed. A pilot study conducted the previous year indicated that four larvae per stem caused an average 65% defoliation of mature plants, with defoliation distributed among all stems although potentially more leaf loss was at the tops of the stems (unpublished data). Therefore, we estimated that three or six larvae per stem should achieve approximately 50 and

207 100% defoliation, respectively. The required number of neonate larvae to infest a given plant,
208 based on intended larval density and stem number, was transferred with a camel's hair brush
209 from hatching dishes into labelled petri dishes containing a prepinned, fresh swallow-wort leaf.
210 The larvae were allowed to settle and feed on the leaf for c. 24 hours before pinning the larval-
211 infested leaf to the appropriate plant. Plants were infested c. 23 d after removal from cold
212 storage, and each pot was covered with a 115 x 25 cm organza sleeve secured to the bottom of
213 the pot with a rubber band and tied closed at the top. The sleeve was supported by a metal hoop
214 secured to the top of three wire stakes.

215 Following pupation of surviving larvae (c. 20 d after infesting), pupae were removed and
216 percentage defoliation was visually estimated by one person for the entire experiment. Stem
217 number was recounted to adjust the number of larvae if needed for infesting plants that were to
218 receive a second round of defoliation. Such plants were infested as previously described
219 approximately 40 d after the first infestation occurred, and pupae were removed and defoliation
220 of new growth estimated c. 22 d after the second infestation. Plants were allowed to grow for an
221 additional 3 wk before harvesting. The maximum stem length was measured, generally of a
222 primary stem although it may have included an axillary branch, and the number of stems,
223 axillary branches and follicles (seed pods) were counted for each plant. Plant biomass was
224 divided into follicle, other above-ground tissue (stems and leaves), and root fractions. The roots
225 were washed of soil and the crown buds were counted. Up to ten follicles were randomly
226 sampled from each plant and total seeds per follicle was counted. Seeds could not be partitioned
227 into filled (usually 95+% viable) or unfilled (nonviable) seeds because several more weeks
228 would be needed for the seeds to mature. The average number of seeds per follicle was

calculated and used to estimate total seed production per plant. The follicle, stem/leaf, and root fractions were dried at 70°C for 5 d and weighed to determine dry mass.

2.2. Impact on seedlings

A separate experiment involving seedlings was conducted simultaneously with the mature plant experiment in the same greenhouse and utilizing the same shade cages. Seeds were collected from the field in late summer 2012 – black swallow-wort from the grounds of the Cornell University Cooperative Extension Office, Millbrook, Dutchess County, New York, USA (N41°46', W73°44'), and pale swallow-wort from Great Gully Preserve (The Nature Conservancy), Cayuga County, New York, USA (N42°48', W76°40'). Mature follicles were collected from at least 50 plants per species. Seeds were cleaned and moist stratified at 4°C for 4 mo. Stratified seeds were germinated in an incubator at 25:20°C and a photoperiod of 14:10 h (L:D). Upon emergence of the radicle, two germinated seeds were transplanted into individual 0.9-L pots in the greenhouse. Pots contained a 2:1 ratio (by volume) of soilless potting mix (Metro-Mix 560) to field soil. The field soil was collected in fall 2012 from active infestations of the two swallow-wort species (Robert G. Wehle State Park for use in pale swallow-wort pots, and Bear Mountain State Park for black swallow-wort pots) and sieved to remove large debris. Field soil was used to allow arbuscular mycorrhizal fungi to inoculate seedlings' roots; this has been shown to improve survival and growth of seedlings (Smith et al., 2008). Pots were watered as needed but no fertilizer was used because of the addition of field soil. Seedlings were grown for 17 d to ensure establishment, and pots were then thinned to a single plant (i.e., single stem), including culling extra seedlings from polyembryonic seeds. Seedlings were grown unshaded for

an additional 2 wk and then randomly assigned to experimental treatments of shading as described previously.

Black swallow-wort seeds are three times greater in mass than the seeds of pale swallow-wort, and thus black swallow-wort produces initially larger seedlings (Milbrath, 2008). In this study, non-experimental seedlings (destructively harvested as described for mature plants, 30 d post-germination) of black swallow-wort had an above-ground dry mass of 48.7 ± 14.1 mg and a root dry mass of 70.1 ± 20.9 mg ($n=17$). Pale swallow-wort seedlings had an above-ground dry mass of 33.4 ± 14.7 mg and a root dry mass of 49.3 ± 15.0 mg ($n=13$). Because initial size is an inherent species difference (no covariate is possible), species was included as an independent variable in analyses. The experimental design was a three-way factorial treatment structure in a split plot design. Light environment (high light forest or low light forest) served as the whole plot treatment and was arranged as six pairs (blocks) of the two light environments in a randomized complete block design. The subplot treatment consisted of a two-way treatment structure in a randomized complete block design, with two swallow-wort species and five combinations of percentage defoliation and defoliation frequency (non-defoliated control, 50% defoliation once or twice, and 100% defoliation once or twice). All subplot treatments were represented by one potted plant in each of 12 blocks for a total of 120 experimental units. Different insect densities were not used because a single larva could defoliate an entire seedling. Instead, three 1st instar larvae were placed on each seedling using a preinfested quarter section of a fresh leaf, observed at least daily, and removed when the approximate percentage defoliation desired was observed (after 1 to 12 d). Seedlings were infested c. 36 d post-transplant, and each pot was covered with a 60 x 12 cm organza sleeve secured to the bottom of the pot with a rubber band and tied closed at the top. The sleeve was supported by a 12 cm diameter plastic lid

attached to the top of a wooden stake. A second defoliation occurred on selected seedlings approximately 41 d after the first infestation, and seedlings were harvested at the same time as mature plants. Harvesting and data collection were similar to that described for mature plants.

2.3. Statistical analyses

One mature plant was omitted from analyses because it was accidentally damaged early in the experiment. Shoot and root biomass and crown bud number were scored as zero for seedlings that had died during the experiment. Data that violated the assumption of normal distribution of residuals were transformed using the arcsine square-root (percentage defoliation), logarithmic (dry mass, seedling stem length) or square-root (all count data) transformations. Standard errors for back-transformed data were approximated using the methods of Deming (1964).

For each species of mature plants, analysis of covariance (PROC MIXED, SAS Institute 2010) was performed on the dependent variables percentage defoliation, maximum stem length; the number of stems at harvest, axillary branches, follicles, seeds, and crown buds per plant; and aboveground (stems, leaves, and follicles) and root dry mass. Light environment and defoliation treatment served as the independent variables, pre-treatment stem number was the covariate, and the blocking factors were treated as random variables. For seedlings, analysis of variance (PROC MIXED, SAS Institute 2010) was performed on the dependent variables maximum stem length; the number of stems, axillary branches, and crown buds per plant; and stem (including leaves) and root dry mass. Swallow-wort species, light environment, and defoliation treatment served as the independent variables, with blocking factors serving as random variables. Stepwise removal of nonsignificant interaction terms was used to determine the best model for each parameter. Means for significant factors were separated using the least-significant difference test with

Bonferroni correction (SAS Institute 2010). Results for follicle number were similar to seeds per plant and are therefore not shown.

3. Results

3.1. Impact on mature plants

Plants of both swallow-wort species receiving three larvae per stem during the first round of defoliation generally experienced less defoliation (62-90%) than plants infested with six larvae per stem, which were mostly to completely defoliated (Table 1). Follicles were also consumed to varying degrees but stems were rarely destroyed. Regrowth of plants primarily came from axillary buds, although 24% of the plants also produced new stems (generally one or two) which were derived from previously noted immature stems or crown buds. The amount of foliage present prior to the second round of defoliation was generally observed to be less than that at the start of the experiment for any larval density. It was especially limited in the low light treatment such that plants were completely defoliated whether they received three or six larvae per stem (Table 1). Many 4th and 5th instar larvae died in pots infested with high larval densities due to a lack of food, especially after the second infestation.

No mortality of mature plants was observed although seven plants had one-four dead stems each and three other plants appeared to be wilting despite adequate soil moisture. These were scattered among both swallow-wort species and damage and light treatments, including control plants. Post-harvest stem number of pale swallow-wort was not affected by light environment or defoliation treatment (6.9 ± 2.6 , $n=60$, Table 2), whereas slight differences in stem number occurred among damage treatments for black swallow-wort (data not shown, overall 1.9 ± 1.4 , $n=59$, Table 2). Maximum stem length at harvest for both species was reduced for plants

receiving six larvae per stem (once or twice) compared with non-defoliated control plants (Fig. 1A, 2A; Table 2). Stem length was longer under high than low light conditions for black swallow-wort plants but not for pale swallow-wort (Table 3). The number of axillary branches produced by black swallow-wort was not affected by light or defoliation (2.8 ± 2.3 , $n=59$, Table 2). In the case of pale swallow-wort, axillary branching was similar among defoliation treatments under high light, whereas plants infested twice with six larvae per stem generally had the fewest branches in low light (Fig. 2C, Table 2).

Aboveground dry mass (including follicles) was less for plants that were defoliated, at any larval density and frequency for pale swallow-wort but only with two rounds of defoliation for black swallow-wort, compared with control plants (Fig. 1B, 2B; Table 2). Root dry mass was not altered by defoliation treatment for either species (Table 2). Aboveground dry mass and root dry mass were greater for plants grown under high than low light conditions with the exception that light levels did not alter root dry mass for black swallow-wort (Table 3). Crown bud number, a type of vegetative reproduction, was not affected by either defoliation treatment or light levels for black swallow-wort (6.0 ± 3.1 , $n=57$) or pale swallow-wort (15.4 ± 6.1 , $n=59$, Table 2).

Both species of swallow-wort flowered and produced follicles in both light environments, but total seeds per plant were often much less in the low than higher light environment (Fig. 1C, 2D; Table 2). For example, comparing control plants only, black and pale swallow-wort produced 90% and 81% fewer seeds, respectively, under low than high light conditions. All combinations of defoliation under high light significantly reduced black swallow-wort seed production by 58-95% compared with control plants. Seed output was reduced 83-100% on average by defoliation under low light conditions, although seed numbers were not significantly different from the control plants (Fig. 1C). A somewhat similar pattern was observed for pale swallow-wort, except

that seed loss was generally not as great within the high light treatment. Apart from plants defoliated twice with six larvae per stem (98% seed loss), the other defoliation treatments caused 21-54% reductions in seed numbers compared with the control plants (Fig. 2D).

3.2. Impact on seedlings

Actual defoliation levels for seedlings of both swallow-wort species ranged from an average 50-63% in the 50% treatments to 100% for the 100% treatments. Once a seedling was completely defoliated, the larvae were usually found off the plant, i.e., the stems were not cut. Most seedlings, regardless of treatment, produced new foliage after the first and second rounds of defoliation. Only eleven defoliated seedlings, all from the low light treatment, died (9%) – one pale swallow-wort and ten black swallow-wort, including all six black swallow-wort seedlings that received two rounds of 100% defoliation. All pale swallow-wort seedlings (n=60) had a single stem at the end of the experiment. Most black swallow-wort seedlings (42 out of 60), including all control plants, had one stem; the remaining seedlings had two or three stems at harvest (Table 4, means not shown). Maximum stem length was longer for black swallow-wort (126.3 ± 7.3 mm, n=60) than pale swallow-wort seedlings (93.7 ± 5.5 mm, n=58) and for plants under high (135.9 ± 8.3 mm, n=58) than low light conditions (87.1 ± 5.3 mm, n=60, Table 4). However, it was not affected by percent defoliation (Table 4). Defoliation of any amount or frequency at least doubled the number of axillary branches compared with control seedlings, but branching did not differ by species or light treatment (Fig. 3A, Table 4).

Stem and root dry mass were generally reduced for seedlings receiving 100% defoliation (once or twice) compared with non-defoliated plants, except for black swallow-wort stem mass under high light (Fig. 3C, 4; Table 4). Root dry mass was reduced under low compared with high

light conditions for both species, but stem mass was less affected by light levels (Fig. 3B, 4). Pale swallow-wort stem mass was only greater than black swallow-wort in the 100% defoliation (twice) treatment, the same treatment in which all the black swallow-wort died (Fig. 4). Pale swallow-wort root mass was greater under low, but not high, light conditions compared with black swallow-wort seedlings (Fig. 3B, Table 4). More crown buds were present on pale swallow-wort (5.6 ± 0.3 , $n=60$) than black swallow-wort (2.6 ± 0.2 , $n=60$) and on high light (4.9 ± 0.4 , $n=60$) than low light-grown seedlings (3.1 ± 0.3 , $n=60$; Table 4). Seedlings completely defoliated twice had fewer buds than most other plants (Fig. 3D). No seedlings of either species flowered during this experiment.

4. Discussion

4.1. Defoliation impact and plant stage

Pre-release impact studies are becoming more common for a variety of candidate agents (e.g., Balciunas and Smith, 2006; Ding et al., 2006; Gerber et al., 2007; Goolsby et al., 2004; Grevstad et al., 2013; Reddy and Mehelis, 2015), although to our knowledge few have involved defoliating moths (Weed and Casagrande, 2010; Weed et al., 2011a; present study). In all cases, within-season reductions in growth rates, biomass, and reproduction have been reported. Such information, whether from pre- or post-release impact studies, can be helpful in understanding future or realized control outcomes, including what densities of agents may need to occur (e.g., Balciunas and Smith, 2006; Berg et al., 2015).

The effect of increasing defoliation levels by *H. opulenta* was mostly negative and generally similar between the two swallow-wort species and, in some cases, between the two light environments as well. Larval defoliation often damaged the apical meristems, which shortened

the stem length in a few cases and tended to increase the production of axillary branches in seedlings and adult plants. Branching is one mechanism of plant compensation to damage that is frequently observed (Schat and Blossey, 2005; Strauss and Agrawal, 1999; Trumble et al., 1993).

Complete defoliation, caused by six larvae per stem in the case of mature plants, was needed to consistently reduce growth and reproduction (compared with non-defoliated plants) for seedlings and adult plants grown under the two lower light regimes we used to simulate different forest habitats. This could often be achieved with a single defoliation event. Contrary to our expectations, two rounds of 100% defoliation usually did not further reduce plant performance, the main exception being seed production for adult plants. This result applied even to seedlings in most cases. Seedlings are expected to be more severely affected by herbivory than mature plants (Strauss and Agrawal, 1999); however, it appears swallow-wort seedlings can tolerate high levels of defoliation. The main difference in plant performance between life stages and swallow-wort species involved the death of all black swallow-wort seedlings that had received two rounds of 100% defoliation under low light (discussed further below). Root mass reductions were not observed, nor necessarily expected, in adult plants given the short-term nature of this study. Several years of damage may be necessary to observe depletions of root reserves in long-lived perennial species such as swallow-worts (Hakansson, 1969; Ringselle et al., 2015). We also included a fairly long recovery period before harvesting plants (3 wk after the end of the second defoliation) because long periods of regrowth may follow the cessation of insect activity (Maschinski and Whitham, 1989). Unless other factors suppress plant regrowth, recovery of plants within a growing season can greatly minimize the impact of defoliators (Syrett, 1983; Winston et al., 2014).

Our results from the high light treatment were similar in some respects to a previous impact study by Weed and Casagrande (2010), which demonstrated a significant effect of a single defoliation on flowering pale swallow-wort, but not black swallow-wort, plants grown under fluorescent lights. Their results were confounded by a plant size difference; pretreatment data indicated that black and pale swallow-wort had 6.4 and 1.5 stems per plant, respectively. Larvae of *H. opulenta*, which were released on a per plant basis in their study, potentially had access to up to four times more leaf biomass on black swallow-wort. Both swallow-wort species can produce a variable number of stems per plant (one to over 20 in the case of black swallow-wort, personal observation). By standardizing defoliation damage across plants of different stem number, we have demonstrated that black swallow-wort biomass and seed production also can be significantly reduced by *H. opulenta*. As a practical matter, infesting multi-stemmed species of plants on a per-stem basis should allow for a more direct comparison of herbivore impact across experimental factors. Unfortunately, we were unable to directly compare adult plants of the two swallow-wort species. It thus remains unclear how different sizes of mature plants, as opposed to their species identity, might influence a plant's response to insect damage (e.g., Cartwright and Kok, 1985; Gerber et al., 2007).

Our results also were in partial agreement with those of a previous artificial defoliation study (Milbrath, 2008), including that the two swallow-wort species generally responded similarly to defoliation damage. However, the artificial defoliation study overestimated biomass and seed losses under low light (the only directly comparable light treatment with the present study). Mortality of defoliated plants under shade also was grossly overestimated (mature plants-64%, seedlings-95%) compared with the present study (mature plants-0%, seedlings-9%). In Milbrath (2008), mature plant rootstocks were transplanted for the experiment immediately following

vernalization of field-collected rootstocks. The rootstocks had lost much of their root mass, and hence reserves for regrowth, at collection. In essence this is a type of simulated root damage, and its combination with defoliation and low light may have contributed to plant mortality. No studies examining artificial or real herbivory on swallow-worts have explicitly combined root and leaf damage, so their combined impact on swallow-wort is unknown (Doubleday and Cappuccino, 2011; Maguire et al., 2011; Weed et al., 2011a). In the present study, mature plants were allowed to reestablish their root systems for one growing season before being defoliated. The plants therefore possessed a realistically larger pool of root reserves from which to regrow. We are unable to explain the difference in seedling survival between the two studies, as seedlings were treated similarly apart from the type of defoliation received (real or artificial). Simulated herbivory studies vary in accurately estimating the magnitude of plant impact from artificial means compared with actual herbivory (Schat and Blossey, 2005; Schooler et al., 2006; Wirf, 2006; Ziganira and Olckers, 2015). Thus, follow-up studies with agents should always be conducted (Raghu and Dhileepan, 2005).

4.2. Light environment

Differing light levels strongly affected plant responses, in many cases more than defoliation itself, and interactions with defoliation treatments were few (see also Milbrath, 2008). It was previously known that pale swallow-wort plants in forest habitats grow smaller, less densely, and have reduced (to no) seed production compared with plants in open fields or light gaps in forests (Averill et al., 2011; Doubleday and Cappuccino, 2011; Hotchkiss et al., 2008; Sheeley, 1992; Smith et al., 2006). However, if light levels within a forest understory or edge are high enough (e.g., 9-30% of ambient light), pale swallow-wort can grow and reproduce at levels comparable

to plants in open fields (Averill et al., 2011). Thus, different forest populations of swallow-wort vary in their productivity and potential tolerance to herbivory. The low light treatment in this study, simulating light levels in a deeply-shaded forest understory, consistently and greatly reduced plant growth and reproduction of pale and black swallow-wort compared with the higher light treatment, which was intended to simulate the light levels of a more open canopy forest understory or forest edge habitat. Larval defoliation further reduced biomass and seed to a similar or greater extent as that in the higher light environment (see also Doubleday and Cappuccino, 2011). Thus, our hypothesis that increased shading would enhance the effect of defoliation is only partially confirmed.

A combination of stressors may be necessary when utilizing some defoliators as biological control agents (Harris, 1981). Low light is a stress to plants and can enhance the effect of defoliation damage on perennial species, including causing plant death if both the light and defoliation stress is severe (Baraza et al., 2004; Blundell and Peart, 2001; Lentz and Cipollini, 1998; Norghauer et al., 2008). Lockett et al. (2012) observed a significant impact of *Chiasmia assimilis* (Warren) (Geometridae) on seedlings of prickly acacia [*Acacia nilotica* (L.) Delile subsp. *indica* (Benth.) Brenan) (Fabaceae) grown under a canopy (shade), but not for seedlings grown under full sun. Thus, herbivory by *H. opulenta* may be more damaging to swallow-wort in low-light environments relative to higher light habitats. In particular, black swallow-wort seedlings appear to be more sensitive to repeated defoliation damage than pale swallow-wort under the lowest light levels we investigated. This does not appear to be the case with established black swallow-wort plants. However, black swallow-wort is less commonly observed than pale swallow-wort in forests in North America, although the mechanism preventing its recruitment

into most forests is unclear. Pale swallow-wort is considered a shade-tolerant species and thus a greater threat to forest environments (Averill, 2009; Hotchkiss et al., 2008; Milbrath, 2008).

Control of swallow-wort populations in open field, high-light environments is particularly desired because the densities and reproductive output of swallow-wort species are the highest in these types of habitats and likely serve as the source of seeds for infesting other habitats (Averill et al., 2011; Smith et al., 2006). Weed and Casagrande (2010) hypothesized that *H. opulenta* may be restricted to forested habitats, and thus not attack open field patches of swallow-wort, but this remains to be documented. However, defoliation by the noctuid moth *Abrostola asclepiadis* (Denis and Schiffermüller) had no effect on swallow-wort plants under full sun conditions one year post-defoliation (Weed et al., 2011a). Also, mechanical control and artificial defoliation studies have demonstrated that seedlings and flowering plants of pale and black swallow-wort are extremely tolerant to aboveground tissue loss under full sun (Averill et al., 2008; Doubleday and Cappuccino, 2011; McKague and Cappuccino, 2005; Milbrath, 2008; Milbrath et al., 2016). It is currently unknown whether this would remain the case with increased intra- or interspecific plant competition (Harris, 1981, 1991; Wise and Abrahamson, 2007).

4.3. Defoliating Lepidoptera as agents

Defoliating insects have commonly been utilized as agents, in part because of their ease of discovery, rearing, and testing. Approximately 54 defoliating species of Lepidoptera (including leaf-tiers, leaf-rollers, and species whose early instars may also mine the leaves but excluding internal feeders like *Cactoblastis cactorum* (Berg)) have been released against c. 33 species of herbaceous and woody plants worldwide (Winston et al., 2014), excluding the recent release in Canada of *H. opulenta* (Young and Weed, 2014). This includes seven species from the family

Erebidae and eight species from the family Noctuidae (which formerly included the genus *Hypena*). Agents from the Erebidae are represented by different subfamilies such as the Hypeninae (e.g., *Hypena*) and Arctiinae (e.g., cinnabar moth, *Tyria jacobaeae* (L.); Zahiri et al., 2012). Of the 38 species that are established, several are considered to provide moderate to substantial control of the target weed, at least in some locations, e.g., *Actinote* spp. (Nymphalidae), *Pareuchaetes pseudoinsulata* Rego Barros and *T. jacobaeae* (Erebidae), and *Evippe* sp. (Gelechiidae) (Winston et al., 2014). For example, although in many areas *T. jacobaeae* is not considered an effective agent of tansy ragwort (*Jacobaea vulgaris* Gaertn., Asteraceae), defoliation damage combined with early frosts can kill rosettes in certain locations in eastern Canada and western U.S. (Harris, 1981; Syrett, 1983; Winston et al., 2014).

Other defoliating lepidopterans have had no to variable impact on their target plants, often because of low populations due, in part, to predation or parasitism (Winston et al., 2014 and references therein). *Hypena laceratalis* Walker (Erebidae), the only congener of *H. opulenta* to be used for biological control, was released against *Lantana camara* L. (Verbenaceae) in the 1950s and 1960s. Its impact is considered moderate at best, likely because of predation and parasitism (Baars, 2003; Winston et al., 2014). Wirf (2006) suggested that *Macaria pallidata* (Warren) (Geometridae) would be a promising agent against *Mimosa pigra* L. (Fabaceae) based on a post-release impact study with potted plants. However, the moth's field populations fluctuate widely, likely due to the availability of food relative to seasonal rainfall (Heard et al., 2010).

Plants targeted for control have also been noted as outgrowing even serious defoliation damage (Winston et al., 2014). In regards to plant impact, Peterson et al. (2005) showed no alteration in photosynthetic rates of Dalmatian toadflax (*Linaria dalmatica* L. Miller,

Plantaginaceae) due to the moth *Calophasia lunula* Hufnagel (Noctuidae), in contrast to marked changes which resulted from the stem-boring *Mecinus janthinus* Germar (Coleoptera: Curculionidae). Thus, abiotic and biotic factors that prevent population increase of an agent or a limited ability to affect plant performance have reduced the effectiveness of many defoliating lepidopteran agents. The addition of other types of stress to the plant, such as low light, may be needed if defoliating moths are to be effective agents.

4.4. Implications for swallow-wort biological control

All life stages of pale and black swallow-wort appear to be able to tolerate severe defoliation damage under full sun conditions (without plant competition), at present based primarily on simulated herbivory studies, which calls into question the efficacy of a defoliating agent in open field habitats (Milbrath, 2008; Milbrath et al., 2016; Weed et al., 2011a). In forests, the relative impact of *H. opulenta* on pale and black swallow-wort plants should increase as levels of canopy shading increase, particularly heavy shading which serves as a source of environmental stress for these invasive species (Averill et al., 2011; Smith et al., 2006). With one complete defoliation event by *H. opulenta*, plants may fully compensate to undercompensate in biomass and undercompensate in seed production within the growing season. Two rounds of complete defoliation, which *H. opulenta* could provide, will further reduce reproduction, potentially eliminating it under low light levels. Only under heavy shade might we expect mortality of black swallow-wort seedlings. Pale swallow-wort (seedlings and adults) and adult black swallow-wort will likely survive, at least in the short term.

Undercompensation in reproductive output is generally considered more important than biomass compensation because overall plant fitness is reduced (Maschinski and Whitham, 1989;

Strauss and Agrawal, 1999). However, in the case of long-lived, polycarpic perennial plants like the swallow-worts, survival is often more influential in the plant's population dynamics than fecundity (Franco and Silvertown, 2004). It may take several seasons of repeated and complete defoliation to reduce the root biomass of mature plants and effect a plant's ability to regrow and ultimately survive in a forest environment (Hakansson, 1969; Ringselle et al., 2015). With more moderate defoliation, seedling and adult pale and black swallow-wort may still be significantly affected if repeated defoliation occurs. Based on this study and Weed and Casagrande (2010), seedlings and mature plants may be able to compensate in most cases for percentage defoliation of less than 50%, although this needs further investigation. Incorporation of these and other impact data into a population matrix model that has been developed for black and pale swallow-wort (Milbrath and Davis, 2014) will allow for projections of population changes in different swallow-wort populations and the creation of testable hypotheses of *H. opulenta*'s post-release efficacy.

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Figure 1. Mean (+ SE) (A) stem length, (B) aboveground dry mass, and (C) seeds per plant of mature black swallow-wort under different larval defoliation and light treatments (see methods for treatment descriptions). Bars denoted by the same letter are not significantly different (Fisher's protected LSD test with Bonferroni correction, $P > 0.05$, $n = 11-12$ except $n = 5-6$ for seeds).

Figure 2. Mean (+ SE) (A) stem length compared at the mean pre-stem count value of 6.5, (B) aboveground dry mass, (C) number of axillary branches, and (D) seeds per plant of mature pale swallow-wort under different larval defoliation and light treatments (see methods for treatment descriptions). Bars denoted by the same letter are not significantly different (Fisher's protected LSD test with Bonferroni correction, $P > 0.05$, $n = 12$ for stem length and dry mass, $n = 6$ for branches and seeds).

Figure 3. Mean (+ SE) (A) number of branches, (B) root dry mass by light, (C) root dry mass by damage, and (D) crown bud number of swallow-wort (SW) seedlings under different larval defoliation and light treatments (see methods for treatment descriptions). Bars denoted by the same letter are not significantly different (Fisher's protected LSD test with Bonferroni correction, $P > 0.05$, $n = 24$ for branches and buds, $n = 30$ for root mass by light, $n = 12$ for root mass by damage).

Figure 4. Mean (+ SE) stem dry mass of black and pale swallow-wort (SW) seedlings under different larval defoliation and light treatments (see methods for treatment descriptions). Bars for any species-light-defoliation treatment combination denoted by the same letter are not

significantly different (Fisher's protected LSD test with Bonferroni correction, $P > 0.05$, $n=6$).

For example, 'a-e' is 'abcde'.

Table 1. Percentage defoliation (mean $\pm$ SE) and analysis of covariance results for reduced models of the effect of different larval densities of *Hypena opulenta* and frequency of defoliation on mature swallow-wort plants under high- and low-light conditions

Table 2. Analysis of covariance results for reduced models of the effect of different larval densities of *Hypena opulenta* and frequency of defoliation (damage) on growth and reproduction of mature swallow-wort plants under high- and low-light conditions (light), with pretest stem number (Pre-count) serving as a covariate^a

Table 3. Growth (mean $\pm$ SE) of mature swallow-wort plants exposed to high- and low-light conditions averaged over different larval defoliation treatments with *Hypena opulenta*^a

Table 4. Analysis of variance results for reduced models of the effect of different defoliation levels by *Hypena opulenta* and frequency of defoliation (damage) on pale and black swallow-wort seedlings (species) under high- and low-light conditions (light)^a

Table 1. Percentage defoliation (mean $\pm$ SE) and analysis of covariance results for reduced models of the effect of different larval densities of *Hypena opulenta* and frequency of defoliation on mature swallow-wort plants under high- and low-light conditions

Damage ^a	First defoliation ^b				Second defoliation ^b			
	Black swallow-wort		Pale swallow-wort		Black swallow-wort		Pale swallow-wort	
	Low light	High light	Low light	High light	Low light	High light	Low light	High light
Control	0 $\pm$ 0 c	^b	0 $\pm$ 0 e	0 $\pm$ 0 e	0 $\pm$ 0 c	0 $\pm$ 0 c	0 $\pm$ 0 c	0 $\pm$ 0 c
1x-3L	64.5 $\pm$ 0.1 b	^b	82.5 $\pm$ 0.1 c	71.2 $\pm$ 0.1 cd	--	--	--	--
2x-3L	62.4 $\pm$ 0.1 b	^b	90.2 $\pm$ 0.1bc	60.1 $\pm$ 0.1 d	100.0 $\pm$ 0.0 a	56.3 $\pm$ 0.1 b	99.9 $\pm$ 0.0 a	65.1 $\pm$ 0.1 b
1x-6L	99.0 $\pm$ 0.1 a	^b	100.0 $\pm$ 0.0 a	100.0 $\pm$ 0.0 a	--	--	--	--
2x-6L	99.6 $\pm$ 0.1 a	^b	99.8 $\pm$ 0.0 a	97.1 $\pm$ 0.1 ab	100.0 $\pm$ 0.0 a	90.6 $\pm$ 0.1 ab	100.0 $\pm$ 0.0 a	100.0 $\pm$ 0.0 a
Factor	F-value (df) ^c	P-value	F-value (df)	P-value	F-value (df)	P-value	F-value (df)	P-value
Light	1.97 _{1,5}	0.219	0.79 _{1,5}	0.414	13.65 _{1,5}	0.014	56.98 _{1,5}	<0.001
Damage	99.08 _{4,42}	<0.001	21.17 _{4,30}	<0.001	153.20 _{2,19}	<0.001	1347.00 _{2,19}	<0.001
Pre-stem count	2.90 _{1,42}	0.096	34.27 _{1,30}	<0.001	0.87 _{1,19}	0.361	0.01 _{1,19}	0.907
Light*Damage	--	--	1.82 _{4,30}	0.151	7.04 _{2,19}	0.005	56.46 _{2,19}	<0.001
Pre*Light	--	--	0.02 _{1,30}	0.887	--	--	--	--
Pre*Damage	--	--	8.72 _{4,30}	<0.001	--	--	--	--
Pre*L*D	--	--	3.03 _{4,30}	0.033	--	--	--	--

^a Control: no larvae, 1x-3L: plants infested once with three larvae per stem, 2x-3L: plants infested twice with three larvae per stem, 1x- 6L: plants infested once with six larvae per stem, 2x-6L: plants infested twice with six larvae per stem

5 ^b For the first round of defoliation with black swallow-wort, means are averaged across both light environments ($n=11-12$); with pale swallow-wort, treatment
6 means compared at the mean pre-stem count value of 6.5 ($n=6$). For the second defoliation, $n=5-6$. For each round of defoliation and swallow-wort species,
7 individual means followed by the same letter are not significantly different (F-protected LSD test with Bonferroni correction, $P > 0.05$).
8 ^c Subscripts are numerator and denominator degrees of freedom. Non-significant interaction terms ($P>0.05$) were iteratively removed beginning with the full
9 (three-factor) model.

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Table 2. Analysis of covariance results for reduced models of the effect of different larval densities of *Hypena opulenta* and frequency of defoliation (damage) on growth and reproduction of mature swallow-wort plants under high- and low-light conditions (light), with pretest stem number (Pre-count) serving as a covariate^a

Effect	Axillary													
	Stem no.		Stem length		branches		Crown buds		Aboveground mass		Root mass		Seeds	
Black swallow-wort														
Light (L)	3.78 _{1,5}	0.110	33.39 _{1,5}	0.002	3.15 _{1,5}	0.136	0.01 _{1,5}	0.941	33.57 _{1,5}	0.002	4.09 _{1,5}	0.099	21.99 _{1,5}	0.005
Damage (D)	4.64 _{4,38}	0.004	7.42 _{4,42}	<0.001	2.05 _{4,42}	0.105	1.31 _{4,40}	0.283	12.87 _{4,42}	<0.001	0.90 _{4,42}	0.472	21.38 _{4,38}	<0.001
Pre-count	147.04 _{1,38}	<0.001	2.76 _{1,42}	0.104	5.64 _{1,42}	0.022	9.98 _{1,40}	0.003	3.82 _{1,42}	0.057	17.57 _{1,42}	<0.001	0.0 _{1,38}	0.971
L*D	--	--	--	--	--	--	--	--	--	--	--	--	4.96 _{4,38}	0.003
Pre*D	10.71 _{4,38}	<0.001	--	--	--	--	--	--	--	--	--	--	--	--
Pale swallow-wort														
Light	0.45 _{1,5}	0.531	6.48 _{1,5}	0.052	3.14 _{1,5}	0.137	0.15 _{1,5}	0.716	131.09 _{1,5}	<0.001	34.43 _{1,5}	0.002	250.68 _{1,5}	<0.001
Damage	1.58 _{4,43}	0.197	2.65 _{4,39}	0.047	3.91 _{4,39}	0.009	0.58 _{4,42}	0.680	27.76 _{4,43}	<0.001	1.52 _{4,43}	0.212	36.06 _{4,39}	<0.001
Pre-count	355.42 _{1,43}	<0.001	16.21 _{1,39}	<0.001	0.60 _{1,39}	0.442	2.67 _{1,42}	0.109	0.39 _{1,43}	0.536	1.80 _{1,43}	0.187	8.23 _{1,39}	0.007
L*D	--	--	--	--	7.15 _{4,39}	<0.001	--	--	--	--	--	--	10.40 _{4,39}	<0.001
Pre*D	--	--	2.91 _{4,39}	0.033	--	--	--	--	--	--	--	--	--	--

^a Values given under each parameter are: F-value, numerator and denominator degrees of freedom (subscript), P-value. For each plant parameter within each swallow-wort species, non-significant interaction terms (P>0.05) were iteratively removed beginning with the full (three-factor) model.

Table 3. Growth (mean $\pm$ SE) of mature swallow-wort plants exposed to high- and low-light conditions averaged over different larval defoliation treatments with *Hypena opulenta*^a

Light ^b	Stem length (cm)		Aboveground dry mass (g)		Root dry mass (g)	
	Black swallow-wort	Pale swallow-wort	Black swallow-wort	Pale swallow-wort	Black swallow-wort	Pale swallow-wort
High	198.2 $\pm$ 8.1 a	158.7 $\pm$ 4.3 a	4.8 $\pm$ 0.6 a	18.5 $\pm$ 1.0 a	4.0 $\pm$ 0.4 a	14.9 $\pm$ 0.9 a
Low	131.4 $\pm$ 8.2 b	143.1 $\pm$ 4.5 a	1.6 $\pm$ 0.2 b	9.0 $\pm$ 0.5 b	3.0 $\pm$ 0.3 a	9.0 $\pm$ 0.6 b

^a Means within each column followed by the same letter are not significantly different (F-protected LSD test with Bonferroni correction, $P > 0.05$, $n=29-30$).

^b High = light levels simulating a high light forest understory, Low = light levels simulating a low light forest understory (see Materials and Methods for values)

Table 4. Analysis of variance results for reduced models of the effect of different defoliation levels by *Hypena opulenta* and frequency of defoliation (damage) on pale and black swallow-wort seedlings (species) under high- and low-light conditions (light)^a

Effect	Stem no.		Stem length		Axillary branches		Crown buds		Stem mass		Root mass	
Species (S)	29.2 _{1,99}	<0.001	19.62 _{1,101}	<0.001	0.34 _{1,103}	0.558	98.65 _{1,103}	<0.001	2.34 _{1,90}	0.130	46.76 _{1,98}	<0.001
Light (L)	0.09 _{1,5}	0.775	33.30 _{1,5}	0.002	6.27 _{1,5}	0.054	16.89 _{1,5}	0.009	106.82 _{1,5}	<0.001	75.75 _{1,5}	<0.001
Damage (D)	2.88 _{4,99}	0.026	1.78 _{4,101}	0.139	7.94 _{4,103}	<0.001	7.33 _{4,103}	<0.001	32.87 _{4,90}	<0.001	15.04 _{4,98}	<0.001
S*L	--	--	--	--	--	--	--	--	3.75 _{1,90}	0.056	12.85 _{1,98}	<0.001
S*D	2.88 _{4,99}	0.026	--	--	--	--	--	--	3.99 _{4,90}	0.005	3.24 _{4,98}	0.015
L*D	--	--	--	--	--	--	--	--	3.45 _{4,90}	0.011	--	--
S*L*D	--	--	--	--	--	--	--	--	4.82 _{4,90}	0.001	--	--

^a Values given under each parameter are: F-value, numerator and denominator degrees of freedom (subscript), P-value. For each plant parameter, non-significant interaction terms (P>0.05) were iteratively removed beginning with the full (three-factor) model.

Figure 2

Figure 3

Figure 4