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SHORT COMMUNICATION



## Establishment of *Hypena opulenta* (Lepidoptera: Erebidae) on *Vincetoxicum rossicum* in Ontario, Canada

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### ABSTRACT

*Hypena opulenta*, a moth native to Ukraine, was released in 2013–2014 as a biocontrol agent for pale swallow-wort *Vincetoxicum rossicum*, an invasive vine, in Ottawa, Ontario, Canada. Blacklight trapping and surveys of feeding damage showed that *H. opulenta* has successfully established, increased in abundance and spread up to 2.0 km.

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Invasive species; weed biological control; dog-strangling vine; swallow-wort; Lepidoptera; Erebidae

European swallow-worts *Vincetoxicum* spp., herbaceous vines in the dogbane family (Apocynaceae), have become serious invasive plants in eastern North America. Pale swallow-wort *Vincetoxicum rossicum*, (Kleopow) Barbar. (syn. *Cynanchum rossicum* (Kleopow) Borhidi), which is commonly known as dog-strangling vine (DSV), is native to southeastern Ukraine and southwestern Russia (DiTommaso, Lawlor, & Darbyshire, 2005). It has become a significant problem in Ontario and Quebec over the past few decades, showing a rapid increase in geographic range, plant cover and density (Bouchier et al., 2013). Black swallow-wort *Vincetoxicum nigrum* (L.) Moench is present in Ontario but to date is not as serious a problem as *V. rossicum*, whereas both species are high invasive problems in the eastern United States, depending on geographic location (DiTommaso et al., 2005). Pale swallow-wort invades forest understories, plantations, field edges and open fields (DiTommaso et al., 2005). Its success is based on the rapid growth of twining stems, high production of wind-dispersed polyembryonic seeds, the presence of anti-feedant and antifungal compounds that affect local habitats, and regenerative root crowns (DiTommaso et al., 2005; Mogg et al., 2008). Costly ongoing efforts using conventional control methods such as mowing, hand pulling, and herbicides have largely been unsuccessful in eliminating established infestations (DiTommaso et al., 2005), which continue to spread with no significant native herbivory (Ernst & Cappuccino, 2005).

*Hypena opulenta* Christoph (Lepidoptera: Erebididae) is a multivoltine moth originating in eastern Europe, which as a larva is a specialist herbivore that only completes its development on *Vincetoxicum* spp. (Hazlehurst, Weed, Tewksbury, & Casagrande, 2012; Weed & Casagrande, 2010). Host-range testing on 82 plant species confirmed that *H. opulenta* was restricted to feeding on the genus *Vincetoxicum* (Hazlehurst et al., 2012; Weed & Casagrande 2010). A joint petition for the release of *H. opulenta* in Canada and the United States was submitted in 2011 (Casagrande et al., 2011) and a letter of release for the insect in Canada was granted in 2013.

Post-release monitoring of weed biocontrol agents is important to verify their establishment, spread, abundance and ability to affect their target weed (Morin et al., 2009). Here, we report on the release, initial establishment and spread of *H. opulenta* at the Central Experimental Farm (CEF, 45.392473°, -75.717402°) in Ottawa, Ontario, Canada.

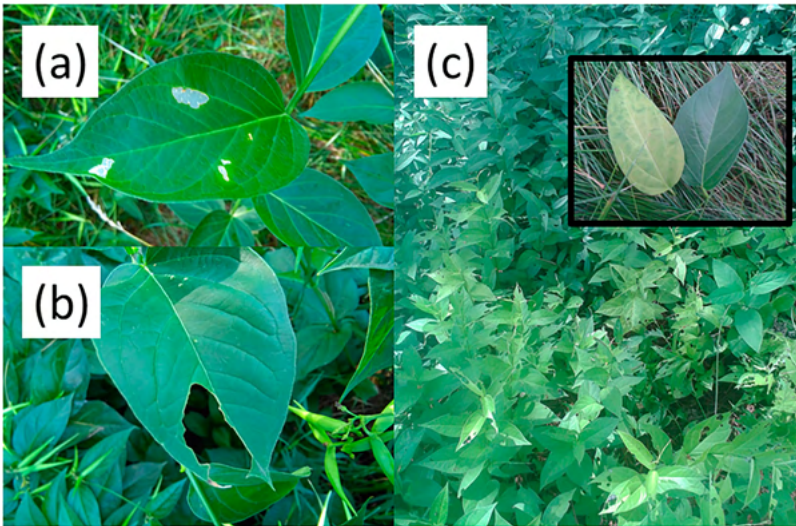
All *H. opulenta* released were from populations collected in Donetsk, Ukraine during 2006 and 2012 and screened in the host-range testing (see Weed & Casagrande, 2010 for site details). *H. opulenta* has been continually maintained in rearing colonies at the University of Rhode Island (URI) (Tewksbury and Casagrande) since 2008 and at Agriculture and Agri-Food Canada (Bourchier) since 2012.

The initial *H. opulenta* release at the CEF took place in September 2013 and included 400 fourth instars and 110 second and third instars that had been reared under short photoperiod (12L: 12D), diapause-inducing conditions at URI. Our objective with this late release date was to see whether the larvae would be able to complete feeding in the field, pupate and overwinter successfully. A 50-m permanent transect with sampling points at 2-m intervals was set up in a DSV stand (15 m × 60 m) under Scots pine (*Pinus sylvestris* L.). The larvae were released in a 1-m<sup>2</sup> quadrat at the northern end the transect.

Between 28 June and 5 July 2014, we released 1445 second and third instars, evenly split between two 1-m<sup>2</sup> quadrats: one at the northern end of the 50-m transect and the second, 30 m to the south of the first. The permanent sampling transect to estimate feeding damage did not include the release quadrats.

Larvae of *H. opulenta* are difficult to monitor directly because they are nocturnal (AR, unpublished data) and cryptic. Late instars often rest away from their feeding damage and they drop off the plant when disturbed. Thus, we monitored the presence of larvae by their distinctive feeding damage on the leaves (Figure 1(a, b)). First and second instars feed from the undersides of leaves without breaking through the topmost leaf membrane, forming an irregular 'windowpane' (Figure 1(a)). Third instars make roughly oval holes in the leaves, while larger instars consume ¼ of the leaf or more in a single feeding bout, generally starting from the distal end and moving towards the petiole (Figure 1(b)). Aside from two-spotted spider mites *Tetranychus urticae* Koch (Acari: Tetranychidae), we have observed almost no herbivores or feeding damage on DSV at the CEF or elsewhere. On the few occasions where damage has been observed, it did not resemble the distinctive feeding damage caused by *H. opulenta*.

To monitor *H. opulenta* and its impact on DSV, we sampled leaves for feeding damage annually along the permanent 50-m transect (excluding the release quadrats) in late August or early September 2014–2018, after the second generation of *H. opulenta* had completed its development but before the leaves had senesced. At each 2-m interval along the transect, 20 leaves were chosen haphazardly by reaching into the mass of



**Figure 1.** Feeding damage on *V. rossicum* (DSV) caused by (a) first and second instars and (b) fourth and fifth instars of *Hypena opulenta*. (c) Yellowing of *V. rossicum* in response to feeding by *H. opulenta*. Inset: leaf on the left is from a stem where other leaves had *H. opulenta* feeding; leaf on the right is from a non-attacked stem.

foliage and removing the first leaf encountered. In late August 2018, in addition to the permanent transect samples, supplemental leaf samples were taken in 20 quadrats in a hedgerow 50 m to the north of the original release site. A second supplemental sample of 20 leaves was taken at a location with an exceptionally high level of feeding damage in a hedgerow approximately 200 m from the release site. Leaves were photographed against a white background and ImageJ 1.x (Schneider, Rasband, & Eliceiri, 2012) was used to estimate percentage leaf area eaten.

Because *H. opulenta* was at the threshold of detectability using random sampling in the initial years after release, we also carried out timed searches for evidence of larval feeding by walking through the release site for a period of 0.5–1.0 h and recording any feeding damage observed. The timed searches were conducted in late July to survey feeding damage caused by the first generation and in late August or early September to survey feeding by the second generation. Searches were also carried out along hedgerows at the CEF and the nearby Dominion Arboretum (45.391019°, –75.703837°), Fletcher Wildlife Garden (45.387288°, –75.703240°) and Hog’s Back Park (45.370757°, –75.697924°).

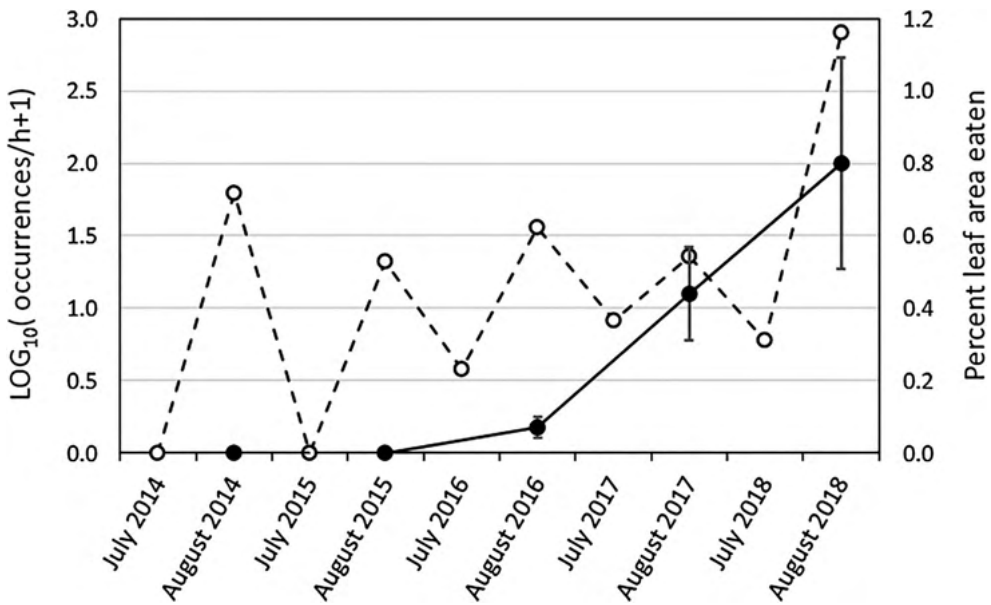
In late August 2014 and again in mid-August 2015, laboratory-reared adults (158 in 2014, 60 in 2015) were released into a large field cage (152 × 152 × 152 cm) placed over patches of DSV foliage to assess the overwintering ability of their offspring. Moths in the cage were provided with vials containing 10% sucrose with a dental wick protruding through a hole in the vial cap from which they could feed. By mid-September of both years, the cages contained larvae, many of which were in the fifth instar; however, it was unknown how many successfully pupated before the foliage quality deteriorated. The mesh of the cage was taken off the frame before the first snow and reinstalled in early May of both years.

On 4 June 2016, we set up a light trap using a 365 nm UV LED emitter (Mouser Electronics, Mansfield, TX) to illuminate a nylon collecting sheet (Bioquip Products, Rancho Dominguez, CA). The trap was placed in an open field at the edge of the main stand of DSV where *H. opulenta* was first released and monitored for 2 h (21:00–23:00 EDT). A second trap was set up in a large stand of DSV approximately 900 m from the release site.

In June 2014, following the initial *H. opulenta* release at the CEF in September 2013, we saw no larvae or evidence of feeding. The second release of larvae in late June and early July 2014, resulted in substantial damage to the plants at the immediate release points: the average percentage of leaf area consumed was  $28.2 \pm 5.1$  (mean  $\pm$  SE) in the northern quadrat and  $21.5 \pm 5.3$  in the southern quadrat. The mobile larvae produced smaller amounts of damage (<5% of leaf area consumed) at transect points 2 m from the release points. Feeding damage estimates from the release points were not included in Figure 2 because the release points were not part of the permanent transects.

The offspring of the released larvae were observed on 24 August 2014, when, over the course of a 1-h search, feeding damage was seen in 10 locations dispersed throughout the site and the nearby hedgerows as far as 50 m from the release points (Figure 2). A fortuitous sighting was made on 8 September, when feeding damage and one fourth instar were observed along a hedgerow 210 m from the northern release point. Despite these observations, no larval feeding damage was found in the leaf samples along the permanent transect in 2014 (Figure 2).

Overwintering success was confirmed on 26 May 2015, when two adults emerged in the field cage. Laboratory-reared adults were then placed in the field cage to allow us to track



**Figure 2.** Indices of *H. opulenta* abundance at original release site on the Central Experimental Farm, Ottawa, Ontario from 2014 through 2018. Percentage leaf area eaten (solid line, filled points) was measured on haphazardly selected leaves in quadrats only along a permanent transect and did not include the original release quadrats;  $\log_{10}$ -transformed number of feeding occurrences (dashed line, open points) are from timed searches at the release site.

phenology. Eggs were observed on foliage in the cage on 6 June and first instars were seen on 11 June. At this time, despite several hours of searching, no naturally occurring larvae or damaged leaves were seen within the release site. The new generation of adults began emerging in the field cages on 15 July. On 4 August, when new larvae were present in the cages, leaves damaged by naturally occurring larvae were also observed at 20 locations throughout the main DSV stand and the nearby hedgerows (Figure 2). As in the previous year, however, this second generation was not abundant enough to detect in the leaf samples (Figure 2).

Successful overwintering was again confirmed in 2016 when three adult *H. opulenta* were attracted to the blacklight trap placed at the release site on 4 June. The second trap in a stand of DSV 900 m from the release site yielded no *H. opulenta*. A single adult emerged in June 2016 in the field cage.

The 2016 season marked the first time we observed a naturally occurring early-summer generation of larvae. Between 5 July and 2 August, a total of 5 hours of searching revealed 14 occurrences of feeding damage (Figure 2). The second generation was more abundant: 70 new occurrences were found on 16 and 18 August in just two hours of searching.

In 2016, *H. opulenta* density was sufficiently high that damage could be detected in the samples along the permanent transect. Feeding damage occurred in seven out of 25 sample locations (Figure 2). For individual leaves that were damaged, the percentage area consumed was <10% ( $3.39 \pm 0.65$ , mean  $\pm$  SE). At the scale of the 20-leaf sample, mean percentage area consumed was <0.5% ( $0.25 \pm 0.06$ ) for quadrats with damage and <0.1% overall ( $0.07 \pm 0.03$ ).

The percentage leaf area eaten along the permanent transect has risen continuously, despite drought and an infestation of two-spotted spider mites in 2018 that caused many plants along the transect to lose their leaves while the summer adults were ovipositing. In early September of 2018, the timed searches in the less drought-stricken areas of the main CEF release site revealed a substantial increase in the number of feeding occurrences (Figure 2). The supplemental sample of 20 locations along a hedgerow approximately 50 m away from the permanent transect yielded a mean percent leaf area eaten of  $1.2 \pm 0.3\%$ , which was slightly higher than the value observed in 2018 on the permanent transect (Figure 2). For the second supplemental sample, in the area with the highest level of damage approximately 200 m from the main DSV stand, 95% of the leaves were damaged and the mean percent leaf area eaten was  $20.5 \pm 3.1$ .

By 2017, feeding damage observations indicated that *H. opulenta* had spread to the Dominion Arboretum (0.5 km) and Hog's Back Park (2.0 km). In 2018, damage and larvae were observed throughout the Arboretum (up to 0.8 km from the release site) and damage was observed in the Fletcher Wildlife Garden (0.6 km).

Based on our monitoring from 2014 to 2018, we can confirm the establishment of the moth, *H. opulenta*, at the Central Experimental Farm in Ottawa. This is the first establishment of this insect in North America and the first biological control agent available for DSV. Confirmation of successful overwintering and establishment is based on successful light trapping of three adult moths on 4 June 2016, as well as the presence of larvae and feeding damage from 2014 through 2018, indicating that there have been two successful generations each year. In addition, larvae and feeding damage observed throughout the hedgerows at CEF, the Dominion Arboretum, the Fletcher Wildlife Garden and Hog's Back Park indicates that *H. opulenta* has spread up to 2 km from the release site.

Although the density of *H. opulenta* increased between 2014 and 2018 to the point at which larval damage was detectable in samples along a permanent transect at the original release site, the level of damage throughout most of the area is still well below that which is necessary to negatively affect plant fitness. Feeding from second instar to pupation by as few as two larvae per plant has been shown to significantly reduce above-ground biomass and seed set in the laboratory (Weed & Casagrande, 2010). However, rather than laying clusters of eggs that might generate high local densities in the field, *H. opulenta* females appear to be spreading their offspring throughout the stand of DSV. The larvae are also quite mobile; fourth and fifth instars commonly move among plants as they feed and even the tiny first instars do not remain long on a single leaf (NC, unpublished data). Both the oviposition behaviour of the females and the movement of the larvae result in damage that is widely dispersed spatially. Thus, the density that must be attained locally to cause substantial damage to the plants is unlikely to be seen on a large scale until the overall density of the population is high throughout the DSV stand. In 2018, we saw the first evidence that higher local densities could be attained in a hedgerow 200 m from the original release site. Our damage estimate of 20.5% of the leaf area eaten in that hedgerow, was much higher than the estimate observed in the quadrats along the permanent transect (Figure 2) and approached the level observed at the original release points from 2014 (21.5% and 28.2%).

In areas where larval density is concentrated at the time of larval releases, we have observed the systemic yellowing of entire DSV stems that have had larval feeding (Figure 1(c)). This yellowing may be a stress response or early senescence of plants similar to the yellowing that happens later in the season. We have observed similar systemic yellowing following other releases (RB, unpubl.); however, the yellowing response was not observed in the highly damaged hedgerow patch during 2018. It is possible that foliage yellowing requires severe early-season defoliation. We have not yet observed patches of DSV severely damaged by the first generation of *H. opulenta*.

Successful weed biological control is often a slow process; for example, reductions in the number of seedheads of spotted knapweed (*Centaurea stoebe* L. spp. *micranthos* (Gugler)) required 30 years and the release of 5 agents (Story, Smith, Corn, & White, 2008). In comparison, the timeline for *H. opulenta* from the first releases in 2013 and 2014, to the first establishment in 2016, and to the first evidence of substantial local damage in 2018 has been relatively fast. However, we are still at an early point in the release programme. With continued sampling and the addition of monitoring for adults using light traps and, if available, pheromones (Jeremy Allison unpubl.), the continued spread of *H. opulenta* from this initial location is expected to be confirmed.

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## Disclosure statement

The authors declare no conflicts of interest.

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