

## RESEARCH ARTICLE

# An experimental application of *Hypena opulenta* as a biocontrol agent for the invasive vine *Vincetoxicum rossicum*

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## Abstract

1. Pre-release testing for biological control agents is focused primarily on assessment of host-range specificity and safety of potential agents. Agent impact is considered pre-release; however, the ultimate assessment of an agent must occur following release in the field under the target population levels and conditions of the invaded ecosystems. The invasive Eurasian vine, *Vincetoxicum rossicum*, has spread aggressively through its invaded range of eastern North America since its initial introduction in the late 1800s. In laboratory tests, the Eurasian moth *Hypena opulenta* has shown great promise as a potential control agent for *V. rossicum*.

2. We were interested in the defoliating ability of *H. opulenta* and its subsequent effect on the seed production of *V. rossicum* under field conditions. To examine this, we established a field site near Kirkfield, Ontario, that consisted of meadow and forest understory plots, both of which were highly invaded by *V. rossicum*.

3. We report highly significant feeding by *H. opulenta* in both light conditions. Unexpectedly, we observed a significant increase in seed production following folivory in shade conditions. We observed no significant effect of larval folivory on seed production under sun conditions, where *V. rossicum* seed production is greater by a factor of 10 as compared to shade conditions.

4. It is unclear how continuous exposure to folivory by *H. opulenta* will affect mature *V. rossicum* stands, although it might be expected that such populations would invest in defenses to herbivory, possibly at the expense of reproductive output. In order to better understand if *V. rossicum* populations in either light condition could exhibit longer-term compensatory growth in response to folivory, further experimental work is needed that examines inter-annual variability in *V. rossicum* reproduction at variable *H. opulenta* densities.

## KEYWORDS

biological control, compensatory growth, folivory, invasive species management, plant–herbivore interactions, plant invasion

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## 1 | INTRODUCTION

Introduced invasive plants can spread rapidly through ecosystems, and in extreme cases result in near monocultures that cause local extirpations of native species and disrupt ecological functioning (Vilà & Hulme, 2017; Livingstone, Isaac, & Cadotte, in press). In these extreme cases, the effort and cost of chemical or physical control can preclude most viable management options (Culliney, 2005; Rejmánek & Pitcairn, 2002). Classical biological control (hereafter 'biocontrol') has been a sustainable solution for many problematic invasive plants (Clewley, Eschen, Shaw, & Wright, 2012; Hinz, Schwarzländer, Gassmann, & Bouchier, 2014) and has been identified as an important component of integrated ecosystem management plans (Denslow & D'Antonio, 2005; Lake, Hough-Goldstein, & D'Amico, 2014; Seastedt, 2015). As with all ecosystem management tactics, it is critical to assess both the potential efficacy and associated risks of biocontrol (Kopf et al., 2017; Simberloff, 2012). While pre-release testing for biocontrol agents can indicate agents that are potentially effective and represent a relatively low-risk of impact on non-target species (Casagrande et al., 2011; Hazlehurst, Weed, Tewksbury, & Casagrande, 2012), their actual efficacy and impact is more difficult to predict and can best be assessed in the field under the conditions of the invaded ecosystems.

The extremely problematic invasive vine, *Vincetoxicum rossicum* (Kleopow) Borhidi (Apocynaceae), commonly known as pale swallowwort or 'dog-strangling vine', has spread aggressively through its invaded range of eastern North America, following its introduction in the late 1800s. With the spread and apparent impact of *V. rossicum*, understanding its distribution and ecology (Averill, DiTommaso, Mohler, & Milbrath, 2011; DiTommaso, Lawlor, & Darbyshire, 2005; Milbrath, Davis, & Biazzo, 2017; Miller & Kricsfalusy, 2007), the mechanisms driving its invasion (Bongard, Butler, & Fulthorpe, 2013; Cappuccino, 2004), its impact on ecosystems (Ernst & Cappuccino, 2005; Bugiel, Livingstone, Isaac, Fulthorpe, & Martin, 2018; Livingstone et al., in press) and the effectiveness of physical and chemical control (Averill et al., 2011) have all been important research priorities. *V. rossicum* is a perennial vine. Its stems typically range from 40 to 200 cm in length, emerge vertically in late April–early May, and then begin to twine together in mid-summer if they do not find a climbing structure (DiTommaso et al., 2005). Its climbing habit is consistent across variable substrates (DiTommaso et al., 2005). Mature *V. rossicum* individuals are multistemmed, producing between 5 and 25 stems per individual (Milbrath, DiTommaso, Biazzo, & Morris, 2016). After twining together, the tangled stems form a dense mat of vegetation that shades the ground. The onset of *V. rossicum*'s flowering typically occurs in early-to-mid June, reaching a peak in early July (Smith, DiTommaso, Lehmann, & Greipsson, 2006). *V. rossicum*'s follicle production has been observed to begin as early as mid-June, but peak follicle production typically occurs in early-to-mid August (Smith et al., 2006). Some inter-annual variation in the timing of flowering and follicle production has been observed (Smith et al., 2006). *V. rossicum*'s fruits are slender follicles (i.e. seed pods) that range from 4 to 7 cm long (Smith et al., 2006). The number of viable seeds contained in each follicle has been found to range from 7 to 10, with lower numbers of seeds being produced in

the shade (Smith et al., 2006). The vast majority of *V. rossicum*'s seeds tend to fall within 1 m of the parent plant (Ladd & Cappuccino, 2005), but the seed's feathery pappus can also facilitate long-distance dispersal during high wind events. *V. rossicum*'s seeds have a high probability of being polyembryonic, a trait which is more pronounced in individuals growing in high-light environments (i.e. each seed can produce one to four individuals; Hotchkiss, DiTommaso, Brainard, & Mohler, 2008; Smith et al., 2006). *V. rossicum* has become dominant in both forest edges and understory habitats in its invaded range; an environmental niche that it also occupies in its native range in Eurasia, but at extremely low densities. It has also come to dominate open fields in eastern North America; an environmental niche that *V. rossicum* does not occupy in its native range (DiTommaso et al., 2005). Following the establishment of *V. rossicum*, the biotic and chemical composition of soil is altered by the plant's release of allelochemicals, via root exudation and leaf leachates. The input of these allelochemicals by *V. rossicum* has been shown to alter soil microbial communities (Douglass, Weston, & Wolfe, 2011; Thompson, Bell, & Kao-Kniffin, 2018); an effect that has been shown in other cases to hinder the growth and establishment of native plants (Schultheis & MacGuigan, 2018). *V. rossicum*'s impact also cascades through local ecosystems by outcompeting native plant species, which in turn limits resources for invertebrate communities and disrupts specialized plant–invertebrate interactions (Livingstone et al., in press).

Attempts to control the spread of *V. rossicum* through clipping have largely proven ineffective, but when paired with the application of chemical herbicides reduced density and seed production have been observed (Averill, DiTommaso, & Morris, 2008). However, the development of a control program using herbicides and clipping remains untenable due to the associated costs of control at the scale of the invasion (Smith et al., 2006). A biological control program was initiated for *Vincetoxicum* spp. in 2002 with the identification of 5 potential biocontrol agents (Tewksbury, Casagrande, & Gassmann, 2002). Following host range testing and laboratory impact studies (Hazlehurst et al., 2012; Weed & Casagrande, 2010), which demonstrated a high degree of specificity and safety with the agent, a petition for the release of the first agent, the moth *Hypena opulenta* Christoph (Lepidoptera: Eribyidae), was submitted to the Canadian Biological Control Review Committee within the Canadian Food Inspection Agency and the United States Department of Agriculture–Animal and Plant Health Inspection Service's Technical Advisory Group for Biological Control of Weeds in 2011 (Casagrande et al., 2011). Subsequently, the federal government of Canada granted regulatory approval for release of *H. opulenta* in 2013 and the USDA permitted release in the United States in 2017. Both sexes of *H. opulenta* are approximately 1 cm in length with a wingspan of approximately 3 cm (Weed & Casagrande, 2010). Larvae develop through five instar stages and reach pupation after approximately 20 days at 20 °C (Weed & Casagrande, 2010). Little is known about the naturally occurring densities and exact phenology of *H. opulenta* in its native range (Hazlehurst et al., 2012), but laboratory studies have found that female moths mature eggs through their lifetime (Seehausen, Timm, Jones, Bouchier, & Smith, 2019) and individuals lay approximately 400 eggs with strong survivorship on *V. rossicum* (Weed

& Casagrande, 2010). *H. opulenta* can be multi-voltine under field conditions with multiple generations overlapping across the growing season. Diapause induction is dependent primarily on photoperiod (Jones, Seehausen, Bouchier, & Smith, 2020; Weed & Casagrande, 2010), and possibly, but to a much lesser extent, the decline of late season foliage quality (Weed & Casagrande, 2010).

Preliminary study of the potential impacts of biocontrol on *V. rossicum* was conducted by Milbrath (2008) who simulated folivory in different light environments, under laboratory conditions, to examine the effect on growth and reproduction. This study found that defoliation significantly reduced the growth and seed production of *V. rossicum* in high and low-light conditions, but with a much greater negative effect on seed production in low-light conditions. Further laboratory work by Weed and Casagrande (2010) found that the reproductive output of *V. rossicum* decreased significantly following folivory by *H. opulenta* (2 to 8 larvae per plant), which was severe. This result was also observed by Milbrath and Biazzo (2016), who additionally found that *V. rossicum*'s reproductive output was significantly lower in low-light conditions following feeding by *H. opulenta* larvae (3 to 6 larvae per plant).

With release permission for *H. opulenta* in Canada, it was possible for the first time to conduct field studies to assess the natural folivory of *V. rossicum* by *H. opulenta* in an established North American population. Here we report the findings from an experimental application of *H. opulenta* to a well-established *V. rossicum* population. The aim of this study was to: 1) assess the ability of *H. opulenta* to act as a defoliating agent for *V. rossicum* in shade and sun conditions; 2) determine the effects of *H. opulenta* larval feeding on the seed production of *V. rossicum* in shade and sun conditions, and 3) determine patterns of *H. opulenta* larval dispersal. We hypothesized that: 1) feeding on *V. rossicum* by *H. opulenta* would occur in both the sun and the shade, but to a greater degree in the shade given that the forest edge/understory is *H. opulenta*'s preferred natural habitat (Weed & Casagrande, 2010); 2) *V. rossicum* seed production would be significantly reduced in both the sun and shade conditions following feeding by *H. opulenta*, but to a greater degree in the shade given the expected differences in degree of folivory and the aforementioned observations of Weed and Casagrande (2010) and Milbrath and Biazzo (2016); and 3) *H. opulenta* larval dispersal would be significantly greater in the shade condition due to lower density of *V. rossicum*.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site

The study site for the experimental release of *H. opulenta* was located near Kirkfield, Ontario and consisted of a deciduous forest and a meadow, both highly invaded by *V. rossicum* (44°36'1.21" N, 78°59'38.20" W). The majority of the forest could be classified as late successional to mature and dominated by *Fraxinus americana* L. and *Acer saccharum* Marsh. There was no evidence of recent significant anthropogenic disturbance in the forest (e.g. grazing or clearing), though the land use history is unclear. Much of the forested area was located in a ravine, so it is likely that it is a remnant system. The forest understory

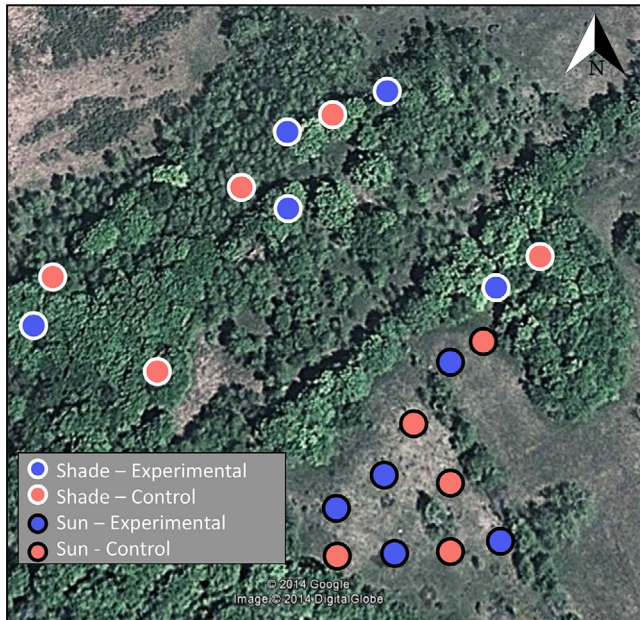
(henceforth, 'shade treatment') was dominated by *V. rossicum*, but we also observed *Carex spp.* L., *Toxicodendron radicans* (L.) Kuntze, *Trillium grandiflorum* (Michx.) Salisb. and many *Fraxinus americana* seedlings below the *V. rossicum* canopy. The meadow, an abandoned agricultural field (henceforth, 'sun treatment'), was also dominated by *V. rossicum*, but we also observed many other herbaceous perennials in and around our plots including; *Bromus inermis* Leyss, *Symphotrichum novae-angliae* (L.) G.L. Nesom, *Solidago spp.* L., *Viccia cracca* L. and *Asclepias syriaca* L. Mean annual precipitation for the region is 932.9 mm/year (709.9 mm rainfall, 223 mm snowfall). Annual temperature in the region ranges from an average of -7.7°C in January to an average of 20.8°C in July (Environment Canada, 2013). This study site was located with the assistance of the Nature Conservancy of Canada.

Consistency of tree canopy coverage across treatments was assessed using hemispheric photos taken on 9 September, 2014. Hemispheric photos, also known as 'fisheye' photos, capture a 180-degree view of the tree canopy, such that all cardinal directions are captured in the image. Proportion of canopy cover was analysed using ImageJ software. Hemispheric canopy photos were converted to black and white binary images, and percentage of black and white pixels were calculated to determine percent coverage. Analysis of variance on the canopy coverage values between sun and shade plots indicated significant differences ( $F_{(1,17)} = 129.89, p < 0.001$ ), but with no significant differences between control and experimental plots in each light treatment ( $F_{(1,17)} = 0.02, p = 0.88$ ).

Twenty 1-m<sup>2</sup> plots were divided equally between shade and sun treatments within a total area of approximately 100 × 130 m for the study site. Shade plots were selected using the criteria that plots have a minimum of 75% foliar coverage by *V. rossicum* and a minimum 75% canopy coverage by overstory trees. Open-field plots were selected using the criteria of a minimum 75% coverage by *V. rossicum* and no overstory shrubs or trees within approximately 10 m of the plot. Mean *V. rossicum* stem density was  $68 \pm 5.43$  ( $n = 5$ ) and  $139 \pm 7.62$  ( $n = 5$ ) stems/m<sup>2</sup> for shade and sun treatments, respectively, with no significant differences between control and experimental plots. Using GPS, plots were located with a between-plot minimum distance of 12.5 m (Figure 1). Within each treatment, five plots were randomly selected as control plots and five plots as experimental for *H. opulenta* release. The number of replicate plots is a product of the aforementioned required criteria for plot selection and the number of insects that could practically be reared for release. The corner of the plot that was closest to our footpath was marked with a primary stake as a reference for sampling.

### 2.2 | *H. opulenta* release

*H. opulenta* larvae were released into each of the 10 experimental plots on 24 July and 1 August 2014. The total number of larvae released was 710 per plot, which was divided approximately equally between the two release dates. This number of larvae was chosen to maximize establishment potential and was also based on previous work by Weed and Casagrande (2010) who found that *H. opulenta* individuals produce approximately 400 eggs, with 85–100% survivorship on *V. rossicum*. In this regard, the number of larvae released approximated the potential



**FIGURE 1** Plot layout for control (pink fill) and experimental (*Hypena opulenta* application) (blue fill) in shade (white outline) and sun (black outline) plots near Kirkfield, Ontario, Canada. Image: Google Earth

ovipositing of two *H. opulenta* individuals in each experimental plot. *H. opulenta* larvae were sourced from laboratory-reared populations from the University of Rhode Island, Agriculture and Agri-Food Canada's Lethbridge Research Centre and the University of Toronto-Scarborough. All lab populations were from the same initial population collected in Donetsk, Ukraine, in 2006 and recollected in 2012, which had been screened for the release petition (Weed & Casagrande, 2010). To control for the variation in larval rearing source and instar level (which ranged from 2–5), larvae from all sources were pooled, then divided into 10 equal batches in the lab (this was done for each of the two release dates). For transport to the field, caterpillars for each release treatment were placed in a lidded 4 L plastic container with a lid. A square grid of stucco wire, covered with sheets of paper towel, was placed at the bottom of the container to allow caterpillars to be easily transferred to release points with minimal disturbance. Stems of *V. rossicum* were added to each container as a food source and to provide habitat during transfer to the field. Bins were held in a cooler for transport. For field release each batch of *H. opulenta* was placed in the quarter of the plot near the primary stake. The wire grids were left in each experimental plot in order to minimize disturbance, ensure release of all larvae and allow larvae to move into the plot on their own.

### 2.3 | Feeding assessment

To assess feeding on *V. rossicum* by *H. opulenta*, we employed three different techniques in the plots: (1) counting leaves with or without feeding, (2) counting total number of leaves and (3) collection and scanning

of leaves. For leaf counting, two 50-cm transects were inserted into each plot, 50-cm along each side of the plot from the primary stake, at an angle of 90° into the plot. Along each of these transects, the five individual plants whose stems were in closest proximity to each 10-cm interval were selected for sampling, totalling 10 sampled plants per plot. For these 10 individuals the total number of leaves were counted as well as the number of leaves showing any feeding damage. Leaf counting was done on 12 August and 22 August 2014. Collection of leaves for scanning was done on 22 August and used the same stems as used for the leaf count. Bottom, middle and top leaves were taken from these 10 individual stems. For leaf selection, the bottom leaf was chosen at random then middle and top leaves from alternating sides of the plant, from the middle and top third of the plant, respectively, were taken. Collected leaves were immediately placed between pages of a notebook book to maintain their form and scanned within 3 hours. Leaf area was determined by scanning bottom, middle and top leaves and analysing total leaf area with ImageJ software (Schneider, Rasband, & Eliceiri, 2012). For leaves showing evidence of feeding, we assessed this by measuring the remaining leaf area (these methods are illustrated in Figure 2). We did not determine the amount of leaf material consumed for each individual leaf because many of the sampled leaves were severely damaged by feeding, which limited our ability to estimate the 'original' leaf area.

### 2.4 | Seed production

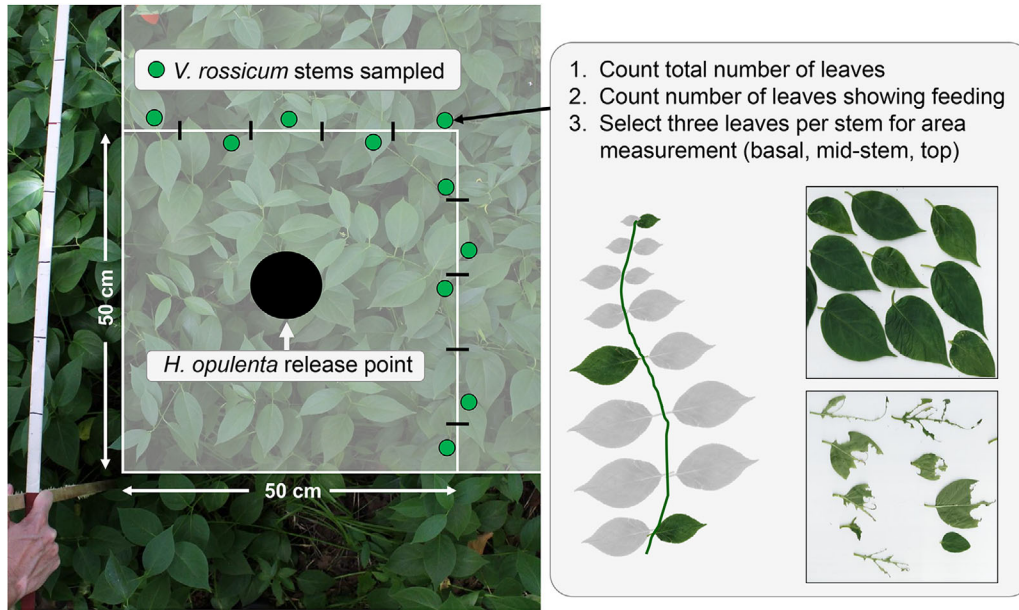
To examine the effects of feeding by *H. opulenta* on the reproductive output of *V. rossicum*, follicles were collected on 9 September 2014 for laboratory analysis. Twenty follicles were randomly sampled from each plot. Fresh follicles were measured for mass, length and diameter on the day after collection. Following these measurements, follicles were opened, pappi were removed and individual seeds were counted and weighed. For individual seed counts and seed mass, seeds were required to meet a minimum threshold of 1 mg to be included in order to avoid including non-viable seeds. For seed mass, the mean mass of seeds per follicle was used as the unit of analysis.

### 2.5 | Larval dispersal

Larval dispersal distances were estimated for each experimental plot on 22 August 2014 by measuring the distance between the primary stake and the furthest leaf showing any feeding damage (along cardinal directions). As such, some of the dispersal distances were measured outside of the plot and some were measured within and through the plot.

### 2.6 | Statistical analysis

Using R statistical software (R Core Team, 2018), we used the 'lme4' package (Bates et al., 2014) to create linear mixed-effects models to

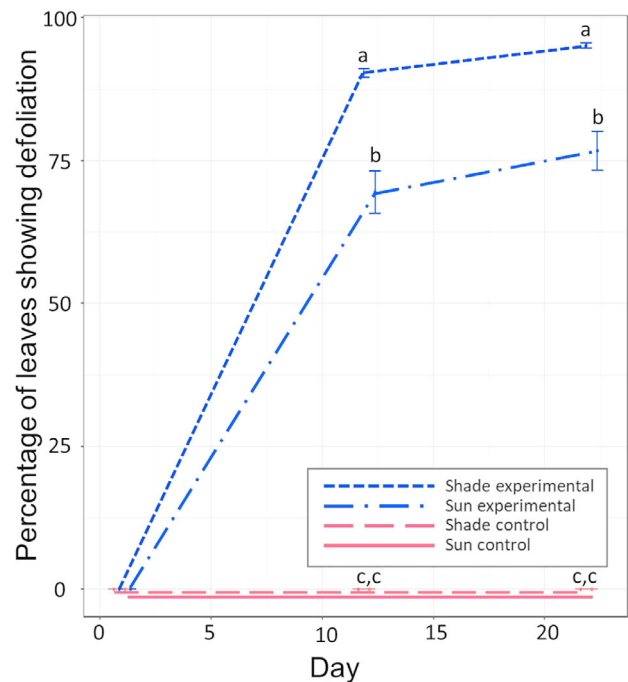


**FIGURE 2** Diagram of sampling design for leaf measurements

analyse differences in leaf area, number of leaves, and seed characteristics of *V. rossicum* at the *H. opulenta* release site. As measurements were taken from multiple individuals both within and across plots, 'plot' was designated as a random effect to resolve the non-independence of sampling (Winter, 2013), with treatment (sun/shade), plot type (experimental/control), and their interaction, designated as fixed effects. Five different models were assessed including combinations of predictor variables and their interaction, and the model with the lowest Akaike Information criterion (AIC) value was used for further analysis. Following model selection, we used the 'car' package in R (Fox et al., 2017) to assess significance across treatments using Wald chi-square tests, which were followed with post-hoc Tukey HSD tests to determine specific differences between treatments. Student's *t*-tests were used to examine differences in larval dispersal distance across treatments.

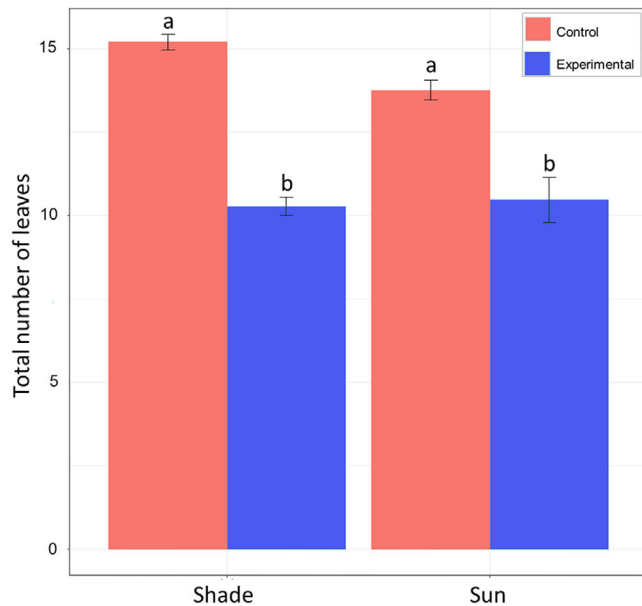
### 3 | RESULTS

Folivory by *H. opulenta* led to significant reduction of *V. rossicum* in both shade and sun conditions, measured by leaf area, percentage of leaves with feeding damage and total number of leaves (Figures 3–5). We found that the percentage of leaves showing feeding damage on *V. rossicum* stems in the experimental plots was highly significant under both treatments (Figure 3; Table 1). The number of leaves showing feeding damage was significantly greater in shade experimental plots compared to experimental plots in the sun (Table 1). After 3 weeks of folivory, we found significantly fewer leaves on sample plants in experimental plots compared to controls under both light conditions ( $\chi^2 = 29.774, p < 0.0001$ ; Figure 4). We also observed significant differences in leaf area between control and experimental plots for bottom, middle and top leaves from both shade and sun treatments (Table 1; Figure 5). And interestingly, we observed a significant increase in leaf



**FIGURE 3** Percentage of *V. rossicum* leaves, per stem, from shade and sun treatments showing any defoliation at 12 and 24 days following the application of *H. opulenta* (mean  $\pm$  SE) in a field experiment near Kirkfield, Ontario. Points at each time interval denoted with different letters are significantly different (Tukeys HSD performed on linear mixed effects model, observations,  $n = 50$ ; groups (random factor),  $n = 5$ )

area for the top leaves of the sun experimental plots ( $\chi^2 = 51.693, p < 0.001$ ; Figure 5). Many authors have noted that *V. rossicum*'s seasonal biomass production, excluding follicles, is typically complete by early July (DiTommaso et al., 2005; Smith et al., 2006). As such, any



**FIGURE 4** Number of leaves (mean  $\pm$  SE) on *V. rossicum* stems in shade and sun treatments following three weeks of folivory by *H. opulenta* in a field experiment near Kirkfield, Ontario. Bars denoted with different letters are significantly different (Tukeys HSD performed on linear mixed effects model, observations,  $n = 50$ ; groups (random factor),  $n = 5$ )

differences observed in leaf area in the current study can be attributed to folivory, or compensatory growth.

We found that *H. opulenta* did not appear to reduce the reproductive output of *V. rossicum*. In fact, we observed a significant increase for all seed parameters (follicle mass, follicle length, seed mass and seed count) in shade plots where *V. rossicum* was fed upon, indicating an increased investment in seed production (Figure 6). But we observed no significant differences in the sun treatment (Figure 6). While feed-

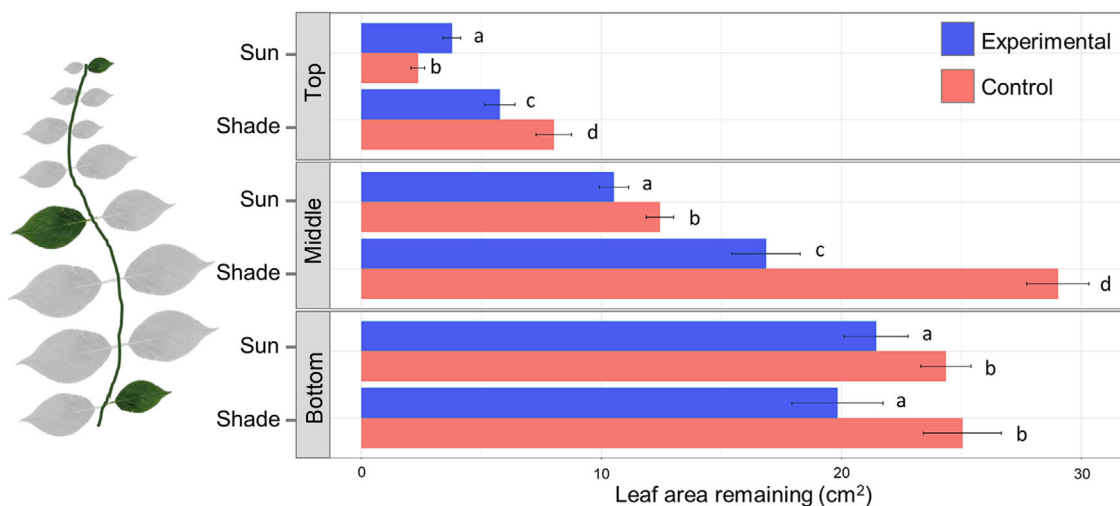
ing by *H. opulenta* did not affect the total number of follicles produced in either light condition, the number of follicles in the sun plots was an order of magnitude higher compared to the shade plots (Figure 6). Furthermore, as *V. rossicum* is polyembryonic, with a greater probability of polyembryony in high-light conditions (Smith et al., 2006), the results are valid for comparison of plots in this study, but they may not represent an assessment of total reproductive output of the plants.

We observed significantly greater larval dispersal distance in the shade compared to the sun treatment for south and east directions;  $t_{(5)} = 5.1, p < 0.01$  and  $t_{(5)} = 6.37, p < 0.01$ , respectively; (Figure 7). Interestingly, in the sun treatment, the directionality and distance of larval dispersal was skewed towards the north and west directions (Figure 7), which correspond to the positioning of the forest cover at the site (Figure 1).

## 4 | DISCUSSION

We observed highly significant feeding by *H. opulenta* on *V. rossicum* compared to control plots (where no foliar damage was observed), an effect that was more pronounced in shade conditions. However, significant folivory by *H. opulenta* did not lead to reductions in seed production in either light habitat. Instead, we observed a significant increase in seed production by *V. rossicum* following folivory in the shade treatment, an unexpected finding given the results of previous studies (Doubleday & Cappuccino, 2011; Milbrath, 2008; Milbrath & Biazzo, 2016; Weed & Casagrande, 2010). We should also reiterate that the observed increase in seed production in our study was dwarfed by the differences in overall seed production between the two light conditions, with the sun treatment producing more than 10 times the number of follicles.

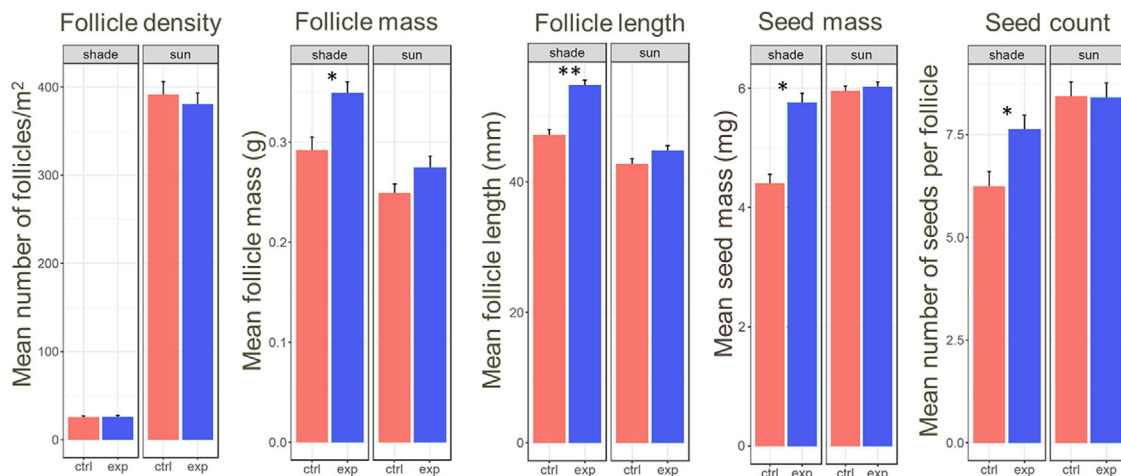
The potential impact of *H. opulenta* as a defoliating agent is perhaps best captured by the total number of leaves on *V. rossicum* stems

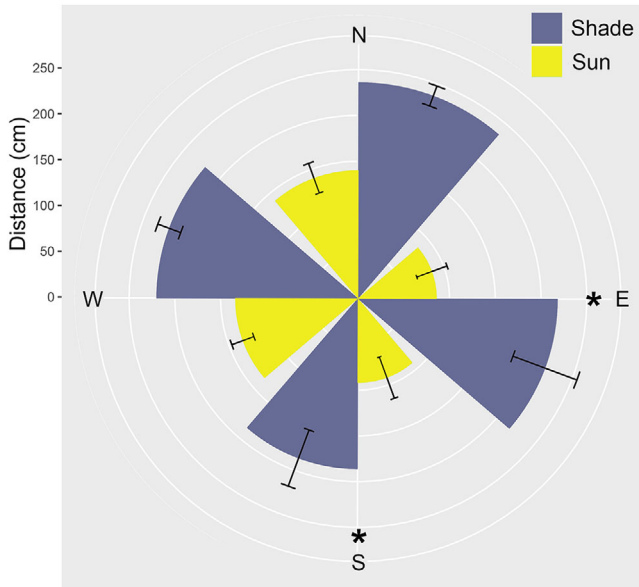


**FIGURE 5** Leaf area remaining of bottom, middle and top leaves (mean  $\pm$  SE) of *V. rossicum* stems following the application of *H. opulenta* in a field experiment near Kirkfield, Ontario. Bars denoted with different letters in each leaf category are significantly different (Tukeys HSD performed on linear mixed effects model, observations,  $n = 50$ ; groups (random factor),  $n = 5$ )

**TABLE 1** Results from linear mixed effects models for all variables showing the effects of experimental treatment on *V. rossicum* seed production, leaf count and leaf area in a field experiment near Kirkfield, Ontario

| Variable            | Model                       | Factor               | df   | $\chi^2$ | p       |
|---------------------|-----------------------------|----------------------|------|----------|---------|
| Seeds               | Seed mass                   | Sun/shade            | 1    | 54.38    | <0.0001 |
|                     |                             | Control/experimental | 1    | 27.39    | <0.001  |
|                     |                             | S/S: C/E             | 1    | 33.88    | <0.0001 |
|                     | Follicle length             | Sun/shade            | 1    | 90.17    | <0.001  |
|                     |                             | Control/experimental | 1    | 34.8     | <0.001  |
|                     |                             | S/S: C/E             | 1    | 16.28    | <0.001  |
|                     | Follicle mass               | Sun/shade            | 1    | 31.17    | <0.001  |
|                     |                             | Control/experimental | 1    | 15.63    | <0.001  |
|                     |                             | S/S: C/E             | 1    | 16.28    | <0.001  |
| Seed count          | Sun/shade                   | 1                    | 0.94 | n.s.     |         |
|                     | Control/experimental        | 1                    | 4.17 | <0.05    |         |
|                     | S/S: C/E                    | 1                    | 6.47 | <0.010   |         |
| Leaf area remaining | Bottom leaves               | Control/experimental | 1    | 4.91     | <0.05   |
|                     |                             | Sun/shade            | 1    | 91.98    | <0.001  |
|                     |                             | Control/experimental | 1    | 34.67    | <0.001  |
|                     | Middle leaves               | Sun/shade            | 1    | 91.98    | <0.001  |
|                     |                             | Control/experimental | 1    | 34.67    | <0.001  |
|                     |                             | S/S: C/E             | 1    | 18.37    | <0.001  |
|                     | Top leaves                  | Sun/shade            | 1    | 51.69    | <0.001  |
|                     |                             | Control/experimental | 1    | 0.63     | n.s.    |
|                     |                             | S/S: C/E             | 1    | 11.84    | <0.001  |
| Leaf counts         | Total number of leaves      | Control/experimental | 1    | 29.77    | <0.0001 |
|                     | Number of leaves defoliated | Sun/shade            | 1    | 3.84     | <0.05   |
|                     |                             | Control/experimental | 1    | 1,140.2  | <0.0001 |
|                     |                             | S/S: C/E             | 1    | 19.43    | <0.0001 |

**FIGURE 6** Plot follicle density ( $n = 5$ ), follicle mass ( $n = 100$ ), follicle length ( $n = 100$ ) and seed mass ( $n = 100$ ) and seed count ( $n = 100$ ) for *V. rossicum* in shade and sun treatments in a field experiment near Kirkfield, Ontario (mean  $\pm$  SE). Note: mean seed mass per follicle was the unit of measure for statistical analysis. "ctrl" and "exp" indicate control and experimental plots, respectively. Significance denoted as; \*\* $p < 0.01$ ; \* $p < 0.05$ ,  $n = 100$  (Wald's Chi Square performed on linear mixed-effect model) (observations: follicle density,  $n = 5$ ; follicle mass,  $n = 100$ ; follicle length,  $n = 100$ ; seed mass,  $n = 100$ ; seed count,  $n = 100$ ; groups (random factor),  $n = 5$ ). Differences between treatments shown in Table 1



**FIGURE 7** Distance and directionality of *H. opulenta* larval movement for sun and shade treatments in a field experiment near Kirkfield, Ontario (mean  $\pm$  SE). Significance denoted as; \* $p < 0.01$  (t-test),  $n = 5$  for comparison between sun and shade in each cardinal direction

following release. Here, our results are indicative of leaf abscission, both damaged and undamaged, by *V. rossicum* after larval feeding began. This appears consistent with an adaptive strategy for *V. rossicum* individuals to abscise leaves in order to reallocate resources while undergoing compensatory growth (Karban & Baldwin, 2007; Zvereva & Kozlov, 2014). This inference is supported by observations that even undamaged leaves underwent abscission (Livingstone, pers. observation). Had only damaged leaves been abscised, the cause might have been simply due to physical strain (Stiling & Simberloff, 1989). The implication here is that minor feeding on *V. rossicum* by *H. opulenta* could disproportionately affect the plant's photosynthetic capacity via a systemic response to folivory. In this regard, future research would do well to examine how varying densities of *H. opulenta*, and associated variability in the degree of folivory, affects leaf abscission by *V. rossicum*.

The eco-physiological mechanisms driving plant response to herbivory vary across species and are also dependent on local environmental conditions, resource availability and competitive interactions (Garcia & Eubanks, 2019; Järemo, Nilsson, & Tuomi, 1996; Maschinski & Whitham, 1989; Muller-Scharer, 1991; Rautio et al., 2005). Greater investment in the number and mass of seeds following herbivory (i.e. overcompensation; Belsky, 1986) has been observed for many species (e.g. *Jurinea mollis* (Asteraceae) L., (Trumble, Kolodny-Hirsch, & Ting, 1993); *Arabidopsis thaliana* (L.) Heynh., (Damgaard, Borksted, & Kjær, 2005; Hawkes & Sullivan, 2001), but many studies have shown negative (Clewley et al., 2012; Hawkes & Sullivan, 2001; van Driesche et al., 2002) or no effect of folivory on seed production (Hawkes & Sullivan, 2001). The 'compensatory continuum hypothesis' (Maschinski & Whitham, 1989), which has received much empirical support (Wise & Abrahamson, 2005), expects that overcompensation as a response

to herbivory should occur more often in resource rich environments. In addition, it is known that plants can compensate for herbivore attack by reallocating carbon and nitrogen assimilates stored in root systems to above-ground biomass (Paige & Whitham, 1987; Zhang et al., 2018), an effect that has been shown to be more pronounced in nutrient-rich conditions (Chapin & Slack, 1979; Trumble et al., 1993). In this regard, with the knowledge that *V. rossicum* individuals are known to produce significantly greater root mass in high-light environments (Milbrath, 2008), we might expect *V. rossicum*'s compensatory response to be more significant under high-light conditions, which was not the case in the current study. However, contrasting results have been found with respect to the effect of severe defoliation, such as that observed in the current study, on the root mass of *V. rossicum* individuals, where significant increases and decreases have been observed in different experiments (McKague & Cappuccino, 2005; Milbrath, 2008). Given that the current study did not excavate *V. rossicum* root structures, we cannot provide clarification on the potential role of resource reallocation from roots to reproductive overcompensation following folivory. Nevertheless, our findings indicate that for mature *V. rossicum* individuals growing in low-light environments, the physiological response to significant folivory differs from those growing in high-light conditions. In a meta-analysis conducted by Hawkes and Sullivan (2001), they found that dicot herbaceous plants (the functional group to which *V. rossicum* belongs) were more likely to exhibit overcompensation as a response to herbivory in low-to-intermediate light conditions but also note that the presence and degree of competition can influence this effect (Irwin & Aarssen, 1996). In this regard, we might expect variable compensatory response of *V. rossicum* to folivory by *H. opulenta* in low-light conditions under different degrees of inter-specific competition.

The current study's finding that *V. rossicum* exhibited overcompensation in seed production following feeding by *H. opulenta* (for a mature stand in shade conditions) has significant implications for large-scale biocontrol and restoration efforts. The greater numbers and sizes of *V. rossicum* seeds that are produced following feeding by *H. opulenta* may enhance germination and seedling recruitment in subsequent years (Cappuccino, Mackay, & Eisner, 2002; Eriksson, 1999; Stanton, 1984), with *V. rossicum* seeds already exhibiting extremely high germination probabilities (Ladd & Cappuccino, 2005). It is difficult to predict the long-term population dynamics since we are not considering the case where *H. opulenta* has established a self-sustaining population that repeatedly feeds on *V. rossicum* in subsequent years (Casagrande et al., 2011). If this were the situation, then folivory of newly recruited seedlings and juvenile plants could actually inhibit recruitment or long-term seed production (Doubleday & Cappuccino, 2011; Weed & Casagrande, 2010). However, it is unclear how long-term exposure to folivory by *H. opulenta* will affect mature *V. rossicum* stands. In addition, recent work by Milbrath et al. (2016) found that an annual application of simulated herbivory and mowing of mature *V. rossicum* stands resulted in no significant reduction in plant biomass or seed production in following years. In fact, they observed overcompensation in the form of increased root mass in mature *V. rossicum* individuals following clipping treatments (Milbrath et al., 2016), but also that multiple applications of simulated herbivory in a single season sometimes inhibited



seed production (Milbrath et al., 2016). This is promising given that in laboratory and field settings *H. opulenta* has been identified as a multi-voltine species with overlapping generations (Bourchier et al., 2019; Jones et al., 2020). Of course, when discussing studies that employ simulated herbivory/folivory, it is necessary to acknowledge that there can be considerable variability in the manner in which plants respond to simulated versus natural herbivory/folivory. The specific biochemical signatures provided by natural herbivory can cause plants to respond differently than they would to simulated herbivory (Baldwin, 1990; Lehtilä & Boalt, 2008). Nevertheless, no consistent trends have been found regarding the directionality of those differences with respect to decreased productivity or overcompensation (Lehtilä & Boalt, 2008).

The fact that our observations contradict laboratory work done with *H. opulenta* could be related to the fact that our study was conducted in a well-established population of *V. rossicum*. Milbrath's (2008) simulated folivory treatments were applied to transplanted *V. rossicum* root stalks grown in a greenhouse setting. It is possible that the added stress from root excavation, or a lack of built-up resource stores skewed the response to simulated folivory, causing declines in seed production that would not have been observed in well-established mature individuals (Mathers, Lowe, Scagel, Struve, & Case, 2007; Muller-Scharer, 1991). In this regard, future work is needed to clarify the extent to which root excavation stress act as a confounding factor when translating laboratory observations to field applications in plant–herbivore interactions. Similarly, where Weed and Casagrande (2010) observed decreases in *V. rossicum*'s seed production following folivory by *H. opulenta*, their experiment was conducted on young *V. rossicum* individuals that were grown from seed in the laboratory. While those findings are informative for the potential effect of folivory on young *V. rossicum* plants, thus influencing population recruitment, our findings indicate that the response of mature well-established individuals could be considerably different, at least after a single generation of intense folivory. Doubleday and Cappuccino (2011) observed significant declines in *V. rossicum* seed production following simulated leaf and root herbivory in a field study, although, the vast majority of the reduction was due to root damage, further supporting the possibility that root excavation was a confounding variable in previous studies. Furthermore, Doubleday and Cappuccino's (2010) experiment was conducted on 'sparser, recently established populations that have been expanding over the past several years'. Again, the conflicting results between our findings and these other studies may be due to differences in root mass, added stress from excavation, the phenological stage at which feeding took place (populations in high-light conditions tend to flower and set seed before those in lower light conditions; Livingstone, pers. observation) and/or differential resource re-allocation strategies (i.e. overcompensation) as a response to herbivory by individual plants at different life stages (McNaughton, 1983; Orcutt & Nilsen, 2000). In order to better understand how populations in these two light conditions may or may not exhibit overcompensation in response to persistent folivory, further experimental field work needs to be conducted on mature populations that integrate the phenological timing at which folivory takes place as well as a root stress treatment.

It is interesting to note that in the sun treatment of the current study, *H. opulenta* larvae tended to move shorter distances (quantified

as observed feeding damage), but that the movement was directed towards the forest edge. The shorter movement distances might be partially explained by the greater stem density of *V. rossicum* in sun conditions. In the shade, there is less food per unit area, leading to greater larval movement. Regarding the directional movement of the larvae, this is likely explained by the larvae having a preference for darker conditions. Paired with our observation of minimal *H. opulenta* larval feeding on the top leaves of *V. rossicum* in the sun treatment, where the larvae would be exposed to bright conditions, this likely indicates a preference for lower light conditions. Generally, there is much uncertainty regarding the extent to which *H. opulenta* larvae can tolerate the higher temperatures of full-sun conditions, despite their nocturnal behaviour. While other studies have noted greater potential for *V. rossicum* biocontrol in shade conditions (Milbrath, 2008; Milbrath & Biazzo, 2016; Weed & Casagrande, 2010), it needs to be noted that *V. rossicum* populations consistently have significantly greater seed output in moderate-to-high light conditions (DiTommaso et al., 2005).

#### 4.1 | Study limitations

Natural folivory, and associated plant response, observed in this study is based on data from a single year where an established population of *V. rossicum* had likely persisted unscathed for multiple decades. It could be the case that consistent folivory by *H. opulenta* across multiple years would negatively affect the plant's stored resources and its ability/strategy to re-allocate to reproductive output. Moreover, as *H. opulenta* field releases occurred relatively late in the season (24 July and 1 August), it is difficult to make robust conclusions about the effect of folivory on seed production. Results may have varied had the release coincided with seasonal emergence of *V. rossicum*. Interestingly, a population of *H. opulenta* has now established at a different field site in Ontario (Bourchier et al., 2019), and individuals will likely be dispersing across the landscape and ovipositing on *V. rossicum* throughout the growing season, perhaps resulting in the same 'late season dynamics' seen in our study. Of course, this would depend on larval abundance and the maturity of the *V. rossicum* population in question. We also may have observed a different response by *V. rossicum* had the *H. opulenta* population produced multiple generations across the full growing season, which is a possibility once populations are able to establish (Casagrande et al., 2011; Jones et al., 2020).

Note: Our long-term objective was to also examine folivory of *V. rossicum* and seed production in sequential years, but *H. opulenta* was not observed at the release site in the following year. This may have been the result of a combination of pupal predation (Livingstone pers. observation) and severe frost events that occurred the following spring.

## 5 | CONCLUSIONS

The implementation of the biocontrol of invasive plant species is increasingly relevant for biodiversity conservation efforts. Host range testing studies required for the release of biological control agents

have a primary focus on agent specificity and safety (Paynter et al., 2015; Schaffner, 2001). Impact studies on laboratory plants might not reflect what the agents encounter in field release situations with high densities of well-established plants, which makes predicting efficacy and impact difficult. With respect to the feeding potential of *H. opulenta* on *V. rossicum*, our results corroborate previous laboratory results (Milbrath, 2008; Milbrath & Biazzo, 2016; Weed & Casagrande, 2010). However, in the current study under field conditions we observed responses to folivory that differed substantially from associated laboratory studies. This divergent response could result from differences in the condition of *V. rossicum* roots in laboratory and field conditions, or differences in the timing of the application of *H. opulenta* (or artificial defoliation). Pre-release efficacy testing of biocontrol agents will be enhanced by examining agent responses across different life stages of the target organism (Doubleday & Cappuccino, 2011) as well as variable environmental conditions. Furthermore, post-release biocontrol agent impacts must be followed by detailed monitoring to assess impacts on fitness and ecological interactions under the invaded habitat conditions (Shea, Possingham, Murdoch, & Roush, 2002).

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#### AUTHORS' CONTRIBUTIONS

SWL, SMS, RSB, KR and MWC conceived and designed the study; SWL, KR and AR collected data; SWL and MWC analysed the data; SWL wrote the manuscript with significant input from all co-authors who gave final approval for publication.

#### CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

#### PEER REVIEW

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#### DATA AVAILABILITY STATEMENT

All data used in this study is available in the publicly accessible repository Dryad: <https://doi.org/10.5061/dryad.prr4xgxjm> (Livingstone et al. 2020).

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