The use of surrogate herbivores for the pre-release efficacy screening of biological control agents of *Lepidium draba*

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**Summary**

Pre-release efficacy assessment has been suggested as a primary selection criterion for potential biological control species to insure the success and safety of biological weed control. Pre-release efficacy of candidate agents is commonly assessed by exposing insects to target plants in the garden or greenhouse (native range) or in quarantine (introduced range) before or in parallel with host-specificity testing. Conducting pre-release impact experiments for several candidate agents simultaneously may be difficult because potential agents are either scarce or may require development of novel culturing procedures. We propose an alternative approach to pre-release efficacy assessment that utilizes oligophagous or polyphagous insect herbivores from the introduced range as surrogates for biological control agents to assess the impact of specific feeding niches on the target weed to direct the search for effective candidate agents. Surrogate herbivores can be cosmopolitan or indigenous insects collected directly from the invasive plant and confamilial species. Insect pest species are particularly well suited to act as surrogates because they are seasonally abundant or easily reared. Based on previous surveys, we identified surrogate herbivores attacking different above-ground plant organs of the biological control target hoary cress (*Lepidium draba* L., Brassicaceae). We tested the density-dependent impact of four oligophagous herbivore species on *L. draba* to demonstrate the applicability of this novel efficacy assessment technique. We found that the endophagous stem miner, *Ceutorhynchus americanus* Buchanan (Coleoptera: Curculionidae), had the highest per-capita effect on hoary cress growth, suggesting candidate agents within this niche should be prioritized.

**Keywords:** herbivore niche, agent selection, generalist insects.

**Introduction**

The biological control of weeds has been a successful, economical and environmentally sound management tool for curbing plant invasions, but McFadyen (2003) estimated that only 55% of biological control agents established contribute to the suppression and control of their target weeds. In addition, more than half of the successful biological control programs can link their success to a single agent (Denoth *et al.*, 2002). Introduction of ineffective biological control agents increases the probability of direct risks to non-target plant species, interference with established agents and indirect effects on ecosystem functions without the benefits gained from reduction of weed dominance or abundance. To minimize the cost of host-specificity screening and risks associated with establishment and proliferation of ineffective agents, improved methods for predicting the impact of candidate agent species before their release are recommended (Sheppard, 2003; McClay and Balciunas, 2005). Pre-release efficacy testing of candidates provides quantitative data to improve the likelihood of selecting the agents most capable of reducing weed abundance.

The most commonly used technique for pre-release efficacy assessment is to expose candidate agents to target plants in garden or greenhouse experiments in their native range or under quarantine conditions in their introduced range. The advantage of these methods is that changes in plant performance can be directly attributed to agents at experimentally controlled densities. However, screening several candidate species

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Materials and methods

*L. draba* ssp. *draba* (=*Cardaria draba* (L.) Desv.) is a Eurasian perennial mustard introduced to North America in the 19th century (Mulligan and Findlay, 1974). *L. draba* is currently considered a noxious weed in 15 western states and three Canadian provinces (Rice, 2007). *L. draba* occurs in a wide range of habitats, including cultivated land, rangeland, wilderness, pastures, roadsides and waste areas, but thrives particularly well in disturbed, riparian or irrigated areas (Mulligan and Findlay, 1974). It reproduces sexually and vegetatively through rhizomes. Seeds usually germinate in early autumn and produce overwintering rosettes, which bolt in spring and flower from April to June in the northwestern USA.

Surrogate insect herbivores were selected based on the feeding modes of two specialist insects currently under consideration for biological control of *L. draba*: the stem-mining weevil, *Ceutorhynchus merkli* Korotyaev (Coleoptera: Curculionidae), and the stem and root-crown feeding flea beetle, *Psylliodes wrasei* Leonard & Arnold (Coleoptera: Chrysomelidae) (Cripps et al., 2006). The native stem-miner, *Ceutorhynchus americanus* Buchanan, was the only insect found mining in the shoots of *L. draba* in North America and was used as a surrogate for *C. merkli*. Adult *C. americanus* feed on foliage and oviposit in the stems of *L. draba* in laboratory no-choice tests (K.P. Puliaticco, unpublished data). The host affinity of this native weevil is unknown, but adults have been collected from *Brassica* and *Lepidium* species and it has only been successfully reared from *Lepidium virginicum* (Buchanan, 1937).

The crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae), was selected as a surrogate to mimic adult feeding of *P. wrasei*. Crucifer flea beetles are introduced Brassicaceae pests, which cause crop damage primarily from feeding on seedling plants but usually have only a minor effect on established plants (Feeny et al., 1970).

Two additional insects were utilized as surrogates to mimic feeding modes for which there are currently no biological control candidate species: a lepidopteran defoliator and a piercing/sucking true bug. The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is considered native to the Mediterranean region but has a cosmopolitan distribution and occurs wherever crucifer crops are grown (Talekar and Shelton, 1993). This defoliating moth is oligophagous within the Brassicaceae (Talekar and Shelton, 1993) and is abundant on *L. draba* in North America (Cripps et al., 2006). The tarnished plant bug, *Lygus hesperus* Knight (Heteroptera: Miridae), is native to North America and was one of the most abundant polyphagous species found on invasive *L. draba* (Cripps et al., 2006). *Lygus* species lacerate plant tissue, inject salivary fluids that digest the tissue extra-orally and then ingest the liquefied tissue (Butts and Lamb, 1990).
This study was conducted at the climate-controlled Manis Entomological Greenhouse, University of Idaho, Moscow, ID. Environmental conditions were maintained with a 15L:9D photoperiod at 24 ± 2°C (day)/18 ± 1°C (night) in the greenhouse throughout all phases of the experiment except for the seedling vernalization described below.

*L. draba* seeds were collected from a population near Moscow, ID (46°44’N, 116°58’W) in August 2003. Seeds were sown into Cornell ‘Peat-Lite A’ artificial potting medium (Hartmann *et al.*, 1990) in the greenhouse. After formation of the first true leaves, seedlings were transplanted into plug trays using the same potting medium. Seedlings were grown for 6 weeks in the greenhouse and were then transferred to a 3 ± 1°C cold room with 12L/12D photoperiod for a minimum of 60 days to initiate bolting and flower production. During vernalization seedlings were fertilized once a week with 0.33 ml of Miracle-Gro® per litre of water (15-30-15 NPK, Scotts Miracle-Gro, Marysville, OH). After cold treatment, plants were transplanted into 3-l plastic pots in artificial potting media (1:2:1 peat moss, vermiculite and perlite mix; augmented with 5% sand, pH stabilizers and trace elements) and fertilized with 2.8 g Osmocote® slow release fertilizer per litre soil (14:14:14 NPK, Scotts Miracle-Gro, Marysville, OH). Plants were then grown in the greenhouse for 6 to 8 weeks before they were used for experimentation.

*C. americanus* was collected from *L. draba* near Vale, Oregon (44°05’N, 117°18’W) on 24 April 2004. Females were tested individually for oviposition on cut *L. draba* stems before the start of the experiment (Harmon and McCaffrey, 1997). *P. cruciferae* adults were collected on 29 April 2004 from *Sinapis alba* seedlings southeast of Genese, ID (46°33’N, 116°55’W). Adult and nymph *L. hesperus* were collected from a mixed field of alfalfa and pasture grasses at the University of Idaho Sheep Research Farm, Moscow, ID (46°44’N, 116°58’W) 2 days before the start of the experiment on 12 August 2004. *P. xylostella* eggs were obtained from Benzon Research Inc. (Carlisle, PA). A laboratory colony was reared on artificial diet in 473-mL Styrofoam cups with plastic lids following the protocols of Shelton *et al.* (1991). Approximately 300 adults of both sexes were caged together, and females were allowed to oviposit for 24 to 48 h on cabbage-juice-coated aluminum foil. Foil was then cut into 1 cm² pieces; eggs were counted and combined into treatment densities, which then were placed at the base of the plants.

Stem number and individual stem lengths were measured for all plants before the start of experiments. Plants were assigned to herbivore treatments in a complete randomized block design with position on greenhouse bench as the blocking factor. Plants were caged with 80-cm-long mesh sleeve cages supported with internal wire frames with the bottom of each sleeve held in place with a metal hose clamp. Herbivores were released according to treatments (see below). All plants were caged for 40 to 44 days and then destructively harvested in blocks. Plants were clipped at the soil surface, the numbers of shoots counted and individual shoot lengths recorded to the nearest 1 cm. Roots were carefully washed to remove soil. Root and shoot biomasses were recorded to the nearest 0.1 g after drying for a minimum of 24 h at 80°C.

The effects of density-dependent herbivory on plant biomass, shoot number and height were determined in two experiments using identical protocols. Insect densities were selected along an exponentially increasing scale and chosen to encompass normal field densities from survey data (Cripps *et al.*, 2006). Experiment 1 started on 29 April 2004 with three insect species with ten replicates for each herbivore and density treatment. Treatments were comprised of either *P. cruciferae* (0, 10, 20, 40 and 80 unsexed adults), *P. xylostella* (0, 75, 150, 300 and 900 eggs on foil) or *C. americanus* (0, 1, 2 and 8 adult females). Male *C. americanus* (0, 1, 1 and 2 and 4 individuals, respectively) were added to the females to ensure continual fertilization of eggs. Experiment 2 started on 12 August 2004 and tested the impact of *L. hesperus* adults (0, 10, 20, 40 and 80) with five replicates and nymphs (0, 20, 40, 80 and 160) with seven replicates.

Data were analyzed using general linear model analysis of repeated measures analysis of covariance models with herbivore treatments as the fixed factor and position on the greenhouse bench as the blocking factor. Pre-treatment shoot length was log<sub>10</sub>-transformed and used as a co-variable for biomass and post-treatment shoot lengths. Pre-treatment shoot number was used as a co-variable for post-treatment shoot number. Upon a significant herbivore effect, means were compared using a Tukey Honestly Significantly Different test, and per-capita herbivore effects on shoot length were examined using regression analysis. All analyses were conducted using Minitab® v15 (Minitab Inc., 2006).

**Results**

*P. xylostella* was the only herbivore that significantly decreased *L. draba* above-ground (*F*<sub>4,35</sub> = 40.09, *P* < 0.001) and below-ground (*F*<sub>4,35</sub> = 47.17, *P* < 0.001) biomass accumulation (Fig. 1). Damage to plants was extensive at all treatment levels of diamondback moth and resulted in total defoliation of plants at the highest egg density. Caterpillars at the highest density starved to death before pupation, allowing compensatory re-growth of plants before harvest.

Defoliation by *P. xylostella* (*F*<sub>4,35</sub> = 6.54, *P* < 0.001), shot-hole feeding by *P. cruciferae* adults (*F*<sub>4,35</sub> = 3.42, *P* = 0.018) and stem-mining by *C. americanus* larvae (*F*<sub>4,35</sub> = 5.24, *P* = 0.002) significantly decreased maximum shoot elongation. Sap feeding by *L. hesperus* had no impact on shoot length by either adults (*F*<sub>4,20</sub> = 0.51, *P* = 0.729) or nymphs (*F*<sub>4,28</sub> = 0.29, *P* = 0.884).
Figure 1. Effect of different *P. xylostella* egg densities on mean above- and below-ground plant biomass (±95% CI) of *L. draba* after 40 days of larval feeding.

*C. americanus* had the highest per-capita effect on maximum shoot length (−1.25 ± 0.46 cm/female, *P* = 0.01, *r*²=0.113), followed by *P. cruciferae* (−0.151 ± 0.038 cm/adult, *P* < 0.001, *r*² = 0.234) and *P. xylostella* (−0.017 ± 0.004 cm/egg, *P* < 0.001, *r*² = 0.217). None of the herbivore treatments reduced the number of vegetative shoots produced per plant (Fig. 2).

*C. americanus* laid eggs in more than 90% of the shoots regardless of female density; however, plant defence responses caused high egg and larval mortality within the stems. Callus tissues were commonly found growing around oviposition holes, eggs and larval mines.

**Discussion**

The principal objective of *L. draba* biological control is to reduce the plant’s vegetative growth capability, thereby limiting stand dominance and rate of spread of hoary cress. We found that above-ground plant architecture was significantly affected by chewing insects in the defoliating (*P. xylostella*), shot-hole feeding (*P. cruciferae*) and stem-mining niches (*C. americanus*). Our results suggest that the introduction of endophagous stem-miners may impact plant performance even at low densities and should be prioritized as candidates for the biological control of *L. draba*. However, none of the insect species investigated decreased vegetative shoot production of *L. draba*, which is an important determinant of the rate of spread for clones.

Despite the lack of direct impacts on vegetative propagules, above-ground feeding may contribute to the cumulative stress on plants and consequently reduce *L. draba*’s ability to tolerate attack by agents in other niches. Defoliators are not currently being considered for the biological control of *L. draba*, but our data suggest that they can have significant impacts on plant biomass. Shot-holes produced by *P. cruciferae* adults had minimal impact on the performance of bolting plants; we therefore expect that adult feeding by *P. wrasei* will not greatly impair bolting plants either. Adult flea beetle feeding on seedlings and spring rosettes may affect hoary cress population density within patches and sexual reproduction success.

Figure 2. Effect of different densities of three surrogate herbivores on maximum shoot length (±95% CI) of *L. draba* after 40 days of feeding.
The use of surrogate herbivores

Faunistic surveys are increasingly being conducted on invasive plants in both their native and introduced ranges (Hinz and Schwarzländer, 2004), providing information on potential surrogate insects. The biogeographic comparison of herbivore faunas from both ranges of L. draba by Cripps et al. (2006), for instance, provided the opportunity to explore the impact of feeding niches potentially important for biological control of this plant. Another example of the potential uses of surrogate herbivory comes from the search for biological control agents for tropical soda apple, Solanum viarum Dunal (Solanaceae), which resulted in the release of the defoliating leaf beetle, Gratiana boliviana Spath (Coleoptera: Chrysomelidae) in the USA in 2003 (Cuda et al., 2004). Ten major crop pests that occupy five distinct feeding guilds have been identified on tropical soda apple in the USA (Cuda et al., 2004), and additional feeding guilds are known from the insect pests of potato (S. tuberosum; Radcliffe, 1982) and tomato (S. lycopersicum; Norris and Kogan, 2005). These pest species could be used as surrogates along with G. boliviana to investigate potential interactions between herbivores on S. viarum before the introduction of any new biological control agents. Our proposed protocol does therefore not intend to introduce an extra step in the agent selection process but could provide a useful alternative technique to identify and prioritize candidate biological control agents based on empirical efficacy data for agents in different feeding niches.

The greatest limitation for the use of surrogate insects to pre-screen candidate biological control agent efficacy is the availability of specialist feeding niches within the invaded range. Although we identified several above-ground feeding niches with available surrogates, we did not find any appropriate below-ground endophagous feeders or shoot gall formers, both niches for which candidate species have been identified (Fumanal et al., 2004; Cripps et al., 2006). The stem-miner C. americanus had high rates of oviposition, but the observed egg and larval mortality may indicate that L. draba is not an ideal host plant. Observed effects of C. americanus may underestimate the potential impact of better adapted specialist stem-miners such as C. merkli and P. wrasei, currently being studied at CABI Europe, Switzerland (Cripps et al., 2006). Surrogate herbivores can be used in numerous invasive plant systems beyond relatives of agronomic species, but the best known generalist insect herbivores are crop pests. Several weeds that are unrelated to crops, including L. draba (Cripps et al., 2006), are recognized as reservoirs of important economic pests; however, suitable surrogates may not be available for all target weed species and potential biological control niches.

Organizing foreign exploration can take a long time due to logistical, financial, political or safety concerns and may require establishment of new collaborations in areas without a tradition of biological weed control. Furthermore, once candidate insects are found, it takes additional time before mass-rearing techniques are developed to produce sufficient numbers for host-testing and pre-release efficacy testing. Testing the efficacy of surrogate herbivores can provide the opportunity to investigate protocols for screening agents before candidates are identified while simultaneously removing ineffective feeding niches from consideration. The use of surrogate herbivores is therefore not intended to replace efficacy testing but could be used at an early stage of a biological control program to assess the impact of specific feeding niches on the target weed in the invaded range to direct the search for effective candidate agents in the area of origin.

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