Excising the ghosts of invasions past: restoring native vegetation to soil infested with invasive swallow-worts

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Abstract

Invasive plants can gain a foothold in new environments by manipulating soil conditions through allelopathy or through the disruption of associations between native plants and their mycorrhizal associates. The resulting changes in soil conditions can affect the recovery of habitats long after the invasive plant has been removed. We conducted a series of greenhouse experiments to examine the effects of soil conditioned by pale swallow-wort [*Vincetoxicum rossicum* (Kleopow) Barbarich; Apocynaceae], on the growth of native plants. Additionally, we tested the effects of aqueous extracts of common milkweed (*Asclepias syriaca* L.; Apocynaceae), a related plant with known allelopathic effects, on the regrowth of *V. rossicum* from transplanted root crowns. Soil from a 15-yr-old *V. rossicum* infestation reduced seedling emergence in *A. syriaca* as well as in *V. rossicum* itself. Conversely, the same soil had no effect on the growth of mature *A. syriaca* plants. Soil conditioned by *V. rossicum* growth in the greenhouse had no effect on the biomass and percentage cover generated by two restoration seed mixes. Soil conditioned by *A. syriaca*, however, yielded lower biomass and percentage cover from both seed mixes. In contrast to the allelopathic effects of *A. syriaca* on seedlings, aqueous extracts of *A. syriaca* increased aboveground plant growth in *V. rossicum*. Our results suggest that the effects of *V. rossicum*–conditioned soil on native plants are concentrated at the seedling establishment phase. Additionally, the use of diverse native seed mixes shows great potential for restoring productivity to ecosystems affected by *V. rossicum*.

Introduction

Many invasive plants gain a foothold in new environments by manipulating soil conditions in their favor. Invasive weeds may undermine native plant growth by producing phytotoxic chemicals (allelopathy) (Bais et al. 2003; Chen et al. 2017), by disrupting mutualisms between native plants and their mycorrhizal associates (Van der Heijden et al. 1998), or by sequestering soil pathogens on their roots (Mangla et al. 2008). Such changes in soil conditions, particularly an altered rhizosphere, can have lasting impacts on the recovery of habitats even after the invasive weed has been removed. We conducted a series of greenhouse experiments, examining the effects of soil conditioned by pale swallow-wort [*Vincetoxicum rossicum* (Kleopow) Barbarich; Apocynaceae] on the growth of native plants. We sought to generate a clearer understanding of how *V. rossicum*–conditioned soil affects native plant growth and to provide direction for the restoration of invaded sites.

Vincetoxicum rossicum is a perennial vine native to southwestern Ukraine (Pobedimova 1952). The species was introduced to North America in the 1800s and has since become highly invasive in Ontario and Quebec, Canada, as well as in the northeastern United States (DiTommaso et al. 2005; Douglass et al. 2009). The weed reduces native plant diversity by smothering neighboring vegetation (Christensen 1998), and the displacement of native plants has cascading effects on native arthropod assemblages (Ernst and Cappuccino 2005). Among the insects affected by *V. rossicum* are monarch butterflies (*Danaus plexippus* L. (Lepidoptera: Nymphalidae). Monarchs regularly lay eggs on *V. rossicum*, a close relative of common milkweed (*Asclepias syriaca* L.; Apocynaceae), but the caterpillars cannot complete development on the weed (Casagrande and Dacey 2007). Invasive swallow-worts (*V. rossicum* and *V. nigrum*) have also been linked to reductions in breeding birds in grassland habitats (DiTommaso et al. 2005) and, of particular concern in Canada, the weeds are encroaching on rare alvar communities in Ontario that are home to multiple species at risk (Lawlor 2000).

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Management Implications

Results of a reseeding experiment suggest that diverse native seed mixes can be a valuable tool for the restoration of productivity and function to ecosystems previously infested by *Vincetoxicum rossicum* (pale swallow-wort). Biomass produced by these native seed mixes was dominated by grass species, including big bluestem, *Andropogon gerardii* Vitman, Canada wild rye, *Elymus canadensis* L., bottlebrush grass, *Elymus hystrix* L., and Virginia wildrye, *Elymus virginicus* L., and the nurse crop Italian ryegrass, *Lolium perenne* L. *spp. multiflorum*. Based on these results, we advocate for the inclusion of these species in seed mixes used for post–*V. rossicum* restoration. In contrast, use of *Asclepias syriaca* (common milkweed) should be carefully considered for restoration of invaded sites, because the plant can have negative effects on native seed germination and can promote *V. rossicum* regrowth from rootstock.

Additionally, results from germination and transplantation experiments suggest that following removal of *V. rossicum* from a site, residual seeds of the weed likely play a secondary role in the resurgence of the invasive species, as germination is inhibited in *V. rossicum*–conditioned soil. The rapid resurgence of the weed, often observed after control, is most likely driven by *V. rossicum* root crowns remaining in the soil. If these root crowns can be successfully removed from infestation sites, then the competitive advantage of the weed over native plants can be substantially reduced.

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Allelopathy has long been considered a "novel weapon" that enhances the competitive abilities of weed species in their introduced ranges (Callaway and Ridenour 2004). Many exotic plants are known to suppress native neighbors and facilitate their invasion by releasing chemicals into the environment (Hierro and Callaway 2003). These chemicals often have stronger negative effects on plants that have not coevolved with the weed (Ridenour and Callaway 2001). Diffuse knapweed (*Centaurea diffusa* Lam.; Asteraceae), for example, has far greater allelopathic effects on plants in its invasive range (North America) than on plants in its native Europe (Callaway and Aschehoug 2000). *Vincetoxicum rossicum* is known to produce allelochemicals, one of which (antofine) has been identified (Gibson et al. 2011; Mogg et al. 2008). The role of allelopathy in *V. rossicum* invasions, however, is unclear. In agar bioassays, *V. rossicum* has been shown to inhibit germination in lettuce (*Lactuca sativa* L.) (Douglass et al. 2011) and reduce root growth in several native species, including *A. syriaca* (Gibson et al. 2011). More recent studies by Gibson et al. (2015), however, showed that antofine is unstable and is often absent from soil samples collected from mature *V. rossicum* infestations. Furthermore, dose–response experiments conducted in nonsterile soil showed that the concentration of antofine needed to reduce lettuce root growth was 20 to 50 times higher than concentrations used in previous agar bioassays.

In addition to allelopathy, invasive plants can manipulate soil conditions in their favor by altering rhizosphere communities (Hawkes et al. 2006; Mangla et al. 2008; Stinson et al. 2006; Vogelsang and Bever 2009). Associations between plants and arbuscular mycorrhizal fungi (AMF) are often host specific (Appoloni et al. 2008; Bever 2004). As such, any disruption to fungal communities brought about by invasive plants can lead to fundamental changes in plant community structure (Vandenkoornhuyse 2002). *Vincetoxicum rossicum* infestations in Canada cause significant shifts in the AMF communities associated with native plant roots compared with those in neighboring noninvaded sites (Bongard et al. 2013). Additionally, *V. rossicum* has been observed to accumulate pathogenic fungal partners that negatively affect native plant species (Day et al. 2016; Dickinson et al. 2021). As with allelopathy, however, the role of soil fungal manipulation in *V. rossicum* invasions is unclear. For example, Dukes et al. (2019) showed that some native plants exhibited increased growth in *V. rossicum*–conditioned soil.

We conducted a series of four controlled greenhouse experiments designed to explore the effects of *V. rossicum*–conditioned soil on native plant growth and to identify effective practices for the restoration of native diversity after *V. rossicum* removal. First, we examined how soil from a mature *V. rossicum* infestation affected seedling emergence and establishment of both *A. syriaca* and *V. rossicum*. Second, we explored the effects of the same soil on mature *A. syriaca* plants. These first two experiments were designed to identify the life stage of representative native plants that are most vulnerable to changes in soil conditions elicited by *V. rossicum*. Third, we conducted a reseeding experiment to determine the success of two native seed mixes in soil conditioned by 13 wk of *V. rossicum* growth from seed. We sought to test the viability of reseeding schemes for the restoration of biodiversity and ecosystem function to *V. rossicum*–infested sites. Finally, we conducted a greenhouse experiment to determine the effects of aqueous *A. syriaca* extracts on the growth of *V. rossicum* from rootstock, to see whether *A. syriaca*, a native plant known to produce allelopathic chemicals (Rasmussen 1975), might represent a potential tool for the control of *V. rossicum*. With these experiments, we aimed to determine (1) how *V. rossicum*–conditioned soil affects native plant establishment, (2) whether native seed mixes are effective for restoring native vegetation to sites previously infested with *V. rossicum*, and (3) whether *A. syriaca*'s allelopathy could be used against *V. rossicum* in the context of ecological restoration.

Materials and Methods

Experiment 1: Assessing the Effects of Vincetoxicum rossicum–conditioned Soil on Seedling Emergence in Asclepias syriaca and Vincetoxicum rossicum

We examined how soil from a mature infestation of *V. rossicum* affects early plant establishment using *A. syriaca* and *V. rossicum* as indicator species. For this experiment, we used a one-way independent-samples design with two treatments: invaded versus uninvaded soil. Forest soil was collected from Uxbridge, ON (44.088694, −79.105694) within (treatment) or 10 m away from (control) a 15-yr-old *V. rossicum* infestation. Treatment and control soil was collected within and adjacent to the same *V. rossicum* infestation to ensure similarities in environmental and edaphic variables and land use history (Cahill et al. 2016; Dukes et al. 2019; Karst et al. 2015). Collected soil was sieved through a 6-mm mesh and placed in 72-cell plug trays (50 ml per cell). Plug trays were watered for 1 wk, and any germinating seeds were removed by hand. After 1 wk, each cell was planted with a single *V. rosscium* seed (field collected from Uxbridge, ON, in February) or an *A. syriaca* seed (collected from the same field site in March). Seeds were all collected from the same site, in late winter, so that they had received natural cold stratification and their germinability would mirror that found in natural conditions. All seeds had been soaked overnight in 0.2% Nutriboost 1 (Nutrilife Plant Products, Abbotsford, BC, Canada) to improve their chances of germination. A total of 1,440 soil plugs were used, and 720 seeds of each species were planted. For each species, exactly half of the seeds were

planted in treatment and control soil. Plug trays were placed in the University of Toronto forestry production greenhouse and watered as needed. Trays were randomly reconfigured weekly to control for any spatial differences in greenhouse conditions. Germination and death were recorded weekly for 4 wk, at which point above- and belowground biomass were separated and roots were washed. Roots and stems were oven-dried at 60 C for at least 3 d before weighing (Day et al. 2015; Ernst and Cappuccino 2005).

Experiment 2: Assessing the Effects of Vincetoxicum rossicum–conditioned Soil on Potted Asclepias syriaca plants

We compared the growth and performance of potted *A. syriaca* plants transplanted into soil from a 15-yr-old *V. rossicum* infestation and into soil from a nearby uninfested site. For this experiment, we used a one-way independent-samples design, comparing growth in infested versus uninfested soil. Treatment soil was collected from a *V. rossicum* infestation in Uxbridge, ON (44.088698, −79.105780), and control soil was collected from a site 10 m away from the *V. rossicum* infestation, as with Experiment 1. All soil was collected in July 2021. Soil was sieved as in Experiment 1 and stored in the greenhouse at the University of Toronto.

Asclepias syriaca plants were grown from seed (SKU: C11570; Wildflower Farm, Coldwater, ON, Canada). In May 2021, seeds were soaked as in Experiment 1, and planted individually in 0.5-L pots containing triple mix (Less Mess Products, Concord, ON, Canada). Seeds were watered as necessary, and pots were rotated weekly to control for any spatial variations in greenhouse conditions. On July 6, 2021, at 10 wk after planting, 92 *A. syriaca* plants were randomly selected, and transplanted into either *V. rossicum*–conditioned soil (46 plants) or control soil (46 plants). Immediately after transplanting, stem height was recorded for each experimental plant to ensure that plant size was relatively even between treatments. Plants were then watered as needed and rotated every 3 d as described earlier. On August 13, 2021, just over 5 wk after transplantation, plant height and number of leaves were recorded again for all 92 experimental plants. All aboveground plant material was removed at the soil level and placed in labeled paper bags. Roots were then removed from the soil before being washed and placed in labeled paper bags. All plant material was dried at 60 C for 72 h before being weighed.

Experiment 3: Assessing the Effects of Soil Conditioned by Vincetoxicum rossicum or Asclepias syriaca on the Success of Native Seed Mixes

We compared coverage and biomass produced by two native seed mixes in control soils and soils conditioned with 13 wk of *V. rossicum* or *A. syriaca* growth from seed. This experiment used a two by three randomized factorial "soil feedback" design (Klironomos 2002; Quinn and Keough 2002). The factorial design tested two factors, seed mix (sun mix and semi-shade mix; Table 1) and soil treatment (*V. rossicum*, *A. syriaca*, and control). Ten replicates of each combination were conducted for a total of 60 replicates. Landscape fabric was placed in the bottom of 60 clear plastic containers (29.8 by 46.3 by 20.8 cm), each with 13 drainage holes cut in the bottom. These containers were filled with a mixture of field soil and root tissue collected from three open meadows in Toronto, ON (43.760989, −79.244806; 43.757751, −79.249238; and 43.803128, −79.183694) (15%) and pro-mix BX (85%; Premier Tech Horticulture, Rivière-du-Loup, QC, Canada). In these soil/potting media mixtures, 32 *V. rossicum* seeds (field-collected from Crother's Wood, Toronto, ON, Canada 43.696812, −79.359441),

32 *A. syriaca* seeds (Wildflower Farm), or no seeds (control) were sown in each container. Containers were positionally randomized each week as in earlier experiments and watered as needed. After 3 wk, 20 additional *V. rossicum* or 7 additional *A. syriaca* seeds were added to each container due to poor initial germination. After 8 wk, containers were thinned to five plants (except for one container in which only four plants were left, and one in which only three plants germinated). After 13 wk, stems of all plants were removed. The roots of two randomly selected plants per container were also removed for molecular analysis of fungal associates (data not included here), while the remaining root tissue was left in the containers as a source of allelochemicals and mycorrhizae.

Each container was then seeded with 1.0 g of one of two native seed mixes (Wildflower Farm) (Table 1) that had been allowed to soak overnight in Nutriboost 1. After 5 wk, plant community cover was measured from overhead photographs (Figure 1; Supplementary Information). Graminoids were harvested for aboveground biomass at 12 wk after seeding, and forbs (a negligible contribution to overall community cover/biomass) were harvested at 13 wk after seeding. Harvested plants were dried and weighed as described previously.

Experiment 4: Assessing the Effects of Aqueous Extracts of Asclepias syriaca on the Growth of Vincetoxicum rossicum from Root Crowns

We compared the growth and fitness of *V. rossicum* plants grown from root crowns treated with extracts of *A. syriaca* versus water controls. For this experiment, we used a one-way independent-samples design comparing growth of treated versus untreated plants. Aqueous *A. syriaca* extracts were prepared based on methods by Wilson and Rice (1968) and Rasmussen (1975). Leaves of *A. syriaca* were collected from Whitby, ON (43.958361, −78.942260) in July and stored at −18C. Aqueous extracts were produced by adding 750 g of leaves to 2,500 ml of distilled water and homogenizing the mixture in a blender. An additional 5 L of distilled water was then added, and the suspension was boiled for 10 min and allowed to cool until safe to handle. The suspension was then filtered through household coffee filters to remove particulates, and diluted 1:1 with distilled water. From this, 100-ml aliquots were prepared and frozen until use. Extracts and distilled water controls were allowed to thaw overnight before being used to treat *V. rossicum* plants.

Vincetoxicum rossicum root crowns were collected from Uxbridge, ON (44.088923, −79.107089), in June 2021 and stored in freezer bags at 5 C. To set up the experiment, 46 *V. rossicium* root crowns were potted in Pro-Mix HP + mycorrhizae medium (Premier Tech Horticulture) using round 750-ml greenhouse pots. Root sections were weighed before planting (7.0 ± 0.1 g, mean ± SE); the mean initial biomass of roots allocated to the treatments was not significantly different (P = 0.173). Planted roots were treated with either 100 ml of *A. syriaca* extract (treatment, $n = 23$) or distilled water (control, $n = 23$) weekly for 5 wk. Experimental pots were positionally randomized each week and watered as needed. Five weeks after the start of the experiment, the tallest stem in each pot was measured for stem height and chlorophyll content index (CCI). Measurement of the CCI was taken as the average of three repeat measurements with a CCM-200 Plus CCI meter (Opti-Sciences, Hudson, NH, USA) from a randomly selected, recently emerged leaf (Parry et al. 2014). Additionally, all above- and belowground plant parts were harvested and dried as described previously for biomass measurements.

Table 1. Two seed mixes (sun and semi-shade) used in Experiment 3

Scientific name	Percentage by count	Percentage by weight	Seed mix[a]
Andropogon gerardii Vitman	5.53	6.88	Sun
Asclepias syriaca L.	0.32	0.98	Sun
Asclepias tuberosa L.	1.69	5.89	Sun
Bouteloua curtipendula (Michx.) Torr.	7.86	9.82	Sun
Coreopsis lanceolata L.	6.72	9.82	Sun
Desmodium canadense (L.) DC.	0.67	1.96	Sun
Elymus canadensis L.	8.22	19.65	Sun
Lespedeza capitata Michx.	2.94	3.93	Sun
Linum lewisii Pursh	3.94	5.89	Sun
Lolium perenne L. spp. *multiflorum*[b]			Sun
Monarda fistulosa L.	3.58	0.98	Sun
Oenothera biennis L.	2.37	0.59	Sun
Oligoneuron rigidum (L.) Small	6.01	1.96	Sun
Panicum virgatum L.	10.24	7.86	Sun
Pycnanthemum virginianum Michx.	3.48	0.20	Sun
Rudbeckia hirta L.	8.38	1.47	Sun
Schizachyrium scoparium (Michx.) Nash	11.86	9.82	Sun
Silphium perfoliatum L.	0.06	0.49	Sun
Sorghastrum nutans (L.) Nash	3.80	3.93	Sun
Sporobolus heterolepis A. Gray	7.59	5.89	Sun
Verbena stricta Vent.	4.74	1.96	Sun
Agastache foeniculum (Pursh) Kuntze	2.88	0.39	Semi-shade
Allium cernum Roth	1.64	1.58	Semi-shade
Anaphalis margaritacea L. Benth.	3.82	0.16	Semi-shade
Aquilegia canadensis L.	3.15	1.58	Semi-shade
Elymus hystrix L.	13.32	15.79	Semi-shade
Elymus virginicus L.	29.44	63.17	Semi-shade
Eupatorium purpureum (L.) E.E. Lamont	2.32	0.79	Semi-shade
Helianthus strumosus L.	0.63	1.18	Semi-shade
Heliopsis helianthoides (L.) Sweet	2.16	4.74	Semi-shade
Liatris aspera Michx.	2.45	1.58	Semi-shade
Lobelia siphilitica L.	13.15	0.24	Semi-shade
Lolium multiflorum Lam.[b]			Semi-shade
Lupinus perennis L.	0.28	3.16	Semi-shade
Ratibida pinnata (Vent.) Barnhart	3.86	1.58	Semi-shade
Symphyotrichum laeve (L.) A. Love & D. Love	5.39	1.18	Semi-shade
Symphyotrichum novae-angliae (L.) G.L. Nesom	2.89	0.39	Semi-shade
Veronicastrum virginicum (L.) Farw.	10.52	0.12	Semi-shade
Zizia aurea (L.) W.D.J. Koch	2.10	2.37	Semi-shade

[a]Seed mixes were obtained from Wildflower Farm (Coldwater, ON, Canada).
[b]Included as a nurse crop.

Statistical Analysis

For Experiment 1, germination was compared between soil types with a one-sided Fisher's exact test. Mean above- and belowground plant biomass were compared between soil types with a Welch's *t*-test for each species. Welch's *t*-tests are more reliable when the populations being compared have unequal variances.

For Experiment 2, we compared plant height immediately after transplantation and after treatment using Welch's *t*-tests. Root biomass and shoot biomass were also compared between soil types using Welch's *t*-tests.

For Experiment 3, the normality assumptions of ANOVA were not satisfied with either biomass or cover. For community biomass, this was addressed by converting data values to ranks that indicate relative magnitude (e.g., Quinn and Keough 2002). For community biomass data, this transformation allowed comparison among treatments using a two-way ANOVA and Tukey's honest significant difference (HSD) post hoc. Percentage plant cover was compared across soil treatments and seed mixes in an exploratory analysis using a Schreirer-Ray-Hare test. We assessed assumptions of data or residual normality and heteroscedasticity using visual inspection of quantile–quantile plots and box plots respectively. Data were borderline even after rank transformation,

so as a conservative measure, we used the Schreirer-Ray-Hare test designed for nonparametric data.

Because neither seed mix nor the interaction of seed mix and soil treatment were significant factors in the model, both were removed. The percentage plant cover was then compared among soil types using a Kruskal-Wallis test with Dunn's multiple-comparison method (Dunn 1964; Midway et al. 2020).

For Experiment 4, we compared biomass, CCI, and stem measurements across treatments with *t*-tests as before. Flowering was compared between treatments using generalized linear models with a binomial logistic family.

All statistical analyses were performed in R v. 4.3.1. (R Core Team 2023) using libraries agricolae for its implementation of Tukey's HSD test (de Mendiburu 2021), MASS for its implementation of generalized linear models (Venables and Ripley 2002), and FSA (Ogle et al. 2021) for implementation of Dunn's (1964) multiple-comparison method.

Results and Discussion

Experiment 1 compared *V. rossicum* and *A. syriaca* seedling emergence using soil from a 15-yr-old *V. rossicum* infestation as
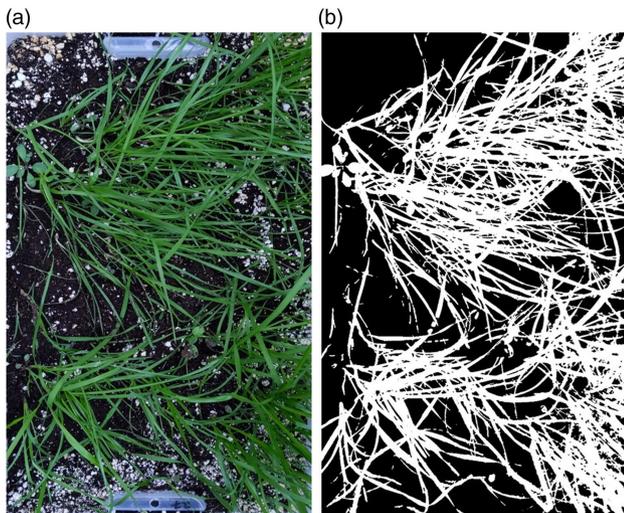
Figure 1. Percentage plant cover assessments for soil feedback experiment. Images were manually cropped to container edges in GIMP (A), then converted to HSV colorspace, masked at [30,25, 25] and [90, 255,255] (determined experimentally to give the best results), decomposed into channels, smoothed with a 5 x 5 gaussian convolution kernel, and binarized with Otsu's, (1979) algorithm in Python (B). From these processed images, percent cover was calculated as the ratio of non-black pixels in the image to the total number of pixels, times 100. In this example, percent cover was 45.7%.

well as control soil from 10 m outside the infested area. The proportion of seeds germinating in invaded soil was lower for both *A. syriaca* (0.161) and for *V. rossicum* (0.033) than for either *A. syriaca* (0.210) or *V. rossicum* (0.067) in uninvaded soil, with the effect being marginal in *A. syriaca* ($N = 720$, $P = 0.058$) and significant in *V. rossicum* ($N = 720$, $P = 0.029$) (Figure 2A). Mean *V. rossicum* above- ($N = 24$, $t = -0.9758_{(23,1)}$, $P = 0.171$) and belowground biomass ($N = 24$, $t = -0.5202_{(23,1)}$, $P = 0.304$) were not significantly different in invaded and uninvaded soil (Figure 2B and 2C). Conversely, in *A. syriaca*, both aboveground biomass (14.8 ± 0.8 mg) (mean $\pm$ SE) ($N = 83$, $t = -2.0333_{(82,1)}$, $P = 0.023$) and belowground biomass (10.4 ± 0.8mg) ($N = 83$, $t = -3.1782_{(82,1)}$, $P = 0.001$) were significantly lower in *V. rossicum*–invaded soil than in control soil (16.8 ± 0.6 mg) (Figure 2B and 2C).

Soil conditioned by a mature *V. rossicum* infestation inhibited seed germination in *V. rossicum* and, to a lesser extent, *A. syriaca*. This is not the first time that *V. rossicum* has been implicated in inhibiting seed germination (Cappuccino 2004; Douglass et al. 2011). Douglass et al. (2011) found that the presence of *V. rossicum* seedlings in agar resulted in reduced germination in lettuce. While these results support those of prior work, the mode of action may be entirely different. Previous experiments involved no soil, and effects on germination were likely related to allelopathy. Our observations of reduced seedling emergence in *V. rossicum*–infested soil could equally be related to differences in the rhizosphere. Infestations of *V. rossicum* are known to cause shifts in AMF communities (Bongard et al. 2013; Dickinson et al. 2021), and such shifts have been associated with changes in seedling emergence in other plant systems (Hartnett et al. 1994; Seiwa et al. 2020). Future work should seek to isolate the effects of common fungal associates on seed germination and seedling emergence in *V. rossicum* and its native competitors. In addition to inhibiting seed germination, soil from a mature *V. rossicum* infestation led to a reduction in above- and belowground biomass in *A. syriaca* seedlings. Invasive plants have previously been shown to reduce

the growth of native plants by eliciting changes in soil conditions. For example, garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara & Grande; Cruciferae], suppresses tree seedlings in North American forests by disrupting their mutualisms with AMF (Stinson et al. 2006). *Vincetoxicum rossicum* infestations have themselves been studied in connection with changes in native plant growth (Bongard et al. 2013; Day et al. 2016; Dickinson et al. 2021; Dukes et al. 2019). A study comparing 54 infested sites across southern Ontario showed that *V. rossicum* biomass production is enhanced by the accumulation of fungal associates, including dark septate endophytes, many of which are pathogenic (Dickinson et al. 2021). The degree to which this enhanced biomass is related to the disruption of fungal associations in native plants is unclear; however, it is likely that changes in fungal communities play a role in *V. rossicum* invasions.

For Experiment 2, we compared the growth of potted *A. syriaca* plants transplanted into soil from a 15-yr-old *V. rossicum* infestation with those transplanted into soil from 10 m outside the infested area. A comparison of stem heights immediately after transplantation showed no difference in plant size between experimental groups before treatment ($N = 91$, $t = -0.416_{(90,1)}$, $P = 0.678$). We observed no difference in *A. syriaca* plant height ($N = 91$, $t = -0.933_{(90,1)}$, $P = 0.177$), belowground biomass ($N = 91$, $W = 1,196$, $P = 0.101$), or aboveground biomass ($N = 91$, $W = 848.5$, $P = 0.070$) between control or *V. rossicum*–invaded soil. Soil conditioned by a mature *V. rossicum* infestation had no negative impact on the growth of transplanted *A. syriaca* plants. Experiments 1 and 2 showed a pattern in which *V. rossicum*–infested soil negatively affected germination and seedling emergence of both *A. syriaca* and *V. rossicum*, but not the growth of established *A. syriaca* plants. These results suggest that any changes in soil conditions brought about by *V. rossicum* infestations, in the case of *A. syriaca*, are more likely to prevent the establishment of new plants than impact the fitness of existing ones. The poor performance of *A. syriaca* seeds casts doubt on their usefulness as a tool for site restoration. However, the effects of *V. rossicum* on native plants is inconsistent across species (Dukes et al. 2019), so we went on to test the efficacy of diverse seed mixes in *V. rossicum*–conditioned soil. It should be noted that the use of a single field site for soil sampling in Experiments 1 and 2 limits the scope of statistical inference that can be drawn from the results (Hurlbert 1984); however, the use of a single site also helped to control for other variations in soil conditions that might have influenced the results.

For Experiment 3, we measured mean plant cover and community biomass produced by two native seed mixes in soils conditioned by *V. rossicum* and *A. syriaca* compared with control soils. Percentage plant cover was not significantly affected by seed mix (Schreirer-Ray-Hare: $n = 10$, $H = 0.238_{(1)}$, $P = 0.626$) or by the interaction of seed mix and soil history (Schreirer-Ray-Hare: $n = 10$, $H = 2.307_{(2)}$, $P = 0.316$). Percentage plant cover was, however, significantly affected by soil history (Kruskal-Wallis test: $n = 20$, $\chi^2 = 22.215$, $P < 0.001$). Soil conditioned with *A. syriaca* produced significantly lower plant cover ($28.760 \pm 2.725\%$) than control soil ($47.632 \pm 2.266\%$) ($z = -4.220$, $P < 0.001$) and soil conditioned with *V. rossicum* ($45.670 \pm 3.559\%$) ($z = -3.929$, $P < 0.001$). Soil conditioned with *V. rossicum* did not differ from control soil in terms of mean plant cover ($z = 0.290$, $P = 0.772$) (Figure 3A).

When measuring rank-transformed community biomass in Experiment 3, we observed a significant effect of soil history ($n = 10$, $F = 15.312_{(2)}$, $P < 0.001$) and a significant interaction
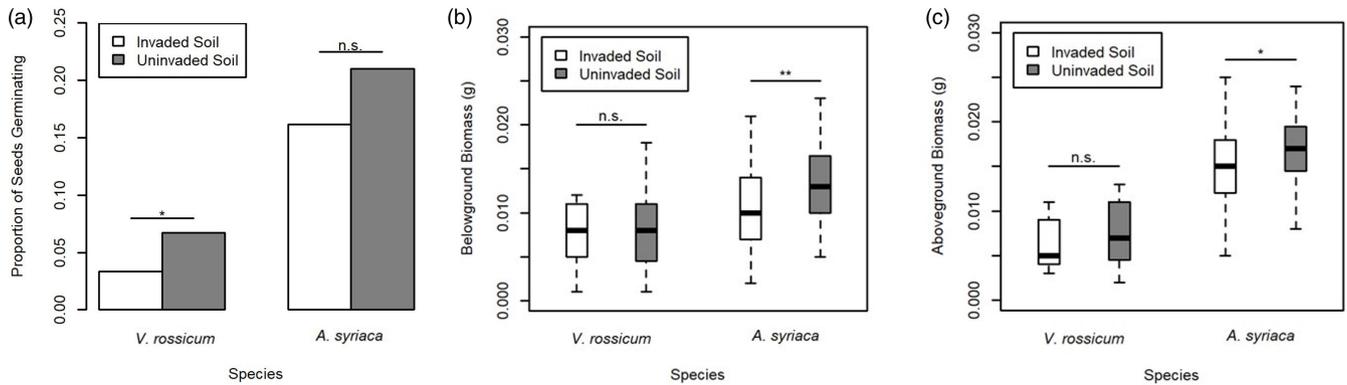
Figure 2. Germination and early establishment of *Vincetoxicum rossicum* and *Asclepias syriaca* in *V. rossicum*–invaded or control (uninvaded) soil. (A) Germination, (B) aboveground biomass, and (C) belowground biomass. n.s., not statistically different (P > 0.1); *significant (P < 0.05) difference; **highly significant (P < 0.01) difference.
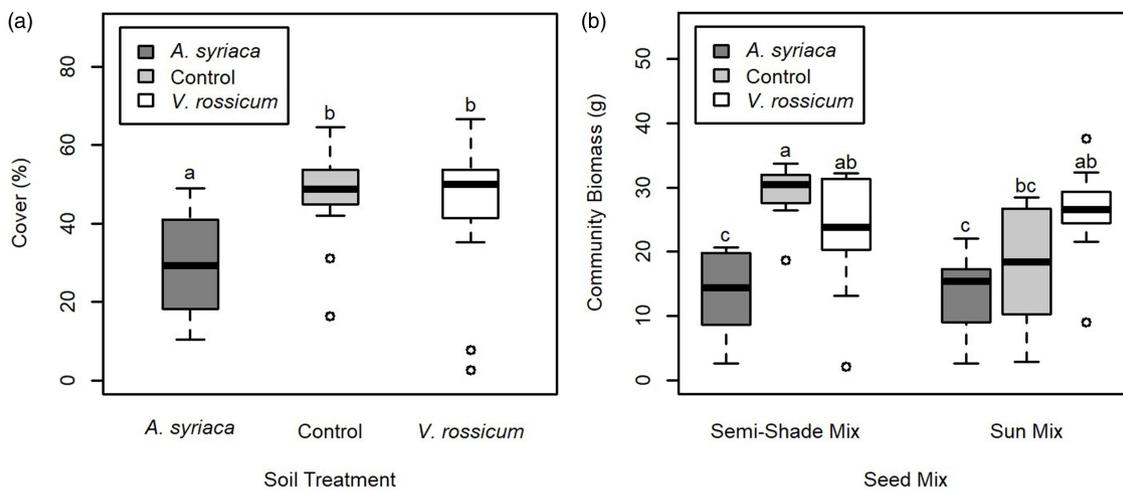

Figure 3. (A) Community percentage cover compared among three soil treatments after 5 wk (data from both seed mixes were combined, as seed mix was not a significant factor in the model). (B) Community biomass after 13 wk of growth in soil conditioned with *Asclepias syriaca*, *Vincetoxicum rossicum*, or control (unconditioned soil). Different letters indicate significant (P < 0.05) differences among treatments.

between seed mix and soil history ($n = 10$, $F = 5.86_{(2)}$, P = 0.005). When the sun seed mix was used, neither soil conditioned with *V. rossicum* nor soil conditioned with *A. syriaca* differed from control soil (P = 0.136 and P = 0.751, respectively). Soil conditioned with *A. syriaca*, however, produced significantly lower community biomass (13.56 ± 2.12 g) than soil conditioned with *V. rossicum* (26.04 ± 2.37) ($n = 10$, difference = −23.4, P = 0.004). When the semi-shade seed mix was used, soil conditioned with *A. syriaca* produced significantly lower community biomass (13.52 ± 2.12 g) than control soil (29.10 ± 1.37 g) ($n = 10$, difference = −31, P = 0.001), and soil conditioned with *V rossicum* (22.98 ± 3.03 g) ($n = 10$, difference = −19, P = 0.03). Community biomass was not significantly different between control soil and soil conditioned with *V. rossicum* ($n = 10$, difference = 12.0, P = 0.362) (Figure 3B).

Overall, the two native seed mixes used in Experiment 3 were not inhibited by soil conditioned by *V. rossicum*, but they were inhibited by soil conditioned with *A. syriaca*. These results indicate that reseeding has potential as a tool for restoring ecosystem function to *V. rossicum*–infested sites, as has proven to be the case in some other systems (Barlow et al. 2020; Cuneo and Leishman 2015). Past research has shown the effects of *V. rossicum*–conditioned soil on native plant growth to be highly inconsistent (Day et al. 2015, 2016; Dukes et al. 2019). By using seed mixes

containing many species, we may provide the functional redundancy required to restore ecosystem function in the face of changes in soil conditions exerted by *V. rossicum*. During our reseeding experiment, much of the native biomass in *V. rossicum*–conditioned soil was made up of grass species including *A. gerardii, E. canadensis, E. hystrix,* and *E. virginicus,* and the nurse crop *L. perenne* spp. *multiflorum*. Based on these results we advocate for the inclusion of these species in seed mixes used for post–*V. rossicum* restoration.

Soil conditioned with *A. syriaca* yielded significantly lower percentage cover and biomass of native plants. The allelopathic effects of *A. syriaca* have been reported previously in Europe, where the plant is an important introduced weed in agricultural systems (Cramer and Burnside 1982; Follak et al. 2021; Nádasy et al. 2018; Rasmussen 1975). *Asclepias syriaca* would seem a logical choice as a species for ecological restoration after *V. rossicum* infestation, not only because of its relatedness, but because its displacement is problematic for monarch butterflies for which *V. rossicum* is an oviposition sink (Casagrande and Dacey 2007). Our results, however, highlight the possible allelopathic effects of *A. syriaca* and caution against its use. For this reseeding experiment, we chose to inoculate experimental soils by growing the test plants in greenhouse conditions for a period of 13 wk, rather than collecting soils from infested habitats. This choice allowed us to compare the effects *V. rossicum* and *A. syriaca* more accurately, because field sites
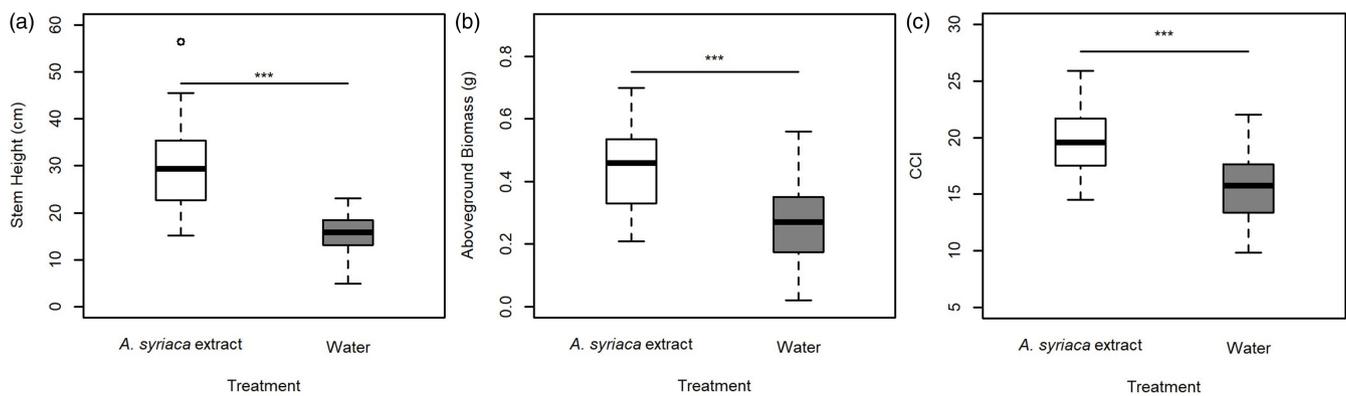
Figure 4. Growth of *Vincetoxicum rossicum* plants grown from rootstock and treated with aqueous extracts prepared from *Asclepias syriaca* leaves (white) or distilled water (gray). (A) Stem height, (B) aboveground biomass, and (C) chlorophyll content index (CCI). Asterisks indicate highly significant differences (***P < 0.0001; *P < 0.01) in group means.

with a monoculture of *A. syriaca* could not be found. Additionally, allelopathic compounds produced by *V. rossicum* have been found to be relatively unstable, persisting only for short periods in collected soil (Gibson et al. 2015). Although 13 wk has been shown to be enough time for plant roots to recruit fungal associates and produce bioactive concentrations of allelopathic chemicals (Day et al. 2015; Dukes et al. 2019, Weißhuhn and Prati 2009), future research should focus on testing seed mixes in soil collected from mature infestations and under field conditions.

Building on the effects of *A. syriaca* on native seed mixes, we tested the effects of aqueous *A. syriaca* extracts on *V. rossicum* plants grown from rootstock (Experiment 4) to determine whether phytotoxins found in *A. syriaca* tissue might be useful as a tool to suppress *V. rossicum* regrowth after removal. Stem height was significantly higher in *V. rossicum* plants treated with *A. syriaca* extracts (29.1 ± 2.0 cm) than in controls (15.0 ± 0.9 cm) ($N = 46$, $W = 489.5$, P < 0.001) (Figure 4A). Aboveground biomass was significantly higher in plants treated with *A. syriaca* extracts (0.433 ± 0.030 mg) than in controls (0.260 ± 0.027 mg) ($N = 46$, $t = 4.7334_{(45,1)}$, P < 0.001) (Figure 4B). CCI was also significantly higher in plants treated with *A. syriaca* extracts (19.7 ± 0.6) than in controls (15.8 ± 0.6) ($N = 46$, $t = 4.3511_{(45,1)}$, P < 0.001) (Figure 4C). In addition to exhibiting increased growth, plants treated with aqueous extracts of *A. syriaca* were significantly more likely to produce flowers (78%) than control plants (4.3%) ($N = 46$, $z = -3.833_{(45,1)}$, P < 0.001).

The positive effects of *A. syriaca* extracts on *V. rossicum* plant growth and fitness were surprising, but not without precedent. Cramer and Burnside (1982) found that decomposing *A. syriaca* tissue enhanced growth in *Sorghum bicolor* L. (Poaceae), even though aqueous extracts of *A. syriaca* are phytotoxic to the same species (Rasmussen 1975). In our experiment, the cause of the increased *V. rossicum* growth is not clear, but may be the result of a fertilization effect if *A. syriaca* extracts contained previously limiting nutrients. Several studies have also demonstrated hormesis in response to low doses of allelopathic chemicals (Abbas et al. 2017). For example, Santa-Maria feverfew (*Parthenium hysterophorus* L.; Asteraceae) is known to produce several phytochemicals, including the sesquiterpene lactone parthenin. Although parthenin is known to inhibit the growth of neighboring plants, some species, including wild mustard (*Sinapis arvensis* L.; Brassicaceae), show enhanced growth when exposed to low doses (Belz 2008). In the future, dose–response studies should be conducted to accurately characterize the relationship between *V. rossicum* and phytochemicals produced by *A. syriaca* (Belz et al. 2007).

The cause of earlier flowering in extract-treated plants is also unclear. Early flowering could be a response to stress (e.g., Takeno 2016), or it could simply be an indication of increased reproductive output in treated plants (Cappuccino 2004). Regardless of the specific mechanism, these results provide further evidence that *A. syriaca* may not be a suitable species for use in the restoration of ecosystems invaded by *V. rossicum*.

Our findings contribute to an understanding of the ecology of *V. rossicum*–invaded sites and can direct restoration efforts in two key ways. First, *V. rossicum*–conditioned soil negatively affected *V. rossicum* seedling establishment, suggesting that residual *V. rossicum* seeds will not necessarily dominate the competition after the removal of mature plants from an infested site. Second, the use of diverse native seed mixes shows great potential for restoring productivity and function to ecosystems affected by *V. rossicum* infestations. Testing the use of similar seed mixes at sites that have been cleared of *V. rossicum* infestations across a range of soil conditions could help to identify the native species best suited for restoration on a site-by-site basis.

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