INFORMING RARE PLANT CONSERVATION WITH EXPERIMENTAL TRANSLOCATIONS AND COMMON GARDEN TRIALS

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DEDICATION

I dedicate this thesis to Walter Muma, who never passed up botanizing a ‘nice ditch’.

ABSTRACT

Conservation translocations are intentional movements of species within or outside their indigenous range with the aim of ensuring population persistence. Despite the rising use of translocations as a recovery tool, many translocations fail because of an inability to identify suitable habitat. In addition, the population differentiation (genetic and phenotypic) of many rare plant species is unknown making seed sourcing decisions challenging. I tested the efficacy of species distribution models (SDMs) and plant community-based models (CBMs) to identify suitable habitat for two rare plant species in southern Ontario, Canada (*Stylophorum diphyllum* and *Symphyotrichum prenanthoides*). First, I conducted a seed addition trial for *S. diphyllum* to determine if the distribution of the species in southern Ontario is limited by dispersal, abiotic conditions (suitable habitat), or biotic interactions (seed predators). Seedling emergence was limited by dispersal coupled with seed predation, whereas survival was predicted by microclimate conditions, and neither were correlated with SDM-predicted habitat suitability. Next, I conducted translocation trials to assess the efficacy of SDMs and CBMs. Neither model was a strong predictor of short-term survival, however both models were positively correlated to one measure of reproductive success. Lastly, I conducted common garden trials to determine the magnitude of phenotypic differentiation among *S. diphyllum* populations. Canadian populations are genetically and phenotypically distinct from nursery sources yet show low phenotypic differentiation among populations despite high genetic differentiation. Using commercial nursery sources for translocations in Canada should be avoided but mixing wild Canadian sources poses little risk and high potential benefits. Together, these experiments elucidate the ecological and evolutionary processes that constrain species occurrences and population persistence with direct implications for improving conservation translocations success rates.

CONTRIBUTIONS OF AUTHORS

Chapter 1: Emma R. Neigel collected, curated, and analyzed the data, co-led project conceptualization, and was the primary writing lead of the manuscript. **J.L. McCune** secured funding for the research, co-led project conceptualization, helped with data collection, advised on data analysis, and supported writing and editing the manuscript. **Timothy D. Schwinghammer** advised on statistical analysis and performed the response surface regressions. This chapter has been published in Journal of Ecology **113**: 2148-2159.

Chapter 2: Emma R. Neigel collected, curated, and analyzed the data, co-led project conceptualization, and was the primary writing lead of the manuscript. **J.L. McCune** secured funding for the research, co-led project conceptualization, helped with data collection, advised on data analysis, and supported writing and editing the manuscript. **Ryan Norris** secured funding for the research, co-led project conceptualization, and supported editing the manuscript. **Axel Moehrenschlager** secured funding for the research. **Nina Hunt** helped to write the propagation methods, conducting seed viability testing, and supported editing of the manuscript.

Chapter 3: Emma R. Neigel collected, curated, and analyzed the data, co-led project conceptualization, and was the primary writing lead of the manuscript. **J.L. McCune** secured funding for the research, co-led project conceptualization, helped with data collection, advised on data analysis, and supported writing and editing the manuscript. **Ryan Norris** secured funding for the research. **Jessenia Buzunis-Delagneau** analyzed the leaf samples and helped to write the leaf analysis methodology.

USE OF GENERATIVE AI

I acknowledge the use of OpenAI's ChatGPT 5.2 (https://chatgpt.com/) to generate R code for nlme models and plots in Chapter 2 and Chapter 3.

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It's been a five-year journey since I first started my PhD. I still remember the first time I spoke to my PhD supervisor, **Dr. Jenny L. McCune**. We talked over Zoom to discuss this project and immediately afterwards I decided it was the perfect fit for me. I was working as a conservation horticulturist at Atlanta Botanical Garden (ABG) in Georgia at the time, where I had gotten a handful of opportunities to help with plant translocations. My previous manager, **Matt Richards**, sparked my interest in translocations when he invited me to help translocate *Platanthera integrilabria* orchids in Tennessee. **Dr. Jess Stephens** later shared Jenny's ad seeking a PhD student focused on rare plant translocations and the rest is history! I thank **Dr. Emily Coffey** for supporting me to pursue my PhD and giving me the *Ex situ plant conservation* book to help kickstart my learning journey (it was especially useful for my comprehensive exams). I can't express how much gratitude I have for the Georgia Plant Conservation Alliance (GPCA) community for helping me grow and enabling me to build the skillset to pursue this endeavor. Thank you to **Jennifer Ceska** for being my biggest cheerleader throughout this process and encouraging me to pursue the next level afterwards.

During my first field season (2021), Jenny and I drove from Lethbridge, Alberta across Canada to the forests of southern Ontario with our undergraduate field assistant, **Kirsty McFadyen**. We stayed in a basement suite together in London, Ontario, better known as the 'icebox'. We spent many long days in the forests, surveying plant communities within multi-flora rose and poison ivy infested areas. Kirsty was a true superstar and helped me construct almost 100 cages for the seed addition trial in the ice box. Her critical thinking and ability to work through tough times with zero complaints is truly admirable. Jenny was the heavy lifter that season and took the lead in the field and at home, pressing many unknown sedges and grasses

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INTRODUCTION

Human-mediated species movements

Humans have a long history of moving species around for food, medicine, and cultural purposes. The domestication of plants and animals can be dated back to around 12,000 years ago when transitions from hunter-gatherer to agricultural societies began (Carey 2023). Ancient settler societies deliberately introduced non-native species to new areas which led to unintentional consequences for native species. For example, Polynesian settlers brought culturally significant 'canoe plants' to the Hawaiian Islands when they settled in approximately 800 to 1200 AD but also introduced rats which contributed to many native species extinctions (Athens et al. 2002, Kirch 2011). In the 1800s, there was a shift to species being moved for the purpose of conservation to help restore native ecosystems. One such case was the reintroduction of Scandinavian red squirrels (*Sciurus vulgaris*), which were moved into Scotland in 1793 after they had become nearly extinct from the United Kingdom largely due to deforestation (Harvie-Brown 1880). Other early cases of these conservation-based movements can be attributed to local people moving plants to restore recently burned or wild-harvested areas (Silcock 2018, Turner et al. 2021). For example, the Aboriginal peoples of Australia replanted "ground-berry" (possibly *Eremophila debilis*) in the early 1900s into areas they had harvested from (Silcock 2018). The use of reintroduction as a conservation tool continued to rapidly rise after the 1980s, leading to the establishment of the 'Reintroduction Specialist Group' (RSG) (now 'Conservation Translocation Specialist Group (CTSG)) by the International Union for the Conservation of Nature (IUCN) Species Survival Commission in 1988. This group developed best practice guidelines for conservation translocations (revised 2013; Seddon et al. 2007, IUCN/SSC 2013).

Conservation translocations (hereafter translocations) are the intentional movement of species within or outside their indigenous range for the benefit of conservation, such as improving species status or ecosystem functioning (IUCN/SSC 2013). Several types of translocations exist, including reintroduction, augmentation, and assisted migration. Translocations have successfully reintroduced extirpated keystone species such as the American Bison (*Bison bison*) and the Eurasian beaver (*Castor fiber*) to their native ranges (Kleiman 1989, Jones and Campbell-Palmer 2014). Translocations can also bolster existing populations (augmentation/reinforcement), as seen in greater sage-grouse (*Centrocercus urophasianus*) translocation efforts which successfully augmented a small population in California over five years (Bell and George 2012). Augmenting populations can also increase genetic diversity and relieve inbreeding depression through introducing new genes from other populations, such as for the Florida panther (*Puma concolor coryi*) populations that received genetic rescue via the translocation of panthers from Texas (Finn et al. 2013). In addition, translocations to establish new populations outside a species' indigenous range (assisted migration/ introduction) may be critical for dispersal-limited species. For example, Barlow and Martin (2004) suggest the Florida Torreya (*Torreya taxifolia*) now occupies climatically unsuitable habitat and will require assisted migration northward to persist. Similarly, many European tree species exhibit an inability to disperse far enough to track near-future climate changes (Svenning and Skov 2004). Although many types of translocations exist, they share the objective to establish resilient, self-sustaining populations (Griffith et al. 1989).

Translocations should never be the first action towards species recovery and should be used in concert with actions that conserve species *in situ.* However, the use of translocations remains controversial by those that fear they undermine the protection of *in situ* populations. For

example, a ‘climate of caution’ (Clements 2013) surrounds the use of plant translocation in Canada due to this fear and also because of the potential genetic risks to recipient populations (i.e., via introducing maladapted genes) and disturbances to recipient plant communities (i.e., disease transfer). These concerns have led to policies opposing the use of translocation for conservation, especially when other viable options remain to conserve species *in situ* (Canadian Botanical Association 2014).

While conserving *in situ* populations remains the highest conservation priority, translocations can be used in tandem to help reduce extinctions (Seddon et al. 2007, Gaywood et al. 2023). Scientists estimate one million species are threatened with extinction, driven by five anthropogenic threats including changes in land and sea use, direct exploitation, climate change, pollution, and invasive species (IPBES 2019). These anthropogenic threats have led to an increase in the use of translocations as a recovery tool, primarily in response to habitat loss and/or habitat degradation (Gaywood et al. 2023). Consequently, many countries now use translocations as a recovery tool and have recommended and/or implemented them as part of species recovery actions. In the U.S., translocations have been performed for at least 42% of listed species and have helped in the recovery of 30% of the species that are now downlisted to lower risk categories (Novak et al. 2021). However, despite many translocation successes, there have also been many past and ongoing translocation failures. This emphasizes their complexity, which is related to many aspects of the species’ biology (Griffith et al. 1989, Godefroid et al. 2011, Morris et al. 2021).

Conservation translocations in the framework of ecological theory

Throughout the 1900s, many translocations were viewed simply as 'management manipulations' or haphazard experiments with little regard to the scientific process (Seddon et al. 2007). Since the 1980s, there has been a substantial gain of knowledge from well-organized translocations and the best practices guidelines developed by the Conservation Translocation Specialist Group (CTSG; previously RSG), which has shifted the mentality towards testing specific research questions (Seddon et al. 2007). Adaptive management is a flexible decision-making process that integrates learning through doing by designing management actions as experiments that are continually evolving based on new knowledge (Holling 1978, Williams et al. 2009). For example, black-footed ferret (*Mustela nigripes*) reintroduction programs implemented adaptive management through experimentally testing prerelease conditions and found that ferrets reared in quasi-natural outdoor pens with the presence of prairie dog (*Cynomys leucurus*) burrows had higher survival rates (Biggins et al. 1999). Future releases were refined to yield higher survival rates by releasing ferrets reared in these conditions (Biggins et al. 1999.). Implementing adaptive management can be difficult and is not always successful (Allen and Gunderson 2011) but treating translocations as experiments has helped identify predictors of translocation success and failure (Godefroid et al. 2011, Berger-Tal et al. 2020, Bellis et al. 2024). Known predictors of success include the life-stage of translocated individuals (Albrecht and Maschinski 2012, Muths et al. 2014), origin of the source population (aka. provenance; Bischoff et al. 2006, Lloyd et al. 2019), and microsite suitability (Dunwiddie and Martin 2016). Overall, translocation design can be optimized by applying ecological theory to meet management objectives and likewise using translocation trials as experiments to test underlying ecological theory.

A central goal in ecology is to understand the processes by which species are included or excluded from a community (Gleason 1927, Clements 1936). Community assembly theory postulates that the presence of species within a local community results from of a series of hierarchical filters (Keddy 1992). Improving translocation success requires understanding the relative influence of each filter by designing experiments to test them. First, the species must be able to arrive (disperse) at a site to become a member of the community. Next, the species must be able to tolerate the abiotic environment at the site, including temperature and light conditions. Lastly, biotic interactions, such as competition, natural enemies (i.e., herbivores, pathogens), and mutualists serve as filters determining the species' establishment and survival.

The relative influence of each filter in limiting species occurrences is an ongoing debate (Gleason 1927, Clements 1936, Ricklefs 1987, Chase 2003). Translocation trials can help to assess the relative influence of each filter. For example, seed addition trials can test the role of dispersal limitation versus abiotic microsite suitability by simulating dispersal to unoccupied habitats and recording recruitment (Eriksson and Ehrlen 1992, Turnbull et al. 2000, Clark et al. 2007). If seedlings emerge and persist, dispersal limitation can be deemed the predominant driver. If seedlings do not emerge or fail to persist, abiotic microsite limitation can be inferred to be the primary constraint. Biotic interactions can be assessed by manipulating competition or predation (Smith et al. 2010, Love et al. 2024). Understanding the relative influence of these ecological filters is important for translocation success but only a few plant translocations studies have tested them simultaneously (Brown and Vellend 2014, Hemrova et al. 2019).

Knowledge gaps in plant conservation translocations

The ecological processes underlying rarity remain poorly understood for many rare plant species. Rarity can be measured by the frequency of occurrences within a species' range in addition to local abundance (Rabinowitz 1981, Gaston 1994). The frequency of occurrences is thought to be primarily limited by low availability of suitable sites (abiotic and biotic conditions) and the species ability to disperse to those sites (Brown et al. 1996). However, the relative influence of dispersal, abiotic conditions, and biotic interactions limiting species occurrences remains uncertain, particularly for rare species at their range edge. Species at their range edge are assumed to be less frequent because they have reached their climatic limits, however rarity may also be related to a species' limited ability to disperse within and beyond its current range limit (Svenning and Skov 2004) or negative biotic interactions (e.g., seed predation; Brown and Vellend 2014).

The inability to identify suitable habitat remains a primary barrier to translocation success (Godefroid et al. 2011, Silcock et al. 2019). Habitat suitability is frequently determined based on the species' historical presence at a site or expert opinion that the site seems suitable, yet is less commonly quantified (Osborne and Seddon 2012). One quantitative method to identify sites with suitable habitat is to estimate habitat suitability with species distribution models (SDMs), which model the relationship between species occurrences and the underlying environment (i.e., climate, soils, and topography) based on statistically derived response surfaces (Guisan and Zimmermann 2000). SDMs emerged in the 1970s and 1980s to quantify species' environmental niches and predict vegetation patterns using regression-based approaches with geospatial data (Franklin 1995). Modern SDMs extend these early approaches to machine-learning methods (i.e., maximum entropy or "MaxEnt") to model complex species-environmental relationships (Elith et al. 2006). SDMs rely on several assumptions, including that the target species is in pseudo-

equilibrium with its environment (Guisan and Theurillat 2000) and that occurrences reflect the species' *realized niche*, constrained by biotic interactions and limiting resources (Guisan and Thuiller 2005). Under these assumptions, SDM predictions have become a common approach for locating new rare plant occurrences (Guisan et al. 2006, McCune 2016), mapping invasive species spread (Peterson and Vieglais 2001, Sheppard 2013), and prioritizing areas for conservation translocations (Maes et al. 2019, Finn et al. 2024). However, few studies have tested the efficacy of SDMs to predict suitable habitat with experimental translocations (Elmendorf and Moore 2008, Draper et al. 2019, Hemrova et al. 2019).

An alternative quantitative method to estimate site suitability is to measure the similarity of the plant community composition between occupied sites and unoccupied sites (Ehrlén et al. 2006, Elmendorf and Moore 2008). Community-based models (CBMs) use the relative abundance of species that commonly occur with the target species to rank site suitability. CBMs assume that unoccupied sites with a community more similar to occupied sites are more suitable than unoccupied sites with a dissimilar community. CBMs directly incorporate biotic interactions through species abundances and indirectly capture abiotic variables based on the assumption that the composition of co-occurring species present reflects the underlying environmental conditions (Elmendorf and Moore 2008). In contrast, SDMs rarely directly incorporate biotic interactions either because these interactions are assumed to play a minor role at macro-scales relative to climate (Pearson et al. 2004) or limitations in the availability of biotic data (Araújo and Luoto 2007). Yet, CBMs remain an underutilized method to predict translocation success, and the efficacy of these two methods has rarely been compared. I found only one study that did so (Elmendorf and Moore 2008), but it was not in the context of conservation translocations. Using quantitative methods to identify suitable habitat, such as SDMs and CBMs, represents a novel

strategy for improving translocation success. This is especially important in Canada, where only a handful of plant species at risk have been translocated despite over one third of the plant species listed under the federal Species at Risk Act (SARA) being translocation-relevant (Swan et al. 2018).

Another ongoing debate surrounding plant translocation success is where to obtain seeds and whether seeds from different sources should be mixed or not (Lesica and Allendorf 1999, Sackville Hamilton 2001, Wilkinson 2001, McKay et al. 2005). Seed sourcing decisions have direct implications for population fitness and long-term persistence. Locally adapted sources may introduce maladapted genes when introduced to foreign sites, potentially leading to outbreeding depression (Knight and Miller 2004, Bischoff et al. 2006). In contrast, populations with small population sizes may exhibit inbreeding depression and benefit from genetic rescue through the introduction of new genotypes (Frankham et al. 2011, Frankham 2015). Mixing sources can increase genetic diversity and thereby enhance climate resiliency (Maschinski et al. 2013, Havens et al. 2015, St. Clair et al. 2020, Goebl et al. 2022). However, the degree of genetic differentiation among populations of rare plant species is frequently unknown prior to restoration (Neale 2012). Reciprocal transplant studies to directly quantify the degree of local adaptation are often unfeasible for rare plant species with few, small populations that may be seed limited and/or have restrictions against source mixing.

Assessing the degree of phenotypic variation using common gardens is an alternative approach to understanding genetic variation and testing for signals of local adaptation (Wallace et al. 2020, Candido-Ribeiro and Aitken 2024). Quantitative traits are polygenic (controlled by multiple genes) and therefore are more likely to capture adaptive variation than neutral molecular markers (i.e., SNPs), which primarily reflect random genetic drift (Vitt et al. 2004). In Canada,

the genetic differentiation among populations is known for only a few at-risk plant species (e.g., Boles et al. 1999, Hoban et al. 2010, Lesica et al. 2016, Lait et al. 2025) and the degree of phenotypic differentiation is even less well known (but see Reiter and McCune 2023). Evaluating the risk versus the benefits of different seed sourcing strategies requires understanding both measures of population differentiation.

Thesis overview and objectives

The study area for this research is southern Ontario, Canada within the Mixedwood Plains Ecozone (Fig. 1.1). This ecozone is characterized by a mild climate, mixed hardwood forests, and high forest fragmentation (Crins et al. 2009). It contains the highest number of rare plant species in Canada and the highest human population density (Haber 1994, Ontario Biodiversity Council 2011). Under Canada's SARA, 80% of plant species listed are peripheral (at their range edge) with many of these species occurring exclusively in southern Ontario (Caissy et al. 2020). Only ~15% of SARA-listed plant species that are translocation-relevant (n = 107) have had translocations implemented based on official government documents (Swan et al. 2018). The 'climate of caution' surrounding plant translocation in Canada seems to be rooted in concern about deprioritizing natural populations and the risks associated with failure (Clements 2013). This thesis focuses on plants, which make up the biggest taxonomic group of species at risk in Canada (~26%; Government of Canada 2025) and also have the highest proportion of translocation-relevant species (64%; Swan et al. 2018).

The study species are *Stylophorum diphyllum* (Michx.) Nutt. [Synonym: *Chelidonium diphyllum* (Michx.)] (Papaveraceae) and *Symphyotrichum prenanthoides* (Muhl. ex Willd.) G.L. Nesom (Asteraceae), both of which are herbaceous woodland perennials and occur at their range

edge in Canada and are listed under SARA as endangered and special concern, respectively (Government of Canada 2022). *Stylophorum diphyllum* has only five known populations in Ontario within forested woodlands (Bowles 2007, McCune 2019). Seeds are ant-dispersed and are eaten by mice (Bowles 2007). Threats include adjacent land use development, recreational pressure (i.e., mountain bikers) and erosion and fill (Bowles 2007). *Symphyotrichum prenanthoides* has 22 known populations in Ontario and occurs along streambanks, flood plains, and roadsides (Environment and Climate Change Canada 2020). Seeds are wind-dispersed; however, unlike *S. diphyllum*, the threats to the species are not well understood, although habitat loss is likely a threat to both species (Environment and Climate Change Canada 2020).

This thesis aims to improve rare plant recovery and test ecological theory through three broad objectives: (1) to test the relative role of dispersal, biotic, and abiotic filters in limiting the occurrence of a rare plant species, (2) to assess the efficacy of using SDMs and CBMs to predict suitable habitat for conservation translocations, and (3) to quantify the magnitude of phenotypic differentiation among populations and the evolutionary mechanisms driving differentiation.

Thesis chapters

In chapter 1, I use a seed addition experiment to test the reasons for rarity of *S. diphyllum* at its range edge. I chose nine, one-hectare unoccupied sites (100 m × 100 m) corresponding to raster cells with varying SDM-predicted habitat suitability based on broad-scale abiotic conditions (i.e., soils, climate, and geology). To simulate natural dispersal, I planted seeds at nine plots within each site. To test the relative influence of biotic interactions, I caged one paired sub-plot to exclude rodents, which are known to eat the seeds (Bowles 2007). I also measured soil moisture, canopy light, and temperature/humidity differentials at each plot to account for the

effects of microsite. I monitored the plots over two growing seasons (2021-2023) and recorded annual survivorship. I predicted that if dispersal is the main limitation, seedling emergence rates would be relatively high regardless of the predicted habitat suitability. Alternatively, if availability of suitable habitat or fine-scale microclimate is the main limitation, I predicted seedling emergence and survival rates would be positively correlated with habitat suitability or microsite conditions. I also predicted that if seed predation is limiting occurrences that emergence rates would be higher in exclosures compared to unprotected sub-plots.

Chapter 2 assesses the efficacy of using broad-scale habitat suitability estimates to predict translocation success. I tested the efficacy of species distribution models (SDMs) and community-based models (CBMs) to predict translocation success for each study species. I chose one-hectare (100 m × 100 m) sites with varying levels of habitat suitability predicted by SDMs. At each site, I quantified plant community composition by measuring the relative abundance of each vascular plant species within the site. Then I compared the similarity of the translocation site community to the community at naturally occurring sites using the Bray-Curtis similarity index (Bray and Curtis 1957). I controlled for other predictors of translocation success including transplant characteristics (i.e., size class, seed source), microsite conditions (i.e., soil moisture, temperature, and canopy gap), and biotic interactions (i.e., herbivory or competition). Within each site, I planted transplants into nine plots and applied a biotic treatment to each paired sub-plot for each species, either excluding large herbivores (*S. diphyllum*) or removing competition (*S. prenanthoides*). I monitored the sites over four years and recorded individual survival, reproduction, and seedling recruitment. I predicted that if SDMs and/or CBMs can effectively estimate habitat suitability, translocation success would be positively correlated with predicted habitat suitability.

In Chapter 3, I aimed to inform future translocation seed sourcing strategies by quantifying trait differences between seed sources. I used common garden trials to quantify the magnitude of phenotypic differentiation among *S. diphyllum* sources and infer the evolutionary mechanisms driving differentiation. I investigated differences among four wild Canadian, one wild U.S., and two commercial nursery sources using two greenhouse trials and one *in situ* field trial (wild Canadian sources only). I compared phenotypic trait distance (P_{ST}) to genetic distance (F_{ST}), geographical, environmental, and ecological distances. Lait et al. (2025) found wild Canadian populations have high genetic differentiation from each other yet are even more genetically distinct from wild U.S. and commercial nursery sources. I predicted that if the magnitude of genetic differentiation correlates with phenotypic differentiation, then commercial nursery sources would have greater phenotypic differentiation from wild populations. I predicted that among Canadian populations, greater geographic separation and larger environmental and ecological distances between populations would be associated with higher phenotypic differentiation.

To conclude, I summarize my research findings and highlight their implications for conservation actions, including future potential research. Translocation use is predicted to continue to rise with increasing pressures from anthropogenic threats, making it crucial to ensure optimal success rates. My research provides guidance for increasing translocation success rates for rare plants by testing the ecological theory regarding what processes limit species occurrences, testing the efficacy of different models to identify suitable habitat, and quantifying phenotypic differentiation among populations in relation to genetic differences to inform seed sourcing.

CHAPTER I

TESTING DRIVERS OF COMMUNITY ASSEMBLY WITH A SEED ADDITION TRIAL

1.1 Abstract

Understanding the causes of species rarity is a central goal in ecology. The three filters thought to predict if a species is present or not in a community are the suitability of abiotic conditions, dispersal limitation, and biotic interactions. Theory emphasizes the importance of the availability of abiotically suitable habitat in determining occurrence frequency, especially for species at their range edge, where the amount of suitable habitat is predicted to decline. However, the relative influence of these filters in driving species rarity is mostly unknown. We used species distribution models (SDMs) to estimate habitat suitability based on broad-scale abiotic predictors for a rare plant species (*Stylophorum diphyllum*) at the northern edge of its global distribution. We tested the role of dispersal limitation by planting seeds in unoccupied sites that varied in their predicted habitat suitability, and measured seedling emergence and seedling survival over two years. To manipulate the biotic interactions, we excluded seed predators by caging half of the seeds. We also measured the microclimate at each microsite, including soil moisture, temperature, and canopy cover. The habitat suitability estimated by the SDMs did not predict seedling emergence or short-term seedling survival. We found dispersal limitation coupled with seed predation was a significant predictor of seedling emergence, while microclimate, specifically microsite temperature, was a significant predictor of short-term seedling survival. Contrary to the assumption that species occur at a low frequency near their range edges due to a lack of suitable habitat, we found that dispersal-limitation coupled with biotic interactions can drive rarity. If this is the case for many rare species at risk of extinction at their range edges, effective conservation

strategies must incorporate assisted dispersal (i.e., translocations) into appropriate microsites and the management of biotic interactions to establish new populations and ensure long-term persistence.

1.2 Introduction

Understanding why many species are rare has long been a central goal in ecology. The frequency of occurrences within a species' range is one of the axes upon which rarity can be measured, in addition to local abundance (Rabinowitz 1981, Gaston 1994). For example, some rare species have large population sizes where they occur, but relatively few occurrences within their geographic range. In comparison, other rare species occur more frequently within their range but have small populations. In the case of the former, two primary processes are thought to limit the frequency of occurrences: low availability of sites with suitable conditions (abiotic and biotic) and the ability of the species to access those sites (dispersal; Moore and Elmendorf 2006).

First, abiotic factors may cause a species to be infrequent on the landscape if its required conditions are restricted, reflecting its fundamental niche (Grinnell 1917). Species that have evolved to specialize in a narrow range of abiotic conditions will occur infrequently. For example, plant species adapted to geological formations restricted to a few areas will occur only in those areas (e.g. Allison and Stevens 2001). In addition, even habitat generalists that occur frequently near the center of their geographic range may become less frequent near their range edges if the combination of environmental conditions they require becomes limited to small isolated pockets or if overall habitat quality gradually decreases towards the periphery (Brown 1984, Holt and Keitt 2000, Holt et al. 2005). However, few studies quantitatively assess the abiotic conditions at central and peripheral site locations in the field (Pironon et al. 2017).

Second, poor dispersal ability can prevent a species from arriving at a site, even if the habitat is suitable. The ability of a species to disperse and colonize can determine its frequency of occurrence. In addition, if a species can disperse quite far, but rarely does so, this can prevent it from recolonizing sites where a population was extirpated (Baur 2014). Low dispersal rates may result from seed limitation, which can include a lack of seed availability (Seabloom et al. 2003, Clark et al. 2007). Additionally, dispersal barriers, such as those created by habitat fragmentation, can reduce colonization rates and exacerbate the effects of dispersal limitation (Primack and Miao 1992). Dispersal limitation can be tested by adding propagules or mature individuals of a species to unoccupied sites. Many seed addition trials have provided evidence for dispersal limitation by documenting successful recruitment in formerly unoccupied sites (e.g., Primack and Miao 1992, Ehrlén and Eriksson 2000, Turnbull et al. 2000, Clark et al. 2007).

Lastly, even if a species can disperse to a site with favourable abiotic conditions, biotic interactions can prevent site occupation. Species may be limited in accessing suitable habitat if natural enemies (i.e. competitors, predators, parasites, and pathogens) are present. The lack of a specific mutualist partner or pollinator can also prevent species from occupying otherwise suitable sites (Williamson 1996, Kraft and Ackerly 2014). For example, some orchid species require specific mycorrhizal fungi to germinate and survive (Reiter et al. 2016, Hemrova et al. 2019). Overall, biotic factors have been understudied relative to abiotic factors as the causes of species occurrence patterns (Wisz et al. 2013, Lamothe et al. 2019, Ge et al. 2024).

Understanding the relative influence of dispersal limitation, abiotic conditions, and biotic interactions on limiting species occurrences is especially important for species of conservation concern close to their range edge because it is often unknown why they are rare. They may have few occurrences due to being near their range edge, where suitable habitat may be less abundant

(patchy) and/or of lower quality (Brown et al. 1996), or their rarity may be related to a lack of time and/or a limited ability to disperse within and beyond their range edge (Svenning and Skov 2004). Anthropogenic habitat fragmentation exacerbates both factors by eliminating suitable habitat and increasing distances between the remaining suitable sites. Uncertainty about whether or not range-edge populations were always rare, even before extensive anthropogenic disturbance, has led to controversies about the need to conserve peripheral populations (e.g., Hunter and Hutchinson 1994, Fraser 2000). For example, Lesica and Allendorf (1995) suggest peripheral populations should only be conservation priorities if they exhibit genetic divergence from central populations.

In our study, we test the influence of dispersal limitation, abiotic conditions, and biotic interactions on the occurrence of the wood-poppy (*Stylophorum diphyllum* (Michx.) Nutt. [Synonym: *Chelidonium diphyllum* (Michx.)], Papaveraceae) an endangered herbaceous perennial with only five known occurrences at the northern edge of its range in southern Ontario, Canada (Government of Canada 2021, 2023). Species distribution models (SDMs) suggest there is an abundance of potentially suitable, unoccupied habitat existing in the region (McCune 2019). The species relies primarily on ants for dispersal, which may limit its ability to colonize sites with suitable habitat, in addition to facing seed predation from rodents (Bowles 2007).

We used a seed addition trial to experimentally test the relative influence of dispersal limitation, abiotic conditions, and biotic interactions on *S. diphyllum* occurrence. First, we estimated the suitability of broad-scale abiotic habitat using an SDM and planted seeds in unoccupied forested sites with variable habitat suitability ratings. We measured canopy cover, soil moisture, and temperature at the planting microsites. To manipulate the biotic interactions, we excluded seed predators by caging half of the seeds. If dispersal limitation is the main

limiting factor, we predicted relatively high rates of seedling emergence and survival regardless of predicted habitat suitability. If the availability of broad-scale suitable habitat is the main cause of the rarity of *S. diphyllum,* we predicted that seedling emergence and survival rates would be positively correlated with habitat suitability. If fine-scale microclimate conditions are more important than broad-scale habitat suitability, we predicted that microsite conditions would be a stronger predictor of seedling emergence and survival, or that they might interact with broad-scale habitat suitability. For example, seedling emergence rates could be high in low suitability sites, but only if planted in a suitable microsite. Finally, if seed predators limit occurrences, we predicted higher rates of seedling emergence in exclosures compared to unprotected controls.

1.3 Methods

1.3.1 Study species

Stylophorum diphyllum is a long-lived herbaceous perennial that occurs in moist deciduous forests and in forested ravines and slopes near streams (COSEWIC 2007). Flowering occurs late April through May and capsule fruits are produced in late May to mid-June through self-fertilization or cross-pollination (Figure S7; COSEWIC 2007). Each capsule contains an average of 68 (between 1 and 146 seeds; personal observation) and each seed is about 2.5 mm long and 1.5 mm wide with an elaiosome (Gunn and Seldin 1976, Gunn 1980). Seeds are ant-dispersed (Bowles 2007) and are also sometimes moved by small slugs (personal observation). *Stylophorum diphyllum* is critically imperiled (S1) near its leading range edge in Ontario and its rear range edge in the states of Georgia and Alabama in the United States (NatureServe 2023). In Ontario, it was first collected in the late 1800s and not re-discovered for nearly 100 years (1987; Bowles 2000). Only four populations were known as of 2010, all within 30 km of the city of London, and a fifth population was discovered in 2015 (McCune 2019). Threats to the wild

populations in Canada include small population sizes with low recruitment, erosion and fill, adjacent land use change, and recreation pressure (Bowles 2007). In addition, mice eat the seeds (Bowles 2007). However, despite its rarity in Canada, *S. diphyllum* is locally common and found in abundance with many occurrences in West Virginia, Kentucky, and Ohio.

1.3.2 Study area

Our study area is located within the Mixedwood Plains ecozone (Fig. 1.1). This is Canada's most densely populated ecozone, exemplified by high land conversion and habitat fragmentation (Crins et al. 2009). It has phanerozoic calcareous bedrock and a mild climate, with average daily temperatures around 17° C in summer and -5° C in the winter, and moist conditions (720-1,000 mm rainfall/ year; Crins et al. 2009). The southernmost part of the Mixedwood Plains contains the Carolinian zone, a biodiversity hotspot dominated by hardwoods (i.e., oaks, hickories, and maples) and a rich diversity of plant and animal species found nowhere else in Canada (Oldham 1990). Most forests in this region are privately owned, although small provincial parks and nature reserves can be found throughout (Government of Ontario 2021).

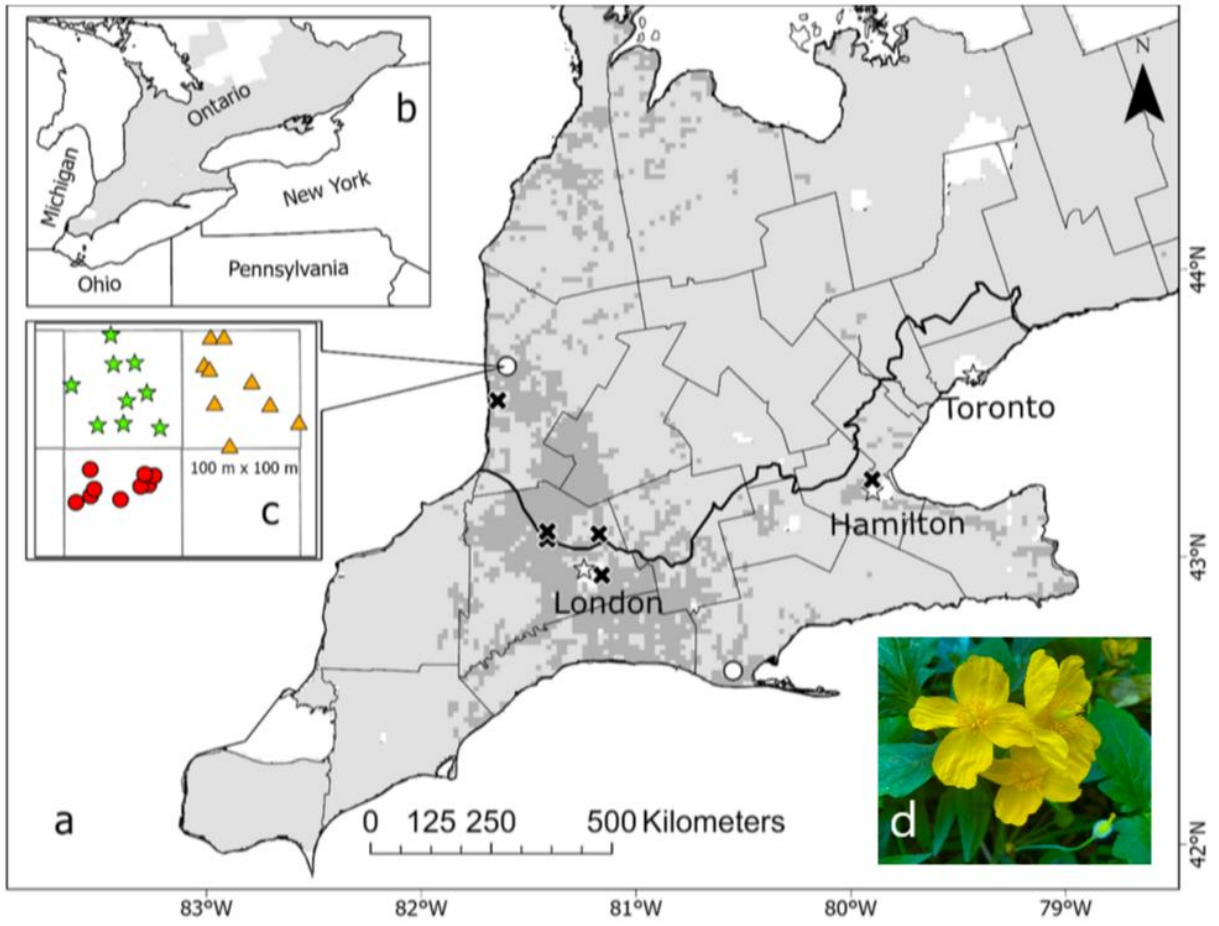

Figure 1.1 Study area in Ontario, Canada.
Panel a: The thin lines represent county borders, and the thick black line shows the approximate northern boundary of the Carolinian zone. Dark grey shading shows areas predicted by the range-edge SDM to be suitable for *S. diphyllum* based on the minimum suitable threshold of 37.9%. Suitable areas have been generalized to 2 km × 2 km cells to increase visibility. Light grey shading shows the extent of the SDM. Circles indicate the location of seed addition properties. Crosses indicate the wild populations and one historically translocated population (near Hamilton). Major cities are represented by stars. Panel b: The entire SDM extent in Ontario (grey shading) and its location in relation to neighbouring states. Panel c: One planting property. Thin lines represent the 100 m × 100 m site boundaries and coloured shapes indicate seed addition plots. High SDM-estimated habitat suitability is represented by green stars, intermediate suitability by orange triangles, and lower suitability by red circles. Panel d: *Stylophorum diphyllum* in bloom with a fruit forming in lower right corner.

1.3.3 Experimental design

To estimate broad-scale habitat suitability across southern Ontario, we used an SDM based on 14 environmental predictors created by McCune (2019). The SDM used MaxEnt (version 3.3.3k), which performs well even with very few occurrences (Hernandez et al. 2006, Phillips et al. 2006). MaxEnt predicts the habitat suitability of each cell across the study extent based on the locations of known occurrences and their associated environmental conditions. The occurrence records were sourced from the Ontario Natural Heritage Information Centre (NHIC) and one new record from McCune's 2015 plant survey (n = 5). The SDM used a resolution of one- hectare (100 m × 100 m grain size; Fig. S1.1) and MaxEnt default settings, except the regularization parameter was set to 0.5. We used MaxEnt's cumulative output, with 0 indicating very low suitability and 100 indicating the highest suitability. Range-edge occurrences are often better predicted by SDMs built at the extent of the range edge only (Stockwell and Peterson 2002, Luoto et al. 2005). However, due to the low number of occurrences in Ontario, we compared the results to a range-wide SDM (Wiedenfeld in prep, 2025) built at the same resolution but with an extent encompassing all *S. diphyllum* occurrences (n = 1329) in North America (GBIF.org 2023; see Appendix A Supplementary Methods S1.1; Table S1.1; Fig. S1.2).

Due to the very small number of *S. diphyllum* occurrences in the study region, we did not have independent presence records with which to test the utility of the SDMs. However, we have confidence that the range-edge SDM provides a reasonable assessment of habitat suitability based on plant community surveys of 295 one-hectare grid cells across southern Ontario (see Appendix A Supplementary Methods S1.2). Grid cells with predicted suitability greater than the lowest suitability of an occupied site (37.8%) had significantly higher relative abundance and number of common associated species of *S. diphyllum* than sites below that threshold (see

Appendix A Supplementary Methods S1.2, Results S1.1; Table S1.2; Figs. S1.3- S1.4). In contrast, we found no significant difference in relative abundance and total number of common associates between sites predicted to be suitable versus unsuitable by the range-wide SDM (see Appendix A Supplementary Results S1.1).

To test the effect of broad-scale habitat suitability on recruitment from seed, we selected nine, one-hectare unoccupied sites (each corresponding to an SDM grid cell) in two different properties owned by the Nature Conservancy of Canada (NCC). Three sites are located within a property located about 100 km southeast of the city of London, ON, comprised of mostly Carolinian forest (44 ha total), and six sites are located at a second property, located approximately 90 km northwest of London, comprised mostly of upland hardwood forest (71 ha). At each property, we selected sites for planting with the aim of representing a range of predicted suitability (Fig. S1.1). The nine selected sites range from 14.23 to 70.42% predicted suitability. We chose sites based on the following criteria: (1) the majority of the site was ecologically similar habitat to wild populations (i.e., we excluded sites with >50% pine plantation, thickets, or non-forest land (i.e., agricultural fields), (2) the site was accessible and relatively close to a trail, and (3) the site had no apparent threats (i.e., evidence of high herbivory rates, encroaching developments, etc.). Within each site, we placed nine plots, selecting the locations nonrandomly with the aim of maximizing the range of canopy light and soil moisture levels. We avoided placing plots directly in standing water (i.e., creeks) or directly on trails.

We collected seeds (n = 109) in late June 2019 from two *in-situ* mother plants of one wild population (one fruit from each plant), much less than 10% of the population's total seed production, as recommended by the CPC (Center for Plant Conservation 2019). We registered seed collection with the Ontario Ministry of Natural Resources and Forestry as *Possession for*

Scientific or Educational Purposes by an Educational Organization (s.23.15) under the Ontario Endangered Species Act. We then planted these seeds in growth chambers in 2020 as part of a germination trial and greenhouse common garden experiment (Reiter and McCune 2023). To obtain seeds for the current seed addition experiment, we harvested fresh seed from 45 adult plants that resulted from the common garden trial starting in late February 2021 until mid-June 2021, and stored seed in moist sand at approximately 5 °C until planting. This ensured an abundant number of seeds for our trial with minimal impact on the wild populations. We sorted the seeds to ensure each plot received the same relative abundance of seeds harvested in different weeks to account for any viability differences between seeds collected at different times during the five-month harvest period. We also conducted a seed viability tests in Petri dishes using a subset of 9-11 seeds from each collection week.

To simulate natural dispersal to the selected, unoccupied sites (breaking dispersal limitation), we planted all nine one-hectare sites over a two-week period between late July and mid-August 2021, approximately one month after the natural seed release period. Before planting, we cleared plots of competing vegetation and removed leaf litter. At each of the nine selected plots within each site, we then carefully hand-planted 50 seeds with tweezers, dividing 25 seeds into each of two sub-plots (each sub-plot ~20 cm in diameter, ~30 cm apart), ensuring that the seeds were at least 2 cm apart and no more than ~3 mm deep. We expected recruitment to occur in spring 2022 because seeds are naturally released in the warm spring-summer months, overwinter, and then germinate the following spring. To test the effect of seed predators (potential biotic limitation), we caged one sub-plot of each pair using a cage made with 23-gage steel mesh wire (¼ inch (0.635 cm) openings), which allows for invertebrate access but excludes mammals, including small rodents (Harrison et al. 2003). We formed each exclosure into a

closed-cube shape (approximately 24 cm diameter and 30 cm high) and inserted each cage approximately 7.5 cm deep into the ground, secured with landscape staples (Supporting Information Fig. S1.1).

To test for the effects of abiotic microsite differences, we measured soil moisture, canopy cover, and temperature at each plot. To quantify soil moisture, we took the average of two soil moisture readings (one in each sub-plot, approx. ~1 m apart) with a Delta-T Devices SM150 two-prong soil moisture kit (model SM150T sensor with a HH150 meter set to the 'mineral soil' setting). To quantify light levels, we captured two canopy photos with a clip-on smartphone fisheye lens attachment and using the freely available 'Gap Light Analysis Mobile App' (GLAMA) to estimate canopy cover, taking the average of two canopy cover index (CaCo) values (Gonsamo et al. 2013). The canopy cover index (%) estimates canopy cover by rescaling hemispherical photographs taken from a single position to perpendicular projections of light gaps in the tree canopy (Tichý 2016). To quantify the microsite temperature, we deployed a Kestrel® drop data logger in each plot (nine plots total per site), measuring the temperature every 0.5 hours for at least two consecutive days between June and August at a height of ~40 cm (the average height of adult plants). We fitted each logger with an opaque, white plastic cup to serve as a solar shield and cut side slits for ventilation.

We did not have enough data loggers to measure all 81 plots at the same time. Therefore, we calculated the mean temperature at each plot ($n = 9$) within each one-hectare site for the same 69-hour period. We then subtracted the mean of the site from the mean of the plot to obtain a 'temperature differential'. This effectively identifies planting plots that were hotter or cooler than the average of all plots in a site. To account for variability in weather conditions at different sites during the logger deployment, we standardized the raw plot differentials by dividing the raw plot

differentials at each site by the site standard deviation. The raw differentials of the plot were highly correlated with these standardized differentials (Pearson's correlation co-efficient; r = 0.98) and therefore we used the raw temperature differentials in all analyses.

We monitored the plots for early seedling emergence in late October 2021 and took two to three censuses throughout the 2022 growing season (late May to late July) to record the total number of seedlings emerged. We monitored again in late May to mid-June 2023 to record year-two survivorship. During each census, we removed any cages without seedlings and opened the tops of the cages with larger plants that could experience restricted growth.

1.3.4 Statistical analyses

We modelled first-year emergence rates and year-two survival rates with generalized linear mixed models (GLMMs) with a binomial distribution (logit link). We modelled emergence with plots nested within site as random effects. We modelled year-two seedling survival with plot only as a random effect because site explained low variance in the response, resulting in singularity of the model fit. We used the predictors cage treatment, SDM-predicted habitat suitability, soil moisture, canopy cover index, and temperature differential (Table 1.2). We fitted soil moisture and canopy cover index with a quadratic term due to the expected hump-shaped relationship between these factors and emergence and survival rates. We took the natural log of soil moisture. We calculated the proportion of seedlings emerged in year-one as the highest number of seedlings observed divided by the 25 seeds planted in each sub-plot (n = 162). We included 14 early recruits from fall 2021 that emerged in the same season as planting. We accounted for zero inflation by applying a single zero inflation parameter to all observations (each sub-plot), where the probability of producing a structural zero is equal for all observations. In the year-two survivorship model, we used the proportion of seedlings that emerged in 2022

(year one) and survived to 2023 (year two) at each sub-plot as the response, including only sub-plots that had at least one seedling emerge (n = 102). We used Pearson's correlation coefficients to ensure that there were no strong pairwise correlations between predictor variables in either model.

We predicted that the broad-scale habitat suitability or the cage treatment could interact with microclimate factors to influence emergence or survival. Therefore, we included two-way interactions of cage treatment and SDM-predicted habitat suitability with each microclimate predictor in both models (Table 1.1). For example, if most uncaged seeds were predated, then the effects of broad-scale habitat suitability or abiotic microsite factors would be detectable only within cages. Similarly, if the SDM accurately predicts overall site suitability but emergence and/or survival require the right abiotic microsite within broadly suitable sites, then the SDM-predicted habitat suitability may interact with the conditions of the abiotic microsite. We also tested microclimatic predictors interacting amongst each other in the emergence model but dropped these interactions in the survivorship model because of model convergence issues due to fewer observations. We repeated the modelling procedure above using the predicted habitat suitability from the range-wide SDM instead of the range-edge SDM (see Appendix A Supplementary Results S1.2, Tables S1.3-S1.4; Figs. S1.5-S1.6).

We built all models with the 'glmmTMB' package (Brooks et al. 2017) in the software R (version 4.1.2; R Core Team 2021). To test the significance of fixed effects and interactions while accounting for other predictors, we conducted single term deletion hypothesis testing using a likelihood ratio chi-square test to perform marginal fitting of terms (i.e., respecting the hierarchy of the terms) with the 'drop1' function in R. The test compares a model without the specified predictor to the full model using Akaike's Information Criterion (AIC). If the test on

the full model showed some of the candidate interactions were not significant, we re-fitted the model without them so we could assess the importance of the individual predictors within the interaction. First, we dropped quadratic term interactions that were not significant, then re-fit the model and dropped non-quadratic interactions that were not significant. To measure the variance explained by each model, we extracted Nakagawa's pseudo R^2 values (conditional and marginal) with the 'performance' package (Lüdecke et al. 2021). We checked for model specification errors using the 'DHARMa' package (Hartig 2024). We confirmed that there is no spatial autocorrelation in the model residuals by plotting spline correlograms using the 'ncf' package (Bjornstad 2022). We used the 'visreg' package to visualize the results of the model using partial regression plots, which show the effect of each predictor or interaction while holding all other predictors in the model constant (Breheny and Burchett 2017).

In addition to GLMMs to test the relative importance of each predictor and interactions, we used SAS software to perform response surface regressions (SAS PROC RSREG) to estimate the combination of conditions that maximized and minimized emergence in 2022, and survival of emerged seedlings to 2023 (SAS Institute Inc. 2024). For the response first-year emergence, we modelled caged treatment separately because the response surface response curves cannot integrate categorical predictors. For year-two survival, we pooled caged and un-caged treatments because there was no significant difference in year-two survival inside versus outside of cages. We modelled the logit-transformed response where μ = seedlings emerged in 2023 and n = seedlings emerged in 2022. The factors in the response surface model were the habitat suitability predicted by the range-edge SDM, soil moisture, canopy cover index, and temperature differential. We used ridge regressions (RIDGE MIN MAX) to explore the response surfaces. The ridge regressions identified the estimated ridges of the minimum and maximum responses

based on the coded data, which indicate environmental optimums for emergence and year-two seedling survival.

1.4 Results

1.4.1 Seedling emergence

Simulated dispersal via seed addition resulted in emergence of seedlings at all one-hectare sites, representing a range of SDM-predicted habitat suitability. Seedling emergence at each sub-plot varied from 0 to 56% (mean = 2.95 ± 0.27 seedlings from 25 seeds). Only 479 seedlings emerged from 4050 total seeds planted (11.8%). The SDM-predicted habitat suitability index was not a significant predictor of seedling emergence rates, regardless of whether we used the range-edge or range-wide SDM estimate (Tables 1.1, S1.3; Figs. 1.2, S1.5). The only significant predictor of seedling emergence rates was the interaction between cage treatment and soil moisture (Tables 1.1, S1.3; Figs 1.2, S1.5). The cage treatment had a large effect on emergence rates. In total, 18.4% of seeds planted in caged sub-plots emerged compared to 5.1% in uncaged sub-plots. Predicted seedling emergence rates were much higher in caged sub-plots, except at the very highest levels of soil moisture (Fig. 1.2). Overall emergence rates in the field were much lower than the rates we observed from our laboratory seed viability test (42.8%). However, field emergence rates neared this rate in caged sub-plots with lower soil moisture levels (Fig. 1.2). The abiotic microsite variables, canopy cover index and the relative temperature differential, were not significant predictors of seedling emergence rates (Table 1.1). The model pseudo R^2 values were 0.17 (marginal) and 0.27 (conditional).

Table 1.1: Results of a generalized linear mixed effects model assessing potential predictors of first-year emergence rates of *S. diphyllum* seedlings.

Predictor	**Coefficient**	***SE***	***df***	**AIC** [a]	***p*** [b]
(Intercept)	-3.65	2.44	*n/a*	644.19	*n/a*
cage (yes)	4.65	0.78	1	*n/a*	*n/a*
SDM habitat suitability index	-0.01	0.01	1	643.24	0.306
soil moisture (log)	0.93	1.60	1	*n/a*	*n/a*
soil moisture (log) ^{2}	-0.05	0.26	1	642.23	0.844
canopy cover index	-0.02	0.08	1	642.25	0.809
canopy cover index ^{2}	0.00	0.00	1	642.19	0.978
relative temperature differential	0.45	0.36	1	643.75	0.213
relative temperature differential ^{2}	-0.91	0.73	1	643.84	0.199
cage × soil moisture (log)	-1.11	0.26	1	659.51	**<0.001**

[a] Akaike's information criterion for the model that includes all factors except the one being tested. To respect marginality, we do not drop individual predictors that are also in an interaction (indicated as *n/a* for those predictors).

[b] *p*-values indicate the significance of each predictor using a likelihood ratio test. We did not drop individual predictors that were also in a significant interaction. Predictors with $p < 0.05$ are indicated in bold text.

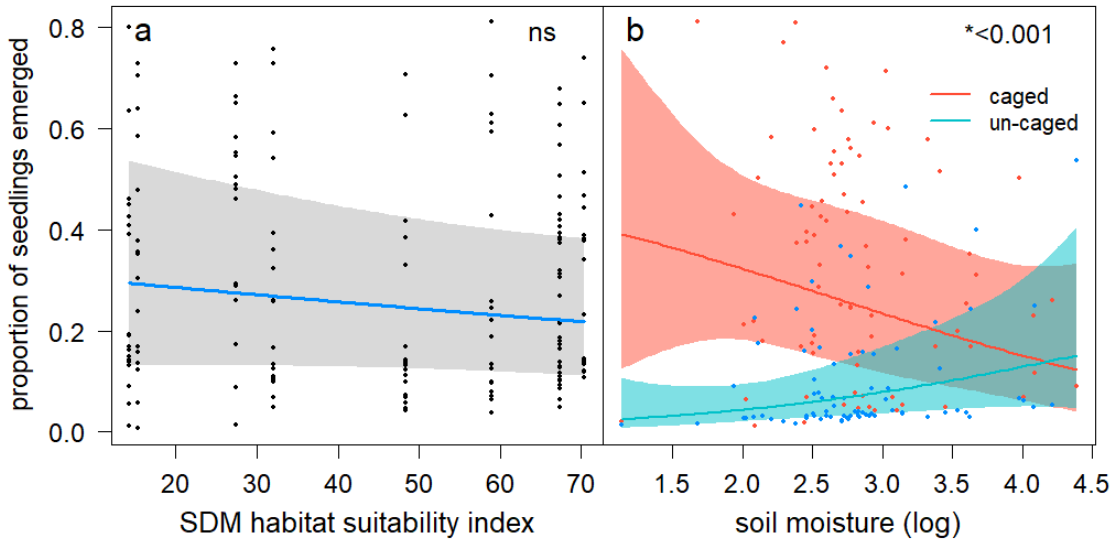

Figure 1.2: Partial regression plots showing the effect of a) the SDM habitat suitability index on seedling emergence and b) the interaction of cage treatment with soil moisture (log) on seedling emergence of *S. diphyllum*. Asterisks indicate significant predictors. All other variables are held

at the median (for continuous variables) and cage treatment is set to caged in panel a. Thick coloured lines represent the prediction and shaded areas represent Wald 95% confidence intervals. Dots represent partial residuals.

1.4.2 Year-two seedling survival

Overall seedling survivorship to year two was ~33% (159 survived to 2023 out of 479 seedlings that emerged in 2022) and 3.9% of all seeds planted in 2021 yielded a living seedling in 2023. As for seedling emergence, the SDM-predicted habitat suitability index was not a significant predictor of year-two seedling survival, regardless of whether we used the range-edge or range-wide SDM estimate (Tables 1.2, S1.4; Figs 1.3, S1.6). The cage treatment also was not a significant predictor of year-two seedling survival with 34.2% of seedlings surviving in caged sub-plots (n = 128) compared to 29.5% of seedlings (n = 31) surviving in uncaged sub-plots (Table 1.2; Fig. 1.3). We found a significant hump-shaped relationship between year-two survival rates and relative temperature differential, where sub-plots with temperatures closer to the site average had higher survivorship than sub-plots with relatively hotter or cooler temperatures (Table 1.2; Fig. 1.3). The other abiotic microsite factors, soil moisture and canopy cover index, were not significant predictors of year-two survival rates (Table 1.2). The model pseudo R^2 was 0.15 (marginal) and 0.51 (conditional).

Table 1.2: Results of the survivorship model assessing potential predictors for year-two survival of *S. diphyllum* seedlings.

Predictor	**Coefficient**	***SE***	***df***	**AIC**[a]	***p***[b]
(Intercept)	-7.50	7.60	*n/a*	303.91	*n/a*
cage (yes)	0.25	0.33	1	302.49	0.448
SDM suitability index	-0.00	0.01	1	301.95	0.851
soil moisture (log)	3.12	3.96	1	302.53	0.432
soil moisture (log)^2	-0.54	0.67	1	302.58	0.412
canopy cover index	0.20	0.23	1	302.78	0.348
canopy cover index^2	-0.00	0.00	1	303.80	0.169
relative temperature differential	-0.36	0.95	1	302.06	0.703
relative temperature differential^2	-5.01	2.24	1	307.62	**0.016**

[a] Akaike's information criterion for the model that includes all factors except the one being tested. To respect marginality, we do not drop individual predictors that are also in an interaction (indicated as *n/a* for those predictors).

[b] *p*-values indicate the significance of each predictor using a drop1 test. We did not drop individual predictors that were also in a significant interaction. Predictors with $p < 0.05$ are indicated in bold text.

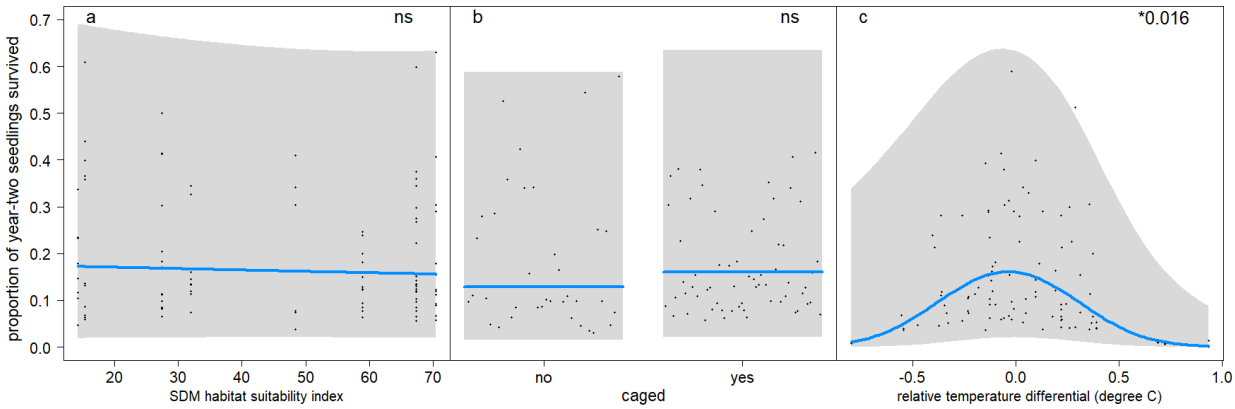

Figure 1.3: Partial regression plots showing the effect of (a) SDM habitat suitability index, (b) cage treatment, and (c) relative temperature differential between plots on year-two seedling survival of *S. diphyllum*. Asterisks indicate significant predictors. In each panel, all other variables are held at the median (for continuous variables), and cage treatment is set to caged in panels a and c. Thick blue lines represent the predictions and grey shaded areas represent Wald 95% confidence intervals. Dots represent partial residuals.

1.4.3 Environmental optimums identified by response surface models

Response surface models showed that the optimal conditions for seedling emergence were quite different than the optimal conditions for survival to the second year (Appendix A Supplementary Results S1.2). For example, the maximum emergence rate occurred inside cages where the canopy cover index was 32% and soil moisture was 65%. In contrast, the maximum survival rate occurred where the canopy cover index was 51% and soil moisture was 28%. The fitted response surface model explained 26.30% of the first-year emergence response under uncaged (cage = no) conditions ($R^2_{\text{uncaged}} = 0.2630$) and 12.14% of the variance of under caged (cage = yes) conditions ($R^2_{\text{caged}} = 0.1214$). The fitted response surface model explained 22.19% of the variance of the survival to year two ($R^2 = 0.2219$). For more details, including uncaged maximum response conditions and caged minimum response conditions, see Appendix A Supplementary Results S1.2.

1.5 Discussion

We coupled a seed addition experiment with a species distribution model to disentangle the influence of dispersal limitation, abiotic habitat suitability, and biotic interactions on the occurrence of a rare plant species near the edge of its geographic range. Community assembly theory places a large emphasis on abiotic habitat suitability filtering out species. In addition, it is hypothesized that near a species' range edge, a scarcity of sites with abiotic conditions within its fundamental niche results in low occurrence frequency (Brown 1984, Holt and Keitt 2000). Yet we found that seedlings emerged and survived at all experimental sites, regardless of estimated relative habitat suitability. This suggests that a lack of available suitable habitat is not the main cause of rarity of *S. diphyllum* in southern Ontario, at least for these early life stages. Instead,

dispersal limitation combined with seed predation are the main limiting factors for seedling emergence (Table 1.1; Fig. 1.2), while microsite temperature influences seedling survival (Table 1.2; Fig. 1.3). Dispersal limitation and seed predation could also influence the frequency of occurrences closer to the center of *S. diphyllum*'s geographic range, where it is more common. Future studies could contrast the relative influence of these factors at different range positions. Nevertheless, our study shows that recovery strategies for *S. diphyllum* at its range edge must include assisted dispersal (i.e., translocations) coupled with seed protection in select microsites to ensure species persistence, rather than habitat protection alone. This may also be true for other plant species of conservation concern, especially near their range edge.

The fact that seedlings emerged at all experimental sites emphasizes the role of dispersal limitation in determining the frequency of occurrences, concordant with many other seed addition studies (Eriksson and Ehrlen 1992, Turnbull et al. 2000, Seabloom et al. 2003). Our overall observed emergence rate (11.8%) is comparable to other seed additions of herbaceous understory plant species (10.3 to 33.8%; Ehrlén and Eriksson 2000) and a global meta-analysis of seed addition trials (~15%; Clark et al. 2007). Only 3.9% of the seeds planted emerged and survived until year two, a rate comparable to short-term survival rates for other woodland perennials planted from seed. For example, Drayton and Primack (2000) found survival rates over three years from 0 to 5.8% for eight forest herb seed additions. Although our results confirm that *S. diphyllum* can emerge and survive when seeds are translocated, we need to measure long-term survival to confirm its ability to establish self-sustaining populations. The few studies that have tracked seed addition experiments over several years have found that the number of initially occupied patches with successful recruitment exponentially decreases over time (Ehrlén et al. 2006, Drayton and Primack 2012).

Broad-scale habitat suitability did not predict seedling emergence rates or short-term seedling survival. This could be due to the failure of the SDMs to predict habitat suitability accurately because they may not always incorporate important predictors or their resolution may be too coarse to define fine-scale habitat heterogeneity relevant to seedling recruitment and survival (Fowler et al. 1981, Guisan and Thuiller 2005, Maschinski et al. 2012). However, the predictions of the range-edge SDM were correlated with the presence and abundance of common wood-poppy associates (Supporting Results S1.1; Table S1.2; Figs. S1.3, S1.4) suggesting the range-edge SDM has some ability to differentiate habitat conditions. Alternatively, SDMs may effectively predict habitat suitability for long-term population viability but not for seedling emergence and short-term survival. Plant niche requirements can change with life stage (Grubb 1977). The SDMs we used are based on conditions at existing occurrences dominated by adult plants, but the optimal conditions for germination can be different than those for establishment (Drayton and Primack 2000, Eriksson 2002). We will continue to monitor the translocated populations to determine if SDM-predicted habitat suitability can determine long-term viability.

Overall the strongest predictor of seedling emergence was the presence or absence of seed predators. Emergence rates were 75 to 79% higher inside cages than in unprotected sub-plots. Bowles (2000) found that 90% of *S. diphyllum* seeds placed on experimental trays at one of the wild Ontario populations were removed within 24 hours, confirmed as rodent activity through mice feces containing *S. diphyllum* seed coats (COSEWIC 2007). Our findings suggest that seed predation is severely limiting seedling emergence rates. *Stylophorum diphyllum* may be prone to elevated levels of seed predation in southern Ontario due to high levels of habitat fragmentation, which can lead to increased rodent density. For example, Tallmon et al. (2003) found that deer mice densities were three to four times higher and trillium seeds were about three

times more likely to be removed in small forest fragments compared to continuous forests. Given these high levels of seed predation, translocation attempts must create or find 'safe sites' that are free of predators (e.g., Jeffries and Lawton 1984, Jusaitis 2005), and seed protection may be required at the existing populations to enable these populations to persist long term.

Although we designed the cages to exclude seed predators, we cannot discount the microclimatic effect of cages as an alternate or additional cause of differences in seedling emergence rates (Davis and Gedalof 2018, Evans et al. 2018). Cages with smaller mesh sizes like ours can influence microclimate by providing a barrier against natural elements (e.g., snow and rain) or change in airflow, resulting in air and ground temperature differences that fluctuate based on other site factors such as canopy cover (Bergez and Dupraz 2000, Evans et al. 2018). However, studies that have tested the microclimatic effects of cages have found that these effects are usually minor compared to the effect of predator exclusion (Pearson et al. 2012, Brown and Vellend 2014, Davis and Gedalof 2018). In addition, cages can influence untargeted biotic factors. We observed a thick accumulation of leaf litter outside cages compared to inside our closed-top cages. Leaf litter can reduce seedling emergence for buried seeds, acting as a mechanical barrier (Rotundo and Aguiar 2005). Therefore, we cannot rule out litter reduction as a contributor to the cage effect we observed.

From a conservation standpoint, our findings suggest that we must take a more proactive approach to recover dispersal-limited species. Recovery strategies for plant species at risk in Canada (almost all of which occur near their northern range limit; Caissy et al. 2020) often focus solely on protecting existing populations under the assumption that occupied sites represent most of the available suitable habitat. For example, many recovery strategies define critical habitat for plants by drawing a buffer around existing populations (e.g., Environment Canada 2014,

Environment and Climate Change Canada 2017). However, our results emphasize the importance of dispersal limitation in combination with biotic factors in limiting the frequency of occurrence rather than the lack of suitable habitat (Tables 1.1, 1.2; Figs 1.2, 1.3). Conserving few, small, isolated populations is insufficient for species that require assisted dispersal to colonize new sites, if increasing the number of populations is the goal (Primack and Miao 1992, Primack 1996). Isolation through anthropogenic habitat destruction and fragmentation exacerbates this situation, because when suitable patches become too isolated, dispersal between sites can no longer occur, leading to a functionally extinct metapopulation (Holt and Keitt 2000, Hylander and Ehrlén 2013). Assisted dispersal may be the best option for such species, especially those relying on a dispersal agent with limited capabilities, such as ants (typical dispersal disperse <10 m; Corlett 2009).

Understanding the causes of species rarity is a fundamental goal of ecological theory that is necessary for the effective conservation of rare species. Our study simultaneously tested the role of dispersal, abiotic, and biotic factors on the emergence and survival of a rare plant species at its range edge. Theory emphasizes the scarcity of habitat with suitable abiotic conditions in limiting species occurrences near their range edge, however, we did not find broad-scale suitable habitat availability to be a limiting factor, at least for seedling emergence and short-term survival. Instead, we found that dispersal limitation coupled with biotic factors and microclimate had a greater relative influence. If other rare, dispersal-limited plant species at their range edge exhibit this pattern, their recovery will require assisted dispersal in combination with mitigation of negative biotic interactions to establish persistent populations.

CHAPTER II

TESTING THE EFFICACY OF SPECIES DISTRIBUTION MODELS AND PLANT COMMUNITY-BASED MODELS TO SELECT SUITABLE SITES FOR RARE PLANT TRANSLOCATIONS

2.1 Abstract

Conservation translocations can be a valuable tool for species recovery but often fail due to an inability to identify suitable habitat, as sites are commonly selected based on expert opinion rather than quantitative approaches. We tested the ability of species distribution models (SDMs) and community-based models (CBMs) to predict translocation outcomes of two rare plant species —the wood-poppy (*Stylophorum diphyllum*) and the crooked stem aster (*Symphyotrichum prenanthoides*)— over three years. SDMs estimate habitat suitability based on broad-scale environmental variables while CBMs quantify the similarity of plant communities at translocation sites to those at wild sites. We planted each species into sites with varying SDM-predicted habitat suitability, measured plant community composition, and controlled for the effects of transplant characteristics (planting size class, seed source), microsite conditions (soil moisture, canopy cover), and biotic interactions (herbivores or competition). Transplant characteristics and microsite conditions were the strongest predictors of early success, rather than SDM- and CBM-predicted habitat suitability. The habitat suitability estimated by the CBMs interacted with microsite variables to influence survival of both species and was independently positively associated with one species flowering. The habitat suitability estimated by the SDM interacted with microsite variables to influence one species flowering and was independently positively associated with seedling production of the other species. The low efficacy of the habitat suitability models to predict early translocation survival likely reflects their coarse spatial

resolution and emphasis on long-term environmental processes, suggesting long-term monitoring is needed to evaluate if these estimates can predict population persistence.

2.2 Introduction

Protecting existing populations of species at risk is key to their persistence, but in an age of rapid biodiversity loss, additional actions may be necessary. Conservation translocations (hereafter "translocations") aim to establish, reestablish, or augment a population via the movement of species within or outside their indigenous range (IUCN/SSC 2013). Translocations can be used as a tool to help reduce extinction risk through mitigating habitat loss, anthropogenic land fragmentation, and reproductive isolation by building population connectivity and resilience (Quinn et al. 1994). Translocations are especially relevant for taxa with limited dispersal ability that exist in fragmented habitats, as is the case for many plant species (Vitt et al. 2010). However, plant translocations can be controversial, as some conservationists fear they undermine the focus on protecting wild populations (Maunder 1992). In addition, many plant translocation attempts fail to produce viable populations (Maunder 1992, Dalrymple et al. 2011, Godefroid et al. 2011). These failures likely contributed to a 'climate of caution' surrounding the use of plant translocation as a recovery tool, at least in Canada (Clements 2013).

Thousands of plant translocations have been carried out worldwide (notably in the USA, the UK, and Australia) and key drivers of translocation success have been identified (Godefroid et al. 2011, Commander et al. 2018). For example, selecting the ideal abiotic microsites by considering soil moisture, temperature, and light availability can improve population growth and persistence (e.g., Renison et al. 2005, Davis and Gedalof 2018). It is also important to mitigate negative biotic interactions by excluding natural enemies such as seed predators and herbivores (e.g., Whitehead et al. 2023, Neigel et al. 2025), or reducing competition (e.g., Jusaitis 2005,

Albrecht and Long 2019). Many studies have shown that characteristics of the founder population affect translocation outcomes (Guerrant and Kaye 2007) and recommend using older propagules (i.e., transplants vs. seeds) and a large founding population (≥ 50 individuals) for optimal success (Guerrant and Kaye 2007, Albrecht and Maschinski 2012). Systematic reviews of experimental plant translocations have repeatedly found that these methods can improve translocation success (Godefroid et al. 2011, Albrecht and Maschinski 2012, Silcock et al. 2019, Bellis et al. 2024).

Although the use of translocations continues to increase and many best practices are now well documented, the inability to identify suitable habitat remains a barrier to success (Godefroid et al. 2011, Silcock et al. 2019). The suitability of a site is related to physical, biological, and historical aspects, making it difficult to quantify suitable receptor sites (Fiedler and Laven 1996). Often translocation sites are selected because the species *used* to occur there or based on expert intuition that the site *seems* suitable (Osborne and Seddon 2012). For example, Primack and Miao (1992) qualitatively matched seed receptor sites for four annual plant species within 1 km from natural populations based on site characteristics (i.e., soil moisture, light availability). Yet only three out of 34 populations persisted over multiple generations (Primack and Miao 1992). Overall, translocation study reviews have found that the inability to choose suitable receptor sites is a leading cause of translocation failure (Godefroid et al. 2011, Silcock et al. 2019).

One way to estimate habitat suitability quantitatively is to use species distribution models (SDMs). Predictions from SDMs have been employed to successfully locate new occurrences of rare plant species (Bourg et al. 2005, McCune 2016), forecast invasive species spread (Peterson and Vieglais 2001, Sheppard 2013), and map suitable habitat for conservation planning (Shapcott and Powell 2011, Elliott et al. 2024). However, very few studies have used *in situ* translocation

trials to validate SDM predictions (Wright et al. 2006, Draper et al. 2019, Hemrova et al. 2019, Bellis et al. 2020). A recent meta-analysis of plant translocation trials used post-hoc SDMs to estimate broad-scale habitat suitability and found that population persistence was best predicted by the climatic suitability of receptor sites (Bellis et al. 2025). These findings suggest SDMs can be a useful tool to select suitable receptor sites, however, more translocation trials are needed that test the use of SDMs for site selection while also incorporating full species life cycles, biotic interactions, and microsite conditions.

An alternative method to identify suitable habitat is to use community-based models (CBMs) which estimate site suitability from the relative abundance of species that co-occur with the target species. Thus, they directly incorporate biotic interactions and indirectly incorporate abiotic variables through the assumption that the species present indicate the underlying environmental conditions (Elmendorf and Moore 2008). If the plant community is indeed a reflection of environmental conditions at a site, then SDM predictions of habitat suitability may correlate to CBM predictions. Alternatively, SDMs and CBMs may provide distinct predictions of habitat suitability. For example, Elmendorf and Moore (2008) found that models using community composition were more effective at predicting reproductive output of sown individuals and site-specific abundance of existing populations compared to models built with environmental data. However, this is the only study we know of that compared CBMs with SDMs as estimates of habitat suitability, and it did not examine translocation of rare plants for conservation purposes.

In our study, we tested the efficacy of SDMs and CBMs to predict translocation success of two rare plant species in Canada: the wood-poppy (*Stylophorum diphyllum* (Michx.) Nutt. [Synonym: *Chelidonium diphyllum* (Michx.)] (Papaveraceae)) and the crooked-stem aster

(*Symphyotrichum prenanthoides* (Muhl. ex Willd.) G.L. Nesom (Asteraceae)). We planted individuals of each target species into receptor sites that varied in their SDM-predicted habitat suitability and measured the similarity of the plant community compared to wild sites. In doing so, we also controlled for other correlates of translocation success including microsite, biotic interactions, size class, and seed source to understand their importance relative to estimates of habitat suitability. If SDMs or CBMs can accurately estimate habitat suitability, then we predicted that translocation success (measured as individual survival and reproduction) would be positively correlated with predicted habitat suitability. While our study ultimately aimed to help recover these two rare species by establishing new, self-sustaining populations, it simultaneously tested a novel approach for translocation site selection that is relevant to plant conservation practitioners globally.

2.3 Methods

2.3.1 Study area and species

Our study area is situated in southern Ontario, Canada, within the Mixedwood Plains ecozone (Fig. 2.1) which is defined by its phanerozoic calcareous bedrock and mild climate, with mean daily temperatures between 18 °C and 22 °C in July and -3 °C and -12 °C in January, along with medium to high annual rainfall (720-1,000 mm/ year; Crins et al. 2009). This ecozone is the most densely populated area in Canada, with significant land conversion and habitat fragmentation, and predominately private forest ownership (Crins et al. 2009, Government of Ontario 2021). This ecozone includes the Carolinian zone (Fig. 2.1), which contains over 40% of Canada's plant species, including species found nowhere else in Canada (Oldham 2017). We chose our two study species because they: (1) are plant species at-risk listed under Canada's Species at risk Act (SARA), (2) have multiple natural populations with reproductive individuals

(ensuring sustainable seed collection), (3) have propagation protocols that ensure individuals can be grown within one year *ex situ* to obtain transplants for outplanting, and (4) have unoccupied, SDM-predicted suitable habitat on protected lands (owned by the Nature Conservancy of Canada).

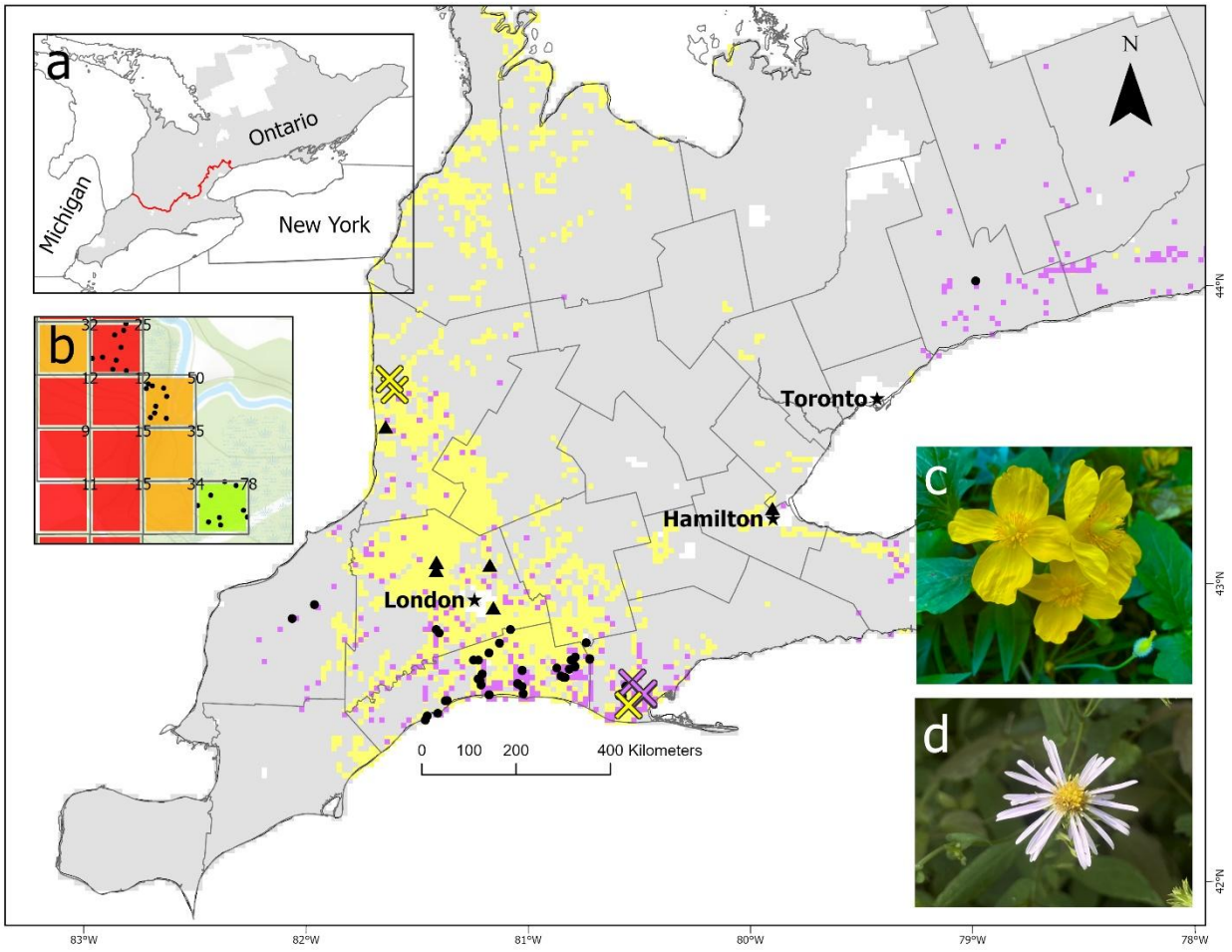

Figure 2.1: Study area in Ontario, Canada. Light grey shading shows the extent of the SDM study area. Yellow and purple shading indicates areas predicted by an SDM to be suitable for *S. diphyllum* and *S. prenanthoides*, respectively, generalized to 2 km × 2 km cells. The grey thin lines represent county borders. Crosses indicate translocation sites (yellow= *S. diphyllum* and purple= *S. prenanthoides*). Triangles indicate *S. diphyllum* natural populations and one historically translocated population near Hamilton; Circles indicate *S. prenanthoides* natural populations. Major cities are represented by stars. Panel a: The entire SDM study area extent in Ontario (grey shading). The red line shows the northern boundary of the Carolinian zone. Panel b: A planting property and the underlying topography. Squares (with SDM-predicted habitat

suitability in top corner) represent the 100 m × 100 m planting sites and circles indicate plots. Panel c: *S. diphyllum* flowering with fruit forming below. Panel d: *S. prenanthoides* flowering.

Stylophorum diphyllum is a long-lived herbaceous perennial with a stout rhizome and is found in deciduous woodlands along forested ravines and streams (Bowles 2007). Flowering occurs late April-May and plants can either outcross or self-fertilize to produce capsules that mature in late May through mid-June (Bowles 2007; Fig. 2.1). Seeds are ant-dispersed and are eaten by mice (Bowles 2007). *Stylophorum diphyllum* is critically imperiled (S1) at its rear range edge in the state of Georgia (USA) and at its leading range edge in Ontario, where it is listed under Canada's SARA as endangered and has only five known populations, plus one historically translocated population (Fig. 2.1; Government of Canada 2021, NatureServe 2023). *Symphyotrichum prenanthoides* is a herbaceous perennial that forms clonal colonies via rhizomes and is found along streambanks, woodland edges, and floodplains (COSEWIC 2012). Flowering occurs late August through early October and flowers are self-incompatible, requiring compatible pollen donors to produce wind-dispersed seeds (COSEWIC 2012; Fig. 2.1). *Symphyotrichum prenanthoides* is ranked S1 at its rear and leading range edges (same as *S. diphyllum*) and is listed as special concern under SARA with 22 extant populations documented (Environment and Climate Change Canada 2020, NatureServe 2023).

2.3.2 Selecting sites with Species Distribution Models (SDMs)

To estimate the broad-scale abiotic suitability of habitat across southern Ontario, we used a separate SDM for each study species based on 14 environmental predictors. For model details see McCune (2019) and Appendix B Supplementary methods S2.1. To validate the accuracy of the *S. prenanthoides* SDM, we used independent presence and absence records (n = 30 and n = 226 respectively) from exhaustive surveys of 256 one-hectare cells across southern Ontario

(McCune 2016; see Appendix B Supplementary methods S2.1, McCune et al. 2017, Rosner-Katz et al. 2020) and new, non-overlapping presence records we obtained from the Natural Heritage Information Centre. We found that the probability of occurrence was strongly correlated with the SDM prediction (Fig. S2.1). Due to the very low number of occurrences for *S. diphyllum* ($n = 5$) we did not have any independent presence records with which to validate the SDM. However, we do have evidence that the SDM is a reasonable measure of habitat suitability because the same dataset of independent surveys showed that sites predicted to be suitable (defined as sites with predicted suitability equal to or higher to lowest valued occupied site) had a significantly higher relative abundance and number of common associated species of *S. diphyllum* than unsuitable sites (Neigel et al. 2025).

To test the effect of SDM-predicted habitat suitability on translocation success, we selected three properties for each species, consisting of unoccupied, one-hectare sites (100 m × 100 m) that correspond to the SDM grid cells (*S. diphyllum*, $n = 12$ sites; *S. prenanthoides*, $n = 9$ sites; Fig. 2.1). At each property we chose sites representing a range of SDM-predicted habitat suitability (3.5-83.8% for *S. diphyllum* and 24.7-78.1% for *S. prenanthoides*). For *S. diphyllum* we determined suitability rankings by arbitrarily dividing the raw SDM output from all properties equally into three bins (<30.57 = low suitability, >30.57 to 66.55 = medium suitability, >66.5 = high suitability). For *S. prenanthoides* we used three bins based on the probability of occurrence calculated by relating the SDM prediction to the independent presence/absence data (<35.68 = low suitability, less than 30% probability of occurrence; >35.68 to 66.54 = medium suitability, greater than 30% but less than 80% probability of occurrence, >66.54 = high suitability, greater than 80% probability of occurrence; Fig. S2.1). We selected the final sites based on three additional criteria: (1) they were primarily composed of naturally

forested land (i.e. excluding areas dominated by pine plantations, thickets, or non-forest land); (2) they were accessible and located near trails; and (3) they lacked obvious threats (i.e. evidence of high herbivory rates, encroaching developments, etc.). Within each site we placed nine plots nonrandomly to capture a broad range of canopy light and soil moisture conditions, avoiding areas in standing water, on trails, and below hazardous trees.

2.3.3 Measuring plant community composition

We measured the plant community composition at each one-hectare unoccupied, translocation site (n = 21) and occupied natural sites (*S. diphyllum*, n = 5 (includes one historic translocation site); *S. prenanthoides*, n = 11) across southern Ontario between May and August. At each site we navigated to the centre of the one-hectare grid cell and delineated four 50 m × 50 m quadrants. We then walked the entire area systematically with a few meters between surveyors and identified all vascular plant species. We estimated the abundance of each species using a coarse ordinal scale of one to five (very rare to dominant). We calculated the Bray-Curtis similarity index (BCI; Bray and Curtis 1957) based on the untransformed species abundance matrices to quantify pairwise similarity in the plant communities among all surveyed sites for each study species. We then calculated the average BCI of each translocation site to all surveyed occupied sites, where a higher BCI indicates a translocation site with a plant community more similar to occupied sites.

2.3.4 Seed collection and planting design

We tested for effects of seed provenance on translocation success because different provenances may have differing performance rates in different conditions (Williams 2001). We collected seeds from two natural populations for both study species, representing two different

seed provenances for each species (~25 km apart for *S. diphyllum*; ~52 km apart for *S. prenanthoides)* in 2021. We tested for effects of seed provenance on translocation success because different provenances may have differing performance rates in different conditions (Williams 2001). Lait et al. (2025) found that the Canadian *S. diphyllum* populations are genetically differentiated from each other. No genetic analysis has yet been conducted on *S. prenanthoides,* but we predict the Canadian populations may also be genetically differentiated because they occur in spatial isolation within highly fragmented habitats. For *S. diphyllum*, we collected from 54 maternal lines (one fruit per plant) from one population, Ilderton, ("IL") and received seeds from a partner (not maternally tracked) from a second population, Meadowlily ("MW"; referred to as "LOND" in Lait et al. (2025). For more details on seed collection see Appendix B Supplementary methods S2.2.

We were interested to know whether natural outdoor stratification could provide viable propagules, resulting in production cost savings associated with not requiring indoor greenhouse space, therefore, we grew seeds from both *S. diphyllum* seed sources indoors (resulting in larger transplants) and outdoors (resulting in smaller transplants). All indoor-grown transplants were ~3 months old and all outdoor-grown transplants were ~2 months old when planted. However, the MW seed source grown indoors had no seeds germinate (likely due to seed rot or delayed emergence due to extended cold storage; see Appendix B Supplementary methods S2.2), meaning we had no larger transplants from the MW source. For *S. prenanthoides* we collected two seedheads per plant from two occupied, natural sites, Central Big Creek ("CBC"; n = 155 maternal lines) and Intven ("IV"; n = 146 maternal lines). We grew both *S. prenanthoides* seed sources indoors, resulting in uniform transplant size. For details on propagation protocols see Appendix B Supplementary methods S2.2.

We planted *S. diphyllum* transplants (n = 1296) from June 13 – 21, 2022, within 12, one-hectare sites (n = 9 plots per site) at three properties: three sites at each of two smaller properties (44 ha and 58 ha) and six sites at one larger property (71 ha; Table 2.1). We divided each plot into two circular 1 m-diameter sub-plots and planted six transplants (n = 12 plants per plot and n = 108 per site) at each sub-plot (Fig. S2.1). We planted each sub-plot with one to six indoor-grown plants and zero to five outdoor-grown, ensuring all sites in each property received the same representation of planting size classes and seed sources (Table 2.1).

Table 2.1: Translocation property code names and number of transplants planted per species (2022) at each one-hectare site (n = 9 plots per site; 18 sub-plots). Seed source codes are listed within brackets.

Property code name	Property size (hectare/ acre)	# of sites	Species planted	# Outdoor plants per sub-plot	# Indoor plants per sub-plot	Total # plants per plot	Total # plants per site
LCB6	44 ha /109 ac	3	*Stylophorum diphyllum*	1 (IL)	5 (IL)	12	108
MTP	58 ha /143 ac	3	*Stylophorum diphyllum*	0	6 (IL)	12	108
JMG	71 ha /175 ac	6	*Stylophorum diphyllum*	4-5 (IL/MW)	1 (IL)	10-12 [a]	90-108
FOT	70 ha /173 ac	3	*Symphyotrichum prenanthoides*	0	25 (IV/ CBC)	50	450
UBC1	25 ha /62 ac	3	*Symphyotrichum prenanthoides*	0	25 (IV/CBC)	50	450
BWB4	34 ha /84 ac	3	*Symphyotrichum prenanthoides*	0	25 (IV/CBC)	50	450

[a] JMG property received an additional 1-2 (IL/MW) outdoor plants and 2-3 (IL) indoor plants in 2023 as a one-time supplemental planting.

We planted only outdoor-grown transplants at two properties because we did not have enough transplants of both seed sources for all three properties (Table 2.1). In addition, we did not have permission to mix seed provenances at all properties, so we planted MW with IL seed source only at the largest property (JMG; Table 2.1) containing two replications of each SDM-

predicted habitat suitability ranking (i.e., two low, medium, and high suitability; $n = 6$ sites total). Due to high mortality rates of the smaller size class, we replaced MW outdoor-grown transplants that did not survive the first month after the primary planting event with individuals from the same source and planting size class.

We also conducted a one-time supplemental *S. diphyllum* planting on June 6, 2023, at the largest property due to the property receiving primarily outdoor-grown plants in 2022, which had very high mortality rates. We planted an additional two indoor-grown plants at each sub-plot and one to two outdoor-grown plants at each sub-plot to match the number of plants per sub-plot ($n = 6$) at the other two properties (Table 2.1). Although supplemental plantings in different years can complicate statistical analyses, staggered plantings are recommended because they increase the likelihood that a population becomes established (Guerrant and Kaye 2007). We planted *S. prenanthoides* transplants ($n = 4050$) from July 5 – 22, 2022, at nine one-hectare sites at three properties ($n = 9$ plots per site). We divided each plot into two 5 m^2 sub-plots and planted 25 plants at each sub-plot ($n = 50$ plants per plot; Table 2.1) in a grid pattern (Fig. S2.2). To test for effects of seed provenance we planted both seed sources ($n = 10$ CBC plants, $n = 15$ IV plants; Fig. S2.2) within each sub-plot. We immediately watered each transplant (one time) with an overhead camp shower. For more planting details see Appendix B Supplementary methods S2.2.

Quantifying the microsite and mitigating negative biotic interactions

To quantify abiotic differences within and between plots, we measured soil moisture and canopy cover in each sub-plot and temperature, humidity, and soil chemistry (i.e., pH, CEC, nutrients) in each plot. We then conducted a Principal Component Analysis (PCA) in the R package 'vegan' (Oksanen et al. 2025) on all soil chemistry variables and retained two or three

Principal Components (PCs; Appendix B Supplementary methods S2.3). To measure canopy light levels we took a single canopy photograph in each sub-plot using a smartphone with a clip-on fisheye lens and the free 'Cover Light Analysis Mobile App' (GLAMA) to calculate the canopy cover index (CaCo; Tichý 2016). The CaCo (%) estimates canopy cover by rescaling hemispherical photographs to perpendicular projections of light gaps (Gonsamo et al. 2013). To measure temperature and humidity in each plot, we deployed one Kestrel® drop data logger at each plot for a minimum of 24 hours. We then calculated a 'temperature differential' and 'humidity differential' respectively for each plot by subtracting the site mean value from each plot's corresponding mean value (Appendix B Supplementary methods S2.3).

To exclude deer from *S. diphyllum* and test the effect of negative biotic interactions, we installed exclosures (one sub-plot per plot; Fig. S2.2) immediately after planting. Canadian wild populations have been susceptible to occasional browsing by deer (COSEWIC 2007). We implemented a competition removal treatment for *S. prenanthoides* because the threats to the species are not well understood (see COSEWIC 2012). However, reducing competition is a management action often used to increase translocation success (Godefroid et al. 2011). Prior to planting, we removed all vegetative competition (including roots) at one sub-plot and cut vegetation height down to soil level (carefully not removing any roots) to increase planting visibility at the other sub-plot.

2.3.5 Measuring translocation success

To quantify translocation success we measured survival and reproduction (number of fruits, flowering status (yes/no) or the number of seedlings produced, and seedling survival). We monitored all plots approximately one month after planting for survival. We monitored *S. diphyllum* during its fruiting period (June 2022 – 2025) and *S. prenanthoides* during the end of

its growing period (August 2022 – 2023), and again during its fruiting period (late October 2022 – 2023). In 2024, we monitored *S. prenanthoides* once for survival in June. For *S. diphyllum*, we measured survival and number of fruits produced for each plant, and the number of new seedlings that emerged within each plot annually from 2023 – 2025. We measured seedling production on the plot-level because we were unable to track the maternal origin of individual seedlings, and many seedlings emerged in between sub-plots. In 2025, we recorded second year survival for *S. diphyllum* transplants planted in 2023 and survival of seedlings to yearlings (2024 – 2025; first year). To ensure we accounted for natural dispersal, we searched for seedlings within ~5 m of each plot. For *S. prenanthoides*, we recorded survival (2022 – 2024) and flowering (yes/no; 2022 – 2023) for each plant but did not record seedlings due to the difficulty in distinguishing them from other Asteraceae seedlings.

2.3.6 Statistical analysis

We built generalized linear mixed models (GLMMs) and generalized linear models (GLMs) to determine the effects of the predictors on translocation success measured as: cumulative individual survival (binomial distribution), individual first-year flowering (yes/no; binomial distribution), cumulative individual number of fruits (Tweedie distribution), cumulative number of seedlings produced at the plot level (negative binomial distribution), and seedling survival to yearling at the plot level (one-year old; beta binomial distribution). We built two versions of each model, one with SDM habitat suitability and one with CBM habitat suitability. Predictors included habitat suitability, abiotic microsite conditions (soil moisture, canopy cover index, soil nutrient PCs, temperature and humidity differentials), the biotic treatment, and transplant characteristics (seed source, size class (stage) at planting (>15 cm = 'large' and ≤15 cm = 'small'; *S. diphyllum* only), and planting year (*S. diphyllum* only)). We predicted that

habitat suitability, biotic treatment, seed source, and planting size class could interact with microclimate factors to influence translocation success. For example, survival rates could be high in low suitability sites, but only if planted in a favourable microsite. Therefore, we included all two-way interactions with these predictors in the individual-level models. We only tested seed source interactions with microclimate factors only for *S. prenanthoides* because different seed sources of *S. diphyllum* were planted at a single property (6 sites).

To account for the nested design, we included sub-plot nested within plots within site as random effects for individual-level models or plots nested within site for plot-level models (Appendix B Supplementary methods S2.3). We excluded random effects from the cumulative seedling survival model (SDM version) due to plot and site explaining low variance in the response. We scaled and centered all predictors and used Pearson's correlation coefficients to check for pairwise correlations between model predictors to ensure they were not highly correlated. We also tested for correlations between the SDM and CBM habitat suitability estimates which were low for both species (r = 0.08 (*S. diphyllum*); 0.40 (*S. prenanthoides*)).

We developed a cumulative individual survival model for each target species from planting to year two (including plants that died in year one). We choose the model family by choosing the distribution with the lowest AIC. For individual-level models, we compared versions with and without quadratic terms for the continuous predictors and selected the model with the lowest Akaike's Information Criteria (AIC), retaining the linear form when AIC <2. Due to the plot-level seedling models having few observations, we excluded quadratic terms from these models to avoid over-fitting. These models also excluded biotic treatment and transplant characteristic predictors because the response is measured at the plot-level. For modelling the cumulative number of seedlings at each plot (2024 – 2025), we included the total

number of fruits produced by individuals in the plot from the year priors (2023 – 2024) as a predictor in both years because the number of seeds can influence the number of seedlings produced, and we wanted to determine effects of other predictors on seedling emergence rates after accounting for the approximate number of seeds produced. We also included total number of plants in the plot as an offset term to account for variation in reproductive opportunity among plots. For modelling survival of seedlings to yearlings (2024 –2025) we additionally excluded the humidity differential predictor due to few observations (n = 60 plots with seedlings in 2024).

We carried out all analyses using R (R Core Team 2021) with RStudio (Posit team 2024).We built all GLMMs with the package 'glmmTMB' (Brooks et al. 2017) .We used the package 'vegan' (Oksanen et al. 2025) to calculate the Bray-Curtis similarity index values. We used single term deletion hypothesis testing with a likelihood ratio X^2 test using the 'drop1' function to perform marginal fitting of terms (respecting the hierarchy of the terms). If this test showed some candidate interactions were not significant in the full model, we re-fitted the model without non-significant interactions so that we could assess the importance of the individual predictors. To measure the variance explained by each final model, we used the 'performance' package (Lüdecke et al. 2021) to extract Nakagawa's pseudo R^2 values (conditional and marginal variance). We checked for model specification errors using the 'performance' package and checked model assumptions using the 'DHARMa' package (Lüdecke et al. 2021, Hartig 2024). We visualized the model results using partial regression plots with the 'visreg' package which shows the effect of each predictor while holding all other predictors constant (Breheny and Burchett 2017). To confirm no spatial autocorrelation in the model residuals we plotted spline correlograms using the 'ncf' package (Bjornstad 2022).

2.4 Results

For both species we found significant interactions between at least one of the habitat suitability predictors (SDM or CBM), abiotic microsite conditions, the biotic treatment, and transplant characteristics (seed source, planting size class, planting year, fruits per plot; Tables 2.2, S1-S2; Figs. 2.2-2.4, S2.5-2.7).

2.4.1 *Stylophorum diphyllum* survival rates

In total, 86.6% of *S. diphyllum* transplants survived their first year, 94% survived their second year, and 84.9% survived both years, indicating that most deaths occurred the first year after planting. The SDM habitat suitability was not a significant predictor of survival but the CBM habitat suitability interacted with abiotic conditions to predict cumulative survival (Tables 2.2, S2.1; Figs. 2.2a, S2.5a). For example, cumulative survival decreased in plots with high soil nutrient PC2 (soils characterized by low pH, Ca, Mg, and Na; Fig. S2.3) as community similarity increased but remained high across levels of community similarity in low to medium soil nutrient PC2 plots (Tables 2.2, S2.1; Fig. 2.2a).

Abiotic microsite conditions had significant effects on survival. Cumulative survival declined as soil moisture increased (Figs. 2.2e, S2.5e) and increased with increasing humidity differentials (Tables 2.2, S2.1; Fig. 2.2f). When SDM-predicted habitat suitability was included as a predictor, cumulative survival increased with increasing soil nutrient PC2 levels (Tables 2.2, S2.1; Fig. S2.5f). Biotic treatment also influenced survival, where significantly more caged plants survived (Tables 2.2, S2.1; Figs. 2.2d, S2.5d). Transplant characteristics, including planting size class and planting year, had the greatest relative influence on survival. Transplants in the small size class (≤15 cm) had significantly lower survival, and those planted in 2023 had

higher survival rates than those planted in 2022 (Tables 2.2, S2.1; Figs. 2.2b-c, S2.5b-c). In total, 93.3% of 2023 transplants survived their first year versus 84.1% of 2022 transplants.

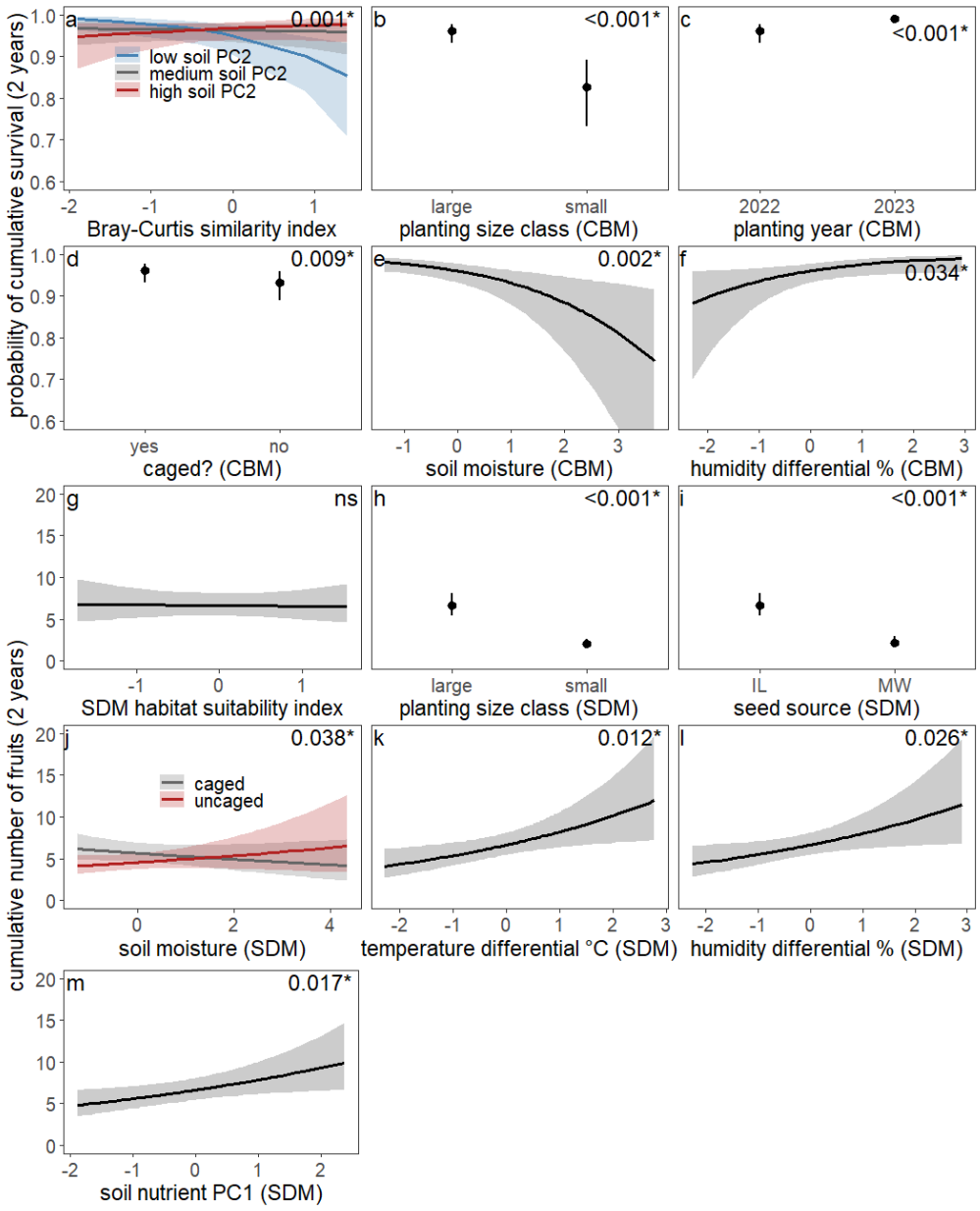

Figure 2.2: Partial regression plots showing effects of predictors on *S. diphyllum* cumulative survival (a-f) and cumulative number of fruits (g-m) over two years. Brackets denote what estimate of habitat suitability was used in the model. Asterisks indicate significant predictors. In each case, all other continuous predictors are held at the mean; categorical predictors are held at the most common category (planting size class = "large", planting year= "2022", source = "IL", and cage = "yes"). Points (±95% CI) show model-predicted responses for categorical predictors, while lines (±95% CI) show model-predicted relationships for continuous predictors (based on fixed-effects only), with all other variables held constant. The pseudo R^2 values for the

cumulative survival model including CBM estimates of habitat suitability and fruiting model including SDM estimates of habitat suitability were 0.552, 0.770 (conditional) and 0.288, 0.506 (marginal), respectively.

2.4.2 *Stylophorum diphyllum* reproduction

In their first year, 55.6% of *Stylophorum diphyllum* individuals produced fruit ($\mu = 1.6$ fruits per individual) versus 68.9% in their second year ($\mu = 3.2$ fruits per individual). The CBM was not a significant predictor of reproduction, whereas the SDM was a significant predictor of the cumulative number of seedlings produced in plots (Tables 2.2, S2.2; Fig. 2.3a).

Abiotic microsite conditions and biotic treatment had significant effects on reproduction. For both models, individuals produced more fruits with increasing temperature and humidity differentials and increasing levels of soil nutrient PCI (Tables 2.2, S2.5; Figs. 2.2k-m, S2.5k-m). For the CBM habitat suitability model, caged individuals produced significantly more fruits than uncaged individuals (Table S2.2; Fig S2.5j). For the SDM habitat suitability model, number of fruits increased with increasing soil moisture in caged sub-plots but decreased with increasing soil moisture in uncaged sub-plots (Table S2.2; Fig. 2.2j). Transplant characteristics including seed source and planting size class, had the greatest influence on fruit production. The IL seed source transplants in the large size class (>15 cm) produced the most fruits (Table S2.2; Fig. 2.2h-i).

In year one (2023), 86.1% of all plots produced fruits and fruit reproduction steadily increased each year from an average of 14.6 fruits per plot (year one; 2023) to 32.67 fruits per plot (year two; 2024) to 43.2 fruits per plot (year three; 2025). Seedlings emerged in 64.5% of plots that had fruits in year one and seedling production increased from an average of 11.2 seedlings per plot in year two to 18.3 seedlings per plot in year three (excludes plots with no

fruits the year prior). Survival of seedlings to yearlings from year two to year three (2024 – 2025) was 6.8% (72 out of 1049 total seedlings). The SDM habitat suitability was a significant predictor of the number of seedlings per plot, where seedling production increased in plots with increasing SDM habitat suitability index (Table S2.6; Fig. 2.3a). The total number of fruits produced in previous years was also a significant predictor of seedling production, where plots with more fruits had more seedlings (Table S2.6; Figs. 2.3b, S2.6b). Microsite soil nutrients were also significant predictors of seedlings, where the number of seedlings decreased with decreasing soil nutrient PC1 (soil characterized by low CEC, Al, and Ni; Figs. 2.3c, S2.6c) and increased with increasing soil nutrient PC2 (soils characterized by low pH, Ca, Mg, and Na; SDM version exclusively; Fig. 2.3d). Seedling to yearling survival was only influenced by the microsite soil nutrients, where survival decreased with decreasing soil nutrient PC1 levels and increasing levels of soil nutrient PC2 (Table S2.6; Figs. 2.3f, S2.6e-f).

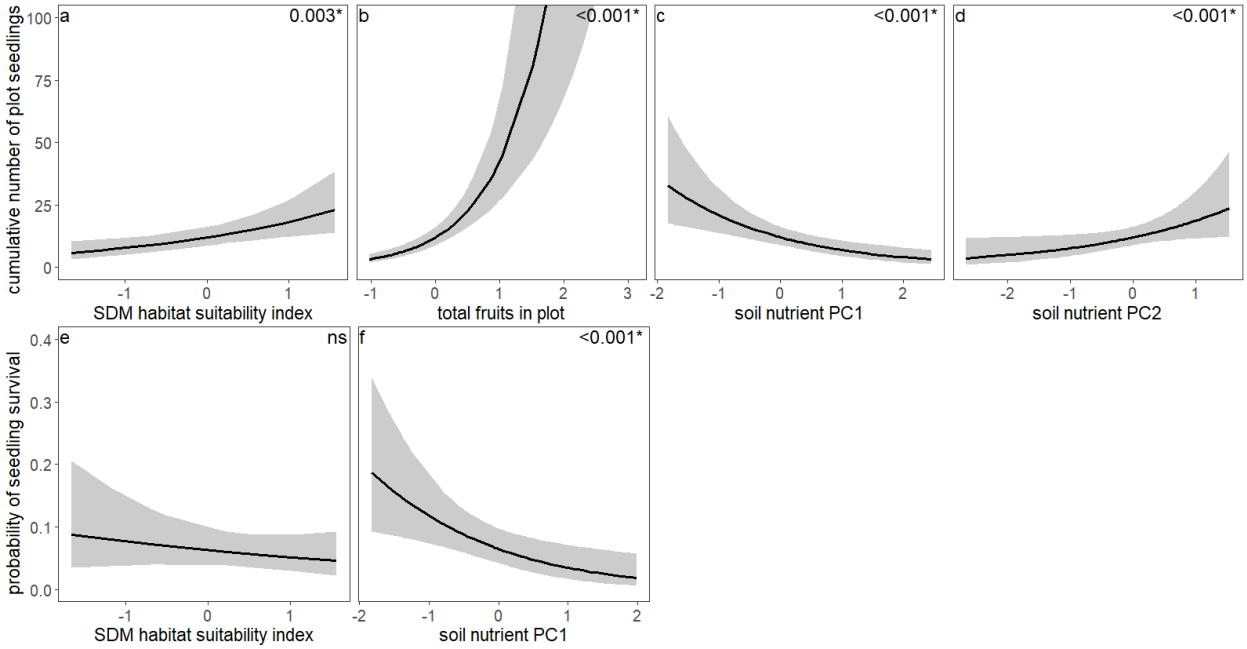

Figure 2.3: Partial regression plots showing the effects of predictors on *S. diphyllum* cumulative number of seedlings (2024 – 2025; a-d) and seedling survival (2024 – 2025; e-f). Asterisks indicate significant predictors. In each case, other continuous predictors are held at the mean. Thick lines represent the model predictions and grey shaded areas represent Wald 95%

confidence intervals based on fixed-effects only. The McFadden's R^2 value for cumulative number of seedlings including SDM estimates of habitat suitability was and R^2 for the seedling survival was 0.094.

2.4.3 *Symphyotrichum prenanthoides* survival rates

In total, 80.2% of transplants survived their first year, 88.0% survived their second year, and 70.5% survived both years, indicating again that most deaths occurred in the first year after planting. The SDM estimate of habitat suitability was not a significant predictor of cumulative survival (Tables 2.2, S2.3; Fig. S2.7a), whereas the CBM estimate of habitat suitability significantly interacted with abiotic conditions (Table S2.3; Fig. 2.4a-c). For example, survival increased with increasing community similarity in plots with low soil moisture but decreased with decreasing similarity in plots with high soil moisture (Table S2.3; Fig. 2.4a).

Abiotic microsite conditions had significant effects on cumulative survival. For models using SDM habitat suitability as a predictor, cumulative survival increased with increasing soil moisture (Table S2.3; Fig. S2.7b) and decreased with increasing soil nutrient PC3 levels (soils characterized by low pH, K, and Na; Figs. S2.4, S2.7c). In both models as canopy cover increased, cumulative survival decreased (Table S2.3; Figs. 2.4d, S2.7d). Weeding significantly increased cumulative survival (Table S2.3; Figs. 2.4e, S2.7e). Seed source was also a significant predictor of cumulative survival, where IV transplants had significantly higher survival rates than CBC transplants (Table S2.3; Figs. 2.4f, S2.7f).

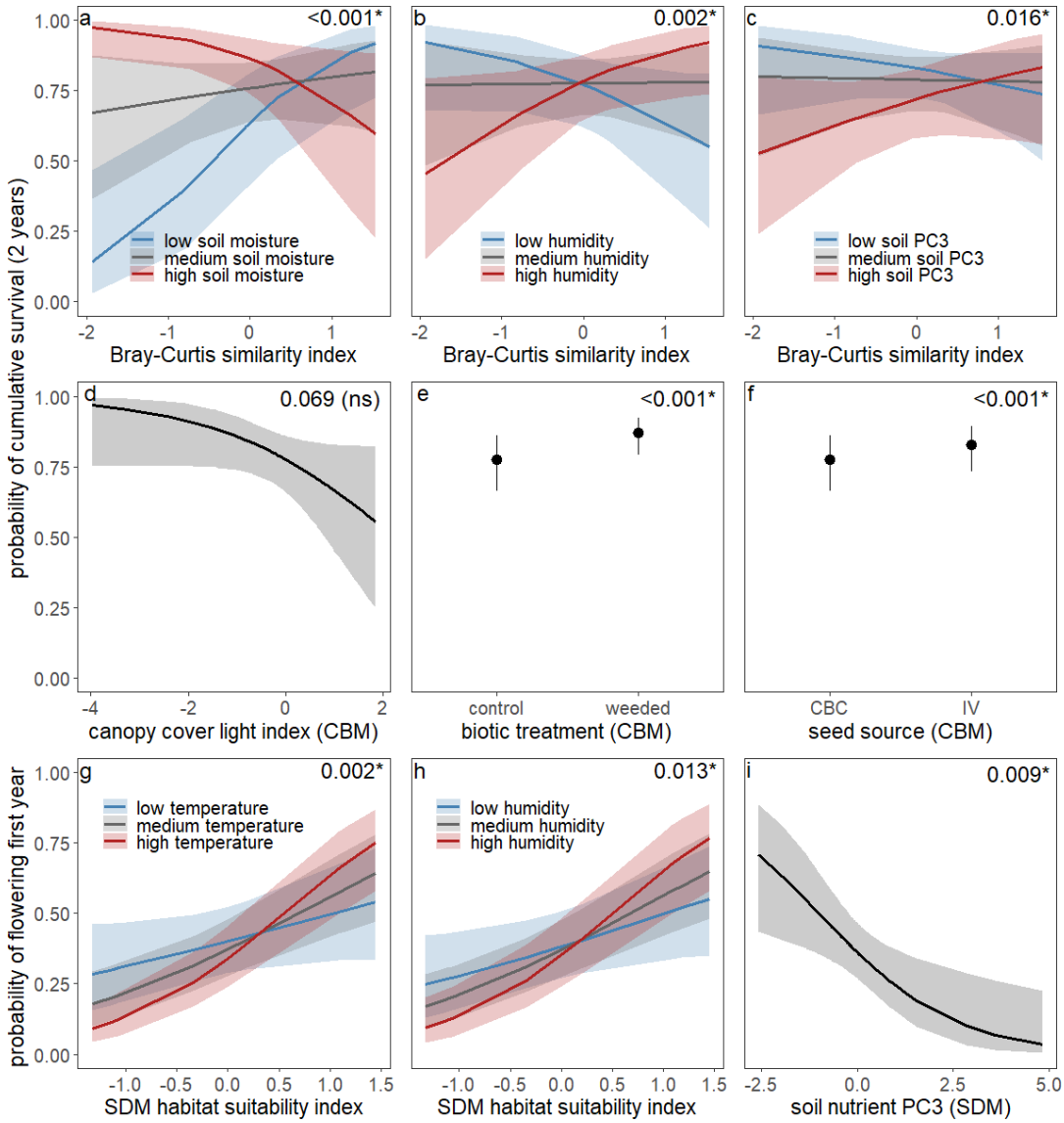

Figure 2.4: Partial regression plots showing the effects of predictors on *S. prenanthoides* cumulative over two years (a-f) and the probability of flowering in the first year (g-l). Brackets denote what estimate of habitat suitability was used in the model. Asterisks indicate significant predictors. In each case all other continuous predictors are held at the mean; categorical predictors are held at the most common category (seed source = "IV" and biotic treatment = "control"). Points (±95% CI) show model-predicted responses for categorical predictors, while lines (±95% CI) show model-predicted relationships for continuous predictors (based on fixed-effects only), with all other variables held constant. The CBM cumulative survival model pseudo R^2 values were 0.69 (conditional) and 0.28 (marginal). The pseudo R^2 value for the probability of flowering in the first year including SDM and CBM estimates of habitat suitability were 0.576, 0.586 (conditional) and 0.236, 0.154 (marginal), respectively.

2.4.4 *Symphyotrichum prenanthoides* reproduction

In year one (2023), 56.1% of *S. prenanthoides* individuals flowered. The SDM-predicted habitat suitability interacted with abiotic conditions, where the probability of flowering increased with increasing habitat suitability only in average to high temperature microsites and in average to high humidity microsites but remained similar in low levels (Tables 2.2, S2.4; Fig. 2.4g-h). The probability of flowering significantly increased as community similarity to the natural sites increased (Table S2.4; Figs. 2.4j, S2.7g).

Abiotic microsite conditions also had significant effects on flowering (Tables 2.2, S2.4). Probability of flowering increased with increasing soil moisture (Table S2.4; Figs. 2.4l, S2.7i). When using SDM habitat suitability as a predictor, high soil nutrient PC3 levels (soils characterized by low Na, Mg, and K) decreased the probability of flowering (Table S2.4; Fig. 2.4i). Significantly more transplants flowered in weeded sub-plots, with more CBC seed source individuals flowering than those from IV seed source; however, in control (un-weeded) sub-plots flowering was higher among IV seed source individuals (Tables 2.2, S2.4; Figs. 2.4k, S2.7h).

Table 2.2: Summary results of models testing the influence of habitat suitability, abiotic factors, biotic treatment, and transplant characteristics on survival and reproduction of *Stylophorum diphyllum* and *Symphyotrichum prenanthoides*.

Species	Response	SDM suitability	CBM suitability	Abiotic factors [c]	Biotic treatment [c]	Transplant characteristics
S. diphyllum	Cumulative two-year survival [a]	*no effect*	▪ Interaction with soil nutrient PC2	▪ Declines with increasing soil moisture ▪ Increases with increasing humidity differentials (CBM) ▪ Increases with increasing soil nutrient PC2 (SDM) and Interaction with BCI	▪ Caged > uncaged	▪ Large > small transplants ▪ 2023 plantings > 2022 plantings
S. diphyllum	Cumulative two-year number of fruits produced per plant	*no effect*	*no effect*	▪ Interaction between soil moisture and cage treatment (SDM) ▪ Increases with increasing temperature and humidity differentials ▪ Increases with increasing soil PC1	▪ Interaction between soil moisture and cage treatment ▪ Caged > uncaged (CBM)	▪ Large > small transplants ▪ IL seed source > MW seed source
S. diphyllum	Cumulative number of seedlings in plot (2024–2025)	▪ Increases with increasing SDM habitat suitability index	*no effect*	▪ Declines with increasing soil PC1 and soil PC2 (SDM)	*no effect*	▪ Increases with increasing total number of fruits in years prior
S. diphyllum	Seedling survival to yearling [b]	*no effect*	*no effect*	▪ Declines with increasing soil PC1 ▪ Increases with increasing soil PC2 (CBM)	*no effect*	*no effect*
S. prenanthoides	Cumulative two-year survival [a]	*no effect*	▪ Interactions with soil moisture, humidity differential, soil nutrient PC3	▪ Declines with increasing canopy cover (SDM) ▪ Increases with increasing soil moisture (SDM) ▪ Declines with increasing soil PC3 (SDM) ▪ Interactions with CBM	▪ Weeded > control	▪ IV source > CBC source
S. prenanthoides	Probability of flowering first year (2023) [a]	▪ Interaction with temperature and humidity	▪ Increases with increasing BCI	▪ Increases with increasing soil moisture ▪ Interactions with SDM	▪ Interaction with seed source	▪ Interaction between seed source and weeding treatment

[a] Model excludes plants that died in the same year as planting (survival models) or plants that died in the same year as flowering.
[b] Model excludes plots with no seedlings the previous year (2024); GLM model.
[c] Brackets designate if result was exclusive to one model estimate of habitat suitability (SDM or CBM).

2.5 Discussion

We tested two different methods of quantifying suitable habitat while controlling for other predictors of translocation success. Neither CBM nor SDM habitat suitability was a strong predictor of the short-term translocation survival, although each model was positively correlated to at least one measure of reproduction success. Overall, transplant characteristics had a much greater relative influence on translocation success, followed closely by microsite conditions. The low efficacy of the habitat suitability models to predict early translocation success likely reflects their coarse spatial resolution which fails to capture critical microsite conditions, or their emphasis on longer-term environmental and ecological processes (i.e., historic climate averages, succession) that do not immediately influence survival and reproduction. Quantifying habitat suitability remains challenging; although broad-scale estimates mostly failed to predict initial survival and reproductive success, long-term monitoring is needed to assess if these estimates can predict population persistence.

2.5.1 Transplant characteristics

Our results confirm the importance of transplant characteristics including planting size class, planting year, and seed source (Table 2.2). It is well known that early growth stages represent a time period of high vulnerability and mortality, therefore, planting later life phases promotes establishment (Drayton and Primack 2000). Although the greatest predictor of cumulative *S. diphyllum* survival was planting size class, planting year also had a significant effect on cumulative *S. diphyllum* survival, indicating 2023 was a better planting year than 2022 (Table S2.1; Figs. 2.2c, S2.5c). This effect of year may be because of climate variability, where milder temperatures and higher precipitation levels promote establishment. For example, during

the planting week total precipitation at one property was 17.9 mm (2023) compared to 0.2 mm (2022) and the mean temperatures were 13 °C and 17 °C respectively (based on the nearest weather station; Environment and Climate Change Canada 2025). Spreading translocation plantings over multiple years allows for a greater likelihood of success by buffering year-to-year climate variability (Guerrant and Kaye 2007).

In both species, seed source influenced at least one metric of translocation success (Tables 2.2, S2.2, S2.3). For *S. diphyllum*, more fruits were produced by IL individuals (Table S2.2; Figs. 2.2i, S2.5i). Within the natural populations, IL produces more fruits per plant on average than the MW population (e.g., 6.8 fruits per plant versus 4.8 fruits per plant in 2022; Wiedenfeld, pers. comm., 2025). However, MW plants in the greenhouse produced more fruits than IL plants (see Chapter 3). For *S. prenanthoides*, IV individuals had significantly greater cumulative survival (Table S2.3; Figs. 2.4f, S2.7f). Although IV seeds had lower germination rates (41%) compared to CBC seeds (49%), the higher survival rates of IV transplants suggest it may be the preferable choice for supplemental plantings at our sites (for details on germination testing see Appendix B Supplementary methods S2.2). In summary, transplant characteristics can strongly influence short-term translocation success, making it critical to consider these aspects when designing a translocation.

2.5.2 Microsite variables

Microsite variables also had strong effects on short-term translocation success (Table 2.2; Figs. 2.2-2.4, S2.1-S2.4). Based on the natural populations of our study species, we predicted the highest survival in medium soil moisture and medium canopy light conditions for S. *diphyllum*, and in high soil moisture and low canopy light conditions for *S. prenanthoides*. Our predictions were generally met. For example, when using SDM-predicted habitat suitability, *S.*

prenanthoides cumulative survival increased with increasing soil moisture and decreased with increasing canopy cover (Table S2.3; Fig. S2.7b). However, *S. diphyllum* cumulative survival increased with decreasing soil moisture instead of having a hump-shaped relationship, indicating lower soil moisture conditions are optimal (Table S2.1; Figs. 2.2e, S2.5e). Soil nutrients were also important predictors for both species (Table 2.2; Figs. 2.2-2.4, S2.5-S2.7). For example, *S. diphyllum* cumulative survival, seedling production, and survival of seedlings to yearlings increased with increasing soil nutrient PC2, indicating soils with low pH and Ca (Tables S2.1, S2.2; Figs. 2.3c-d, 2.3f, S2.5f, S2.6f). This suggests acidic soils are optimal, which matches the mean pH of the natural populations ($\mu = 6.58$), even though *S. diphyllum* is known to tolerate slightly alkaline, calcareous soils as well (Native Plant Information Network 2013). In models using SDM-predicted habitat suitability, cumulative survival for *S. prenanthoides* decreased with increasing soil nutrient PC3, indicating soils with high CEC and low pH (Table S2.3; Fig. S2.7c). The natural populations we measured had relatively high CEC ($\mu = 23$ meq/ 100g) and pH ($\mu = 7.3$), however, *S. prenanthoides* is known to tolerate a range of soil textures from rich sand to clayey soils (COSEWIC 2012). The influence of microsite on translocation success emphasizes the importance of refining microsite selection to meet species preferences within larger, heterogenous sites (Dunwiddie and Martin 2016).

2.5.3 Interactions between habitat suitability and microsite

We expected a potential tradeoff between habitat suitability and microsite, where favourable microsite conditions would minimize the influence of broad-scale habitat suitability and unfavourable microsite conditions would maximize it. We found frequent interactions between broad-scale CBM habitat suitability and microsite variables (Table 2.2) but these interactions were only partly explained by species preferences. For *S. prenanthoides* survival,

soil moisture interacted with community similarity, where survival was highest in high soil moisture plots (favourable) but only in low community similarity, whereas in high community similarity, survival was highest in low soil moisture plots (unfavourable; Tables S2.3; Fig. 2.4a). This suggests that increasing community similarity is a proxy for underlying abiotic conditions we did not measure that buffer the negative effects of unfavourable microsites, suggesting dissimilar sites have more favourable conditions compared to natural sites. However, it is difficult to explain why there were negative relationships between habitat suitability and survival in favourable microsites. It could be because more dissimilar communities lack dominant competitors found in natural populations, reducing resource competition and increasing success. We suggest a future study could conduct fine-scale community surveys (within 1 m of plantings) to assess how species interactions (i.e., competition, facilitation) influence translocation success relative to naturally occupied sites.

2.5.4 Biotic treatments

Within the microsite, biotic treatments also influenced short-term translocation success. For *S. diphyllum*, caging significantly increased cumulative survival and number of fruits (Tables 2.2, S2.1, S2.2; Figs. 2.2d, S2.5d, S2.5j). Herbivory decreases survival, growth and flowering (Fletcher et al. 2001, Fenu et al. 2016, Albrecht and Long 2019) by reducing plant height and number of leaves, which are positively correlated to reproduction probability (Anderson 1994, Kraft et al. 2004). We observed very few instances of herbivory so we suspect herbivores may be browsing uncaged plants after monitoring in the late summer to early fall when other understory plants are declining (i.e., senescing). In addition to excluding herbivores, the cages seem to provide support and protect transplants from falling debris, including trees and large branches. The significant interaction between soil moisture and cage treatment (Table S2.2; Fig 2.2j) may

reflect microclimate effects of the cages acting as windbreaks (Evans et al. 2018), thereby reducing transpiration loss and conferring an advantage to transplants in drier sub-plots where water availability limits production, whereas in wetter sub-plots the cages offer no benefit except protection.

Reducing competition by removing surrounding plants is also known to increase success (Godefroid et al. 2011). We found removing competition increased *S. prenanthoides* cumulative survival and found competition removal interacted with seed source affecting flowering (Tables 2.2, S2.4; Figs 2.4k, S2.7h). In un-weeded subplots, the probability of flowering was slightly higher for IV transplants than CBC transplants, suggesting individuals from the IV population may be better adapted to more competitive environments. Local adaptation is typically more evident under reduced competition, especially in later reproductive life stages, but high competition can reduce the local advantage (Sambatti and Rice 2006, Rice and Knapp 2008). Our results reinforce the benefit of including biotic treatments in translocations, despite their high labour demands and costs.

2.5.5 CBMs versus SDMs

After accounting for microsite conditions, neither habitat suitability estimate independently predicted more than one measure of translocation success (Tables 2.2, S2.2, S2.4; Figs. 2.3a, 2.4j). The CBMs in combination with microsite variables predicted survival of both species across two years and was independently positively associated with *S. prenanthoide*s probability of flowering. Elmendorf and Moore (2008) suggest community composition is a more robust measure of broad-scale habitat suitability because it is the outcome of multiple species interactions (biotic) and their relationships with the underlying environment (abiotic), whereas SDMs characterize only the environmental conditions. For example, plant community

composition may reflect factors not available for inclusion in the SDMs, such as site disturbance history (Brown and Boutin 2009). While SDM-based habitat suitability was not associated with survival, it was significantly positively correlated to the number of *S. diphyllum* seedlings and showed significant interactions with microsite influencing *S. prenanthoides* flowering (Tables 2.2, S2.2, S2.4; Figs. 2.3a, 2.4g-h).

The SDM predictions failed to predict short-term survival but may align more closely with long-term success (5 – 10 years) because they are based on established populations and long-term climate averages. For example, across 235 plant translocations monitored over an average of 8.4 years, post-hoc SDMs based on climate best predicted population persistence, whereas management techniques (i.e., founder size, number of source populations) and niche metrics more strongly influenced next-generation recruitment (Bellis et al. 2025). To better link habitat suitability to fitness, Rusconi et al. (2022) recommend a two-step modeling and field approach; broad-scale niche and distribution modelling followed by refining site selection using fine-scale field measurements of natural populations to identify microsites with conditions that correlate to high fitness. Combining environmental and community-based estimates can further improve predictions. For example, Hemrova et al. (2019) predicted suitability for orchids using SDMs based on fine-scale abiotic and biotic factors (i.e., vegetation cover) and found that habitat suitability was positively correlated with germination for three out of four orchid species. Ultimately, improving translocation success by quantifying habitat suitability requires a long-term commitment to monitoring, coupled with investigating the effects across different spatial scales and species.

2.6 Conclusion

Quantitative assessments of broad-scale habitat suitability using SDMs or CBMs are a potential tool for site selection to increase translocation success and improve recovery outcomes for plant species of conservation concern. However, we found short-term survival of our study species was mainly determined by transplant characteristics and microsite conditions, rather than these broad-scale estimates of site suitability. Yet both models predicted at least one measure of reproduction success and model efficacy may improve over time as the translocated populations establish and show whether or not they can maintain stable populations. In the meantime, we must continue to monitor translocated and natural populations so we can understand what site characteristics affect population viability and how these effects change over time. Maximizing early translocation success requires prioritizing optimal transplant characteristics, careful microsite selection, and minimizing negative biotic interactions, while continuing to evaluate the role of broad-scale habitat suitability in shaping long-term outcomes.

CHAPTER III

ASSESSING POPULATION DIFFERENTIATION IN WOOD-POPPY (*STYLOPHORUM DIPHYLLUM*) USING PHENOTYPIC TRAITS

3.1 Abstract

A central debate in restoration ecology is where to source seeds and whether sources should be mixed or not. Informed seed sourcing requires knowing the magnitude of genetic differentiation and corresponding trait differences between potential source populations to infer the evolutionary mechanisms underlying population differentiation. When phenotypic divergence exceeds neutral genetic divergence, this signals directional selection and potential local adaptations, implying sources should remain separate. Conversely, if phenotypic divergence is equal or lower than neutral genetic divergence this signals genetic drift or stabilizing selection, suggesting mixing sources poses low risk. The wood-poppy (*Stylophorum diphyllum*; Papaveraceae) populations in Canada are genetically distinct from one another and commercial nursery sources but phenotypic differentiation has not been assessed among these populations. We used common garden trials to assess phenotypic differentiation among four wild Canadian populations, one U.S. population, and two commercial nursery sources. We measured differences in germination, growth, leaf morphology, and reproduction-related traits to quantify phenotypic differentiation and then compared phenotypic trait distance (P_{ST}) to genetic distance (F_{ST}; measured in a previous study) to infer the evolutionary mechanism underlying population differentiation. We also compared trait distance to geographic, environmental, and ecological distances. Trait distance was positively correlated to genetic distance between wild Canadian populations and commercial nursery sources, indicating that nursery sources are genetically and phenotypically divergent from wild populations. Canadian populations exhibited stabilizing trait selection, whereas

nursery sources showed genetic drift acting on reproduction-related traits. Overall, trait differences among populations were greatest for reproduction-related traits. Despite high genetic differentiation among Canadian populations, low phenotypic differentiation in fitness-related traits suggests low risk of local adaptation. Commercial nursery sources should not be used for restoration in Canada, but mixing locally-sourced wild populations poses low genetic risks and may provide the benefit of increased genetic diversity.

3.2 Introduction

Selecting appropriate seed sources is a central challenge in restoration ecology (Lesica and Allendorf 1999, Sackville Hamilton 2001, Wilkinson 2001, McKay et al. 2005). Seed sourcing decisions are important when augmenting existing populations or establishing new ones because genetic differentiation among and within source populations can affect fitness and long-term persistence. For example, local genotypes will perform better in their home environment compared to non-local genotypes if there is local adaptation (Knight and Miller 2004, Bischoff et al. 2006). Introducing propagules from non-local sources can lead to reduced fitness through outbreeding depression caused by the introduction of maladapted genotypes (Montalvo and Ellstrand 2001). Therefore, a single source provenance from a site ecologically similar to the target site is often recommended to preserve locally adapted gene complexes (Center for Plant Conservation 2019). However, studies investigating the effect of provenance on success have found mixed results (e.g., Montalvo and Ellstrand 2000, Bischoff et al. 2006, Fedriani et al. 2019). While a recent meta-analysis of 275 plant translocations found reintroductions using a single source had a higher probability of reaching next-generation maturity (Bellis et al. 2024), too little genetic diversity can reduce population fitness (Hufford and Mazer 2003, Neale 2012). For example, small, inbred populations may require genetic rescue to counter inbreeding

depression (Frankham et al. 2011, Frankham 2015). Mixing seed sources can increase genetic diversity and fitness, promoting ecological resilience in an era of rapid environmental change (Maschinski et al. 2013, Havens et al. 2015, St. Clair et al. 2020, Goebl et al. 2022). In general, making seed sourcing decisions for restoration is difficult because there is not one recommendation that fits all species.

An informed seed sourcing strategy requires evidence of the magnitude of genetic differentiation among populations and whether there are corresponding trait differences that are adaptive. Reciprocal transplant studies directly assess fitness to test for local adaptation (e.g., Gordon and Rice 1998, Montalvo and Ellstrand 2000) and controlled tests of single versus mixed sources directly assess the benefits compared to the risks of source mixing (e.g., Lesica and Allendorf 1999, Goebl et al. 2022). Yet such testing is infrequently conducted for at-risk plant species prior to establishing new populations. Many rare plant populations occur on restricted private lands, are seed-limited, and/or are constrained by regulations that prohibit mixing sources at natural populations. Common gardens offer an alternative approach to measure phenotypic variation among sources in traits that influence fitness and drive adaptation. Phenotypic variation can be genetically determined (i.e., local adaptation or random drift), a plastic response to the current environment, or a combination of both. Common garden experiments rule out phenotypic plasticity by removing the confounding effects of the environment and testing for signals of local adaptation (e.g., Knapp and Rice 1998, Wallace et al. 2020). Phenotypic variation among source populations can be quantified as trait distance (i.e., Q_{ST} or P_{ST}) and correlated to genetic distance (i.e., F_{ST}), or geographic, environmental and ecological distances to identify the driver(s) of differentiation (e.g., Kawakami et al. 2011, Toczydlowski and Waller 2022). The strength of local adaptation is generally expected to increase with increasing geographic distance

between populations as gene flow is reduced and the magnitude of environmental differentiation increases (Galloway and Fenster 2000, Montalvo and Ellstrand 2000, Becker et al. 2008).

We measured phenotypic differentiation among populations of the wood-poppy (*Stylophorum diphyllum* (Michx.) Nutt. (Papaveraceae) [Synonym: *Chelidonium diphyllum* (Michx.)]), an herbaceous woodland perennial. *Stylophorum diphyllum* is listed as endangered in Canada under the Species at Risk Act (SARA), with only five populations, all in southern Ontario (Fig. 3.1; Government of Ontario 2016, Government of Canada 2022). Canadian populations exhibit genetic differences from one another and from the core United States (US) populations and commercial nursery sources based on Bayesian clustering analyses and population pairwise F_{ST} values (Lait et al. 2025). Genetic differentiation is lower among Canadian populations than between Canada–US and either US-US or US-nursery sources (Lait et al. 2025). All wild populations exhibit low levels of gene flow, and low to moderate levels of genetic diversity with peripheral populations having the lowest heterozygosity (Lait et al. 2025).

It is unknown if the observed genetic differences correspond to trait differences in *S. diphyllum*, or if trait differences correlate to geographic, environmental or ecological distances among populations. Reiter and McCune (2023) found that plants from one Canadian population grew faster and produced more leaves compared to two commercial nursery sources in a common garden experiment. However, phenotypic differentiation in *S. diphyllum* reproductive traits (i.e., number of seeds and fruits) have not been assessed. Translocation trials are currently underway to establish new populations but planting admixture sources is prohibited at some nature reserves due to the unknown genetic consequences (see Chapter 2). Seed sourcing for future translocations and population augmentations can be informed by quantifying the

magnitude of phenotypic differentiation among populations and testing for signals of local adaptation.

We quantified *S. diphyllum* phenotypic differentiation by growing seeds from two commercial nursery sources, four wild Canadian sources, and one wild US source in two greenhouse common garden trials. We also used an *in situ* common garden to test for differences in germination and survival of four wild Canadian sources. We addressed three main questions: (1) What is the magnitude of phenotypic differentiation between sources? (2) Is the magnitude of trait differences among sources correlated to genetic, geographic, environmental or ecological distances? (3) Does phenotypic differentiation suggest random drift, directional selection, or stabilizing selection?

3.3 Methods

3.3.1 Study species

Stylophorum diphyllum is a long-lived, woodland herb that produces four-petaled yellow flowers from April to May, which later develop into hairy, dehiscent capsules (Gleason and Cronquist 1993). The species is capable of self-pollination or outcrossing (Bowles 2007). Despite have lower levels of heterozygosity compared to US populations (Lait et al. 2025), Canadian populations have little evidence of inbreeding depression (based on seed:ovule ratios at three populations; Bowles 2007). The population size of the four Canadian sources used in this study ranges from 76 to 1287 individuals (Wiedenfeld, pers. comm.). The center of the species' global distribution occurs in Kentucky, where the species is apparently secure (S5), with the distribution trailing from northwest Georgia and leading into southern Ontario where it is critically imperiled (S1; Fig. 3.1; NatureServe 2023). It is sold as a garden plant in the eastern

North-American nursery industry, and although the exact origins of nursery stock are often unknown, Lait et al. (2025) found that plants grown from seed from two commercial nursery suppliers were genetically more similar to U.S. than to Canadian populations.

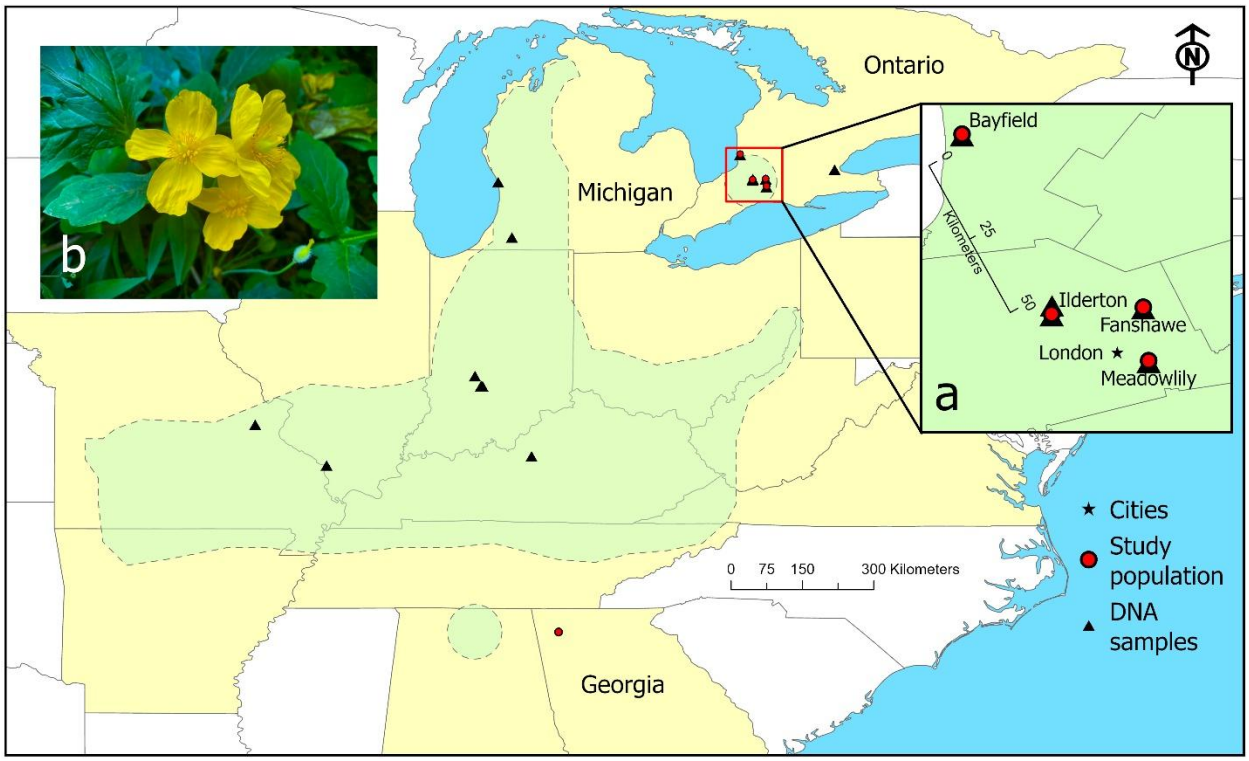

Figure 3.1: The distribution of *Stylophorum diphyllum*. Red circles represent approximate locations of one US and four Canadian wild populations used in greenhouse common garden trials and black triangles represents populations with DNA samples from Lait et al. (2025). Blue shading represents water bodies. Thin lines represent state and provincial boundaries. Black stars represent cities. Yellow shading indicates that the species is native to the state or province based on the Biota of North America Plant Atlas (BONAP 2014) and green shading represents the Flora of North America distribution map. Panel a: Inset map shows the four wild Canadian populations with county lines denoted by the thin lines. Panel b: *Stylophorum diphyllum* in bloom.

3.3.2 Common garden trials and seed collection

We conducted three common garden trials to test for differences among *S. diphyllum* individuals from commercial nursery sources and wild Canadian and US source populations. The first *ex situ* greenhouse trial (2022-2023) included two commercial nursery sources, two wild

Canadian sources, and one wild US source. We conducted a second greenhouse trial (2023-2024) including four wild Canadian sources because we were unable to obtain enough seed from all four viable populations in 2022 and had low germination rates of two Canadian sources in the first trial. The third trial (2022-2023) was an *in situ* common garden using seed from four wild Canadian sources planted at an unoccupied forest site within 2 km of one wild population.

For our 2023 common garden trials, we collected seed on June 24-25, 2022 from four of the five *S. diphyllum* wild source populations (known as Bayfield, Fanshawe, Ilderton, and Meadowlily) from 5, 31, 53, and 41 maternal lines (mother plants) respectively (Table 3.1), based on natural seed availability and following the less than 10% seed collection rule (Center for Plant Conservation 2019). We could not collect seeds from the fifth population because it had no seed-producing plants in 2022. We excluded Bayfield from the 2023 greenhouse trial and used all seed for the *in situ* trial due to limited seed availability (except for a few seeds ($n = 5$) withheld to test seed viability in the growth chamber). We also collected seed from plants grown in the greenhouse from seeds purchased from two commercial nursery sources (three Toadshade plants and five Botanically Inclined (BotanyCa)) plants in late March through mid-July 2022 as part of a previous common garden experiment (Reiter and McCune 2023). We collected seed from one US natural population in Georgia from two mother plants on April 20, 2021.

For our 2024 greenhouse trial we collected seed on June 21-28, 2023, from four of the five Canadian source populations (Bayfield, Fanshawe, Ilderton, and Meadowlily) from 31, 15, 18, and 10 maternal lines respectively, following the same collection protocols we used in 2022 (Table 3.1). We collected Meadowlily seeds from *ex-situ* Meadowlily plants in raised beds at the Environmental Sciences Western Experimental Field Station (University of Western Ontario) because we did not have permission to re-access the wild population (under new, private

ownership). We were again unable to obtain seeds from the fifth population due to the population having no seed-producing plants in 2024.

Table 3.1: Provenance and seed source of *S. diphyllum* in common garden greenhouse trials (number and maternal line).

Provenance	**Seed source**	**Number of plants in 2023 trial**	**# maternal lines in 2023 trial**	**Number of plants in 2024 trial**	**# maternal lines in 2024 trial**
Canadian-wild	Bayfield (BW)	0 [a]	*n/a*	37	6 [b]
Canadian-wild	Fanshawe (FW)	29	5[b]	38	6 [b]
Canadian-wild	Ilderton (IL)	0 [a]	*n/a*	37	6 [b]
Canadian-wild	Meadowlily (MW)	13	3	40	6 [b]
US-wild	Georgia (GA)	9	2	0	*n/a*
US-nursery (Bowman's Hill Wildflower Preserve, PA)	BotanyCa / Botanically Inclined (BI)	16	5	0	*n/a*
Unknown	Toadshade (TS)	10	3	0	*n/a*
TOTAL	----------	81(11) [c]	18	152(23) [c]	24

[a] We used all 2022 Bayfield seeds for the *in situ* common garden trial and excluded Ilderton plants due to low germination ($n = 4$) in 2023.
[b] We chose a random sub-set of available maternal lines with high germination rates to ensure adequate replication within lines.
[c] Numbers in brackets indicate the number of plants excluded from analyses due to early mortality from overwatering or high levels of pest damage.

3.3.3 Greenhouse common garden trials

Seed treatments, growth chamber conditions, and transplanting

We stored seeds in plastic containers with moist sand and kept them refrigerated at ~4 °C. We tested two different seed treatments in the 2023 trial: (1) warm-cold stratification and (2)

cold stratification. Baskin and Baskin (1984) suggest a warm stratification period is unnecessary for *S. diphyllum* to germinate but Deno (1993) and commercial nurseries (e.g., Prairie Moon Nursery 2025) propose that the species require a warm-cold cycle to break dormancy. For seeds that received the warm-cold stratification we used two month intervals as per Reiter and McCune (2023), starting with a warm period (mid-October) at 25 °C, followed by a cold period (mid-December) at 4 °C. We used only the warm-cold stratification treatment in the 2024 trial because no seeds that received the cold stratification only treatment in 2023 germinated.

We sowed all seeds mid-February into ITML #162 Deep (4.5 cm) square plug trays (*n* =162 cells) with PRO-MIX® general purpose mycorrhizae potting media (peat moss-based) with play sand added for drainage [1 soil bale: 0.5 sandbag mix ratio]. We planted each seed into its own cell (~1 cm deep) labelled with a unique ID code to identify the source, maternal line, and tray cell position. We placed each tray in a liner so we could bottom-water and ensure equal water distribution throughout the tray. After sowing, we immediately moved trays to a single temperature-controlled growth chamber set to 12 °C with a 12-hour photoperiod. We used a Conviron® growth chamber (model no. BDR16) with lighting set to level one (~334 µmol/m²/s) in 2023 and a Percival growth chamber (model no. PCG-10) with LED lighting set to 50% intensity (~282 µmol/m²/s) in 2024 based on availability.

We checked seed trays daily for emergence and kept trays continuously moist. To account for any microclimate conditions within the growth chamber, we rotated each tray to a different position within the chamber approximately once a week. As seedlings started to emerge (~2 weeks after planting) we increased the temperature to 15 °C to simulate warming spring temperatures. We transplanted seedlings to a single bay at the University of Lethbridge greenhouse in individual 4” deep pots (10.16 cm) with the same media mix at 30 days (± 5 days;

Canadian sources) or 40 days (+/- 5; US sources) from the emergence of the first true leaf (2023 trial) or from the germination date (2024 trial). We transplanted the Canadian sources earlier in the 2023 trial due to earlier emergence of their first true leaf compared to all other sources. In the 2024 trial, we transplanted all plants at 40 days old (± 5 days).

3.3.4 Experimental design

The greenhouse bay was set to a day temperature range of 16-24 °C and a night temperature range of 12-24 °C (based on the local sunrise/ sunset times) with a humidity target of 55% (dehumidify target of 70%). We used no supplemental lighting and deployed all available shade curtains (ceiling and west-facing wall) to mimic natural forest shade conditions. We fertilized all plants weekly with Miracle-Gro water soluble, all-purpose plant food (24-8-16) and watered as needed. We treated pests (i.e., whiteflies, thrips, spider mites, and mealy bugs) using a combination of organic bio-controls, sticky traps, and a botanic insecticide (Doktor Doom 0.2% Pyrethrin spray). In the 2023 trial, we used a randomized incomplete block design (due to low germination rates of some sources) with 17 blocks of five plants per block. We spaced plants in an alternating arrangement on trays to ensure growth was not restricted. In the 2024 trial, we used a randomized complete block design and set up 7 blocks with 20 plants per block.

Germination, growth, and reproduction measurements

We measured ten variables related to germination, growth, and reproduction (including phenological timing of emergence and first fruit) for each individual over the course of the experiment. After sowing, we checked for germination daily and recorded the emergence date as the first day the hypocotyl was visible. We measured plant height (taken from the plant base at soil-level to the tallest growth point) and the number of leaves each week until measurements remained stable. We recorded weekly number of fruits until the end of the experiment, including

date of first fruit. In 2023, we bagged all fruits to capture any seeds, and after the fruit dehisced, we counted and weighed all dried seeds produced by each individual plant. We calculated an average weight per seed for each plant by dividing the total number of seeds by the total weight of all seeds produced by the individual. In 2024, we used the same procedure, except we weighed 100 seeds from a sub-set of five individuals from each source (each representing a different maternal line) to get a representative sample and then divided the total seed weight by the number of seeds to obtain an average weight per seed. We also calculated the average number of seeds per fruit per individual based on the ratio of total seeds to total fruits produced.

3.3.5 Biomass measurements

At 200 days of age (+/- 7 days) we harvested the plants and carefully rinsed the roots to remove any soil. We separated each plant's above-ground and below-ground biomass and placed each component into a paper bag for drying. We dried biomass samples in a drying oven set at 60-70 °C for a minimum of 48 hours before weighing.

3.3.6 Leaf trait measurements

We also measured trichome density and leaf lobing (number of rounded leaf divisions), as these traits are associated with environmental adaptations related to heat, herbivory and disease (Nicotra et al. 2011, Hauser 2014). Prior to drying, we clipped a single leaf tissue sample (1" (2.54 cm) diameter) from the biggest leaf of each plant and stored it in a coin envelope. To capture a picture of part of each clipping, we used an Olympus SZ61 microscope equipped with a AXioCam ERC 5s camera under a 10x objective lens (4× in 2024) with an exposure time of 150 ms in the Zen 2.3 Lite program (photo size 2.47 × 1.85 mm in 2023 and 1.41 × 1.06 mm in 2024 due to microscope availability).We analyzed the photos using ImageJ (Schneider et al.

2012) and traced each trichome using the freehand tool, adding each tracing event to the Region of Interest (ROI) manager and used the Multi-Measure tool within the ROI manager to count the number of trichomes per photo area. We calculated trichome density as the number of trichomes per square millimeter (mm^2) by dividing the total number of trichomes counted by the area assessed (mm^2).

We also captured a single leaf scan of the biggest leaf from each plant with a Brother DCP-L25500W flatbed scanner set to 600 dbi resolution colour setting (300 dbi black and white setting in 2025) for leaf shape analysis. Prior to analysis in 2023, we edited photos in GIMP (GNU Image Manipulation Program) to remove petioles (petioles were removed prior to scanning in 2024). We used the Fiji distribution of ImageJ (Schindelin et al. 2012) and the Multiple Leaf Sample Extraction System (MuLES) plugin (Bowman et al. 2023) to quantify leaf lobing, measured as circularity and solidity (Kuan et al. 2022). Circularity quantifies the degree a shape overlaps a circle (4π area/perimeter^2) where circularity = 1 denotes perfect roundness. Solidity quantifies the overall concavity of a shape (area/ convex hull area) where solidity = 1 denotes zero concaveness. For example, an irregular jigsaw shape could have an elongated perimeter (low circularity) and shallow indentations (high solidity).

3.3.7 Distance metrics

We measured trait, genetic, geographic, environment and ecological distances between each source pair. To measure trait distance we calculated the magnitude of phenotypic divergence (P_{ST}; Equation 1) as a proxy for Q_{ST} (Leinonen et al. 2006). Q_{ST} is a quantitative genetic analogue of F_{ST}, which is the standardized genetic differentiation among populations based on neutral marker loci (Wright 1951). Q_{ST}- F_{ST} comparisons give evidence for the underlying evolutionary mechanism of differentiation. If $Q_{ST} > F_{ST}$ this suggests directional selection, if Q_{ST}

= F_{ST} this suggests genetic drift, and if $Q_{ST} < F_{ST}$, this suggests stabilizing selection (Merilä and Crnokrak 2001). Unlike Q_{ST}, P_{ST} is strictly based on phenotypic trait data and does not require knowing the genetic relationships of individuals within a population (i.e., the additive genetic variance), therefore, we used P_{ST} to approximate Q_{ST} (Leinonen et al. 2006, Brommer 2011). We calculated P_{ST} as in Brommer (2011) using equation (1),

$$(1)\ P_{ST} = \frac{c\sigma_B^2}{c\sigma_B^2 + 2h^2\sigma_w^2}$$

where σ_B^2 (V_{source}) is among-population variance, σ_w^2 ($V_{residual}$) is within-population variance, h^2 is the heritability of a trait, and c is the proportion of total variance from additive genetic effects among populations. As heritability was unknown, we made the common null assumption (Brommer 2011) that the proportion of total phenotypic variance from additive effects is the same for among- and within-population variance ($c = h^2$).

To measure genetic distance, we used F_{ST} values from Lait et al. (2025), calculated using the Weir and Cockerham (1984) method based on single nucleotide polymorphism (SNP) data from DNA of 19 individuals per wild population and 11 individuals per commercial nursery. We did not measure genetic distance for Georgia because no genetic data is yet available.

To measure geographic distance, we used population coordinates and measured the geodesic distance between points with the 'geodist' package (Padgham and Sumner 2025). We converted all geodesic distances to kilometers. To measure environmental distance, we calculated dissimilarity in multivariate climate and soil conditions. We used the 'raster' package to extract the values of 19 bioclimatic variables (30 arc second resolution) from WorldClim (Fick and Hijmans 2017) and 17 soil characteristics from the United North American Soil Map (Liu et al. 2013) for each population. We then scaled all climate and soil variables and calculated the Euclidean distance between each pair of populations for climate and soil separately using the

'dist' function in R (R Core Team 2021). We excluded commercial nursery sources from geographic, climate and soil distance metrics due to their unknown provenance. To measure ecological distance we conducted plant community surveys to calculate dissimilarity in community composition among wild Canadian sources. We surveyed a 100 m × 100 m area from each source population's centroid and assigned ordinal abundances for all vascular plant species within the survey plot (see Chapter 2). We then calculated Bray-Curtis dissimilarity based on the untransformed species abundance matrices to quantify pairwise differences in the plant communities among all populations using the 'vegan' package (Oksanen et al. 2025). We measured ecological distance in the 2024 greenhouse trial only because this metric does not apply to cultivated sources and we did not have plant community data from the Georgia source population.

3.3.8 *In situ* common garden trial

We used an *in situ* seed addition trial to test for differences between wild Canadian sources in seedling emergence and survival in natural conditions. We stored a sub-set of seeds collected from Canadian populations in 2022 in plastic bags within moist paper towels in the refrigerator at ~4 °C until planting in early August. We selected 11 planting plots at an unoccupied, forested site ~1.75 km from the Meadowlily population. To test whether seedling emergence and survival varies in different microsite conditions we chose each plot non-randomly with the aim of maximizing the range of environmental conditions (i.e., soil moisture, canopy cover, slope and aspect) and avoided trails and creeks. At each plot, we carefully planted seeds from each source (n = 20 seeds per source; 17 seeds for Bayfield due to limited seed availability) in their own respective sub-plot (~30 cm apart). We planted seeds (n = 847 total) ~0.5 cm deep in a dispersed, circular pattern and caged each sub-plot with an enclosed wire exclosure (~24 cm

diameter, 30 cm height, 0.635 cm mesh openings) to deter seed predators and increase the probability of emergence (Neigel et al. 2025).

3.3.9 *In situ* measurements

We measured soil moisture at each plot using a Delta-T Devices SM150 two-prong soil moisture kit (model SM150T sensor with a HH150 meter set to the 'mineral soil' setting). We quantified canopy cover with a photo taken with a clip-on smartphone fisheye lens attachment and using the freely available 'Gap Light Analysis Mobile App' (GLAMA) to estimate the canopy cover index (Gonsamo et al. 2013). Our partners monitored the plots for any premature emergence in early October 2022. We took two seedling censuses throughout the 2023 growing season (late May and mid-August), and recorded year one survival on June 20, 2024.

3.3.10 Statistical analysis

Modelling phenotypic differentiation

We tested for differences in 15 phenotypic traits related to phenological timing (germination, emergence, first fruit), reproduction, growth rate, leaf morphology, and biomass using linear, generalized linear, nonlinear and mixed-effect models depending on the distribution and hierarchical structure of the data (Table S3.1). We specified all models with source and block as fixed effects unless models used a subset of all available plants (i.e., leaf morphology models and 2024 seed weight model) in which case we removed block due to low replication. We included maternal line as a random effect to account for variation due to differences in maternal lines in 2024 analyses but excluded maternal line in the 2023 analyses because of low replication within maternal lines due to low germination of some sources. To test for pairwise

differences in each trait, we tested for significant differences in estimated marginal means using a Tukey HSD adjustment (Tukey 1949).

To model the S-shaped (sigmoidal) curve pattern of plant growth we used nonlinear mixed-effect models. We used a self-starting nonlinear least squares four-parameter logistic growth model 'SSLogis' based on the logistic growth curve formula defined as:

$$(2)\ f(x) = \frac{Asym}{1+exp\left(\frac{xmid-x}{scal}\right)}$$

where $f(x)$ denotes the independent input variable (age as days since germination), *Asym* denotes the upper asymptote (maximum height), *xmid* denotes the inflection point (age at which growth is half of the asymptote), and *scal* denotes the scale (the inverse of the growth rate constant, where a lower scale denotes a faster growth rate). We fitted random intercepts for asymptotic size and scale grouped by individual to allow for growth parameters to vary from individual to individual and to account for repeated measurements (Table S3.1). To test model significance, we used an ANOVA to compare the model with source as a fixed effect to a null model without source to determine if source had a significant effect on growth rate.

Distance metrics analysis

We estimated P_{ST} for six traits (maximum height, number of leaves, total seeds, total fruits, dried shoot weight and root weight) from linear mixed models with source and block fitted as random effects. For trait models that did not fail to converge with the inclusion of maternal line (family) as a random effect we also calculated P_{ST} incorporating the random variance from within-population family effects (P_{STall}), which increases the estimated within-population variance relative to the common null assumption ($c = h^2 = 1$) that all additive genetic variance

comes from source (Brommer 2011). This typically produces a lower P_{ST} estimate because more within-population variance ($V_{residual}$ and V_{family}) inflates the denominator, therefore, decreasing the proportion of total variance due to among-population (V_{source}) differences. We estimated P_{ST} (mean ± 95% CI) for each of the six traits from 500 non-parametric bootstrap replicates. We classified selection for each trait as divergent, stabilizing, or neutral when the CIs landed entirely above, below, or overlapped the mean F_{ST}, respectively (Brommer 2011). We then calculated combined P_{ST} pairwise source values based on the mean pairwise value across traits. We compared trait distance among sources with genetic, geographic, climate, soil, and ecological distances using mantel tests in the 'vegan' package (Oksanen et al. 2025).

Modelling in situ emergence and survival

We used generalized linear mixed models to model *in situ* emergence and year one survival using the predictors source, soil moisture, and canopy cover index, with plot as a random effect to account for multiple seed cages nested within each plot. We used a beta-binomial family distribution for the seedling emergence model and for the seedling survival model to address overdispersion and obtain a lower model AIC, respectively. In the seedling emergence model we applied quadratic terms to the environmental predictors to account for hump-shaped relationships. We excluded plots with no emergence or survival past August from the year one survival model and used source as the sole predictor due to the limited number of observations (n = 18).

We built all models in the software R (version 4.5.1; R Core Team 2021) using the packages 'lme4' (linear mixed models; Bates et al. 2015), 'glmmTMB' (generalized linear mixed models; Brooks et al. 2017), and 'nlme' (nonlinear mixed effect models; Pinheiro and Bates

2000, Pinheiro et al. 2025). To assess significance of each predictor we used the 'drop1' function with F-tests (Type II ANOVA) for linear models with Gaussian family distribution or likelihood ratio tests (χ^2) for generalized linear models with non-Gaussian distributions. For linear mixed models we used the 'anova' function with F-tests (Type II ANOVA) with Kenward-Roger's method for calculating denominator degrees of freedom. We conducted post-hoc comparisons amongst sources with the 'emmeans' package to obtain estimated marginals means (EMMs) (Lenth 2025). We checked for model specification errors with the 'DHARMa' package (Hartig 2024) and ensured no spatial autocorrelation of the model residuals with spline correlograms using the 'ncf' package (Bjornstad 2022). To visualize estimated marginal means plots and nonlinear growth models we used the 'ggplot2' (Wickham 2016) package and the 'visreg' package (Breheny and Burchett 2017) for partial regression plots.

3.4 Results

3.4.1 Greenhouse common garden experiment

Phenological timing: Germination, emergence, and first fruit

In 2023, 36% of seeds germinated (n = 379 planted; excludes BW) under the cold-warm treatment and 0% of seeds germinated (n = 435 planted) under the cold only treatment (Table S3.2). Overall, few Ilderton seeds germinated likely due to seed rot caused by seed storage in plastic bags instead of plastic containers. The cold-warm treatment germination rate of wild seed sources (excluding IL) was 65.5% (median = 83%), compared to 15.6% (median = 0%) for nursery seed sources. Fanshawe had significantly higher mean germination rates (90%) than all sources except for Georgia, and Toadshade had the lowest mean germination rate (8.9%; Tables S3.2, S3.3; Fig. 3.2). In 2024, 76% of seeds germinated (n = 639 planted) under the cold-warm treatment. Fanshawe and Ilderton had the highest mean germination rates (87.6% and 96.7%

respectively), which were significantly higher than Bayfield or Meadowlily (Tables S3.2, S3.3; Fig. 3.2).

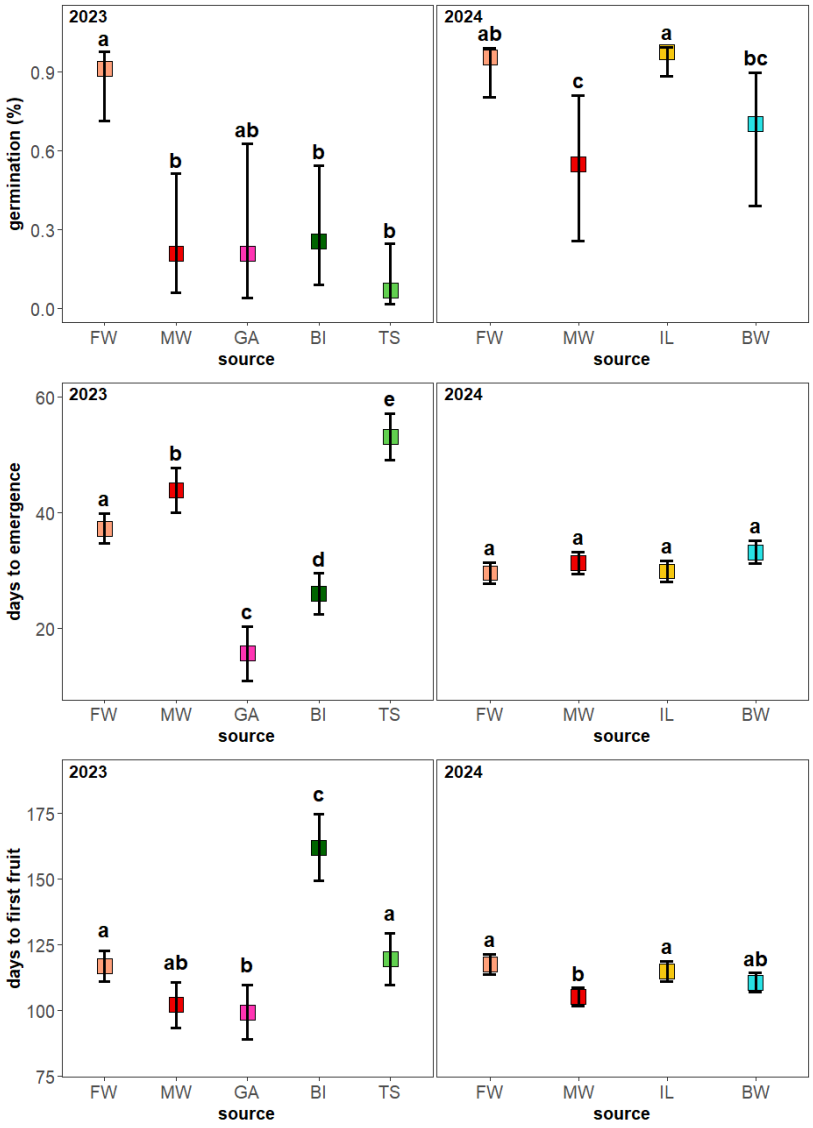

Figure 3.2: Estimated marginal means plots showing differences in *S. diphyllum* phenological timing amongst sources in 2023 and 2024 common garden trials. Coloured squares represent the estimated marginal means for each source (± 95% CI) and shared letters indicate no significant difference between sources within years according to Tukey-adjusted pairwise tests. FW = Fanshawe, MW = Meadowlily, GA = Georgia, BI = Botanically Inclined, TS = Toadshade. For germination (2023), IL and BW seeds are excluded from the analyses due to extremely low germination ($n = 4$) and limited seed availability, respectively.

Days to emergence significantly varied among sources with Toadshade taking the longest to emerge (μ = 53 days) and Georgia the earliest to emerge (μ = 16 days; Table S3.4; Fig. 3.2). In 2023, Fanshawe plants emerged significantly earlier than Meadowlily seeds (~1 week on average) but in 2024 there were no significant differences (Table S3.4; Fig. 3.2).

Days to first fruit significantly varied among sources with Botanically Inclined having the longest days since germination to first fruit compared to all other sources (Table S3.4; Fig. 3.2). Georgia had the earliest first fruit timing, which significantly differed from Toadshade. Amongst all Canadian sources in 2024, Meadowlily had significantly earlier fruit set than all sources and Fanshawe had the latest fruit set, which was significantly later than Bayfield and Meadowlily (Table S3.4; Fig. 3.2).

Reproduction (total fruits and seeds)

Nursery sources produced significantly fewer fruits than all other sources except for Fanshawe, which produced a similar number of fruits to Toadshade (Tables S3.6; Fig. 3.3). Fanshawe produced significantly fewer fruits than other wild sources in 2023 (Georgia and Meadowlily) but had the highest average number of seeds per fruit and significantly more seeds per fruit than Georgia or Toadshade. In 2024, Meadowlily produced significantly more fruits than all other Canadian sources but there were no significant differences in average seeds per fruit amongst sources. Meadowlily produced significantly more seeds than all sources in 2023, and significantly more seeds than Ilderton in 2024. Wild sources all produced significantly more seeds than nursery sources in 2023. There were no significant differences in seed weight amongst sources in 2023 but in 2024, Fanshawe and Meadowlily seeds were significantly heavier than Bayfield seeds (Table S3.6; Fig. 3.3).

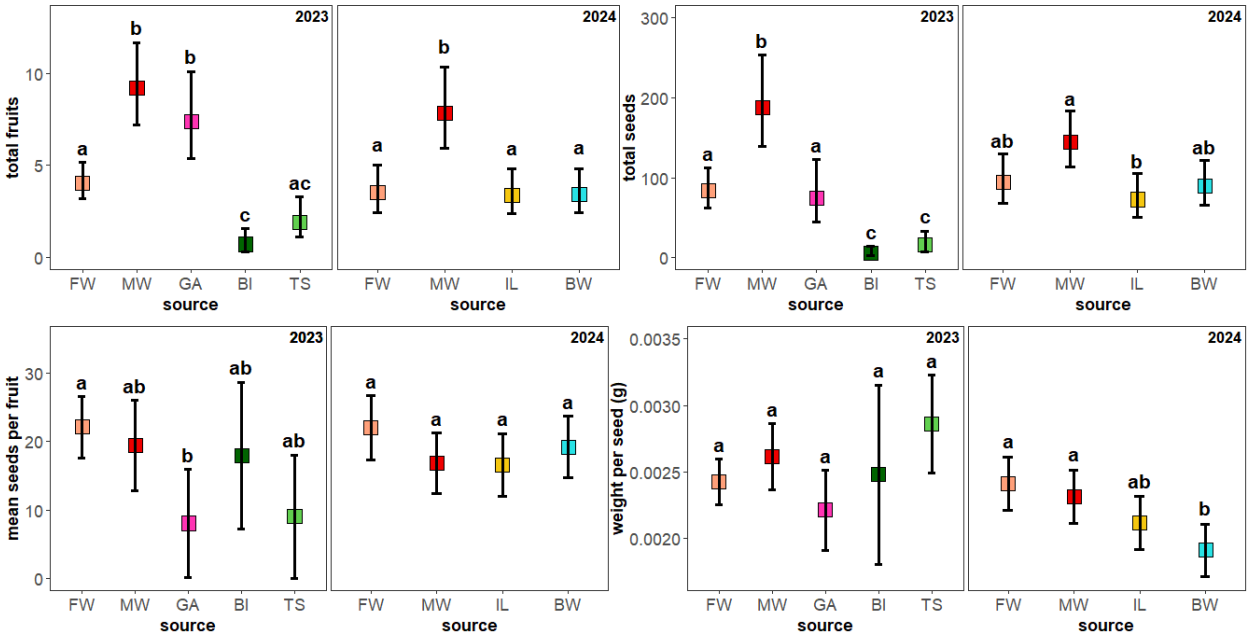

Figure 3.3: Estimated marginal means plots showing differences in *S. diphyllum* reproduction amongst sources in 2023 and 2024 common garden trials. Coloured squares represent the estimated marginal means for each source (± 95% CI) and shared letters indicate no significant difference between sources according to Tukey-adjusted pairwise tests. For meaning of source abbreviations see Figure 3.2.

Growth rate

In 2023, Botanically Inclined had significantly lower asymptotic height (maximum height) and growth rate (higher inverse growth rate constant) than all other sources (Fig. 3.4; Table S3.7). Georgia had a significantly higher growth rate (lower inverse growth rate constant) than all other sources and there were no significant differences between Toadshade, Fanshawe or Meadowlily growth rate constants. In 2024, Ilderton had a significantly higher asymptotic height than Meadowlily, although there were no differences in growth rate constants amongst Canadian sources (Table S3.7; Fig. 3.4). The nlme models including source as a fixed effect had a significantly better fit than the null models without source effect in both years (2023: L. Ratio = 108. 33, p = <.0001, ΔAIC = 92.32; 2024: L. Ratio = 18.82, p = 0.027, ΔAIC = 0.82).

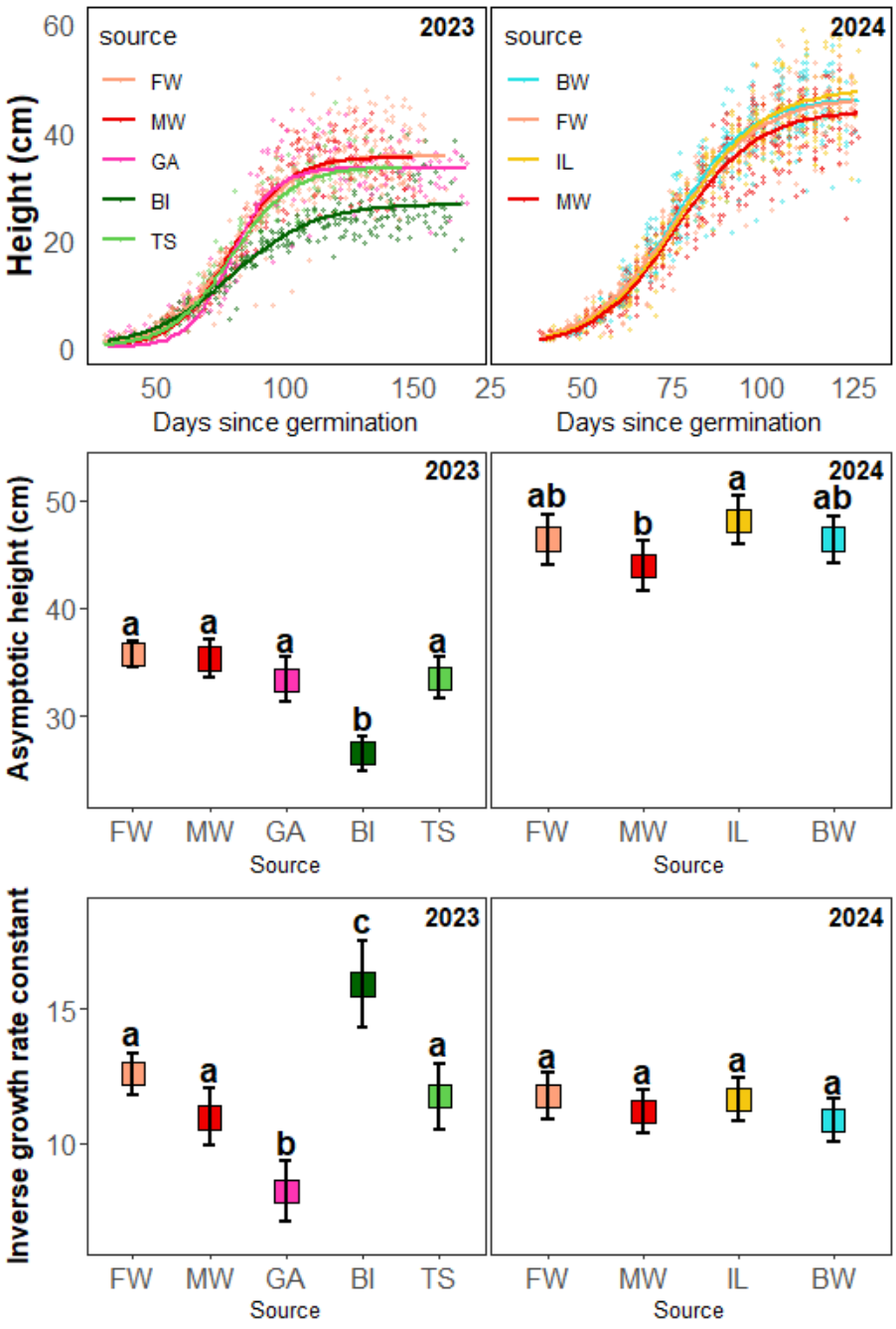

Figure 3.4: Source growth rates for *S. diphyllum* common garden experiments fitted with logistic growth curves from nonlinear mixed effect models (SSlogis form) for 2023 and 2024 trials (top row). Thick coloured lines represent the fitted model predictions of each source and coloured circles represent individual observations. Estimated marginal means plots (middle and bottom rows) showing differences in Asymptotic (maximum) height (cm) and the inverse growth rate constant (scal) among sources. Coloured squares represent the estimated marginal means for each source (± 95% CI) and shared letters indicate no significance according to Tukey-adjusted pairwise tests. For meaning of source acronyms see caption of Figure 3.2.

Leaf traits

In the 2023 trial, wild sources (Meadowlily, Fanshawe, and Georgia) had significantly more leaves than nursery sources (Tables S3.8, S3.9; Fig.3.5). Meadowlily had the highest number of leaves in both trial years, however this difference was not significant in 2023. In 2024, Meadowlily had significantly more leaves than Fanshawe and Ilderton. Meadowlily and Georgia had significantly higher leaf solidity than Botanically Inclined and significantly higher leaf circularity than Toadshade (Tables S3.8, S3.9; Fig. 3.5). Canadian sources did not differ significantly in leaf solidity or circularity. Commercial sources and Georgia had significantly higher trichome density than Fanshawe or Ilderton. The effect of source on trichome density was also significant in 2024, however, this is likely due to high within-group variance, where no Fanshawe plants had trichomes and a few Ilderton plants were extremely hairy. The pairwise Tukey comparisons showed no significant differences in trichome density among Canadian sources (Tables S3.8, S3.9; Fig. 3.5).

Biomass

Shoot biomass was significantly higher for Fanshawe and Bayfield plants compared to Toadshade (Tables S3.10, S3.11; Fig. 3.5). Root biomass was significantly higher for Fanshawe plants compared to Georgia and Toadshade, while Botanically Inclined plants also had significantly higher root biomass than Toadshade. No significant differences were found between Canadian sources in shoot or root biomass (Tables S3.10, S3.11; Fig. 3.5).

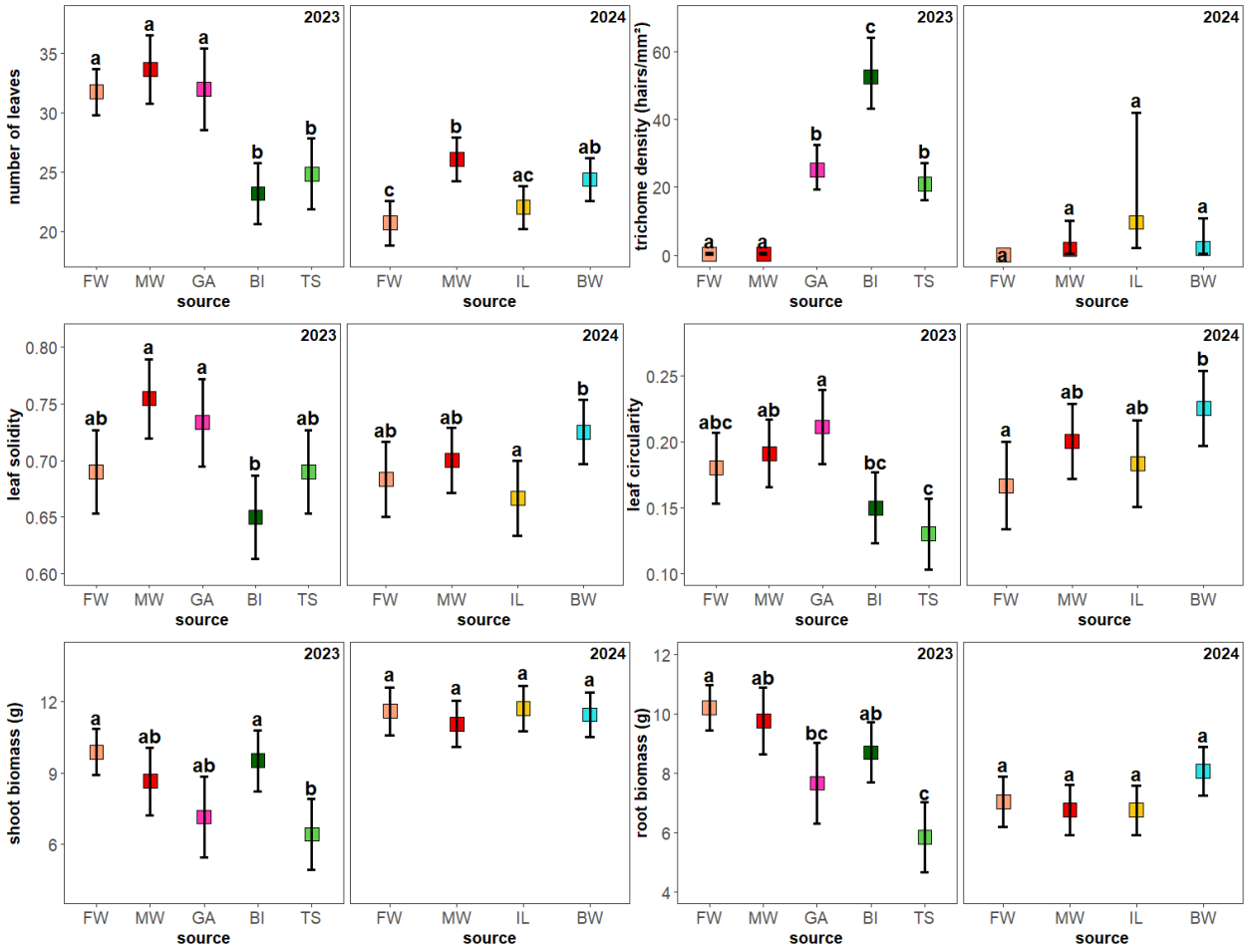

Figure 3.5: Estimated marginal means plots showing differences in *S. diphyllum* leaf traits and biomass among sources in 2023 and 2024 common garden trials. Coloured squares represent the estimated marginal means for each source (± 95% CI) and shared letters indicate no significant difference between sources according to Tukey-adjusted pairwise tests. No CI is given for FW in 'trichome density' (2024 plot) due to having a zero trichome count causing an infinite upper-CI estimate (log-link model). For meaning of source acronyms see caption of Figure 3.2.

Distance metrics

We found a significant positive correlation between trait distance and genetic distance among all sources in the 2023 greenhouse trial (Fig. 3.6). In 2023, maximum height, maximum number of leaves, shoot and root biomass had evidence for stabilizing selection ($P_{ST} < F_{ST}$), but total fruits and seeds had evidence for neutral selection/ drift ($P_{ST} \sim F_{ST}$; Table S3.12; Fig. 3.7).

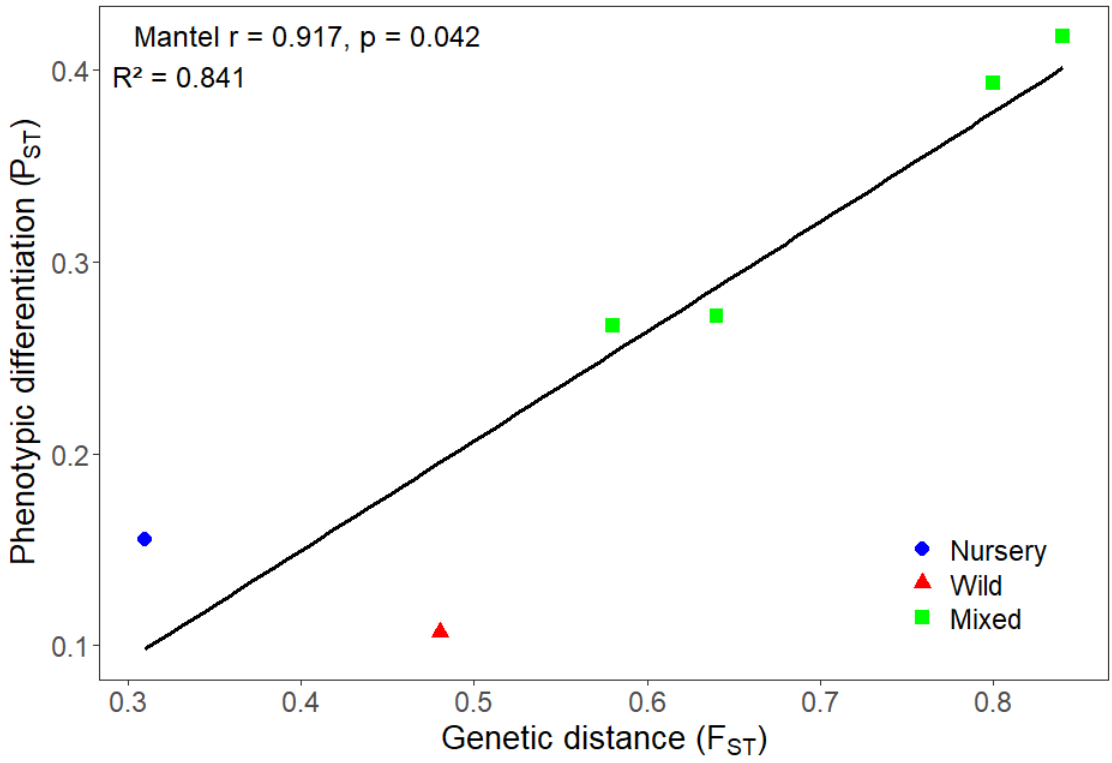

Figure 3.6: Mantel test for association between *S. diphyllum* phenotypic trait distance (P_{ST}) and genetic distance (F_{ST}) for two Canadian wild populations, one US wild population, and two nursery-sources. Symbols represent pairwise comparisons between different sources; blue circle = nursery-nursery, red triangle= wild-wild, green square= mixed (nursery-wild). Thick black line represents fitted linear regression trendline.

We did not have enough wild population pairs (n = 3) to test correlation between trait distance and geographic, climate or soil distances in the 2023 trial. There was no significant correlation of trait distance with genetic distance or with geographic, environmental, or ecological distance metrics among the Canadian populations in the 2024 greenhouse trial. Climate distance showed a positive association with trait distance (Mantel's r = 0.46, p = 0.375). All six traits showed evidence of stabilizing selection ($P_{ST} < F_{ST}$; Table S3.12; Fig. 3.7).

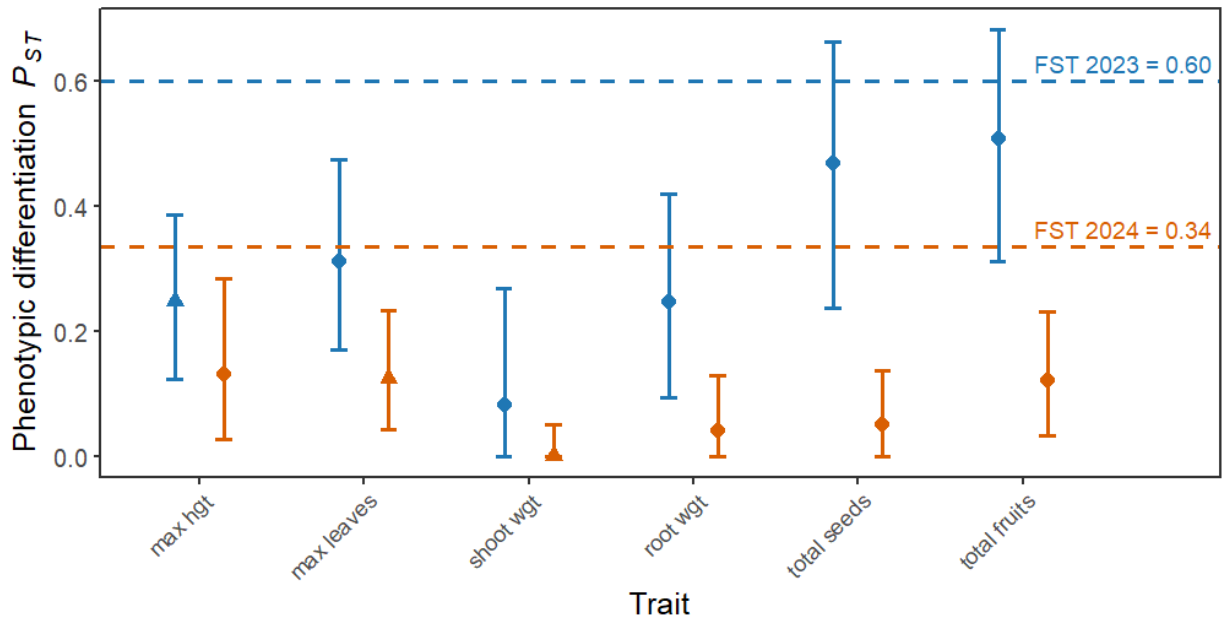

Figure 3.7: Phenotypic trait differentiation (± 95% CI) versus FST for *S. diphyllum* traits in two common garden trials. Dotted lines represent the global mean F_{ST} for each respective trial year (2 nursery and 2 Canadian wild sources in 2023, 4 Canadian wild sources in 2024). Triangles represent P_{ST} calculated including family (maternal line) variance and circles represent P_{ST} calculated without family variance due to model convergence failure (blue = 2023, red = 2024). For trait variances (source, family, block, residual, and VPOP) see Table S3.13.

3.4.2 *In situ* common garden

There were no significant differences in seedling emergence or year one survival between Canadian sources planted *in situ*. Soil moisture was not a significant predictor of seedling emergence. Significantly more seedlings germinated as canopy cover decreased, representing higher light levels (Table S3.13; Fig. 3.8). Emergence rates *in situ* were much lower than in the greenhouse trials with a median *in situ* emergence rate of 8% (μ = 17.7 %), compared to 83% and 88% for Canadian sources in the 2023 and 2024 common garden trials, respectively. The survival rate of seedlings that emerged to year one was 32% (n = 23). Source had a near-

significant effect on seedling survival to year one (p = 0.08; Table S3.13), where seeds sourced from Bayfield had the highest mean survival rates (Fig. 3.8).

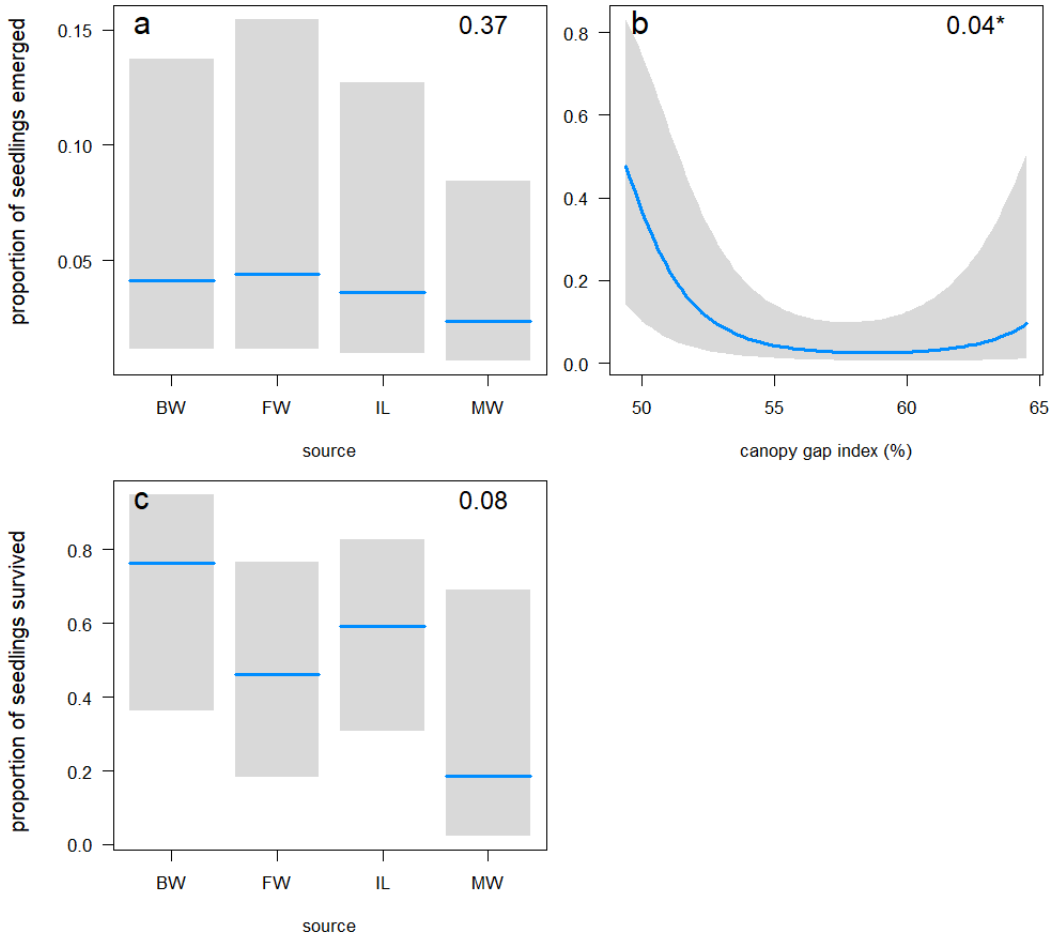

Figure 3.8: Partial regression plots showing the effect of (a) source (b) canopy gap index on the proportion of *S. diphyllum* seedlings emerged, and (c) source on year one seedling survival. In each panel, all other variables are held at the median (for continuous variables). Asterisks indicate statistical significance based on likelihood ratio tests (χ^2). Thick blue lines represent the model predictions and grey shaded areas represent Wald 95% confidence intervals. For meaning of source acronyms see caption of Figure 3.2.

3.5 Discussion

Seed sourcing decisions are challenging and depend on understanding the genetic differentiation and corresponding trait differences between potential source populations, which

remains unknown for many species. Using common gardens for *S. diphyllum*, we found that commercial nursery sources have a high magnitude of trait differentiation from wild source populations that positively correlates with genetic distance (Fig. 3.6). Canadian populations showed significant phenotypic differentiation in traits related to germination, growth, leaf morphology, and reproduction (Tables S3.1-11; Figs. 3.2-3.5). However, we found no significant correlations between trait distance and genetic, geographic, environmental, or ecological distances among wild Canadian source populations. Although wild Canadian populations exhibit high genetic differentiation, trait variability was lower than neutral genetic divergence, indicating stabilizing selection ($P_{ST} < F_{ST}$) as the primary evolutionary mechanism (Table S3.12; Fig 3.7), rather than drift or divergent selection as suggested by (Lait et al. 2025). Our results suggest restorations could benefit from mixing wild sources due to low risk of introducing maladapted genotypes, but commercial nursery sources should be avoided due to their high magnitude of phenotypic and genetic differentiation.

We found the greatest magnitude of trait differentiation between commercial nursery sources and wild Canadian populations. Cultivated sources are often distinct from wild sources because they undergo several stages of artificial selection throughout the production process. For example, particular maternal lines may be underrepresented in an *ex situ* collection if they have low germination rates and are then unintentionally selected against. Cultivation can also lead to unique nursery cultivars with trait differentiation from wild types, such as faster and more abundant germination rates, and loss of seed dormancy due to artificial selection and unnatural seed storage conditions (Schröder and Prasse 2013, Ensslin et al. 2018). Although *S. diphyllum* commercial nursery sources did not have significantly lower germination rates, they had distinct phenological timings compared to wild sources (Table S3.3; Fig. 3.2). Botanically Inclined plants

emerged earlier than all other sources (except Georgia) but grew slower and more horizontally (significantly less vertical height), and took longer to fruit than all other sources, whereas Toadshade plants were the latest to emerge. Both commercial nursery sources exhibited distinct leaf morphology from wild sources, including producing fewer leaves with higher trichome density and a greater degree of lobing (Tables S3.8, S3.9; Fig. 3.5). The leaf trait differences we quantified between commercial nursery sources and wild Canadian sources match the qualitative observations by Reiter and McCune (2023). The reduced leaf count of Botanically Inclined plants could be an artificial selection for compactness, as we observed they all grew in a horizontal-creeping growth habit. Although the exact source populations are unknown, fewer leaves and higher trichome density may be adaptations to reducing water loss and increasing drought tolerance in warmer origin sites (Hauser 2014, Zia et al. 2021).

Phenotypic trait differentiation of commercial sources from wild sources was positively correlated to genetic distance (Fig. 3.6). In the 2023 trial, P_{ST} matched F_{ST} for reproductive traits (total seeds and fruits), suggesting neutral divergence (random drift) acting on these traits (Table S3.12; Fig 3.7). Among-population phenotypic variance was driven by the low fecundity of commercial sources which produced significantly fewer fruits and seeds than most wild sources (Tables S3.5, S3.6; Fig. 3.3). The reduced fecundity of commercial nursery sources may reflect genetic erosion and increased genetic load arising from small population sizes, inbreeding, and adaptation to nursery conditions. These processes intensify with cultivation duration leading to increased genetic differentiation from wild populations and rapid loss of genetic diversity within only a few generations (Ensslin et al. 2011, Ensslin and Godefroid 2019, Forgiarini et al. 2023). For example, repeatedly collecting seeds from the same individuals reduces the effective population size and causes genetic drift and increased relatedness through inbreeding. In

contrast, the 2024 trial excluding commercial sources yielded $P_{ST} < F_{ST}$ indicating reproductive traits among the Canadian populations are relatively uniform and under stabilizing selection (Table S3.12; Fig 3.7). High genetic differentiation (F_{ST}) reflects the neutral genetic divergence caused by the combined effects of random drift and restricted gene flow, typical of isolated populations (Wright 1951, Merilä and Crnokrak 2001).

Trait differentiation was lowest among wild sources and Canadian sources showed more trait similarities to each other than to the US wild source (Georgia). Georgia plants emerged significantly earlier and had a faster growth rate (lower scale) than all sources (Tables S3.7, S3.8; Fig. 3.2, 3.4), suggesting a latitudinal cline in which the number of days for germination decreases and growth rate increases moving towards the equator. Many plant species exhibit specific latitudinal clines in germination timing (i.e., emergence rates), with the direction of the cline varying depending on the selection pressures (e.g., Webb and Farmer 1968, Zettlemoyer et al. 2017, Muir et al. 2022). For example, northern sources may emerge earlier as an adaptation to short growing seasons. In contrast, early emergence in southern sources (i.e., Georgia plants) may be an adaptive strategy to survive because despite the longer growing season, earlier germination timing can reduce susceptibility to common late-season droughts (Muir et al. 2022). Georgia plants also had significantly more trichomes than Canadian sources, comparable with Toadshade (Tables S3.8, S3.9; Fig 3.5). The high trichome count of Georgia plants and commercial nursery sources (suspected to be of US origin), may be an adaptation to warmer southern temperatures. In contrast, Georgia had a similar number of leaves and leaf circularity to Fanshawe and Meadowlily (Tables S3.8, S3.9; Fig 3.5).

Differentiation among wild Canadian sources was highest in germination and reproduction-related traits (Tables S3.2-S3.6; Figs. 3.2-3.3). Meadowlily had significantly lower germination rates and fewer days to first fruit than all Canadian sources except for Bayfield and produced significantly more fruits than all Canadian sources (Tables S3.2-S3.6; Figs. 3.2, 3.3). However, fitness advantages in the greenhouse may not directly translate to fitness advantages in field conditions because a particular source may have high fitness in low-stress, controlled environments but perform relatively poorly in high-stress field conditions. For example, Allphin and Windham (2002) found that the highest producing population of a rare daisy in cultivation produced significantly fewer flower heads in the field than four out of six populations. Similarly, Meadowlily plants produced fewer fruits on average than two of the other Canadian populations in a 2022 census (Wiedenfeld, pers. comm.). In addition, in their first reproductive year Meadowlily plants translocated in the wild had significantly lower average fruit numbers per individual than Ilderton plants (see Chapter 2 results). However, the relative fruit production of Ilderton and Meadowlily individuals depended on the microsite, where Meadowlily plants produced more fruits than Ilderton plants in some microsites. Source effects on emergence also differed between environments, where *in situ* there was no significant source effect (Table S3.13; Fig 3.8) but in the 2024 greenhouse common garden trial, Fanshawe and Ilderton had significantly higher emergence rates than Meadowlily and Bayfield (Table S3.2-S3.4; Fig. 3.2). In contrast, Bayfield had the highest *in situ* seedling survival rates to year one ($p = 0.08$; Table S3.13; Fig. 3.8) but as fitness differences can accumulate over time (Montalvo and Ellstrand 2000), long-term monitoring is needed. Although a particular source may emerge as a 'winner' in common garden trials, this advantage may not translate *in situ*; therefore, planting multiple sources can serve as a bet-hedging strategy to increase restoration success.

We detected no correlations between trait distance and genetic distances between wild Canadian populations grown in a common garden environment. In contrast to our results, global meta-analyses of comparisons of trait and genetic diversity find that quantitative trait distance (Q_{ST}) weakly correlates with genetic distance (F_{ST}) and that Q_{ST} typically exceeds F_{ST} in plants as well as other taxa (Merilä and Crnokrak 2001, Leinonen et al. 2013). In addition, a meta-analysis of 44 plant species (excluding cultivated sources) found Q_{ST} exceeded F_{ST} in ~72% of cases for plants, suggesting many plant populations exhibit adaptive differentiation (De Kort et al. 2013). However, this is dependent upon the traits being measured and the plant species' lifespan, such that long-lived perennials often show weaker adaptive divergence than short-lived annuals, especially in reproductive and physiological traits (De Kort et al. 2013, Chung et al. 2023). In general, annual species invest in rapid reproduction and are more likely to show local adaptation in reproductive traits, whereas perennial species maximize resource conservation, such that phenological and biomass-related traits are more likely to be selection targets (Grime 1977, De Kort et al. 2013).

Wild Canadian *S. diphyllum* populations appear to exhibit stabilizing trait selection despite high genetic divergence. High genetic differentiation can yield similar phenotypes because quantitative traits are controlled by many loci with small additive effects (Lande 1976) and different genotypes may favour similar phenotypes across populations under similar environmental conditions (Loveless and Hamrick 1984). For example, the wild Canadian *S. diphyllum* populations occur in locations that receive similar annual rainfall amounts (985 to 1012 mm/year historic average) and differ in mean average temperature by less than 1 °C (Fick and Hijmans 2017), which may be limiting trait divergence. The low phenotypic differentiation despite high genetic divergence may also reflect recent fragmentation events, where rapid genetic

drift has occurred, but insufficient time has elapsed for selection to drive corresponding phenotypic divergence (Musche et al. 2008, Gentili et al. 2018).

Trait distance among Canadian populations was also decoupled from geographic, environmental, and ecological distances. These results align with stabilizing selection, whereas evidence for local adaptation would include positive correlations between phenotypic traits and geographic or environmental distances. The effect of divergent selection on population differentiation typically increases with increasing geographic distance (> 200 km; Becker et al. 2008, De Kort et al. 2013), with the strength of adaptation being more variable at smaller geographic scales and less likely result in consistent adaptive divergence (Leimu and Fischer 2008). The lack of correlation of trait distance and geographic distance could be caused by wild Canadian populations occurring within a relatively small geographic area (~80 km). The relative environmental homogeneity among wild Canadian populations may further contribute to the absence of trait-distance relationships. For example, the wild Canadian populations have a mean sub-soil pH of 7.5 (range 7.45 to 7.63) and receive 985-1012 mm of annual precipitation compared to the Georgia population which has a lower sub-soil pH (5) and receives higher annual precipitation (1483 mm). Geographic and environmental distances were positively correlated when the Georgia population was included, indicating environmental dissimilarity covaries with geographic distance. However, among the Canadian populations these gradients are weak which aligns with the low magnitude of phenotypic differentiation. Overall, the lack of trait-distance correlations reflects the small geographic area and relatively homogenous environment occupied by the Canadian populations.

3.6 Conclusion

Resolving the debate around seed sourcing in restoration ecology remains a complicated challenge due to species-specific patterns of population differentiation. Incorporating quantitative trait measures into conservation decisions provides a more complete understanding of population differentiation instead of solely relying on neutral molecular markers (Vitt et al. 2004, Leinonen et al. 2008). Although quantitative traits can be neutral, they are more likely to be under selection and capture adaptive variation because they are polygenic (controlled by multiple loci), unlike neutral markers which typically only reflect random drift, potentially leading to underestimates of population differentiation (Vitt et al. 2004). Our results suggest that mixing wild Canadian *S. diphyllum* sources poses low risk of introducing maladapted genotypes or unique local adaptations because despite having high genetic divergence, populations exhibit relatively low phenotypic differentiation, consistent with stabilizing selection maintaining similar trait optima across populations. In addition, incorporating multiple sources can increase the probability that at least some populations will perform well in different restoration sites or even in within different microsites (e.g., Leimu and Fischer 2008) and boost ecological resilience (Maschinski et al. 2013, Bucharova et al. 2019). Translocations using commercial nursery *S. diphyllum* sources should be avoided but mixing wild sources is likely to offer greater benefits than risks for long-term population persistence.

CONCLUSION

"*And yet for conservation to work into the longer term, in this human-dominated world, we need to bring people with us and widen the 'ownership' of projects, as ultimately it is the local champions and land users who remain to follow through the necessary action on the ground, long after the professional conservationists have moved on*."

-M.J. Gaywood, J.G Ewen, P.M Hollingsworth, A. Moehrenschlager, in *Conservation Translocations* (2023, pp. xxv)

The above quote reminds me of an important conservation lesson my friend Houston Snead taught me that conservation is about working with people. Developing testable hypotheses and integrating adaptive management into translocation science is key to improving success rates. However, the foundation of success starts with building trust and a sense of ownership for conservation projects with the local community. I saw the positive effects firsthand of having a local champion involved with the wood-poppy translocations when I met George the fisherman (GTF) along the roadside on the first day my team went out to monitor in spring 2022. After giving him an initial rundown of the project he quickly jumped on board as an advocate and shared the importance of our work with the local cabin community. GTF dedicated his time over two planting seasons to transport plants, people, and fencing into the woods. He also invited members of the Maitland Trail Association to get involved in this project. Building partnerships in the local community is an important underlying predictor of translocation success. Although I did not directly assess the effect of community engagement, my thesis set out to investigate many predictors of translocation success.

The main goal of my thesis was to improve plant translocation success. I had three main objectives: (1) to test the relative role of dispersal, biotic, and abiotic filters in limiting the occurrence of a rare plant species, (2) to assess the efficacy of using SDMs and CBMs to predict suitable habitat for conservation translocations, and (3) to quantify the magnitude of phenotypic differentiation among populations and the evolutionary mechanisms driving differentiation. To address objectives (1) and (2) I used translocation trials to test the efficacy of predicted habitat suitability based on SDMs (Chapters 1 and 2) and CBMs (Chapter 2) for site selection. I also tested the relative influence of dispersal (Chapter 1), abiotic factors and biotic interactions (Chapters 1 and 2) on translocation success. To address objective (3) I used common garden trials and measured fitness-related phenotypic traits. I then related trait distance to genetic distance to infer the underlying evolutionary mechanism. Collectively, my aim was to improve translocation success by clarifying the ecological and evolutionary processes that constrain species occurrences and population persistence.

Chapter 1

In Chapter 1, I used a seed addition trial to test the relative influence of dispersal, abiotic conditions, and biotic interactions on *S. diphyllum* seedling emergence and survival over two years at its range edge. Theory suggests that the availability of suitable habitat plays a large role in limiting species occurrences and that habitat suitability declines and/or becomes less frequent for species at their range edges (Brown et al. 1996). I used SDMs to estimate broad-scale habitat suitability and planted seeds into unoccupied habitat with varying levels of predicted suitability. To test the effect of seed predators, I caged half of the seeds. I also selected a variety of microsites stratified by soil moisture and canopy light. I predicted that if habitat suitability was

limiting *S. diphyllum* occurrences, seedling emergence and survival would increase with increasing habitat suitability. If fine-scale microsite conditions are more important than broad-scale habitat suitability, I predicted that seedlings would only establish within suitable microsites. If dispersal and biotic interactions (seed predators) are the main limiting filters of *S. diphyllum* occurrences, I predicted high seedling emergence regardless of habitat suitability but only in caged sub-plots.

The SDM-predicted habitat suitability failed to predict seedling emergence or short-term survival, suggesting a lack of suitable habitat was not limiting the frequency of occurrences, but rather dispersal limitation reduces the species' ability to colonize new sites. Seed predation was a significant predictor of seedling emergence, while microclimate was a significant predictor of seedling survival. However, it is important to note that niche requirements can change with life stage (Grubb 1977) and the SDM predictions may align better with later life stages or may simply be too coarse to capture fine-scale habitat heterogeneity (Guisan and Thuiller 2005). Other rare species at their range edge may exhibit this pattern and require assisted dispersal via translocation into suitable microsites in combination with reducing negative biotic interactions to support their recovery.

Chapter 2

In Chapter 2, I tested the efficacy of SDMs and CBMs to estimate suitable habitat for translocation of *S. diphyllum* and *S. prenanthoides*. I chose one-hectare (100 m × 100 m) sites with varying SDM-predicted habitat suitability and translocated plants into each site. I measured plant community composition at each translocation site and occupied sites by recording the relative abundance of all vascular plants. I then calculated the Bray-Curtis similarity of the plant

community at each translocation site compared to the community at occupied sites. I measured success as transplant survival, reproduction, and next-generation seedling emergence and survival over two to three years. I controlled for known predictors of translocation success by varying seed source, transplant size class, and choosing a variety of microsites with each site. I predicted that if SDMS and CBMs can accurately estimate habitat suitability, then translocation success would be positively correlated with predicted habitat suitability.

Neither estimate of habitat suitability was a strong predictor of early translocation survival. However, CBM-predicted habitat suitability in combination with microsite variables frequently influenced survival and reproduction, and the CBM-predicted habitat suitability had a significant positive correlation with the probability of *S. prenanthoides* flowering, while the SDM-predicted habitat suitability showed a positive correlation with plot seedling production. The inability of the SDM-based habitat suitability to predict short-term survival of translocated plants may be due to the models being built based on the locations of long-established populations and long-term climate averages. For this reason, SDM-based habitat suitability may be a better predictor of long-term translocation success. Transplant characteristics (i.e., transplant size class) and microsite conditions had the greatest relative influence on early translocation success, which is consistent with other studies reviewing predictors of early translocation success (Guerrant and Kaye 2007, Albrecht and Maschinski 2012). Quantifying habitat suitability for translocation site selection remains challenging, and long-term monitoring is needed across different spatial scales and species to assess the efficacy of SDMs and CBMs for population persistence.

Chapter 3

In Chapter 3, I used common garden trials to quantify the magnitude of phenotypic differentiation among four wild Canadian, one wild US, and two commercial nursery sources of *S. diphyllum*. Understanding the magnitude of population differentiation and the underlying mechanisms can help inform seed sourcing decisions, including whether or not to mix seed sources. I measured 15 traits related to growth, reproduction, biomass, and leaf morphology, and tested for correlations between trait distance and genetic, geographic, environmental, and ecological distances. I compared phenotypic trait distance (P_{ST}) to genetic distance (F_{ST}) to detect signals of local adaptation. Lait et al. (2025) found commercial sources had high genetic differentiation from wild sources, and that Canadian wild populations have high genetic differentiation from each other. I predicted that if the magnitude of genetic differentiation correlates with phenotypic differentiation, then commercial nursery sources would have greater phenotypic differentiation from wild populations. I predicted that among Canadian populations, greater geographic separation and larger environmental and ecological distances between populations would be associated with higher phenotypic differentiation. I also conducted an *in situ* trial at one unoccupied site to test for differences in seedling emergence and survival among wild Canadian sources in natural conditions.

I found that wild sources had significant phenotypic differentiation from commercial nursery sources. However, despite the wild Canadian populations having high genetic differentiation from each other, there were few significant trait differences between them. Comparisons of phenotypic with genetic differentiation showed that the wild Canadian populations are exhibiting stabilizing selection for all traits, with no evidence for local adaptation. Trait distance was correlated to genetic distance when all sources were included

(Canadian, U.S., and commercial nursery), but not when I considered Canadian populations alone. In the *in situ* common garden, canopy light influenced seedling emergence but there was no significant effect of seed source on emergence or survival. These results indicate that restorations of *S. diphyllum* in Canada should not use commercial nursery sources due to their significant phenotypic and genetic differentiation. Mixing wild Canadian seed sources poses low risk of outbreeding depression because these populations occur within similar environments and exhibit low phenotypic differentiation consistent with stabilizing selection.

Study limitations and future research

Issues of scale and time

Applying an ecological framework to translocation science requires an understanding of both regional and local constraints. Regional environmental conditions determine species distributions at broad geographic scales (e.g., global and landscape scales; Willis and Whittaker 2002) while local constraints (i.e., biotic interactions, microsite environment; Dunwiddie and Martin 2016) influence species establishment within local sites. The inability of the SDM-based habitat suitability to predict short-term translocation success could be because fine-scale habitat heterogeneity (i.e., microsite conditions, biotic interactions) is a primary driver of early plant life stages. Alternatively, the SDMs may be missing an important predictor we did not measure, such as historic land-use or disturbance history. Future research could build SDMs incorporating these factors (if available) to test if model efficacy improves.

Similarly, the CBMs capture plant community composition across a one hectare area, which reflects underlying abiotic conditions at the site and historical disturbances. However, fine-scale variation in the plant community composition may be a more important determinant of

the early success of individual transplants. Interactions between plants and their neighbours are spatially localized (i.e., competition or facilitation) and performance is most strongly affected often within tens of centimeters (Pacala and Silander 1985, Caldwell et al. 1996, Stoll and Weiner 2000). A future study could investigate the effects of plant community composition within a few meters of each translocated plant to see if capturing these fine-scale interactions improves the ability of the CBM habitat suitability to predict translocation outcomes. However, the efficacy of the SDMs and CBMs to predict long-term persistence remains unknown and requires the continued monitoring of the translocated populations over multiple generations. Long-term demographic rates of the translocated populations could also then be directly compared to the wild populations to evaluate their relative success.

Improving future common garden trials

Seed sourcing for translocation is an ongoing debate because the magnitude of population differentiation among many rare plant species remains poorly understood, creating uncertainty about whether mixing sources may lead to genetic risks (i.e., outbreeding depression, genetic swamping). Common gardens measure quantitative traits and help evaluate genetic risks associated with mixing sources, particularly when levels of genetic differentiation are also known. Our common garden did not measure all sources at the same time and future common garden trials of *S. diphyllum* could be expanded to also including wild populations throughout its range, especially those that have genetic data available (i.e., U.S. populations from Lait et al. 2025). These wild populations could be used to test for signals of local adaptation by testing correlations between trait distance and geographic, environmental, or ecological distances,

thereby informing whether or not wild U.S. populations and wild Canadian populations should be mixed.

A future *in situ* common garden could be conducted using juvenile plants instead of seeds, as the very low emergence and survival of the seedlings in my trial reduced the power to detect differences in performance between the sources. Long-term monitoring of this trial could also test temporal shifts in source performance, as early 'winners' may be outperformed by another in subsequent years. In addition, if permissions are granted to mix sources at the wild Canadian populations, reciprocal transplant studies could directly assess fitness to determine if any populations exhibit local adaptations to their home sites to inform seed sourcing decisions. Overall, the recovery of many at-risk species could be strengthened by measuring the magnitude of genetic and phenotypic differentiation among their populations, as these patterns remain unknown for most listed species.

Concluding remarks

There is no one-size-fits-all for ensuring translocation success. Some species can thrive with minimal management, while others require frequent care. Early life stages may be particularly vulnerable, as is the case for the wood-poppy seeds eaten by mice. To ensure translocation success and work towards species recovery it is important to understand these threats and know how to mitigate them. Experimental translocations offer a way to assess the relative influence of dispersal, abiotic factors, and biotic interactions limiting species occurrences. This is especially important for species that do not have well-documented threats, including the crooked-stem aster. We must continue to treat translocations as experiments and apply adaptive management practices to ensure populations persist.

Identifying suitable habitat for translocations remains a challenge. The SDMs and CBMs did not predict short-term success well for *S. diphyllum* or *S. prenanthoides*, but their predictions may align with long-term population persistence. However, future models may benefit from incorporating fine-scale data, such as plot-level community surveys and finer resolution geospatial rasters. The efficacy of using quantitative modeling approaches to identify suitable habitat for translocations is in its infancy and remains unexplored for many species.

We also need a better understanding of the magnitude of genetic and phenotypic differentiation among rare plant populations so we can make informed decisions on their management, including seed sourcing decisions to establish new populations. Incorporating quantitative trait measures into conservation decisions provides a more comprehensive understanding of population differentiation that may not be evident from genetic markers alone.

Ultimately, evaluating the success of translocations requires making a long-term commitment to monitoring, which is essential for determining whether these adaptive management actions are paving the way towards species recovery. Translocation success can be improved by continuing to clarify the ecological and evolutionary processes that constrain species occurrences and population persistence.

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APPENDIX A

Supplementary material for Chapter 1

A.1 Supplementary Methods

A.1.1 Supplementary Methods S1.1

Species Distribution Model

To estimate broad-scale habitat suitability, we used an SDM for southern Ontario only (range-edge extent) based on the five known natural occurrences of *S. diphyllum* using 14 environmental predictors (see McCune 2019), and a resolution of one-hectare (Figure S1.1). While habitat suitability near the edge of a species range is often improved by reducing the SDM extent to the region of the range edge only, the low number of occurrences available at this extent could hinder the model's ability to predict suitable habitat. Therefore, we also tested a range-wide SDM built by Wiedenfeld (in prep, 2025) based on 10 environmental predictors (Table S1.1) and using occurrences throughout the species' North American range (n = 1329). The occurrence records were sourced from the Global Biodiversity Information Facility (GBIF.org 2023), the Ontario Natural Heritage Information Centre (NHIC), and one new record from McCune's 2015 plant survey. The range-wide SDM (Figure S1.2) also used a resolution of one-hectare and MaxEnt default settings, except the regularization parameter was set to 0.5 (the same as the range-edge SDM), and the area under the receiver operating curve (AUC) was 0.830. Also concordant to the range-edge SDM, we defined the threshold for a cell being suitable as the lowest value predicted for a wild site in Ontario, which was 30.90% for the range-wide SDM. As an independent test of the ability of the SDMs to rank habitat suitability in southern Ontario, we used plant community surveys of one-hectare cells across southern Ontario and compared the

relative abundance and number of plant species known to be associated with *S. diphyllum* in 'suitable' vs. 'unsuitable' sites, as described below.

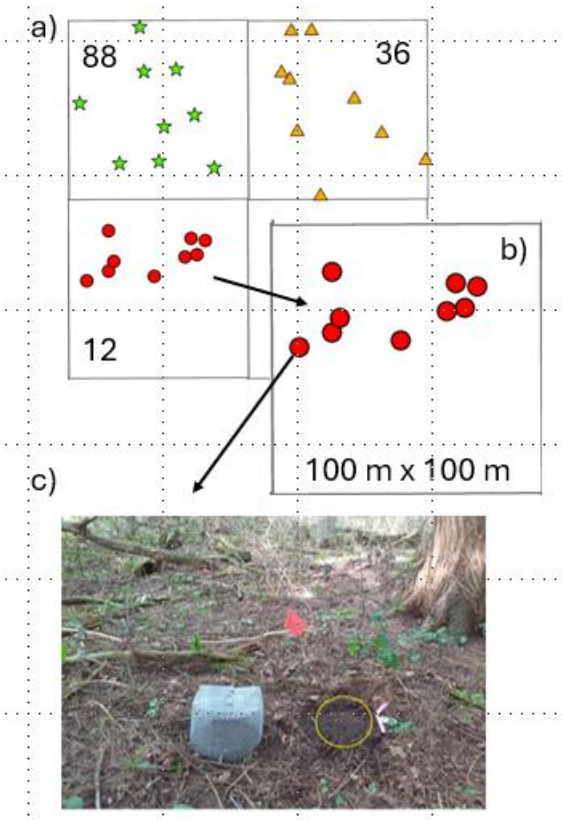

Figure S1.1: Experimental design of *S. diphyllum* seed additions. Panel a: Three adjacent one-hectare sites with SDM-predicted habitat suitability value shown in the corners. Site boundaries (100 m × 100 m) are represented by solid lines, coloured shapes represent plot locations within each site. Panel b: A single site with low SDM-predicted suitability and nine plots (red circles) nested within site. Panel c: A seed addition plot with a caged (left) and uncaged (right; yellow circle) treatment.

Table S1.1: Range-wide SDM data layers and data source from Wiedenfeld (in prep, 2025).

Data layer	Data source
Digital Elevation Model (DEM)	USGS APPEARS: https://lpdaac.usgs.gov
Slope*	USGS APPEARS: https://lpdaac.usgs.gov *Extracted from the DEM layer
Depth to Bedrock	ISRIC World Soil Information https://files.isric.org/soilgrids/former/2017-03-10/data/ Accessed March 21, 2023
Soil organic carbon	ISRIC World Soil Information https://files.isric.org/soilgrids/former/2017-03-10/data/
Soil pH	ISRIC World Soil Information https://files.isric.org/soilgrids/former/2017-03-10/data/
Soil texture	ISRIC World Soil Information https://files.isric.org/soilgrids/former/2017-03-10/data/
Surficial geology	USGS: https://ngmdb.usgs.gov/gmna/#gmnaImages https://pubs.er.usgs.gov/publication/ds424
Growing degree days	ClimateNA (https://climatena.ca/); Wang et al. (2016)
Relative humidity	ClimateNA (https://climatena.ca/); Wang et al. (2016)
Summer heat-moisture index	ClimateNA (https://climatena.ca/); Wang et al. (2016)

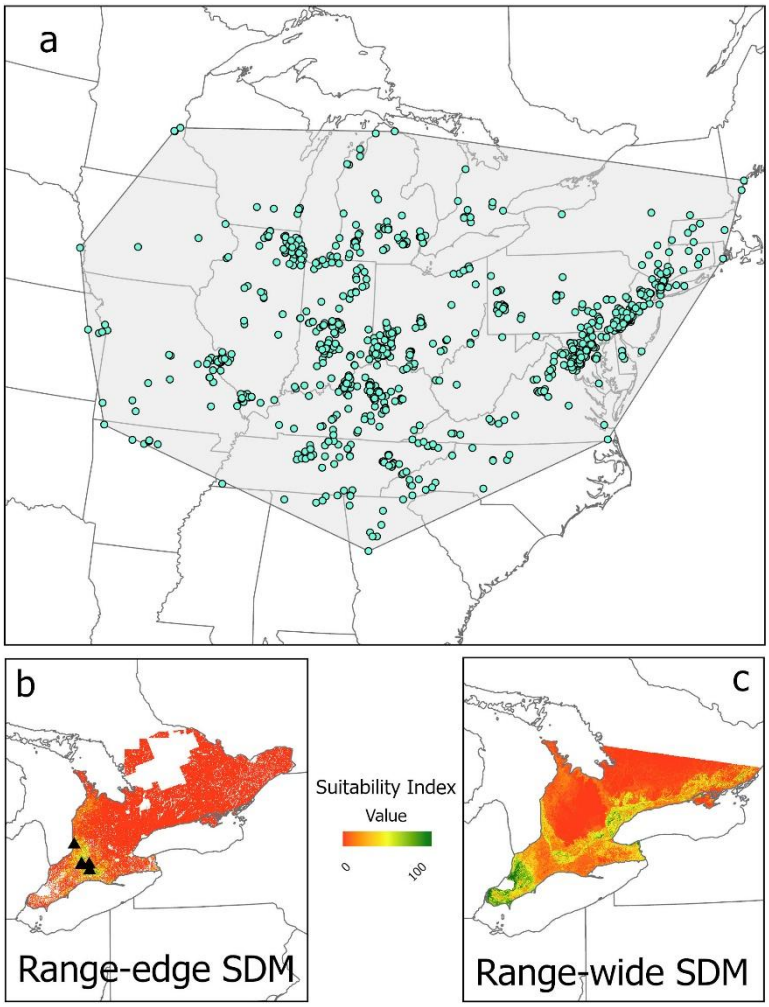

Figure S1.2: SDM predictions for *Stylophorum diphyllum*. Panel a: Map of Eastern North America showing GBIF records (blue dots) of *S. diphyllum* (GBIF.org 2023) used to build a range-wide SDM. The light grey shading indicates the extent of the SDM. Note that GBIF includes herbarium records, research-grade iNaturalist records, and naturalized occurrences outside the original native range of the species. Panel b: Range-edge SDM prediction. Black triangles represent the five wild S. diphyllum Canadian occurrences used to build the model. Panel c: Range-wide SDM prediction for southern Ontario only. Suitability index value bar shows SDM-predicted habitat suitability from low (red) to high (green).

A.1.2 Supplementary Methods S1.2

Plant community surveys

Our survey database consisted of 295 one-hectare (100 m × 100 m) forest sites (grid cells) across southern Ontario (surveyed between May–August of each year). We used 154 surveyed sites

from 2014 and 2015 by McCune (2016), 70 surveyed sites from 2017 by Rosner-Katz et al. (2020), 56 surveyed sites from 2018 by McCune, nine surveys of our seed addition sites, plus three unoccupied sites and three sites containing wild Canadian *S. diphyllum* populations which we completed from 2021 to 2023. At each site we navigated to the centre of the 100 m × 100 m area and used flagging tape to delineate four 50 m transects in each cardinal direction from the centre point. We then walked the entire area systematically with not more than a few meters between surveyors and identified all vascular plant species. We estimated the abundance of each species using a coarse ordinal scale of one to five (very rare (i.e., only two individuals) to dominate). For species that could not be reliably identified we lumped them to the genus level.

Comparisons of common species associated with S. diphyllum

We classified sites as 'suitable' (n = 61 cells) if the SDM-predicted suitability value was greater than or equal to the lowest predicted value at an extant wild *S. diphyllum* population. For the range-edge SDM this value was 37.8%, resulting in 61 'suitable' sites and 234 'unsuitable' sites. For the range-wide SDM this value was 30.9%, resulting in 78 'suitable' sites and 200 'unsuitable' sites. We used less sites (n = 278) compared to the range-edge SDM (n = 295) because 17 of the surveyed sites were outside the range-wide SDM's extent (Fig. S1.2). If the SDM was accurately estimating relative habitat suitability for *S. diphyllum*, we predicted that SDM-predicted 'suitable' sites would have a significantly higher number of common plant species usually associated with *S. diphyllum* and a higher relative abundance of these species compared to 'unsuitable' sites. We compiled a list of 23 common plant species known to be associates of *S. diphyllum* in Ontario (Table S1.2) as described in the recovery strategy (Bowles 2007) and COSEWIC assessment and update status report (2007). For each surveyed site, we

calculated the total number of associated species and then calculated the relative abundance of associated species by dividing the total abundance of associated species in the site by the total abundance of all species in the site. We then used a Wilcoxon rank sum test (for non-parametric data with un-equal variance) to determine if the total number of associated species and their relative abundance was significantly higher in 'suitable' sites than 'unsuitable' sites. We used a Welch's two sample t-test (for parametric data with equal variance) to determine if the relative abundance of associated species was significantly higher in 'suitable' sites than in 'unsuitable' sites for the range-wide SDM only. We used the software R for all statistical tests (R Core Team 2021).

Table S1.2: Name of common plant species associated with *S. diphyllum*, their life-form, and reference(s)

Associated species name	Life-form	Reference(s)
Acer saccharum	Tree	Recovery strategy, COSEWIC assessment
Alliaria petiolate	Herb	Recovery strategy
Arisaema triphyllum	Herb	Recovery strategy, COSEWIC assessment
Asarum canadense	Herb	Recovery strategy, COSEWIC assessment
Carya cordiformis	Tree	Recovery strategy
Caulophyllum thalictroides	Herb	Recovery strategy, COSEWIC assessment
Celtis occidentalis	Tree	Recovery strategy, COSEWIC assessment
Cornus alternifolia	Shrub	Recovery strategy
Elymus hystrix	Herb	COSEWIC assessment
Erythronium americanum	Herb	COSEWIC assessment
Fagus grandifolia	Tree	Recovery strategy, COSEWIC assessment
Fraxinus americana	Tree	Recovery strategy, COSEWIC assessment

Geranium maculatum	Herb	Recovery strategy
Hydrophyllum canadense	Herb	COSEWIC assessment
Hydrophyllum virginianum	Herb	Recovery strategy, COSEWIC assessment
Impatiens capensis	Herb	COSEWIC assessment
Maianthemum racemosum	Herb	COSEWIC assessment
Prunus serotina	Tree	COSEWIC assessment
Prunus virginiana	Shrub	Recovery strategy
Rhus typhina	Shrub	Recovery strategy
Solidago flexicaulis	Herb	Recovery strategy, COSEWIC assessment
Tilia americana	Tree	Recovery strategy
Ulmus americana	Tree	Recovery strategy

A.2 Supplementary Results

A.2.1 Supplementary Results S1.1

For the range-edge SDM (southern Ontario extent) we found SDM-predicted 'suitable' sites had a significantly higher number of associated species (W = 398.5, p = <0.001) and a significantly higher relative abundance of associated species (t_{94} =-2.51, p = <0.05) compared to 'unsuitable' sites (Figs. S1.3-S1.4). For the range-edge SDM, there was no significant difference in the number of associated species (W = 8596, p = >0.05) or the relative abundance of associated species (W = 8184, p = >0.05) between 'suitable' and 'unsuitable' sites.

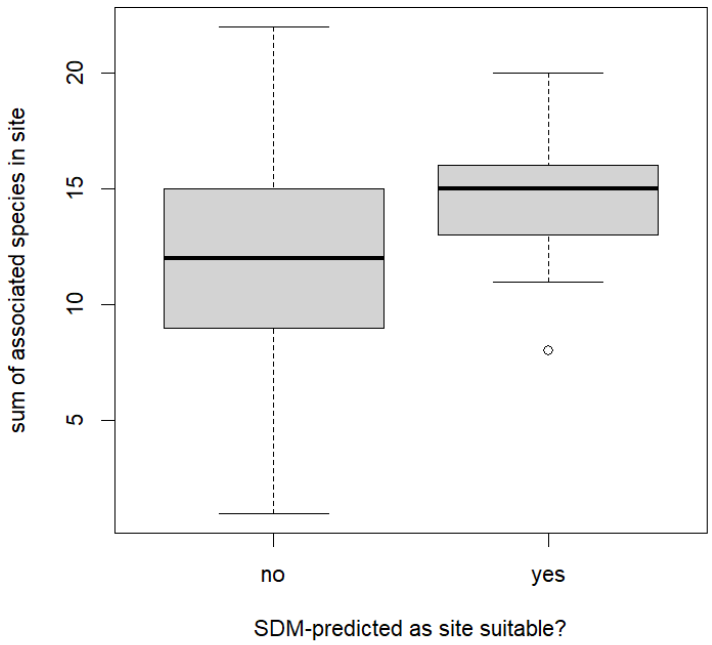

Figure S1.3: The total number of *S. diphyllum* associated species in sites predicted to be suitable, "yes" (n = 61), or unsuitable, "no" (n = 234) according to the range-edge SDM. We classified sites as suitable if the SDM-predicted suitability value was greater than or equal to the lowest extant wild *S. diphyllum* population's estimated suitability (37.8%) and unsuitable if the value was below the minimum threshold. Solid lines represent the upper and lower quartiles. Thick lines represent the medians. Dots represent outliers.

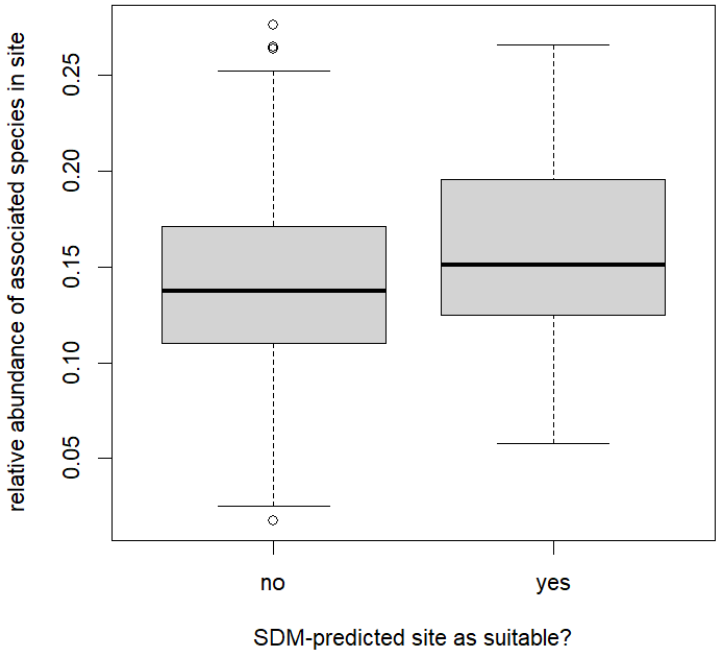

Figure S1.4: The relative abundance of *S. diphyllum* associated species in sites predicted to be suitable, "yes" (n = 61), or unsuitable, "no" (n = 234) according to the range-edge SDM. We classified sites as suitable if the SDM-predicted suitability value was greater than or equal to the lowest extant wild *S. diphyllum* population's estimated suitability (37.8%) and unsuitable if the value was below the minimum threshold. Solid lines represent the upper and lower quartiles. Thick lines represent the medians. Dots represent outliers.

A.2.2 Supplementary Results S1.2

GLMMs with range-wide SDM predictions

To test whether the range-wide SDM estimate of habitat suitability could predict seedling emergence or survival, we re-ran the GLMMs following the same methodology as the range-edge SDM models (see main manuscript), except substituting the range-wide SDM estimates of habitat suitability. Note that the predictions of the two models for the nine, one-hectare sites were not strongly correlated (Pearson's r = 0.32; Fig. S1.2). However, corresponding to the

range-edge SDM models, we found no significant effect of SDM-predicted habitat suitability on seedling emergence or survival (Tables 1.1-1.2, S1.3-S1.4; Figs. 1.2-1.3, S1.5-S1.6).

Table S1.3: Results of a generalized linear mixed effects model assessing potential predictors of first-year emergence rates of *S. diphyllum* seedlings using a range-wide SDM.

Predictor	Coefficient	***SE***	***df***	**AIC** [a]	***p*** [b]
(Intercept)	-1.69	2.33	*n/a*	644.67	*n/a*
cage (yes)	-3.27	0.55	1	*n/a*	*n/a*
SDM habitat suitability index	0.01	0.01	1	643.24	0.448
soil moisture (log)	0.24	1.59	1	*n/a*	0.887
soil moisture (log) ^{2}	-0.04	0.27	1	642.69	*n/a*
canopy cover index	-0.02	0.08	1	642.71	0.831
canopy cover index ^{2}	-0.00	0.00	1	642.67	0.981
relative temperature differential	0.45	0.36	1	644.19	0.217
relative temperature differential ^{2}	-0.94	0.72	1	644.38	0.191
cage × soil moisture (log)	0.78	0.18	1	659.74	**<0.001**

[a] Akaike's information criterion for the model that includes all factors except the one being tested. To respect marginality, we do not drop individual predictors that are also in an interaction (indicated as *n/a* for those predictors).

[b] *p*-values indicate the significance of each predictor using a likelihood ratio test. We did not drop individual predictors that were also in a significant interaction. Predictors with $p < 0.05$ are indicated in bold text.

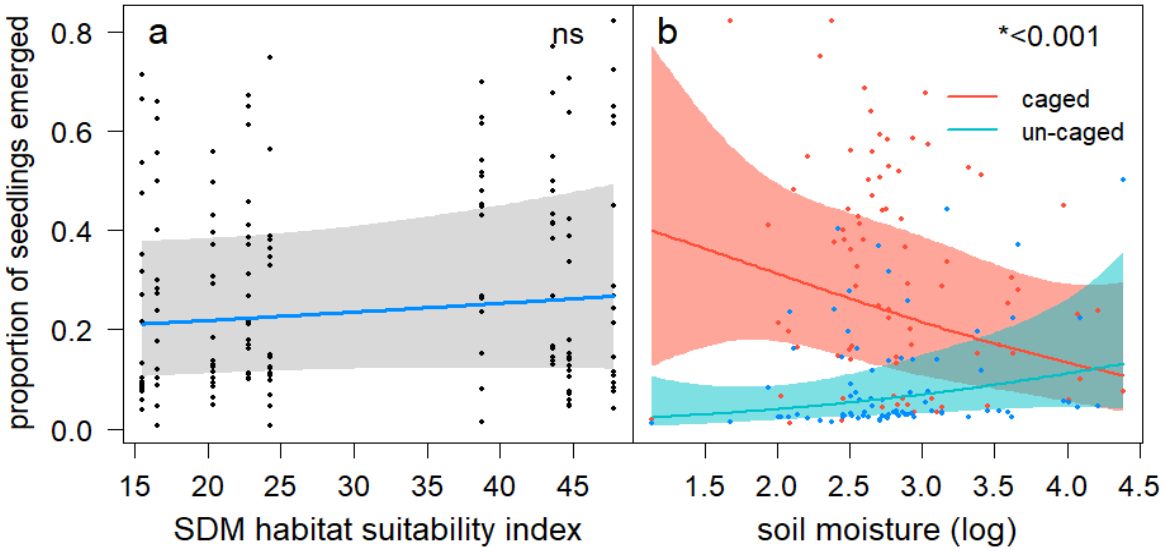

Figure S1.5: Partial regression plots showing the effect of a) the SDM (range-wide) habitat suitability index on *S. diphyllum* seedling emergence and b) the interaction of cage treatment

with soil moisture (log) on seedling emergence. Asterisks indicate significant predictors. All other variables are held at the median (for continuous variables) and cage treatment is set to caged in panel a. Thick coloured lines represent the model prediction and shaded areas represent Wald 95% confidence intervals. Dots represent partial residuals.

Table S1.4: Results of a generalized linear mixed effects model assessing potential predictors of second-year survival rates of *S. diphyllum* seedlings using a range-wide SDM.

Predictor	Coefficient	***SE***	***df***	**AIC** [a]	***p*** [b]
(Intercept)	-8.16	7.55	*n/a*	302.56	*n/a*
cage (yes)	0.24	0.34	1	301.10	0.464
SDM habitat suitability index	-0.02	0.02	1	301.95	0.240
soil moisture (log)	4.39	4.05	1	301.73	0.281
soil moisture (log) ^{2}	-0.75	0.68	1	301.78	0.270
canopy cover index	0.19	0.23	1	301.27	0.400
canopy cover index ^{2}	-0.00	0.00	1	302.21	0.199
relative temperature differential	-0.49	0.95	1	300.83	0.606
relative temperature differential ^{2}	-5.25	2.21	1	307.03	**<0.05**

[a] Akaike's information criterion for the model that includes all factors except the one being tested. To respect marginality, we do not drop individual predictors that are also in an interaction (indicated as *n/a* for those predictors).

[b] *p*-values indicate the significance of each predictor using a likelihood ratio test. We did not drop individual predictors that were also in a significant interaction. Predictors with $p < 0.05$ are indicated in bold text

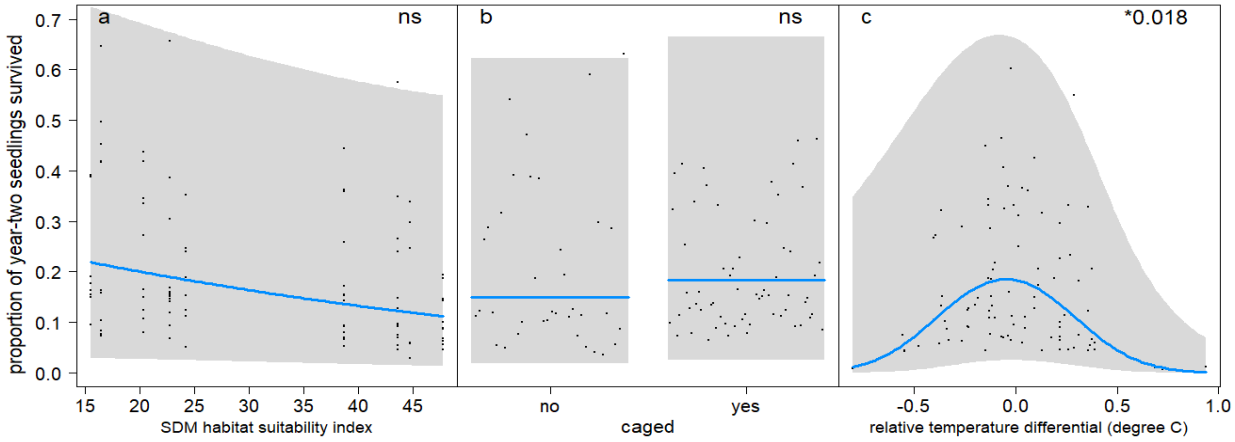

Figure S1.6: Partial regression plots showing the effect of (a) SDM (range-wide) habitat suitability index (b) cage treatment and (c) relative temperature differential between plots on *S. diphyllum* year-two seedling survival. Asterisks indicate significant predictors. In each panel, all other variables are held at the median (for continuous variables), and cage treatment is set to caged in panels a and c. Thick blue lines represent the model prediction and grey shaded areas represent Wald 95% confidence intervals. Dots represent partial residuals.

Response surface regression results

First-year emergence model

The results were suggestive but inconclusive, and the total models were not statistically significant ($p_{uncaged} = 0.0876$, $p_{caged} = 0.8205$). Inside cages, the maximum emergence rate was estimated to be 33 ± 32% of seedlings where SDM-predicted habitat suitability was 26.7%, canopy cover index was 31.66%, soil moisture was 64.91%, and temperature differential was -0.01° C. Under uncaged conditions, the maximum emergence rate was estimated to be 23 ± 7% of seedlings, when SDM-predicted habitat suitability was 43.33%, canopy cover index was 30.2%, soil moisture was 49.8%, and temperature differential was +0.75°C. The minimum emergence rate was estimated to be -5 ± 13% of seedlings where SDM habitat suitability index was 33.50%, canopy cover index was 41.47%, soil moisture was 58.70%, and temperature differential was -0.64°C. Inside cages the maximum emergence rate was estimated to be 33 ±32% of seedlings where SDM-predicted habitat suitability was 26.7%, canopy cover index was 31.66%, soil moisture was 64.91%, and temperature differential was -0.01°C. The minimum emergence rate was estimated to be -5 ± 26% of seedlings where SDM habitat suitability index was 49.00%, canopy cover index was 31.15%, soil moisture was 18.6%, and temperature differential was -0.35°C.

Year-two survival model

The total model was not statistically significant ($p = 0.8793$). The lack of fit test was not statistically significant ($p = 0.6223$), which indicated that the model adequately captured the relationship between the predictors and the response variable without significant unexplained variation. The maximum survival rate occurred when SDM-predicted habitat suitability was 25.95%, canopy cover index was 50.64%, soil moisture was 28.35%, and temperature

differential was -0.27°C. The estimated minimum survival rate occurred where SDM habitat suitability index was 40.28%, canopy cover index was 59.12%, soil moisture was 31.89%, and temperature differential was 0.74°C.

Supplementary Figure

Figure S1.7: *Stylophorum diphyllum* seeds released from a fruit capsule.

APPENDIX B

Supplementary material for Chapter 2

B.1 Supplementary Methods

B.1.1 Supplementary Methods S2.1

SDM model details

The models used MaxEnt (version 3.3.3k), a method that demonstrates good performance with limited species occurrences (Hernandez et al. 2006, Phillips et al. 2006). MaxEnt uses known species occurrences and their associated environmental conditions to predict the habitat suitability of each cell (100 m × 100 m resolution) across the study extent (Figure 2.1). The occurrence records used to build the SDMs (*S. diphyllum, n* = 5; *S. prenanthoides*, *n* = 20) we sourced from the Ontario Natural Heritage Information Centre (NHIC) and one *S. diphyllum* record from McCune's 2015 plant surveys (McCune 2019). The SDMs used MaxEnt default settings (except a regularization parameter of 0.5). We used the cumulative output, with 0 indicating the lowest suitability and 100 indicating the highest suitability. The AUC from cross-validation was 0.974 (*S. diphyllum*) and 0.987 (*S. prenanthoides*).

These SDMs were built with an extent of southern Ontario only, as SDMs built at the extent of the range edge often have more accurate predictions compared to range-wide extents (Stockwell and Peterson 2002, Luoto et al. 2005). Concordantly, we found that site suitability as predicted by a range-wide SDM for *S. diphyllum* was not significantly correlated with the presence or abundance of common associated species (Neigel et al. 2025). Therefore, we used the range-edge SDM to predict habitat suitability.

To validate the accuracy of the *S. prenanthoides* model, we used independent presence and absence records (*n* = 30 and *n* = 226 respectively) from exhaustive surveys of 256 one-

hectare cells across southern Ontario (McCune 2016, McCune et al. 2017, Rosner-Katz et al. 2020) and new, non-overlapping presence records we obtained from the Natural Heritage Information Centre. We found that the probability of occurrence was strongly correlated with the SDM prediction (Figure S2.1).

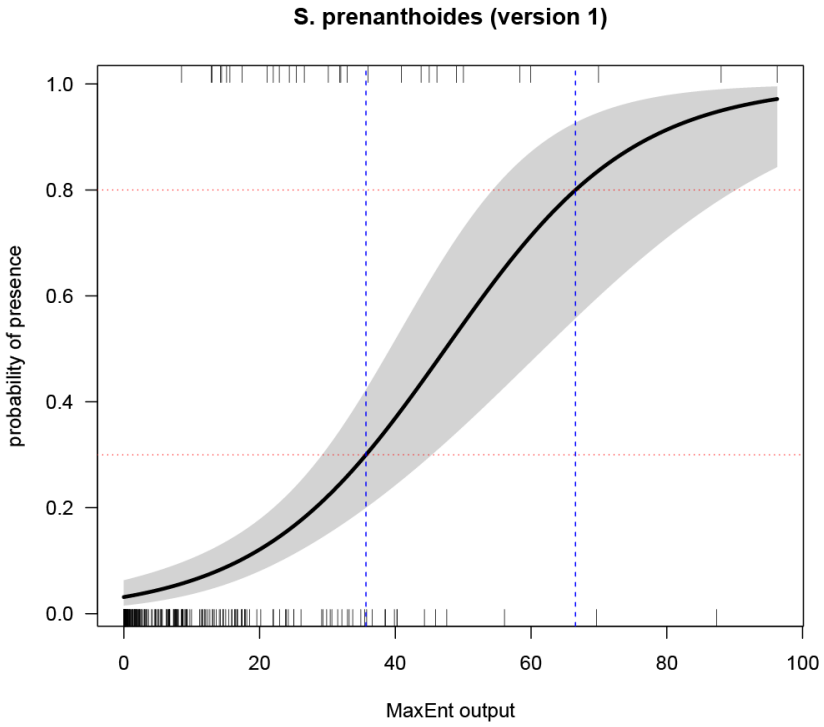

Figure S2.1: Relationship between probability of presence of *Symphyotrichum prenanthoides* and Maxent output based on 256 independent surveys. The red lines show probability of presence 'bins': 0-0.3 = low, 0.3 to 0.8 = medium, and 0.8 and above = high. Blue lines show the corresponding MaxEnt SDM-predicted habitat suitability based on the relationship with independent presences/absences.

B.1.2 Supplementary Methods S2.2

Stylophorum diphyllum propagation protocols

We collected *Stylophorum diphyllum* seeds from two occupied, natural sites. These two collections represent two different seed provenances about 25 km apart, one near Ilderton, ON

(IL) and one near London, ON (MW). We collected from 54 maternal lines (one fruit per plant) from one population, Ilderton, ("IL") on June 23, 2021 and received seeds (not maternally tracked) from a second population, Meadowlily ("MW") on July. 25, 2021 that were collected by a partner with the exact collection date unknown but likely from the 2021 season. Ilderton seeds were stored in plastic containers in moist sand upon collection and held at 4 °C until their stratification treatment began. Meadowlily seeds were stored in plastic bags containing moist moss and kept refrigerated. We grew Meadowlily seeds indoors and outdoors as we were interested to know whether natural outdoor stratification could provide viable propagules, as it is associated with cost savings of not requiring indoor greenhouse space. Therefore, we counted a subset of Meadowlily seeds to directly sow into pots outdoors (natural outdoor stratification) and counted another subset for indoor sowing following an artificial cold-warm stratification. We transferred the indoor seeds into plastic bags filled with moist sand prior to treatment.

We followed the indoor artificial stratification protocol by Reiter and McCune (2023) giving the seeds a two-month warm period (21 °C from Oct. 8 to Dec. 7, 2021) followed by a two-month cold period (4 °C from Dec. 7, 2021 to Feb. 9, 2022). We planted seeds (2391 Ilderton seeds and 2800 Meadowlily seeds) ~1 cm deep into PRO-MIX BX potting soil in open trays on February 9, 2022. Trays were kept moist until germination occurred. Germination occurred February 23 through March 3, 2022. Ilderton seed germination rates were 33% (n = 701) but no Meadowlily seeds germinated, likely due to seed rot or a prolonged dormancy period initiated by a longer cold storage period after collection. For our second propagation protocol (outdoor natural sowing), we planted seeds (2368 Ilderton seeds and 2835 Meadowlily seeds) ~1 cm deep into grower pots with PRO-MIX BX potting soil on August 18, 2021. We overwintered the pots in a wooden cold frame, surrounded by hay bales for insulation within an unheated,

outdoor greenhouse. We covered the pots with a 1-2” layer of mulch, protected them with chicken wire (0.5” openings), and wrapped everything over with burlap. All materials were removed from the pots in the spring to allow for germination.

Germination occurred April 7 through May 4, 2022. Ilderton seeds had a germination rate of 16% (n = 341) and Meadowlily seeds had a germination rate of 25% (n = 710). Seedlings received regular watering as needed and we fertilized them weekly with Miracle Gro® Water Soluble Plant Food 20-20-20 (N-P-K). In late March 2022, all indoor-grown plants were transferred to the University of Guelph greenhouse and fertilized weekly (~200 ppm N). All seedlings were transplanted into 3” (7.5 cm) deep pots in April.

Symphyotrichum prenanthoides propagation protocols

We collected *Symphyotrichum prenanthoides* seeds from two natural, occupied sites on October 25 and 28, 2022. These two collections represent two different seed provenances, source “IV” (n = 146 maternal lines) from along Kettle creek (near St. Thomas, ON) and source “CBC” (n = 155 maternal lines) from along Venison creek (near Walsingham, ON; ~52 km apart). We collected two seedheads per plant. Due to the difficulty of counting aster seeds, we counted a subset of 10 seedheads per source (~46 seeds per seedhead from IV and ~28 seeds per seedhead from CBC) and estimated the total number of seeds planted by multiplying the number of seedheads collected by the average number of seeds per seedhead (~13,432 from IV and ~8,680 from LJ). Seeds were stored in plastic bags with moist sand at room temperature before receiving a cold stratification at 4 °C from December 14, 2021, to February 12, 2022 (60 days). Seeds were surface sown March 31, 2022 into germination trays with PRO-MIX BX potting soil under misting benches at the University of Guelph greenhouse until germination was complete.

Germination occurred April 5 to April 25, 2022 and concluded with a total of 1,985 CBC seedlings and 2,890 IV seedlings. Based on our estimated number of seeds planted, both sources had an average germination rate of ~22%. To get a more precise measurement of germination, a separate germination test from a subset of 100 seeds was conducted resulting in 41% germination for IV seeds and 49% germination for CBC seeds. Seedlings were transplanted into 4" (10.64 cm) deep, 50-cell plug trays late April and fertilized weekly with liquid fertilizer (~200 ppm N).

Stylophorum diphyllum planting methods

We delicately barerooted all transplants to ensure excess soil was removed without damaging the roots (except for outdoor-grown *S. diphyllum* where some soil was left intact due to their fragility). We planted each transplant in a circular pattern (Figure S2.2). We planted transplants to root depth (~10 cm deep) with about 30 cm equal spacing between plants and the sub-plot border. Immediately after planting, we watered transplants for ~5 sec (~150 ml of water), mimicking "moist" conditions. To exclude herbivores from *S. diphyllum*, we formed each exclosure into a 1 m-diameter cylinder (3 ft (0.91 m) tall) using 14-gauge galvanized wire (5.08 cm × 2.54 cm openings) secured with four landscape staples.

Symphyotrichum prenanthoides planting methods

Prior to planting, we cleared competition in one sub-plot by removing all vegetation (including roots) and cut down all vegetation in the adjacent sub-plot. We carefully loosened each plug's root ball immediately before planting and planted in a grid pattern (Figure S2.2), following the same spacing guidelines as for *S. diphyllum*. Immediately after planting, we watered transplants for ~10 sec (~300 ml of water), mimicking "wet" conditions. We re-planted

10 transplants at one *S. prenanthoides* sub-plot ~1 month after the main planting event due to rodent activity that resulted in several plants being uprooted and destroyed.

Planting design

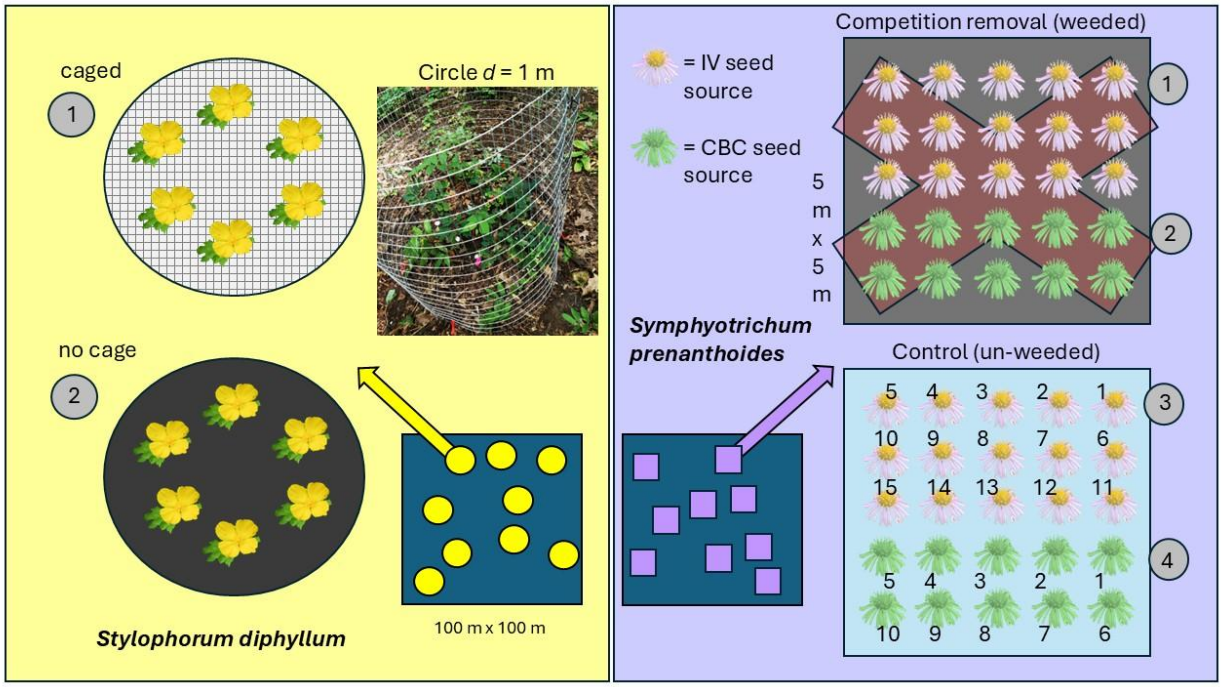

Figure S2.2: Plot planting design for *Stylophorum diphyllum* and *Symphyotrichum prenanthoides*. Blue square boxes represent one-hectare (100 m x 100 m) translocation sites, each with nine plots (coloured symbols). Each plot has two paired sub-plots with a biotic treatment. Permanent numbered metal ID tags are represented by the grey circles. *Stylophorum diphyllum* (left side; yellow box) sub-plot (d = 1 m) "1" represents the caged sub-plot (lower number) and "2" represents the un-caged plot (higher number). Each *S. diphyllum* plant received a unique numbered ID tag. *Symphyotrichum prenanthoides* (purple box; right side) sub-plot numbering represents which seed source is planted in the row with the lower number representing IV seed source (e.g., "1" = IV seed source, "2" = CBC seed source). Only the first row of each seed source was numbered (i.e., first three rows correspond to IV, fourth and fifth row correspond to CBC). Individual number (not marked) corresponds to grid position (e.g., "3.2" = second IV plant in row one). The red cross denotes the sub-plot with competition removed.

B.1.3 Supplementary Methods S2.3

Quantifying soil chemistry

We measured soil moisture with a Delta-T Devices SM150 two-prong soil moisture kit (model SM150T sensor with a HH150 meter set to the 'mineral soil' setting) and took the average of the soil moisture reading(s) in each sub-plot (one reading in each *S. diphyllum* sub-plot; two readings in *S. prenanthoides* sub-plots due to the larger area). We avoided taking soil moisture readings within 48-hr of heavy rainfall. During the monitoring seasons of 2023-2024, we collected two soil cores (1.91 cm × 15.24 cm deep) from each sub-plot and combined into a composite sample for each plot. We also collected soil samples from several known populations; 7 samples total from four populations for *S. diphyllum* (including one historically translocated population) and 20 samples total from two *S. prenanthoides* populations. All samples were labelled, stored at room temperature, and air-dried in paper bags. We used a 100-mesh sieve (1.4 mm openings) to remove rocks and large organic debris (i.e., roots) and weighed each dried sample to ensure at least 50 g of soil was collected for analysis. We sent the samples to the Natural Resources Analytical Laboratory at the University of Alberta to be quantified using the standard methods for pH (1:2 water analysis), organic matter (loss on ignition method), CEC (Ammonium Acetate method at pH 7.0 (Lavkulich 1981)) and nutrients (Al, B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Zn; ICPOES and the EPA method 6010d (modified)).

Soil PCs

We included the least correlated (Pearson's $r = <0.70$) soil nutrient variables in each PCA and log transformed all variables prior to conducting the PCA. We selected principal components (PCs) that had greater eigenvalues than those generated by the broken stick method (Borcard et

al. 2011) for use as predictors in the models, resulting in the first two scores for *S. diphyllum* (Figure S2.3) and the first three scores for *S. prenanthoides* (Figure S2.4) .

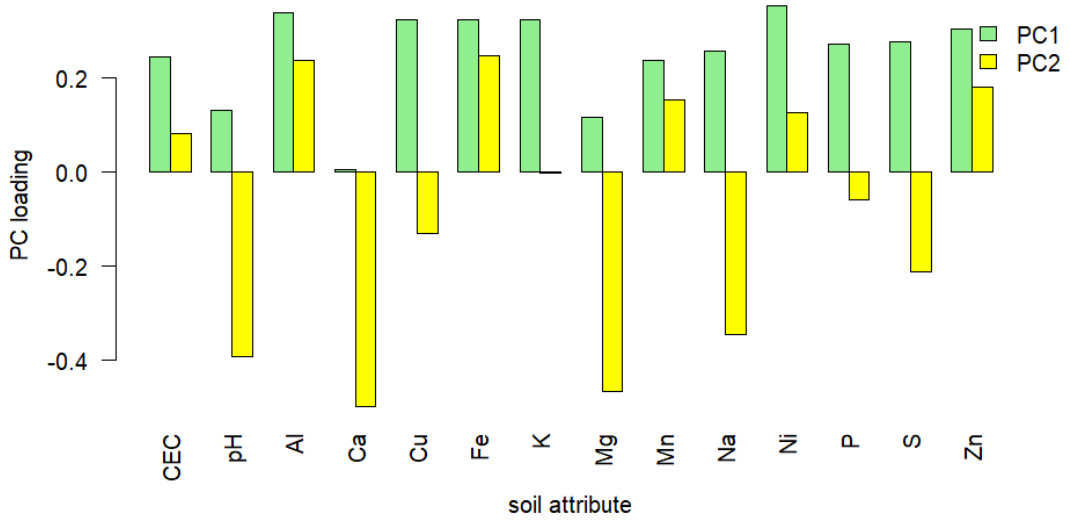

Figure S2.3: Principal Component (PC) loadings of soil nutrient attributes for *S. diphyllum* from a Principal Component Analysis (PCA).

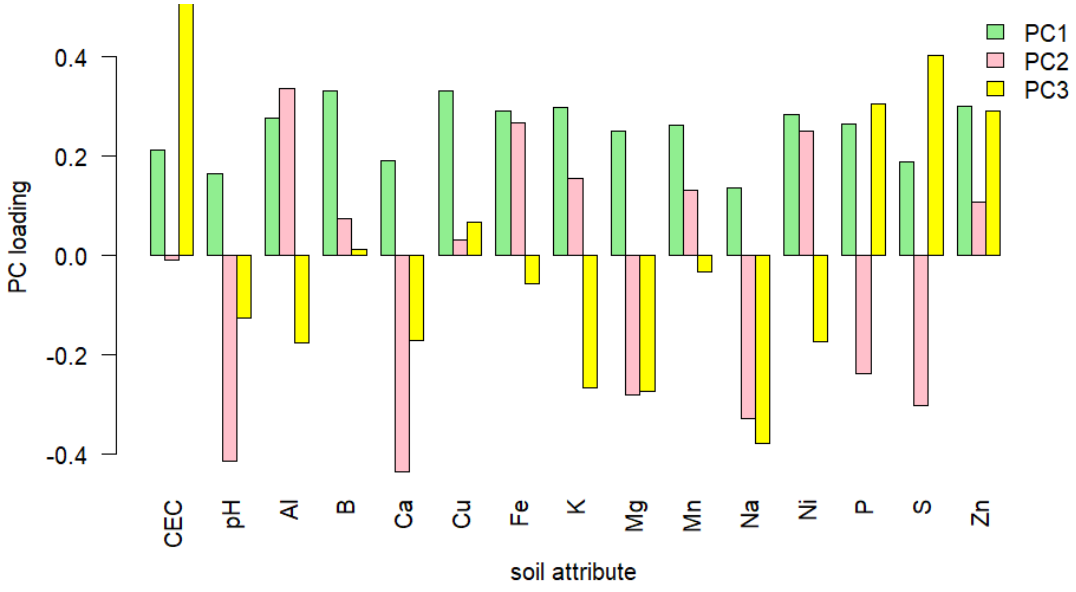

Figure S2.4: Principal Component (PC) loadings of soil nutrient attributes for *S. prenanthoides* from a Principal Component Analysis (PCA).

Temperature and humidity differentials

We deployed Kestrel® drop data loggers between June and August and set the logging interval for every 30 minutes. We did not have enough data loggers to deploy at all plots at the same time, therefore, we deployed all 27 loggers (one per plot) at one property at a time (summer 2022 for *S. diphyllum* and summer 2023 for *S. prenanthoides*). In low canopy closure plots we enclosed each logger within a ventilated, opaque white plastic cup to serve as a solar shield. We calculated site-level mean temperature and humidity for each one-hectare site by averaging the plot-level means from the nine plots sampled during the same time period. The time period chosen was the overlapping period for which all loggers gave successful, continuous readings (69-hr for *S. diphyllum* and 45-hr for *S. prenanthoides*). We then calculated a 'temperature differential' and 'humidity differential' respectively for each plot by subtracting the site mean value from the corresponding plot mean. To account for inter-site weather variation at different sampling periods, we standardized the raw plot differentials using the site-specific standard deviation. We used the raw differentials in subsequent analyses because the raw differentials of the plot were highly correlated with these standardized differentials.

Model specifications

We used the responses: (1) individual survival and (2) reproduction (individual number of fruits, individual flowering status (yes/no), plot number of seedlings produced, and seedling survival). Our predictors included: predicted habitat suitability index (SDM or CBM), microsite conditions, biotic treatment, and transplant characteristics (planting size class, planting year). We selected the final random effects structure based on how much variance was explained by each random term and when variance explained was negligible (<0.001), we dropped the term to

avoid singularity of model fit and convergence issues. We modeled sub-plots nested within plots as random effects for the individual-level models and we did not use any random effects in the plot-level seedling models (except site for the CBM cumulative number of seedlings) because of the extremely low variance explained by plot or site. For *S. prenanthoides* individuals flowering (2023) we added a single zero inflation parameter to all observations (SDM version only).

B.2 Supplementary Results

Stylophorum diphyllum survival rates

Table S2.1: Final model results for *S. diphyllum* using SDM- and CBM-predicted habitat suitability as a predictor for cumulative two-year survival.

Model	**Predictor**	**Coefficient**	***SE***	***df***	**AIC** [a]	***p*** [b]
Cumulative (two-year) survival (SDM) [c]	Intercept	3.09	0.28	*n/a*	929.04	*n/a*
	SDM-predicted habitat suitability	0.02	0.18	1	927.05	0.637
	Cage (no)	-0.48	0.22	1	931.93	**0.013**
	Seed source (MW)	-0.20	0.28	1	927.54	0.260
	Planting size class (small)	-1.82	0.26	1	981.54	**<0.001**
	Planting year (2023)	1.47	0.32	1	952.72	**<0.001**
	Canopy light index	-0.05	0.17	1	927.14	0.757
	Soil moisture	-0.43	0.17	1	933	**0.030**
	Temperature differential	0.30	0.25	1	928.55	0.119
	Humidity differential	0.35	0.25	1	929.06	0.051
	Soil nutrient PC1	0.11	0.18	1	927.42	0.575
	Soil nutrient PC2	0.30	0.18	1	929.82	**0.034**
Cumulative (two-year) survival (CBM) [c]	Intercept	3.20	0.29	*n/a*	944.5	*n/a*
	BCI	-0.26	0.20	*n/a*	*n/a*	*n/a*
	Cage (no)	-0.58	0.23	1	949.3	**0.009**
	Seed source (MW)	-0.24	0.28	1	943.25	0.386
	Planting size class (small)	-1.63	0.25	1	987.41	**<0.001**
	Planting year (2023)	1.41	0.31	1	965.69	**<0.001**
	Canopy light index	-0.05	0.16	1	942.58	0.772

	Soil moisture	-0.58	0.18	1	952.37	**0.002**
	Temperature differential	0.42	0.25	1	945.39	0.089
	Humidity differential	0.51	0.24	1	946.97	**0.034**
	Soil nutrient PC1	0.12	0.18	1	942.96	0.496
	Soil nutrient PC2	0.25	0.18	*n/a*	*n/a*	*n/a*
	BCI × soil nutrient PC2	0.64	0.20	1	952.92	**0.001**

[a] Akaike's information criterion for the model that includes all factors except the one being tested. To respect marginality, we do not drop individual predictors that are also in an interaction (indicated as *n/a* for those predictors).
[b] *p*-values from a likelihood ratio test. We did not drop individual predictors that were also in a significant interaction. Predictors with $p < 0.05$ are indicated in bold text.
[c] Model includes plants planted in 2022 and 2023 (excludes plants that died in the same year as planting).

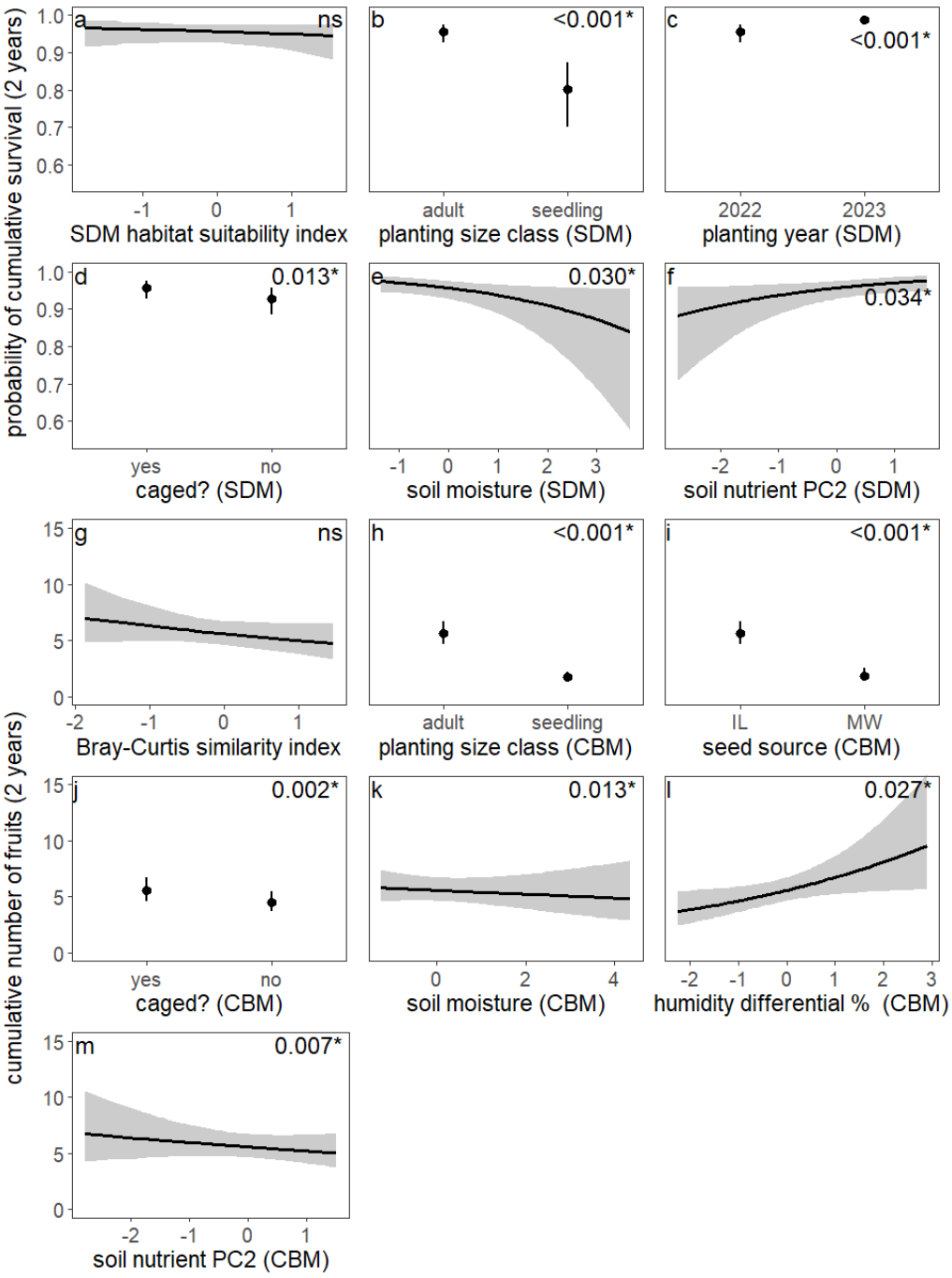

Figure S2.5: Partial regression plots showing the effects of predictors on *S. diphyllum* cumulative survival (a-f) and cumulative number of fruits (g-m) over two years. Brackets denote what estimate of habitat suitability was used in the model. Asterisks indicate significant predictors. All continuous predictors are scaled. In each case, all other predictors are held at the mean (for continuous predictors) and the most common category for categorical predictors (planting size class is held at "large", planting year at "2022", seed source at "IL", cage treatment is held at 'caged'). Points (±95% CI) show model-predicted responses for categorical predictors, while lines (±95% CI) show model-predicted relationships for continuous predictors (based on fixed-

effects only), with all other variables held constant. The pseudo R^2 values for the cumulative survival model including SDM estimates of habitat suitability and fruiting model including CBM estimates of habitat suitability were 0.542, 0.776 (conditional), 0.236, 0.528 (marginal), respectively.

Stylophorum diphyllum reproduction

Table S2.2: Final model results for *S. diphyllum* using SDM-predicted habitat suitability as a predictor for: A) cumulative number of fruits (2023-2024), B) cumulative number of seedlings in plot (2024-2025), and C) cumulative seedling survival (2024-2025).

Model	**Predictor**	**Coefficient**	***SE***	***df***	**AIC** [a]	***p*** [b]
A) Cumulative number of fruits (SDM) [c]	Intercept	1.73	0.10	*n/a*	5036.20	*n/a*
	SDM-predicted habitat suitability	-0.01	0.09	1	5034.20	0.944
	Cage (no)	-0.22	0.06	1	5122.70	*n/a*
	Seed source (MW)	-1.11	0.13	1	5271.80	**<0.001**
	Size class (small)	-1.18	0.08	1	5034.80	**<0.001**
	Planting year (2023)	0.06	0.07	1	5034.30	0.424
	Canopy cover light index	0.02	0.06	1	5040.50	0.777
	Soil moisture	-0.07	0.06	1	5039.10	*n/a*
	Temperature differential	0.21	0.08	1	5039.90	**0.012**
	Humidity differential	0.19	0.08	1	5034.40	**0.026**
	Soil nutrient PC1	0.17	0.07	1	5038.50	**0.017**
	Soil nutrient PC2	-0.03	0.07	1	5036.20	0.686
	Cage (no) × soil moisture	0.15	0.07	1	5034.20	**0.038**
A) Cumulative number of fruits (CBM) [c]	Intercept	1.72	0.09	*n/a*	5037.00	*n/a*
	BCI	-0.12	0.09	1	5036.50	0.219
	Cage (no)	-0.22	0.07	1	5045.10	**0.002**
	Seed source (MW)	-1.10	0.13	1	5122.10	**<0.001**
	Size class (small)	-1.18	0.08	1	5270.00	**<0.001**
	Planting year (2023)	0.07	0.07	1	5035.90	0.335
	Canopy cover light index	0.00	0.06	1	5035.00	0.947
	Soil moisture	-0.03	0.06	1	5035.30	0.593
	Temperature differential	0.21	0.08	1	5041.20	**0.013**
	Humidity differential	0.19	0.08	1	5039.90	**0.027**

	Soil nutrient PC1	0.19	0.07	1	5042.30	**0.007**
	Soil nutrient PC2	-0.07	0.08	1	5035.80	0.379
Model	**Predictor**	**Coefficient**	***SE***	***df***	**AIC** [a]	***p*** [b]
B) Cumulative number of seedlings in plot (2024-2025; SDM)	Intercept	-0.60	0.15	*n/a*	737.28	*n/a*
	Total number of fruits (2023-2024)	1.26	0.19	1	782.31	**<0.001**
	SDM-predicted habitat suitability	0.43	0.14	1	743.70	**0.004**
	Canopy light index	0.20	0.21	1	736.21	0.335
	Soil moisture	0.40	0.22	1	738.93	0.056
	Temperature differential	0.19	0.25	1	735.81	0.464
	Humidity differential	0.09	0.21	1	735.48	0.652
	Soil nutrient PC1	-0.55	0.15	1	747.56	**<0.001**
	Soil nutrient PC2	0.44	0.21	1	739.60	0.038
B) Cumulative number of seedlings in plot (2024-2025; CBM)	Intercept	-0.60	0.22	*n/a*	744.88	*n/a*
	Total number of fruits (2023-2024)	1.13	0.24	1	763.81	**<0.001**
	BCI	-0.28	0.24	1	744.12	0.265
	Canopy light index	0.22	0.23	1	743.77	0.346
	Soil moisture	0.27	0.25	1	744.12	0.266
	Temperature differential	0.07	0.27	1	742.95	0.792
	Humidity differential	0.04	0.22	1	742.92	0.843
	Soil nutrient PC1	-0.42	0.18	1	747.82	**0.026**
	Soil nutrient PC2	0.27	0.24	1	744.1	0.268
C) Seedling to yearling survival (2024-2025; SDM) [d]	Intercept	-2.77	0.26	*n/a*	139.10	0.353
	SDM-predicted habitat suitability	-0.22	0.23	1	137.96	0.360
	Soil moisture	0.26	0.27	1	137.94	0.110
	Canopy light index	0.58	0.37	1	139.65	0.272
	Temperature differential	0.32	0.28	1	138.30	0.353
	Soil nutrient PC1	-0.66	0.24	1	143.88	**0.009**
	Soil nutrient PC2	0.49	0.27	1	140.42	0.068
C) Seedling to yearling survival (2024-2025; CBM) [d]	Intercept	-2.78	0.24	*n/a*	139.90	*n/a*
	SDM-predicted habitat suitability	0.06	0.23	1	137.96	0.801
	Soil moisture	0.25	0.28	1	138.69	0.372
	Canopy light index	0.48	0.26	1	141.10	0.073
	Temperature differential	0.33	0.28	1	139.19	0.256

	Soil nutrient PC1	-0.76	0.26	1	145.19	**0.007**
	Soil nutrient PC2	0.52	0.24	1	142.66	**0.029**

[a] Akaike's information criterion for the model that includes all factors except the one being tested. To respect marginality, we do not drop individual predictors that are also in an interaction (indicated as *n/a* for those predictors).
[b] *p*-values from a likelihood ratio test. We did not drop individual predictors that were also in a significant interaction. Predictors with $p < 0.05$ are indicated in bold text.
[c] Model includes plants planted in 2022 and 2023 (excludes individuals that died same year).
[d] Model excludes plots with no seedlings the previous year (2024).

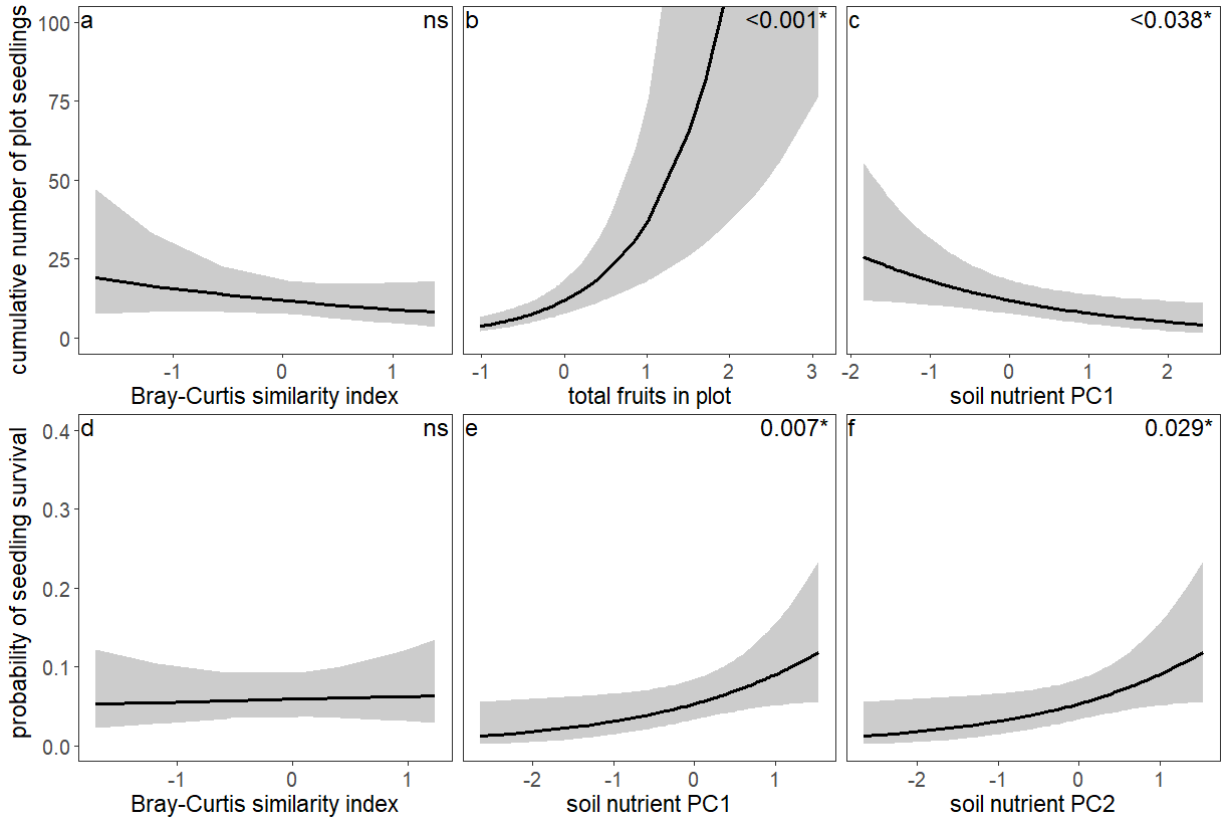

Figure S2.6: Partial regression plots showing the effects of predictors on *S. diphyllum* cumulative number of seedlings (plots a-c) and seedling survival (2024 – 2025; plots d-f). Asterisks indicate significant predictors. All continuous predictors are scaled. In each case, other continuous predictors are held at the mean. Thick lines represent the model predictions and shaded areas represent Wald 95% confidence intervals (based on fixed-effects only). The pseudo R^2 values for cumulative seedlings and seedling survival models including CBM estimates of habitat suitability were 0.621 and 0.088 (conditional) and 0.563 and 0.059 (marginal), respectively.

Table S2.3: Final model results for S. *prenanthoides* using SDM-and CBM- predicted habitat suitability as a predictor for cumulative two-year survival.

Model	**Predictor**	**Coefficient**	***SE***	***df***	**AIC** [a]	***p*** [b]
Cumulative (two-year) survival [c] (SDM)	Intercept	0.92	0.28	*n/a*	2991.40	*n/a*
	SDM-predicted habitat suitability	0.36	0.27	1	2991.20	0.183
	Biotic treatment (weeded)	0.67	0.20		3000.60	**<0.001**
	Seed source (IV)	0.33	0.10	1	3000.00	**<0.001**
	Canopy light index	-0.70	0.30	1	2994.90	**0.019**
	Soil moisture	0.87	0.39	1	2994.20	**0.029**
	Temperature differential	-0.38	0.31	1	2990.90	0.215
	Humidity differential	0.25	0.32	1	2990.00	0.443
	Soil nutrient PC1	-0.42	0.30	1	2991.30	0.174
	Soil nutrient PC2	-0.02	0.31	1	2989.40	0.960
	Soil nutrient PC3	-0.76	0.30	1	2995.70	**0.012**
Cumulative (two-year) survival [c] (CBM)	Intercept	1.22	0.29	*n/a*	2981.20	*n/a*
	BCI	0.06	0.30	*n/a*	2990.30	*n/a*
	Biotic treatment (weeded)	0.67	0.20	1	2989.80	**<0.001**
	Seed source (IV)	0.33	0.10	1	2982.50	**0.001**
	Canopy light index	-0.55	0.30	1	2980.40	0.069
	Soil moisture	0.74	0.37	*n/a*	2979.30	*n/a*
	Temperature differential	-0.32	0.28	1	2979.90	0.260
	Humidity differential	0.06	0.31	*n/a*	2993.40	*n/a*
	Soil nutrient PC1	-0.11	0.33	1	2988.30	0.741
	Soil nutrient PC2	-0.28	0.32	1	2985.00	0.382
	Soil nutrient PC3	-0.78	0.29	*n/a*	2981.20	*n/a*
	BCI × soil moisture	-1.24	0.32	1	2990.30	**<0.001**
	BCI × humidity differential	1.05	0.35	1	2989.80	**0.002**
	BCI × soil nutrient PC3	0.97	0.40	1	2982.50	**0.016**

[a] Akaike's information criterion for the model that includes all factors except the one being tested. To respect marginality, we do not drop individual predictors that are also in an interaction (indicated as *n/a* for those predictors).
[b] *p*-values from a likelihood ratio test. We did not drop individual predictors that were also in a significant interaction. Predictors with $p < 0.05$ are indicated in bold text.
[c] Model excludes plants that died in the same year as planting.

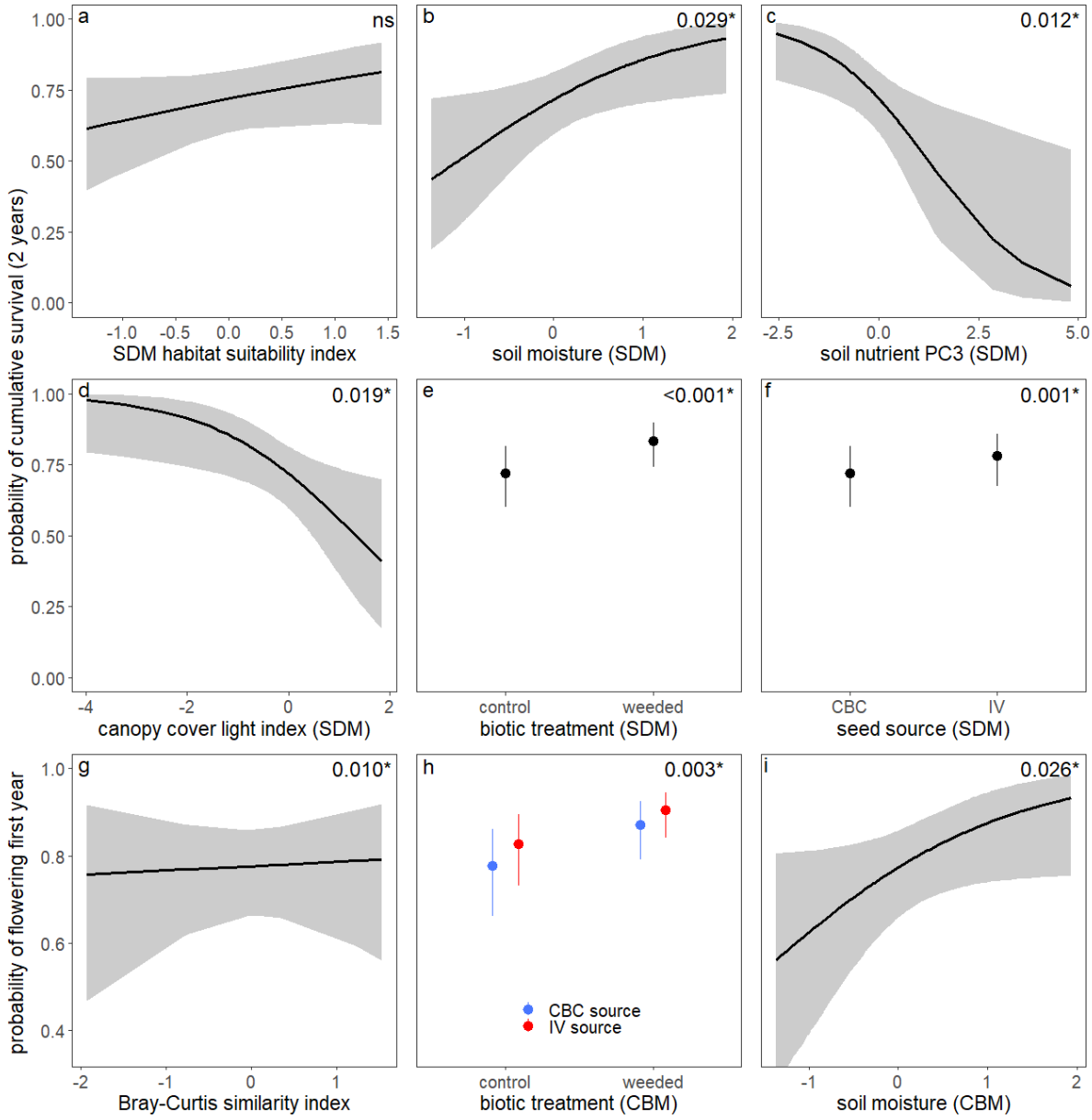

Figure S2.7: Partial regression plots showing the effects of predictors on *S. prenanthoides* cumulative survival (a-f) over two years and first year probability of flowering (g-i). Brackets denote what estimate of habitat suitability was used in the model. Asterisks indicate significant predictors. Brackets denote what estimate of habitat suitability was used in the model. All continuous predictors are scaled. In each panel, all other predictors are held at the mean (for continuous predictors) and the most common category for categorical predictors (seed source held at 'IV' and biotic treatment is held at 'control'). Points (±95% CI) show model-predicted responses for categorical predictors, while lines (±95% CI) show model-predicted relationships for continuous predictors (based on fixed-effects only), with all other variables held constant.

The pseudo R^2 values for the cumulative survival model including SDM estimates of habitat suitability and first year probability of flowering including CBM estimates of habitat suitability were 0.681, 0.586 (conditional) and 0.181, 0.154 (marginal), respectively.

Symphyotrichum prenanthoides reproduction

Table S2.4: Final model results for *S. prenanthoides* using SDM- and CBM-predicted habitat suitability as a predictor for probability of first year flowering (2023).

Model	**Predictor**	**Coefficient**	***SE***	***df***	**AIC** [a]	***p*** [b]
Probability of first-year flowering [c] (2023; SDM)	Intercept	-0.74	0.22	*n/a*	3199.2	*n/a*
	SDM-predicted habitat suitability	0.79	0.19	*n/a*	*n/a*	*n/a*
	Biotic treatment (weeded)	1.12	0.23	*n/a*	*n/a*	*n/a*
	Seed source (IV)	0.40	0.13	*n/a*	*n/a*	*n/a*
	Canopy light index	-0.38	0.21	1	3200.4	0.074
	Soil moisture	1.12	0.30	1	3210	**<0.001**
	Temperature differential	-0.23	0.21	*n/a*	*n/a*	*n/a*
	Humidity differential	-0.10	0.22	*n/a*	*n/a*	*n/a*
	Soil nutrient PC1	-0.30	0.22	1	3199.1	0.167
	Soil nutrient PC2	-0.08	0.22	1	3197.3	0.721
	Soil nutrient PC3	-0.57	0.21	1	3204	**0.009**
	SDM-predicted habitat suitability × temperature differential	0.73	0.23	1	3207.1	**0.002**
	SDM-predicted habitat suitability × humidity differential	0.56	0.22	1	3203.4	**0.013**
	Biotic treatment (weeded) × Seed source (IV)	-0.58	0.19	1	3206.3	**0.002**
Probability of first-year flowering [c] (2023; CBM)	Intercept	-0.77	0.25	*n/a*	3212.7	*n/a*
	BCI	0.67	0.26	1	3214.4	**0.010**
	Biotic treatment (weeded)	1.12	0.23	*n/a*	*n/a*	*n/a*
	Seed source (IV)	0.40	0.13	*n/a*	*n/a*	*n/a*
	Canopy light index	-0.27	0.23	1	3212	0.253
	Soil moisture	0.73	0.33	1	3215.7	**0.026**
	Temperature differential	-0.08	0.24	1	3210.8	0.735

	Humidity differential	0.14	0.26	1	3211	0.591
	Soil nutrient PC1	-0.29	0.27	1	3211.8	0.288
	Soil nutrient PC2	-0.11	0.26	1	3210.9	0.661
	Soil nutrient PC3	-0.18	0.25	1	3211.2	0.485
	Biotic treatment (weeded) × Seed source (IV)	-0.57	0.19	1	3219.7	**0.003**

[a] Akaike's information criterion for the model that includes all factors except the one being tested. To respect marginality, we do not drop individual predictors that are also in an interaction (indicated as *n/a* for those predictors).
[b] *p*-values from a likelihood ratio test. We did not drop individual predictors that were also in a significant interaction. Predictors with $p < 0.05$ are indicated in bold text.
[c] Model excludes plants that died in 2023.

APPENDIX C

Supplementary material for Chapter 3

C.1 Supplementary Results

Table S3.1: Summary of traits analyzed across greenhouse common garden trials (2023 and 2024), specifying the model type, corresponding family distribution, and the fixed and random effects structure.

Trait	**Year**	**Model type**	**Family distribution**	**Fixed effects**	**Random effects** [a]
Germination (%)	2023	GLMM	Beta binomial	Source, tray	Maternal line
Germination (%)	2024	GLMM	Beta binomial	Source	Maternal line
Days to emergence	2023	LM	Gaussian	Source	*n/a*
Days to emergence	2024	LMM	Gaussian	Source	Maternal line
Days to first fruit	2023	LM	Gaussian	Source, block	*n/a*
Days to first fruit	2024	LMM	Gaussian	Source, block	Maternal line
Total fruits	2023	GLM	Negative binomial (1)	Source, block	*n/a*
Total fruits	2024	GLMM	Negative binomial (1)	Source, block	Maternal line
Total seeds	2023	GLM	Negative binomial (1)	Source, block	*n/a*
Total seeds	2024	GLMM	Negative binomial (1) [b]	Source, block	Maternal line
Average seeds per fruit [c]	2023	LM	Gaussian	Source, block	*n/a*
Average seeds per fruit [c]	2024	LMM	Gaussian	Source, block	Maternal line
Weight per seed (g)	2023	LM	Gaussian	Source, block	*n/a*
Weight per seed (g) [d]	2024	LM	Gaussian	Source, block	*n/a*
Maximum number of leaves	2023	LM	Gaussian	Source, block	*n/a*
Maximum number of leaves	2024	LMM	Gaussian	Source, block	Maternal line
Trichome density [e]	2023	GLM	Tweedie	Source	*n/a*
Trichome density [e]	2024	GLM	Tweedie	Source	*n/a*
Leaf solidity [e]	2023	LM	Gaussian	Source	*n/a*
Leaf solidity [e]	2024	LM	Gaussian	Source	*n/a*

Trait	Year	Model type	Family distribution	Fixed effects	Random effects [a]
Leaf circularity [e]	2023	LM	Gaussian	Source	*n/a*
Leaf circularity [e]	2024	LM	Gaussian	Source	*n/a*
Above-ground (shoot) biomass (g)	2023	LM	Gaussian	Source, block	*n/a*
Above-ground (shoot) biomass (g)	2024	LMM	Gaussian	Source, block	Maternal line
Below-ground (root) biomass (g)	2023	LM	Gaussian	Source, block	*n/a*
Below-ground (root) biomass (g)	2024	LMM	Gaussian	Source, block	Maternal line
Asymptotic height (*Asym*)	2023 [f]	NLMM	*n/a*	Source	~1 | ID
Growth rate constant (*scal*)				Source	~1 | ID
Asymptotic height (*Asym*)	2024	NLMM	*n/a*	Source	~1 | ID
Growth rate constant (*scal*)				Source	~1 | ID

[a] A single global (constant) mean for a parameter is represented by ~1 and ~1 | ID accounts for repeated individual measures and allows specified growth parameters to vary by individual.
[b] Includes a zero-inflation term applied to all observations.
[c] Excludes plants with zero fruits.
[d] Seed weight measured from a sub-set of five maternal lines per source (100 seeds per line).
[e] Leaf clippings analyzed from a sub-set of all available plants.
[f] Inflection point (*xmid*) varies by ~1 in 2023 trial due to not enough observations to make the model converge to allow it to vary by source for a fixed effect.

Table S3.2: Seed germination rates for *Stylophorum diphyllum* across sources and seed treatments in common garden greenhouse trials.

Trial year	Source code	Seed treatment	Seeds Planted	Seeds germinated	Percent of seeds germinated	Mean source germination (%) 2023-2024
2023	BI	cold-warm	81	19	23	*n/a*
2023	GA	cold-warm	36	10	28	*n/a*
2023	TS	cold-warm	117	12	10	*n/a*
2023	BW	cold-warm	5[a]	0	0	67
2024	BW	cold-warm	162	108	67	
2023	FW	cold	136	0	0	*n/a*
2023	FW	cold-warm	90	81	90	89
2024	FW	cold-warm	162	142	88	
2023	IL	cold	170	0[b]	0	*n/a*

2023	IL	cold-warm	82	4[b]	5	51
2024	IL	cold-warm	153	148	97	
2023	MW	cold	129	0	0	*n/a*
2023	MW	cold-warm	55	14	25	40
2024	MW	cold-warm	162	88	54	

[a] Seeds planted for assessing seed viability for *in situ* experiment.
[b] Plants excluded from greenhouse experiment due to low sample size from low germination suspected to be a result of rot from storing seeds in plastic bags instead of containers.

Table S3.3: ANOVA summary table for *Stylophorum diphyllum* germination, emergence, and days to first fruit.

Trait	**Predictor**	***df***	**AIC**	**LRT**		***p*** [d]	
Germination (2023) [a]	Full model	*n/a*	152.21	*n/a*		*n/a*	
	source	4	164.97	20.76		**<0.001**	
	tray [b]	3	151.57	5.36		0.147	
Germination (2024) [a]	Full model	*n/a*	228.30	*n/a*		*n/a*	
	source	3	235.78	13.49		**0.004**	
Emergence (2023)	----------	***df***	**AIC**	**Sum of squares**	**RSS**	***F***	***p***
	Full model	*n/a*	313.38	*n/a*	3676.50	*n/a*	*n/a*
	source	4	407.57	9726.40	13402.90	**48.94**	**<0.001**
Emergence (2024)	----------	***ndf***	***ddf***	**Sum of squares**	**Mean Square**	***F***	***p***
	source	3	15.98	0.09	0.03	3.34	**0.046**
First fruit (2023) [a]	----------	***df***	**AIC**	**Sum of squares**	**RSS**	***F***	***p***
	Full model	*n/a*	363.25	*n/a*	12775.00	*n/a*	*n/a*
	source	4	411.55	17600.50	30375.00	18.94	**<0.001**
	block	5	358.07	983.30	13758.00	0.85	0.523
First fruit (2024) [c]	----------	***ndf***	***ddf***	**Sum of squares**	**Mean Square**	***F***	***p***
	source	3	15.20	2073.73	691.24	12.13	**<0.001**
	block	6	83.64	485.24	80.87	1.42	0.217

[a] Likelihood ratio tests (χ^2) for fixed effects from a generalized linear mixed model using maternal line as a random effect with a beta-binomial distribution.
[b] Tray included as a fixed effect due to sources being interplanted within trays and not evenly represented in all trays versus in 2024 where each source was given its own respective tray.

[c] Type II ANOVA F-tests for fixed effects from a linear mixed model using maternal line as a random effect; denominator degrees of freedom approximated by the Kenward–Roger method.
[d] p-values indicate the significance of each predictor using a likelihood ratio test. Predictors with $p < 0.05$ are indicated in bold text.

Table S3.4: Pairwise comparisons of estimated marginal means (emmean) for *Stylophorum diphyllum* germination rates, days to emergence, and days to first fruit.

Trait	**Source**	**emmean ± SE** [a]		***df*** [b]	**Lower CL** [c]	**Upper CL** [c]	**Tukey group** [d]
Germination (2023)	FW	0.91	0.06	*Inf*	0.72	0.98	a
	MW	0.21	0.12	*Inf*	0.06	0.51	b
	GA	0.21	0.16	*Inf*	0.04	0.63	c
	BI	0.26	0.12	*Inf*	0.09	0.54	d
	TS	0.07	0.05	*Inf*	0.02	0.25	e
Germination (2024)	FW	0.91	0.06	*Inf*	0.80	0.99	ab
	MW	0.21	0.12	*Inf*	0.26	0.81	c
	IL	0.21	0.16	*Inf*	0.89	1.00	a
	BW	0.26	0.12	*Inf*	0.39	0.90	bc
Days to emergence (2023)	FW	37.2	1.31	74	34.6	39.8	a
	MW	43.8	1.95	74	40	47.7	b
	GA	15.6	2.35	74	10.9	20.2	c
	BI	25.9	1.76	74	22.4	29.4	d
	TS	53.1	2.03	74	49	57.1	e
Days to emergence (2024)	FW	29.5	0.857	16.8	27.7	31.3	a
	MW	31.2	0.895	16.1	29.4	33.2	a
	IL	29.8	0.851	15.7	28	31.7	a
	BW	33.1	0.939	15.4	31.1	35.1	a
Days to first fruit (2023)	FW	116.9	2.98	55	110.9	123	a
	MW	102.1	4.31	55	93.5	111	ab
	GA	99.2	5.17	55	88.9	110	b
	BI	162	6.32	55	149.3	175	c
	TS	119.6	4.89	55	109.8	129	a
Days to first fruit (2024)	FW	118	1.82	19.3	114	122	a
	MW	105	1.52	11.3	101	108	b
	IL	115	1.78	18	112	119	ac
	BW	110	1.66	14.2	107	114	bc

[a] Estimated margin means (emmean) are reported on the original response scale.
[b] Degrees of freedom (*df*) are determined using the Kenward-Roger method for linear models. Infinite degrees of freedom (*Inf*) are based on asymptotic Wald z-tests for generalized linear models.
[c] Confidence level set at 95%.
[d] Tukey-adjusted significance; different letters indicate a significant difference ($p < 0.05$) between sources within years.

Table S3.5: ANOVA summary table for *Stylophorum diphyllum* reproduction using source and block as fixed effects.

Trait	**Predictor**	***df***	**AIC**	**LRT**			***p*** [e]
Total fruits (2023) [b]	Full model	*n/a*	350.37	*n/a*			*n/a*
	source	4	415	72.63			**<0.001**
	block	5	349.41	9.039			0.108
Total seeds (2023) [b]	Full model	*n/a*	728.54	*n/a*			*n/a*
	source	4	805.74	85.20			**<0.001**
	block	5	724.34	5.80			0.326
Total fruits (2024) [c]	Full model	*n/a*	664.83	*n/a*			*n/a*
	source	3	673.08	14.26			**0.003**
	block	6	676.26	23.44			**<0.001**
Total seeds (2024) [d]	Full model	n/a	1342.30	*n/a*			*n/a*
	source	3	1349.00	12.74			**<0.01**
	block	6	1346.40	16.15			**0.013**
Average seeds per fruit (2023) [a]	**Predictor**	***df***	**AIC**	**Sum of squares**	**RSS**	***F***	***p***
	Full model	*n/a*	313.24	n/a	7022.40	*n/a*	*n/a*
	source	4	319.90	1872.72	8895.10	3.47	**0.014**
	block	5	308.23	588.14	7610.60	0.87	0.507
Weight per seed (2023) [a]	Full model	*n/a*	313.24	n/a	7022.40	*n/a*	*n/a*
	source	4	319.90	1872.72	8895.10	3.47	**0.014**
	block	5	308.23	588.14	7610.60	0.87	0.507
Average seeds per fruit (2024) [c]	----------	***ndf***	***ddf***	**Sum of squares**	**Mean Square**	***F***	***p***
	source	3	16	358.63	119.55	1.35	0.294
	block	6	88	540.38	90.06	1.02	0.418
Weight per seed (2024) [a]	----------	***df***	**AIC**	**Sum of squares**	**RSS**	***F***	***p***
	Full model	*n/a*	-334.90	*n/a*	<0.001	*n/a*	*n/a*
	source	3	-325.69	<0.001	<0.001	5.70	**0.008**
	block	1	-336.41	<0.001	<0.001	0.37	0.553

[a] Type II ANOVA F-tests for fixed effects from a linear model.

[b] Likelihood ratio tests (χ^2) for fixed effects from a generalized linear model with a negative binomial (nbinom1) error distribution.
[c] Type II ANOVA F-tests for fixed effects from a linear mixed model using maternal line as a random effect; denominator degrees of freedom approximated by the Kenward–Roger method.
[d] Model summary uses Type II ANOVA F-tests for fixed effects using a generalized linear model with maternal line as a random effect with a negative binomial (nbinom1) error distribution and a single intercept term for zero applied across all observations.
[e] *p*-values indicate the significance of each predictor using a likelihood ratio test. Predictors with $p < 0.05$ are indicated in bold text.

Table S3.6: Pairwise comparisons of estimated marginal means (emmean) for *Stylophorum diphyllum* total fruits, total seeds, mean seeds per fruit, and mean weight per seed.

Trait	**Source**	**emmean ± SE** [a]		***df*** [b]	**Lower CL** [c]	**Upper CL** [c]	**Tukey group** [d]
Total fruits (2023)	FW	4.05	0.50	*Inf*	3.19	5.16	a
	MW	9.18	1.13	*Inf*	7.22	11.67	b
	GA	7.38	1.18	*Inf*	5.40	10.09	b
	BI	0.72	0.29	*Inf*	0.33	1.56	c
	TS	1.92	0.53	*Inf*	1.12	3.29	ac
Total seeds (2024)	FW	1.25	0.182	*Inf*	0.899	1.61	a
	MW	2.06	0.142	*Inf*	1.78	2.34	b
	IL	1.22	0.178	*Inf*	0.871	1.57	a
	BW	1.23	0.177	*Inf*	0.882	1.58	a
Mean seeds per fruit (2023)	FW	22.12	2.24	52.00	17.62	26.60	a
	MW	19.46	3.29	52.00	12.86	26.10	ab
	GA	8.06	3.95	52.00	0.14	16.00	b
	BI	17.95	5.35	52.00	7.22	28.70	ab
	TS	9.07	4.48	52.00	0.08	18.10	ab
Mean seeds per fruit (2024)	FW	22.00	2.21	17.50	17.40	26.70	a
	MW	16.80	2.05	13.30	12.40	21.30	a
	IL	16.60	2.18	17.20	12.00	21.20	a
	BW	19.20	2.11	15.00	14.70	23.00	a
Mean weight per seed (2023)	FW	0.00243	8.45E-05	48	0.00226	0.0026	a
	MW	0.00261	1.24E-04	48	0.00236	0.00286	a
	GA	0.00221	1.49E-04	48	0.00192	0.00251	a
	BI	0.00248	3.35E-04	48	0.00181	0.00315	a
	TS	0.00286	1.81E-04	48	0.0025	0.00323	a
Mean weight per seed (2024)	FW	0.00241	9.43E-05	15	0.00221	0.00262	a
	MW	0.00232	9.37E-05	15	0.00212	0.00252	a
	IL	0.00212	9.30E-05	15	0.00192	0.00232	ab
	BW	0.00191	9.30E-05	15	0.00171	0.00211	b

[a] Estimated margin means (emmean) are reported on the original response scale.

[b] Degrees of freedom (*df*) are determined using the Kenward-Roger method for linear models. Infinite degrees of freedom (*Inf*) are based on asymptotic Wald z-tests for generalized linear models.
[c] Confidence level set at 95%.
[d] Tukey-adjusted significance; different letters indicate a significant difference ($p < 0.05$) between sources within years.

Table S3.7: Pairwise comparisons of estimated marginal means (emmean) for *Stylophorum diphyllum* asymptote (maximum height) and scale (inverse growth rate constant).

Trait	**Source**	**emmean ± SE** [a]		***df*** [b]	**Lower CL** [c]	**Upper CL** [c]	**Tukey group** [d]
Asymptote (2023)	FW	35.7	0.62	1421	34.5	36.9	a
	MW	35.3	0.925	1421	33.5	37.1	a
	GA	33.3	1.07	1421	31.2	35.5	a
	BI	26.6	0.823	1421	25	28.2	b
	TS	33.5	0.978	1421	31.6	35.4	a
Asymptote (2024)	FW	46.4	1.18	1393	44.1	48.7	ab
	MW	43.9	1.15	1393	41.7	46.2	a
	IL	48.1	1.14	1393	45.9	50.4	b
	BW	46.4	1.11	1393	44.2	48.6	ab
Scale (2023)	FW	12.58	0.387	1421	11.8	13.34	a
	MW	10.96	0.54	1421	9.9	12.02	a
	GA	8.21	0.566	1421	7.1	9.32	b
	BI	15.9	0.809	1421	14.3	17.49	c
	TS	11.75	0.626	1421	10.5	12.98	a
Scale (2024)	FW	11.8	0.431	1393	10.9	12.6	a
	MW	11.2	0.417	1393	10.4	12	a
	IL	11.6	0.405	1393	10.8	12.4	a
	BW	10.9	0.395	1393	10.1	11.6	a

[a] Estimated margin means (emmean) are reported on the original response scale.
[b] Degrees of freedom (*df*) are determined using the Kenward-Roger method for linear models.
[c] Confidence level set at 95%.
[d] Tukey-adjusted significance; different letters indicate a significant difference ($p < 0.05$) between sources within years.

Table S3.8: ANOVA summary table for *Stylophorum diphyllum* leaf traits using source and block as fixed effects. Bolded *p*-values indicate significant predictors.

Trait	**Predictor**	***df***	**AIC**	**Sum of squares**	**RSS**	***F***	***p*** [d]
Maximum number of leaves (2023) [a]	Full model	*n/a*	267.41	*n/a*	1810.20	*n/a*	*n/a*
	source	4	302.06	1295.97	3106.10	12.35	**<0.001**
	block	5	262.20	113.31	1923.50	0.86	0.510
Leaf solidity (2023) [a]	Full model	*n/a*	-280.37	*n/a*	0.15	*n/a*	*n/a*
	source	4	-311.68	0.07	0.08	5.16	**0.002**
Leaf circularity (2023) [a]	Full model	*n/a*	-280.37	*n/a*	0.15	*n/a*	*n/a*
	source	4	-299.07	0.04	0.12	5.74	**<0.001**
Total trichomes (2023) [b]	**---------**	***df***	**AIC**	**LRT**			***p***
	Full model	*n/a*	462.40	*n/a*			*n/a*
	source	4	546.11	91.72			**<0.001**
Maximum number of leaves (2024) [c]	**---------**	***ndf***	***ddf***	**Sum of squares**	**Mean Square**	***F***	***p***
	source	3	15.85	439.78	146.60	7.49	**0.002**
	block	6	105.47	80.03	13.34	0.68	0.665
Leaf solidity (2024) [a]	**---------**	***df***	**AIC**	**Sum of squares**	**RSS**	***F***	***p***
	Full model	*n/a*	-177.87	*n/a*	0.04	*n/a*	*n/a*
	source	3	-175.38	0.01	0.05	2.83	0.060
Leaf circularity (2024) [a]	Full model	*n/a*	-177.87	*n/a*	0.04	*n/a*	*n/a*
	source	3	-175.38	0.01	0.05	2.83	0.060
Total trichomes (2024) [b]	**---------**	***df***	**AIC**	**LRT**			***p***
	Full model	*n/a*	105.50	*n/a*			*n/a*
	source	3	111.02	11.52			**0.009**

[a] Model summary uses Type II ANOVA F-tests for fixed effects from a generalized linear model.

[b] Model summary reports likelihood ratio tests (χ^2) for fixed effects from a generalized linear model with a negative binomial (nbinom1) error distribution.
[c] Type II ANOVA F-tests for fixed effects using a linear mixed model fitted with maternal line as a random effect; denominator degrees of freedom approximated by the Kenward–Roger method.
[d] *p*-values indicate the significance of each predictor using a likelihood ratio test. Predictors with $p < 0.05$ are indicated in bold text.

Table S3.9: Pairwise comparisons of estimated marginal means (emmean) for *Stylophorum diphyllum* maximum number of leaves, leaf solidity and circularity, and trichome density.

Trait	**Source**	**emmean ± SE** [a]		***df*** [b]	**Lower CL** [c]	**Upper CL** [c]	**Tukey group** [d]
Maximum number of leaves (2023)	FW	31.70	0.97	69.00	29.80	33.70	a
	MW	33.60	1.44	69.00	30.80	36.50	a
	GA	31.90	1.73	69.00	28.50	35.40	a
	BI	23.20	1.30	69.00	20.60	25.80	b
	TS	24.80	1.50	69.00	21.80	27.80	b
Maximum number of leaves (2024)	FW	20.70	0.89	17.80	18.80	22.60	c
	MW	26.10	0.86	16.00	24.20	27.90	b
	IL	22.00	0.85	15.20	20.20	23.80	ac
	BW	24.40	0.84	14.60	22.60	26.20	ab
Leaf solidity (2023)	FW	0.69	0.02	45.00	0.65	0.73	ab
	MW	0.76	0.02	45.00	0.72	0.79	ab
	GA	0.73	0.02	45.00	0.70	0.77	ab
	BI	0.65	0.02	45.00	0.61	0.69	b
	TS	0.69	0.02	45.00	0.65	0.73	ab
Leaf solidity (2024)	FW	0.68	0.02	24.00	0.65	0.72	ab
	MW	0.70	0.01	24.00	0.67	0.73	ab
	IL	0.67	0.02	24.00	0.63	0.70	a
	BW	0.73	0.01	24.00	0.70	0.75	b
Leaf circularity (2023)	FW	0.18	0.01	44.00	0.15	0.21	abc
	MW	0.19	0.01	44.00	0.16	0.22	ab
	GA	0.21	0.01	44.00	0.18	0.24	a
	BI	0.15	0.01	44.00	0.12	0.18	bc
	TS	0.13	0.01	44.00	0.10	0.16	c
Leaf circularity (2024)	FW	0.17	0.02	24.00	0.13	0.20	a
	MW	0.20	0.01	24.00	0.17	0.23	ab
	IL	0.18	0.02	24.00	0.15	0.22	ab
	BW	0.23	0.01	24.00	0.20	0.25	b
Trichome density (2023)	FW	0.28	0.14	*Inf*	0.11	0.74	a
	MW	0.20	0.11	*Inf*	0.07	0.57	a
	GA	25.02	3.30	*Inf*	19.32	32.41	b
	BI	52.55	5.26	*Inf*	43.20	63.94	c
	TS	20.94	2.77	*Inf*	16.16	27.13	b

Trichome density (2024)	FW	0.00	1.13	22.00	-2.35	2.34	a
	MW	1.05	0.82	22.00	-0.66	2.75	a
	IL	1.88	0.55	22.00	0.73	3.02	a
	BW	1.08	0.83	22.00	-0.64	2.81	a

[a] Estimated margin means (emmean) are reported on the original response scale.
[b] Degrees of freedom (*df*) are determined using the Kenward-Roger method for linear models. Infinite degrees of freedom (*Inf*) are based on asymptotic Wald z-tests for generalized linear models.
[c] Confidence level set at 95%.
[d] Tukey-adjusted significance; different letters indicate a significant difference ($p < 0.05$) between sources within years.

Table S3.10: ANOVA summary table for *Stylophorum diphyllum* biomass traits using source and block as fixed effects. Bolded *p*-values indicate significant predictors.

Trait	**Predictor**	***df***	**AIC**	**Sum of squares**	**RSS**	***F***	***p*** [c]
Shoot biomass (2023) [a]	Full model	*n/a*	156.43	*n/a*	444.24	*n/a*	*n/a*
	source	4	168.38	127.66	571.90	4.96	**0.001**
	block	5	156.54	60.70	504.94	1.89	0.108
Root biomass (2023) [a]	Full model	*n/a*	119.10	*n/a*	276.98	*n/a*	*n/a*
	source	4	150.19	177.28	454.26	11.04	**<0.001**
	block	5	118.27	34.08	311.06	1.70	0.147
Shoot biomass (2024) [b]	----------	***ndf***	***ddf***	**Sum of squares**	**Mean Square**	***F***	***p***
	source	3	15.82	6.89	2.30	0.30	0.826
	block	6	105.74	146.86	24.48	3.19	**0.006**
Root biomass (2024) [b]	source	3	15.93	17.98	5.99	1.77	0.194
	block	6	104.52	27.12	4.52	1.33	0.249

[a] Model summary uses Type II ANOVA F-tests for fixed effects from a generalized linear model.
[b] Type II ANOVA F-tests for fixed effects using a linear mixed model fitted with maternal line as a random effect; denominator degrees of freedom approximated by the Kenward–Roger method.
[c] *p*-values indicate the significance of each predictor using a likelihood ratio test. Predictors with $p < 0.05$ are indicated in bold text.

Table S3.11: Pairwise comparisons of estimated marginal means (emmean) for *Stylophorum diphyllum* above-ground (shoot) and below-ground (root) biomass.

Trait	**Source**	**emmean ± SE** [a]		***df*** [b]	**Lower CL** [c]	**Upper CL** [c]	**Tukey group** [d]
Shoot biomass (2023)	FW	9.89	0.48	69.00	8.93	10.85	a
	MW	8.66	0.72	69.00	7.23	10.09	ab
	GA	7.16	0.86	69.00	5.45	8.87	ab
	BI	9.53	0.64	69.00	8.24	10.81	a
	TS	6.43	0.74	69.00	4.95	7.91	b
Shoot biomass (2024)	FW	11.60	0.53	18.00	10.50	12.70	a
	MW	11.10	0.51	16.10	9.99	12.20	a
	IL	11.70	0.50	15.20	10.64	12.80	a
	BW	11.50	0.50	14.40	10.39	12.50	a
Root biomass (2023)	FW	10.20	0.38	69.00	9.44	10.96	a
	MW	9.76	0.57	69.00	8.64	10.89	ab
	GA	7.66	0.68	69.00	6.31	9.01	bc
	BI	8.69	0.51	69.00	7.68	9.71	ab
	TS	5.84	0.59	69.00	4.67	7.01	c
Root biomass (2024)	FW	7.04	0.48	17.10	6.03	8.06	a
	MW	6.76	0.47	16.00	5.75	7.76	a
	IL	6.75	0.47	15.60	5.76	7.75	a
	BW	8.06	0.47	15.20	7.07	9.00	a

[a] Estimated margin means (emmean) are reported on the original response scale.
[b] Degrees of freedom (*df*) are determined using the Kenward-Roger method for linear models. Infinite degrees of freedom (*Inf*) are based on asymptotic Wald z-tests for generalized linear models.
[c] Confidence level set at 95%.
[d] Tukey-adjusted significance; different letters indicate a significant difference ($p < 0.05$) between sources within years.

Table S3.12: Summary of *Stylophorum diphyllum* common garden trials P_{ST} trait values, variances, and P_{ST} : F_{ST} comparison. Variances (V) extracted from REML models and represent source, family (maternal line), block, residual, and population (pop) variance.

Trait	**Trial year**	**V_{source}**[a]	**V_{family}**	**V_{block}**	**V_{resid}**	**V_{POP}**[b]	**P_{ST}**[c]	**P_{STall}**[d]	**F_{ST}**[e]	**P_{ST}: F_{ST}**
Maximum height	2023	11.37	*N/A*	*N/A*	19.58	0.37	0.23	*N/A*	0.60	$P_{ST} < F_{ST}$
	2024	4.97	1.64	4.16	27.69	0.13	0.06	0.08	0.33	$P_{ST} < F_{ST}$
Maximum leaves	2023	23.96	1.44	0.09	26.85	0.46	0.30	0.30	0.60	$P_{ST} < F_{ST}$
	2024	4.94	*N/A*	*N/A*	19.82	0.20	0.11	*N/A*	0.33	$P_{ST} < F_{ST}$
Total fruits	2023	0.63	0.01	0.04	0.31	0.64	0.51	0.50	0.60	$P_{ST} \sim F_{ST}$
	2024	0.13	0.06	0.09	0.50	0.16	0.10	0.10	0.33	$P_{ST} < F_{ST}$
Total seeds	2023	3.56	0.01	0.08	2.01	0.63	0.47	0.47	0.60	$P_{ST} \sim F_{ST}$
	2024	0.25	0.41	0.44	2.44	0.07	0.05	0.04	0.33	$P_{ST} < F_{ST}$
Shoot biomass	2023	0.86	0.95	0.14	6.09	0.11	0.11	0.06	0.60	$P_{ST} < F_{ST}$
	2024	0.00	*N/A*	*N/A*	8.43	0.00	0.00	*N/A*	0.33	$P_{ST} < F_{ST}$
Root biomass	2023	2.58	0.50	0.22	3.74	0.37	0.29	0.23	0.60	$P_{ST} < F_{ST}$
	2024	0.18	0.59	0.05	3.38	0.04	0.03	0.02	0.33	$P_{ST} < F_{ST}$

[a] V_{source} represents the raw variance among populations on the trait's original scale.
[b] V_{pop} represents the proportion of source variance to total variance (V_{source} / V_{total}).
[c] P_{ST} is estimated from source and residual variances.
[d] P_{STall} is estimated from source, family, and residual variances.
[e] F_{ST} values from Lait et al. (2025) are the global mean derived from sources in the respective trial.

Table S3.13: Results of a generalized linear mixed effects model assessing potential predictors of emergence rates and survival rate (year one) of *S. diphyllum* seedlings.

Response	**Predictor**	**Coefficient**	***SE***	***df***	**AIC** [a]	***p*** [b]
Seedling emergence	(Intercept)	140.50	62.45	*n/a*	178.97	*n/a*
	Source (FW)	0.06	0.39	3	176.09	0.373
	Source (IL)	-0.14	0.40	3		
	Source (MW)	-0.58	0.40	3		
	Soil moisture	-0.04	0.04	1	177.88	0.341
	Soil moisture ^2	0.00	0.00	1	177.78	0.367
	Canopy cover index	-4.88	2.21	1	180.96	**0.046**
	Canopy cover index ^2	0.04	0.02	1	180.79	0.051
Seedling survival (year one)	(Intercept)	-0.35	0.84	*n/a*	51.25	*n/a*
	Source (FW)	-1.33	0.83	3	51.90	0.084
	Source (IL)	-0.79	0.84	3		
	Source (MW)	-2.66	1.26	3		

[a] Akaike's information criterion for the model that includes all factors except the one being tested.

[b] *p*-values indicate the significance of each predictor using a likelihood ratio test. Predictors with $p < 0.05$ are indicated in bold text.