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Biogeography of Biological Control: Spatial Variation in Agent-Host Interactions

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BIOGEOGRAPHY OF BIOLOGICAL CONTROL: SPATIAL VARIATION IN AGENT-HOST INTERACTIONS

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ABSTRACT

Management of plant invasions using biological control has the potential to generate spatial patterns which reflect geographic or genetic variation in invader or control agents. Despite its rarity in practice, investigations into the biogeography of interacting species (i.e., plant invader and control agent) in the context of biological control can lend insights into species distribution-abundance patterns and provide predictions for spatial variation in control success. I explored spatial variability in biological control agent-plant interactions using two wetland weed study systems with large geographic distributions: flowering rush (*Butomus umbellatus* L.) and alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb). Through literature and database review, I found that geographic variability in biological control success is relatively common, and abiotic factors are more often implicated than biotic factors. For flowering rush, I explored whether genetic and geographic variation in susceptibility to fungal pathogens could cause variation in plant performance and biological control. I found that patterns of disease varied between flowering rush cytotypes in field (higher disease rates in diploid plants) and laboratory (higher disease impacts in triploid plants) studies and were spatially variable along a latitudinal gradient for triploid plants only. I hypothesized that variation in alligatorweed biological control among sites and seasons in Louisiana was due to variation in plant quality (foliar nitrogen; FN). Over four years, I found that FN varied among sites and seasonally, with peak FN in spring and fall. Foliar nitrogen decreased the duration of larval development across a range of temperatures and slightly reduced dispersal at moderate conspecific densities. Finally, I explored the role of weather on biological control of alligatorweed across an environmental (climate) gradient in Louisiana. Biological control agent mean and maximum density decreased with latitude, population variability increased, and host (alligatorweed) density increased with latitude, likely

due to low agent abundance at higher latitudes. Agent phenology and variability were influenced by weather variables and better explained alligatorweed density than weather or beetle densities alone. By combining literature review on geographic variation in biological control success with complementary studies in the above systems, my work is an important addition to the invasion biology and biological control literature and lends insights into how a biogeographical approach can be applied to study biological control of plant invasions and make predictions about the success of future agents.

CHAPTER 1.
INTRODUCTION

Biological invasions are among the most important threats to earth's biodiversity, along with climate change and human disturbance (Mainka and Howard 2010, Maxwell et al. 2016, Tilman et al. 2017, Díaz et al. 2019). Management of biological invasions can require a major initial investment of funding and manpower to offset economic and ecological impacts to recipient ecosystems (Pimentel 2011, Martin and Blossey 2013). Plant invasions, in particular, damage natural ecosystems and human infrastructure by obstructing navigation (Gopal 1987), altering fire regimes (D'Antonio and Vitousek 1992), altering soil biogeochemical pathways (Ehrenfeld 2003, Scherer-Lorenzen et al. 2007), reducing habitat or food quality for wildlife (Pimentel et al. 2005), and displacing native species (Pimentel et al. 2005). Additionally, many of these impacts are expected to increase in frequency and magnitude with climate change (Hellmann et al. 2008, Rahel and Olden 2008, Wu and Ding 2019).

Large-scale plant invasions are expected to result in spatially heterogeneous invader impacts in recipient ecosystems because of introduction history, differences in regional or local site and climate characteristics, spatial variation in biotic resistance (Schaffner et al. 2011, Stricker et al. 2016a), and genotypic variation in invader populations that result in invasive trait differences among them (Richardson and Pyšek 2006, Van Kleunen et al. 2010a, Harms 2020). Biogeographical investigations of invader ecology and evolutionary history are now front and center as invasion biologists attempt to better understand mechanisms (e.g., introduction pathways, evolution of increased competitive ability) for invasion success in order to prevent future introductions or impacts, or to mitigate ongoing impacts through control programs (Blossey and Notzold 1995, Gaskin et al. 2013, Cronin et al. 2015, Kwong et al. 2019). In particular, biological control is a common approach to management of invasive plant species

wherein a host-specific herbivore or pathogen agent, often sourced in the native range of the invader, is introduced in order to reduce invasiveness and associated impacts of the invader (Van Driesche et al. 2009). Although biological control has a recent history of successes with limited off-target impacts (Hinz et al. 2019), there have been a number of cases where effectiveness varies considerably within and among introduction areas (Winston et al. 2017). The goal of this dissertation was to examine the importance of geographic variation in biological control successes by reviewing evidence for this phenomenon in historical biological control programs and to explore, experimentally, various aspects of geographic variability on biological control success. As a result of this research, I advocate for a biogeographical approach to biological control, much in the same way that invasion biologists now commonly work within this type of framework to predict, explain, and manage invasions (e.g., Hierro et al. 2005, Cronin et al. 2015, Bhattarai et al. 2017a, Lu et al. 2018).

In this dissertation, I aimed to investigate the ecology and management of plant invaders and their biological control agents using a combination of experimental and observational studies in two complementary weed systems. My overall research question was: How does invader success relate to biogeographic variation in invader or control agent interactions, genetics, and abundance? I demonstrate how management of invasive weeds, particularly with biological control, fits into a biogeographic framework and how studying biological control within this framework can lead to novel insights into variability in management success. Below, I outline my study systems and provide a synopsis of each dissertation chapter.

STUDY SYSTEMS

In order to investigate biogeography of biological control systems, I chose to examine invasive weed systems that were in stages before or after the introduction of biological control

agents. Examination of weed systems prior to implementation of biological control may offer insight into baseline impacts caused by the target plant (Blossey 1999, Forrest and Taylor 2002), whether weed impacts vary spatially or by lineage in weed populations (Cronin et al. 2015), whether biotic resistance is present and at what magnitude (and genetic identity interacts with biotic resistance; Chapter 3), and to identify potential interactions between biotic resistance and future introductions of control agents (Chapter 3). These pre-introduction studies can be used to develop hypotheses about future control success once agents are introduced. After agent introductions, programs can be examined to identify spatial or temporal variability in control, including gradients in control that may be related to environmental limitations on distributions of the agent or host. This is particularly relevant to systems where the host plant has a large geographic distribution but the control agent does not, which is a common phenomenon (Chapter 2). Using this approach of studying systems in different stages of management implementation (i.e., before or after introduction of biological control agents) allowed me to make predictions about the importance of biogeographic variation in control based on genetic structure in invader populations, climate, or other factors.

I focused on two invasive aquatic weeds, flowering rush (*Butomus umbellatus* L.) and alligatorweed (*Alternanthera philoxeroides* Mart. Griseb.), and their management by biological control. Flowering rush originates from Europe and has been introduced multiple times in North America (Anderson et al. 1974), with at least two cytotypes (diploid, triploid), and several genotypes (Gaskin, unpublished data) present. Flowering rush primarily impacts recipient aquatic ecosystems by increasing suitable habitat for pond snails, an intermediate host organism for the swimmer's itch parasite (Parkinson et al. 2010), competing with native plant species for space and nutrients, and colonizing unvegetated habitats which previously supported native fish

species (e.g., cutthroat and bulltrout) (Parkinson et al. 2010, Jacobs et al. 2011). A biological control program for flowering rush in the US was undertaken in 2013 and now consists of testing four potential agents: three insects (*Bagous nodulosus* Gyllenhal and *B. validus* Rosenhauer, Coleoptera: Curculionidae; *Phytoliriomyza ornata* Meigen, Diptera: Agromyzidae) and one pathogen (*Doassansia niesslii* Fungi: Doassansiaceae) (Hafliger et al. 2017).

Alligatorweed is a semiaquatic perennial weed common to the southeastern US, successfully established by 1897 (Zeiger 1967). A biological control program for alligatorweed was initiated in 1959 as a collaboration between the US Department of Agriculture (USDA) and US Army Corps of Engineers (USACE) (Zeiger 1967). This program led to introduction of three host-specific agents (*Agasicles hygrophila* Selman & Vogt, Coleoptera: Chrysomelidae; *Amynothrips andersoni* O'Neill, Thysanoptera: Phlaeothripidae; *Arcola* (=*Vogtia*) *malloi* Pastrana, Lepidoptera: Pyralidae), of which *A. hygrophila* (alligatorweed flea beetle; AFB) has received the most attention because of the control it provides in most areas (Buckingham 1996b). Although the distribution of alligatorweed includes Arkansas, Tennessee, Kentucky, and Virginia, AFB overwintering is mostly restricted to coastal areas and where winters remain warm (Figure 1.1). However, dispersal into cooler areas occurs and may provide some control (Spencer and Coulson 1976, Julien et al. 1995, Harms and Shearer 2017). Although it is widely understood that control by the AFB varies with overwintering and ability to attack plants early in the year (Harms and Shearer 2017), the relationship between winter severity and control across the range of the AFB has not been fully investigated.

Figure 1.1. State-level distribution of A) alligatorweed (*Alternanthera philoxeroides*) and B) the alligatorweed flea beetle (*Agasicles hygrophila*) in the United States.

Although geographic variability in biological control effectiveness may be important, particularly when the target weed has a large geographic distribution, its explicit reporting has been minimal. In Chapter 2, I conducted a review using the World Catalogue of Agents and Their Target Weeds (WCATW; Winston et al. 2014) to determine whether geographic variability in weed biological control was common, and whether biotic or abiotic factors were most important for generating this variability. The WCATW is a comprehensive database of all weed biological control programs worldwide, agent releases, and qualitative estimates of individual program control successes since the 1800s. From this database, I categorized programs by the biotic or abiotic factors that were likely to result in observed geographic variability in control success through their effects on agents, plants, or both. I further reviewed these factors and provided case studies to illustrate how each could contribute to control variability on a large-scale. I then discussed potential ecological and evolutionary outcomes of this variability.

In Chapter 3, I examined genetic and geographic variation in susceptibility of flowering rush to biotic resistance (i.e., negative interactions with the recipient community) in the form of generalist fungal pathogens (Harms et al. 2019). Spatial variation in the strength of biotic

resistance may arise because of genetic variability in flowering rush populations, different climates where the infestations occur, and resident communities over its introduced range (Maron and Vilà 2008, Beaury et al. 2019). In particular, future biological control agents may perform differently on plants of different lineages or in different parts of the range. I tested whether flowering rush cytotypes differed in disease attack and whether spatial (latitudinal, climate) patterns emerged for either cytotype. I surveyed 27 populations (17 triploid populations, 10 diploid populations) across the US range to document disease occurrence and, with a colleague, pathogen species associated with plants. For a subset of populations, we isolated and cultured pathogenic foliar fungi and then tested pathogenicity of three different isolates in laboratory (excised-leaf) assays. I report results of field and laboratory studies and discuss implications for future introductions of biological control for flowering rush.

Host plant quality can vary spatially and temporally, and may have dramatic effects on performance of biological control agents. In Chapter 4, I used a combination of field measurements and laboratory experiments to study the range of foliar nitrogen (N) that larvae of the AFB are exposed to in the field and its importance to larval development and density-dependent dispersal. I first assessed seasonal variability in foliar N at field sites spanning a large portion of the latitudinal range of AFB (southern to northern Louisiana) every 2-3 weeks during the growing season for four years. Then, in a series of laboratory experiments, alligatorweed foliar N was manipulated to examine its influence on larval development and survival (under different temperature regimes), adult biomass, and dispersal of the AFB. I addressed the question about whether foliar N, as a result of seasonal and spatial variation in temperature and plant nutrition at field sites, could contribute to observed variation in AFB efficacy in the field and have important effects on biological control of alligatorweed.

In Chapter 5, I studied alligatorweed biological control over four years across a climate gradient that encompasses a large portion of the latitudinal range of the AFB in the US. Climate change is expected to shift some species' distributions poleward but how interacting species (such as AFB and alligatorweed) may fare in the future is relatively understudied. Studying interacting organisms near their leading edges (i.e. at the range margin) may provide insight into the importance of biotic and abiotic factors on their distributions (e.g., spatial patterns of abundance, variability) (Fourcade and Öckinger 2016). These insights may be particularly relevant for biological control of weeds, in which host-specific herbivores or pathogens are introduced to suppress target weed populations. Because the geographic ranges of alligatorweed and the AFB incompletely overlap in the southeastern US, spatial heterogeneity in control is generated. I used this system to test whether latitudinal variation in alligatorweed abundance (higher in high latitudes) was best explained by the biological control agent's abundance (lower at high latitudes), phenology (date of first activity), or a combination of biological control and climate-driven weather variables (temperature, precipitation). I used structural equation modelling to analyze four years of data collected in Louisiana field sites to determine direct and indirect effects of latitude and weather on agent and host. The results of this study may assist other programs in which variable control is observed, especially where agent and host geographic distributions are not fully-overlapping and limiting environmental gradients are suspected.

Finally, in Chapter 6, I summarize and synthesize my major research findings and discuss their importance for biological control of weeds. Additionally, I present directions for future research that would further advance the biogeographical approach to biological control.

CHAPTER 2.
BIOGEOGRAPHY OF WEED BIOLOGICAL CONTROL: CAUSES AND CONSEQUENCES OF GEOGRAPHICAL VARIABILTY IN CONTROL SUCCESS

INTRODUCTION

Although biological control has a long history of documented successes (Room et al. 1981, Buckingham 1996a, Fowler et al. 2000, McFadyen 2000, Seastedt 2015), these are often overshadowed by rare but high-profile failures, including issues of non-target impacts (Louda et al. 2003, Pearson and Callaway 2003) or the lack of agent establishment (Cullen 1995, Baars 2003, Milan et al. 2006, Bean et al. 2007). The failure of agents to adequately reduce the abundances of target species has been linked to phenological, climatic, or genetic mismatches between agent and host, although the frequency of this outcome has been drastically reduced since modeling and molecular techniques have become more accessible during program development (Van Driesche et al. 2009, Yeates et al. 2012). For systems in which the weed has a broad distribution, a biogeographic research emphasis may provide a better understanding of spatial variability in past management success and more realistic expectations for future control across regions. This approach is similar to that employed in modern invasion biology (e.g., Sax et al. 2005, Pyšek and Richardson 2006, Wilson et al. 2009, Cronin et al. 2015) and could be valuable for studying systems in which the invader has been established for a long time before management, potentially with introductions from multiple source areas (or hybridization; Williams et al. 2005, Kwong et al. 2017a), leading to a predicament in decision-making about where to source effective agents (DeLoach et al. 2003, Van Driesche et al. 2009).

Variable outcomes in weed biological control programs may be attributed to limited establishment of agents due to poor release procedures (e.g., too few individuals released, an inadequate number of releases made, or poor spatial coverage of releases) (Grevstad 1999, Shea

and Possingham 2000, Lockwood et al. 2005), inadequate host plant quality (Van Hezewijk et al. 2008), Allée effects during establishment of the agent (Hopper and Roush 1993, Courchamp et al. 1999), genetic incompatibility of agents and hosts (Boughton and Pemberton 2011), competition with other established agents (Ehler and Hall 1982), dispersal limitations and variable spread of agents (Heimpel and Asplen 2011, Lake et al. 2018), novel associations with predators or disease (Goeden and Louda 1976, Christensen et al. 2011, Parys and Johnson 2012, Coon et al. 2014), or climate and related physiological limitations of the agents (Hill and Olckers 2000, Milan et al. 2006, Zalucki and Van Klinken 2006). Given the ecological complexity of reuniting natural enemies and their hosts with the aim of suppressing target populations in a novel range, it is perhaps surprising that so much success has been achieved in managing weeds with biological control (McFadyen 2000, Heimpel and Cock 2018). Nonetheless, geographic variability in weed control, particularly for those species with large distributions, may be expected due to differences in climatic requirements between agents and hosts and associated latitudinal or elevational gradients in biotic or abiotic factors.

Species' distributions reflect the biotic (e.g., predation, competition, symbioses) and abiotic (e.g., geology, climate) conditions they experience at varying spatial and temporal scales relative to their niche requirements (Brown 1995, Gaston and Blackburn 2008). When patterns of herbivore abundance and associated impacts reflect spatial variability in biotic or abiotic factors, weed populations may experience corresponding variability in enemy-release, leading to patterns in a number of plant traits (e.g., defensive chemistry, phenology or reproductive strategy) that reflect this variability (Rasmann and Agrawal 2011, Ågren et al. 2013, Cronin et al. 2015, Lehndal and Ågren 2015, Allen et al. 2017). These patterns may reflect predictable

environmental gradients (e.g., temperature) or less predictable variability (e.g., stochasticity in extinction-colonization dynamics).

Theory predicts plant trade-offs between growth and defense against herbivores or disease (i.e., growth-defense trade-offs), reflecting allocation of limited resources (Huot et al. 2014, Züst and Agrawal 2017). Differences in plant growth-defense tradeoffs between native and introduced weed populations are well known, thought to be an adaptive response to enemy-release in the introduced range (Pan et al. 2013), and forms the basis of a number of hypotheses that attempt to explain invader successes (e.g., Evolution of Increased Competitive Ability hypothesis; EICA; Blossey and Notzold 1995). However, there are also (potentially rapid) evolutionary consequences of spatial variability in the interactions between introduced biological control agent and host, such as generation of genetically-based plant chemical or structural defensive responses specific to biological control agents (Liu et al. 2018). Although spatial gradients in biological control and associated clines in target weed defenses are not widely reported, there is recent evidence of such patterns (Kollmann and Bañuelos 2004, Kambo and Kotanen 2014, Stastny and Sargent 2017, Xiao et al. 2019). It is unclear whether incomplete geographical overlap between agent and weed is common, or whether observed variability in control is due to predictable environmental variation, but it is probably quite common given that the ranges of monophagous herbivores must be contained within the range of their hosts (Gaston 2003). Nonetheless, spatial variability of biological control is rarely studied in a biogeographic framework (however, see Zalucki and Van Klinken 2006). In a rare example, Zulucki and Van Klinken (2006) demonstrated that predictions about agent population dynamics, geographic distribution, and potential spatial variability in establishment success could be made based on

data that are commonly collected during exploratory surveys or pre-release laboratory developmental studies of potential biological control agents.

The goal of this review is to identify causes and consequences of geographic variability of weed biological control and determine whether it is a commonly reported problem. We reviewed worldwide biological control programs through 2005 (The World Catalogue of Agents and Their Target Weeds; hereafter referred to as, 'the catalogue'; Winston et al. 2017) to determine which range-limiting factors explain variability in agent impacts. The catalogue is an extensive database of all weed biological control programs implemented around the world and includes information such as weed species, agent species, limiting factors (if known), non-target impacts (Hinz et al. 2019), country where releases were made, and the organization that conducted the releases (Winston et al. 2017). We address the following questions: 1) what proportion of weed biological control programs are geographically variable in control outcomes? 2) Do spatial patterns of control reflect biotic or abiotic factors? And, 3) which factors are responsible for generating variable success? We provide biological control case studies and discuss modern approaches used by biological control researchers to understand the biogeography of their systems. This type of approach can provide a useful framework for understanding past variability in success and give managers more realistic expectations in current and future programs.

MATERIALS AND METHODS

Review of the World Catalogue of Agents and Their Target Weeds

To determine whether geographic variability in weed biological control is common, we reviewed the catalogue for all biological control programs through the year 2005 in which variable or inconsistent impacts were reported (Winston et al. 2017). The WCBCW is a

comprehensive accounting of all weed biological control programs between the late 1800s and the present with curated information on program location, year, and the current status of the program (e.g., agent abundance, severity of impact, geographic scale of impact). The curators of the catalogue compiled information on release programs obtained through extensive literature review and expert interviews to determine the impact severity and geographic extent of control by introduced agents. We searched entries of intentional introductions of biological control agents (List #1) where general impact was reported as "variable", or the geographic extent of impact by introduced agents was "variable", "local", or "regional". From this list, we excluded newer (releases conducted after 2005) programs to allow time for establishment and assessment of impacts across the potential range. Although the 2005 cutoff is largely arbitrary, 10-15 years should be a sufficient time to allow for a determination of program success. Although we could determine the age of the program based on initial release dates, we could not estimate whether effort towards establishment was continuous or periodic. We also excluded those programs in which the geographic extent of damage was largely or wholly unknown. For each combination of weed, agent, and release country, we assessed factors associated with limiting the distribution of the agents. Although the curators of the catalogue had largely assigned important limiting factors to programs, we attempted to verify entries and further categorize them to one or more of distinct sub-factors. Our categories included: climate, habitat, genetic incompatibility with host, parasitism, predation, competition with other agents, and phenological asynchrony with hosts. For climate and habitat, we further assigned agents to the following categories: precipitation, temperature (climate); flooding, soil, moisture, and shade (habitat). We attempted to verify designations, but in many cases that was not possible because the original information was obtained through extensive interviews with control practitioners. Therefore, we used the

catalogue as the authority. We omitted duplicate entries if an agent was released multiple times (e.g., over several years, potentially as distinct biotypes) in the same country. Multiple introductions of the same agent in the same country are difficult to differentiate in the field, so we merged all instances of a weed-agent combination within a country into a single entry for examination. If multiple important factors were reported for an introduction, those are discussed separately. If a program has been reported as variable with regards to success, but reasons for variability were not previously reported, we reviewed the literature on the program to assess whether the information was currently available. After assigning programs to biotic or abiotic factor categories, we examined each program to determine whether data were available to make comparisons about the relative importance of each on control effectiveness. This ultimately proved to be fruitless, as many programs lack quantitative spatial data on control outcomes, so we discuss the outcome of the survey qualitatively.

Causes and Consequences of geographic variability

Based on the categories above, we reviewed the literature on the importance of each category for limiting the distribution of organisms. Although our focus is largely on regional-scale variation in success of biological control, we briefly discuss some factors that may limit efficacy at the local habitat scale, such as soil, shade, nutrients, etc.

RESULTS

Of the 1,014 combinations of agents and target plants reported in List #1 of the catalogue, 38% (380 combinations) are reportedly geographically variable in their impact and ultimate reduction in target weed populations. Of those, 39.5% were categorized as at least partly limited by abiotic factors, 27.1% partly by biotic factors (Table 1). Additionally, 55% of variable

programs reported *only* biotic (27%) or abiotic (28%) factors as important. A large proportion of programs (44.5%) did not have adequate information available to determine causes of variability. Unfortunately, it was not possible to identify whether geographic variability in biological control outcome was more common in some countries over others. The most programs reported with variable outcomes came from the countries with the most active biological control programs historically (i.e., the contiguous United States: 78 cases South Africa: 41 cases, Australia: 74 cases). Among the three countries with the most programs, 56%, 44%, and 44% of US, South African, and Australian programs, respectively, display geographic variation in control success. In the rest of the world (excluding those three countries), 36% of programs have reported variable outcomes. That the three most active countries have a greater proportion of variable programs is likely due to their large sizes relative to other countries, which may promote geographic variation in control due to biogeographic processes acting on agents and hosts across large areas.

Table 2.1. Results from review of the World Catalogue of Agents and Their Target Weeds to determine causes for variable effectiveness of introduced agents. For programs and agents in which variability was determined, we categorized the limiting factors by biotic and abiotic types. Programs could fall under more than one category. For the analysis, agents released in different countries or that were assessed on multiple target plants were considered separate programs.

Limiting factor	**No. of agents**	**Percent of *variable* programs**	**Percent of *all* programs**
Biotic	103	27.11%	10.16%
Predation	39	10.26%	3.85%
Parasitism	37	9.74%	3.65%
Disease	3	0.79%	0.30%
Competition with native spp.	0	0.00%	0.00%
Competition with other agents	18	4.74%	1.78%
Genetic incompatibility with host	21	5.53%	2.07%
Anthropomorphic disturbance	2	0.53%	0.20%

Phenological asynchrony		4	1.05%	0.39%
Abiotic		150	39.47%	14.79%
Climate		121	31.84%	11.93%
	Precipitation	69	18.16%	6.80%
	Temperature	53	13.95%	5.23%
Habitat		49	12.89%	4.83%
	Soil	3	0.79%	0.30%
	Wind	1	0.26%	0.10%
	Flooding	4	1.05%	0.39%
	Moisture	15	3.95%	1.48%
	Shade	16	4.21%	1.58%
	Nutrients	5	1.32%	0.49%
Effect on host*		17	4.47%	1.68%
Factors not known/ determined		169	44.47%	16.67%

*It is unclear from the literature whether the limiting factor acts directly on the agents or creates an environment where the agents are more effective because the host is impacted by the factor. These cases were not included in the other categories.

Abiotic factors

Climate- temperature

Climate provides perhaps the most important limitation on species' distribution directly (humidity/precipitation and temperature) or indirectly if host plants, competitors, or predators respond to climate in ways that ultimately impact control agent population dynamics (e.g., photoperiod or temperature-cued plant senescence in some areas but not others, outbreaks of predator or competitor species, etc.) (e.g., Crawley et al. 1986, Cullen 1995, Newman et al. 1998, Zalucki and Van Klinken 2006). Almost 14% of the management programs with variable success were attributed to temperature. Lower thermal limits restrict agent establishment and survival in a number of control programs where cold winters are common (Cowie et al. 2016). For example, the giant salvinia weevil, *Cyrtobagous salviniae* Calder and Sands, is not able to survive in temperate areas of the US, despite the presence of its host plant year-round (Mukherjee et al. 2014), requiring the annual release of agents. Likewise, the alligatorweed flea beetle, *Agasicles*

hygrophila Selman & Vogt, is restricted by cold winter temperatures to warm coastal areas in the southeastern US which leads to areas outside the overwintering range of *A. hygrophila* in which alligatorweed remains largely uncontrolled (Vogt et al. 1992, Harms and Shearer 2017). The ways in which biological control practitioners typically approach climate limitations on agents are multifold. Commonly, new native-range exploration is undertaken in climates that better match areas in the introduced range where agent abundance is low, with the intention to locate new genotypes (or species) of agents that are better adapted (Buckingham et al. 1983). For example, putatively cold-tolerant alligatorweed flea beetles collected in temperate South America were introduced in the US during the early 1980s in response to the overwintering temperature limitations experienced by established beetle populations (Buckingham and Boucias 1982b, Buckingham et al. 1983). Not typically undertaken, but potentially useful, are surveys in other parts of the introduced range to identify whether sufficient genetic variation exists in the biological control agent to encourage locally adapted populations (e.g., Reddy et al. 2019). Reddy et al. (2019) identified an introduced population of the water hyacinth weevil *Neochetina eichhorniae* Warner in Australia that performed better at cool temperatures than either introduced populations in California or native populations from Uruguay or Argentina. What is particularly interesting about this case is that the Australian introduction of *N. eichhorniae* was made from source populations in the US, which suggests there may be better cold-adapted *N. eichhorniae* populations already in the US.

Changing climate trajectories have impacts on species distributions through rising average temperatures, the variability of temperature extremes, and increased frequency of extreme weather events (Easterling et al. 2000b, Parmesan and Yohe 2003, Parmesan 2006). Host plant availability is generally not limiting for the distribution of biological control agents,

but if increasing global temperatures promote poleward shifts in the distribution patterns (i.e., location and abundance) of both agents and hosts, then unequal expansion rates may lead to increased importance of host-limitations for predicting agent occurrence. Although this has not been addressed explicitly for weed biological control agents and their hosts, there are a number of other systems in which phenological mismatches between herbivores and plants are likely to occur as a result of climate change (Tylianakis et al. 2008, Blois et al. 2013)

Climate- precipitation

Eighteen percent of programs that were deemed variable in the catalogue were affected by variation in precipitation. Precipitation can have limiting effects on introduced agents by changing local or regional humidity, host quality, or physical damage to agents (Moran and Hoffmann 1987, Dhileepan and McFadyen 2012). For example, South African populations of the highly successful cochineal cactus (*Opuntia stricta* (Haw.) Haw) agent, *Dactylopius opuntiae* (Cockerell), are reduced by heavy rainfall because the rain dislodges the immobile females and nymphs from plants at a time when the additional moisture encourages vigorous host growth (Paterson et al. 2011). Control by *D. opuntiae* resulted in 90% biomass reduction within a decade after introduction, but it is thought that the reduction would have occurred much more rapidly if not for a period of rainfall and flooding shortly after introduction (Paterson et al. 2011). In contrast, the rust *Puccinia abrupta* var. *partheniicola* was introduced for control of Parthenium weed (*Parthenium hysterophorus* L.) in Australia, but was only established in areas with sufficiently wet winters (Dhileepan and McFadyen 2012).

Habitat

Habitat type and quality (i.e., flooding, shading, moisture, nutrients, soil) may be important for biological control, particularly if it varies regionally. Variability of success in 13% of evaluated programs was at least partly attributed to habitat variation. For instance, the tansy ragwort (*Jacobea vulgaris* Gaertner) flea beetle, *Longitarsus flavicornis* (Stephens), cannot persist in areas prone to flooding (e.g., floodplain sites) due to high larval mortality (Potter et al. 2007). *Listronotus setosipennis* (Hustache), introduced for control of Parthenium weed in Australia, is more abundant on plants in areas with alluvial and black soils than clay and sand. As larval *L. setosipennis* mature, they move from feeding in the stem to roots, ultimately exiting to create a pupal chamber in the soil, which is thought to be the limiting feature of soil type (Dhileepan et al. 2018). Available nutrients may vary regionally and have large impacts on control agents' impacts on host plants. Although there are few examples where nutrients are thought to limit the distribution of agents, nutrients have direct effects on agents through altered life history traits and population dynamics (Room et al. 1989, Wheeler and Center 1997, Center and Dray Jr 2010, Uyi et al. 2016, Harms and Cronin 2019a), and indirect effects through moderation of host-plant defenses (Tomley 1990, Nybakken et al. 2018). Although not an example sourced from biological control, Bravo and Harms (2017) demonstrated geographic variability in sodium content in tropical fig species, highlighting the potential influence on herbivore populations.

Biotic factors

Biotic resistance (predation, parasitism, disease, competition)

Biotic resistance has received little attention for its role in determining the geographic distribution of introduced biological control agents but has been considered in a number of other systems, particularly in light of potential climate change impacts on trophic interactions (Van der Putten Wim et al. 2010, Louthan et al. 2015, Beaury et al. 2019). In 20% of variable programs (8% of *all* programs), support for the role of biotic resistance in generating variable control was evident. Strong biotic pressures (predation, competition) in the native range are often absent or much-reduced in the novel range during invasion (i.e., enemy-release) (Keane and Crawley 2002, Torchin and Mitchell 2004). Moreover, native predators or parasites should benefit from the potential food subsidy that comes with introduction of large numbers of biological control agents (Carlsson et al. 2009).

Acquisition of new predators or parasites is detrimental to some biological control programs, but evidence for their role in determining distributional boundaries of agents is lacking. For instance, a native acquired aquatic parasitoid (*Trichopria columbiana* Ashmead) exerts substantial pressure on the introduced hydrilla (*Hydrilla verticillata* L.f. Royle) biological control agents, *Hydrellia pakistanae* Deonier and *H. balciunasi* Bock in the US (Coon et al. 2014). Whether *T. columbiana* is partly responsible for restricting the geographical distribution of agents to the southern US is unclear and has so far not been tested (Grodowitz et al. 1997, Coon et al. 2014). *Trichopria columbiana* is broadly distributed in the northern US, associated with common native hosts, so may provide some resistance to northward range expansion of agents, though incompatibility between introduced *Hydrellia* spp. and northern hydrilla genotypes is more likely (see section below). Additionally, the native egg parasitoid,

Kolpolynema ema (Schauff and Grissell), parasitizes eggs of the water hyacinth planthopper (*Megamelus scutellaris* Berg) at field-measured rates up to 26% in the US, though parasitism in *M. scutellaris* rearing cultures was much higher (Minteer et al. 2016). As with *T. columbiana* and introduced *Hydrellia* spp., evidence is lacking for the influence of *K. ema* on *M. scutellaris* distribution. A number of generalist predators have been implicated in reducing impacts of biological control agents. For instance, the red imported fire ant (RIFA), *Solenopsis invicta* Buren (Dray et al. 2001), limits control of common salvinia (*Salvinia minima* Baker) in Louisiana by feeding on populations of the introduced biological control agent, *C. salviniae* (Parys and Johnson 2012). That RIFA limits geographic distribution of giant salvinia agents is unlikely though, because successful control of *S. minima* regularly occurs in the southern US, where densities of fire ants can be high (Morrison and Porter 2003). Predation by RIFA may act synergistically with climate to limit northern spread of *C. salviniae*, but this has not been tested. In another example of severe ant predation, the tamarisk (*Tamarix* spp.) leaf beetle *Diorhabda elongata* Brullé were heavily predated by ants (RIFA and native spp.) in Texas, limiting establishment only to some of the original release sites (Knutson and Campos 2019).

Perhaps understudied, but important, is the influence of interspecific competition with other herbivores on the performance and distribution of introduced biological control agents. In a recent example, Groenteman et al. (2007) found that after introduction of the nodding thistle (*Carduus nutans* L.) seed fly (*Urophora solstitialis* (L.)) in New Zealand, gall numbers were reduced by 46–93% when the earlier-established seed weevil *Rhinocyllus conicus* Frölich was present. In a simulation of nodding thistle growth rates under attack by one or both of the introduced agents, it was found that at high densities of both agents, nodding thistle growth rate would be 27% and 18% higher than when *U. solstitialis* or *R. conicus* occurred alone,

respectively (Groenteman et al. 2007). In a rare example of investigation into the compatibility of using insect and pathogen agents to control a weed, Ray and Hill (2016) found that heavy feeding by the introduced mirid, *Eccritotarsus catarinensis* (Carvalho), increased subsequent time for infection by the water hyacinth pathogen, *Acremonium zonatum* (Sawada) W. Gams. On melaleuca (*Melaleuca quinquenervia* (Cav.) S.F. Blake) in Florida, fitness of the rust fungus *Puccinia psidii* G. Winter and weevil *Oxyops vitiosa* Pascoe were reduced when both agents were present, with infection lowering larval survival and feeding by *O. vitiosa* reducing available leaves for infection by *P. psidii* (Rayamajhi et al. 2006).

However, interactions between agents can be complex. Marlin et al. (2013) found both negative and positive interactions between three introduced biological control agents on water hyacinth leading to variability in plant biomass reduction depending on the combination of agents. Although they did not approach their work through a geographic lens, there has been a fair amount of effort to better understand distributional patterns of water hyacinth insects relative to thermal physiology of the agents, given that some are more cold-hardy than others (Hill and Olckers 2000, May and Coetzee 2013). By connecting previous work on water hyacinth agent distributions (and abundance within their distributions) to interactions between agents, it is likely that field measurements would confirm that the importance of agent-agent interactions varies with location and agent abundance. This should be examined in the future and could provide valuable insight into geographic variation in control relative to agent-agent interactions. Furthermore, indirect competition between agents may occur through herbivore-mediated changes in plant chemistry (i.e., induced defenses). Because different natural enemy species (Liu et al. 2018) or guilds (i.e., chewing, piercing-sucking, pathogen) (Felton et al. 1994, Felton and Korth 2000) may induce different responses in their host plants, use of multiple agents in the

same feeding guild may be more likely to generate a stronger negative competitive interaction than agents in multiple guilds. With such sparse data available on interactions between introduced biological control agents, these types of studies should be commonly included in testing when additional agents are under consideration for release.

Genetic variability in host populations and agent-host incompatibilities

Incompatibilities between agents and hosts or genetic variability in host populations was implicated in nearly 6% of variable programs identified in the catalogue. Biological control agent performance may vary geographically because of spatial variation in the genetic makeup of host-plant populations. For example, *Hydrellia pakistanae* was released in the US for control of the aquatic weed hydrilla (Center et al. 1997), but has been mostly restricted to the southeastern US where a dioecious genotype of hydrilla occurs (Grodowitz et al. 2004, Grodowitz et al. 2010, True-Meadows et al. 2016). In the northeastern US, the fly has been unsuccessful in establishing and impacting hydrilla populations of a different, monoecious genotype (Grodowitz et al. 2010). The lack of establishment and impact to monoecious hydrilla has been suggested to result primarily from the overwintering habitat requirement (plant stems in the water column) of the agent rather than palatability (Dray and Center 1996) or climate differences, because *H. pakistanae* has been collected as far as N 46° in its native range (Deonier 1993). If the northern genotype expands southward, the distribution of *H. pakistanae* may shrink further if the two genotypes co-occur but the northern population is more successful because of differential impacts by the agent (i.e. through apparent competition, the negative impact of one species on another mediated by a shared predator or herbivore). The difference between hydrilla genotypes is the result of introductions from different source populations, but genetic differences between

some invasive plant populations in the introduced range may also result from evolutionary processes acting during or after the invasion process (e.g., genetic bottlenecks, hybridization).

Novel host plant genotypes may be formed during the invasion process through hybridization between different source populations or between introduced and native lineages or species (Ellstrand and Schierenbeck 2000, Lambertini et al. 2012, Moody et al. 2016). Hybrids represent novel genotypes which are absent from the native range of the invasive parent plant and complicate the process of biological control development (Moody et al. 2016). For instance, in the US both native and introduced *Myriophyllum* co-occur and have produced a number of hybrid offspring and backcrosses throughout their range (Borrowman et al. 2014, Moody et al. 2016). In addition to problems with predicting biological and ecological interactions between hybrids, their susceptibility to biological control is not always as would be predicted from tests with parental genotypes (Roley and Newman 2006, Borrowman et al. 2014). In the case of the native North American milfoil weevil, *Eurhychopsis lecontei* (Dietz), variable performance was found on the multiple new lineages of *Myriophyllum*. Additionally, the high level of genetic diversity in *Phragmites* populations in the US (Lambertini et al. 2012) and occurrence of native-invasive hybrids may complicate biological control agent development and lead to differences in impacts between populations when agents are introduced (Cronin et al. 2015). In addition to performance of the agent, variable control may result from genotypic differences in performance of the host plant that manifest through increased resource acquisition or growth rate (i.e. hybrid vigor) (Ellstrand and Schierenbeck 2000, Lee 2002).

Phenological asynchrony between agent and host

Although not commonly reported (approximately 1% of programs identified as variable), geographic differences in agent and host phenologies (i.e., the timing of important life history events) may limit control in some cases, particularly when the primary impact of the agent is related to seasonality or life stage of the host. For example, the tamarisk leaf beetle, *Diorhabda carinulata* (Desbrochers), was widely introduced into North America from China, but southern introductions in the US experienced day-length related premature diapause, which reduced their impacts on host trees (Bean et al. 2014). Subsequently, evolution of day-length diapause initiation was recorded; agents are now better-synchronized with their hosts in southern environments, and as a result efficacy of *D. carinulata* has increased (Bean et al. 2012). Phenologies of many organisms are expected to change under future climate regimes (Scranton and Amarasekare 2017, Chmura et al. 2019). If phenologies of interacting species (e.g., biological control agent and host) shift at different rates (Forrest 2016, Renner and Zohner 2018), there may be an increase in important periods of time where agent abundance is low relative to susceptible host stages. Additionally, some insects target reproductive structures of host plants which may only occur during a short window (e.g., flowers, seeds), and disruption of the agent-host synchrony may reduce the impact of the agent to nearly zero. On the other hand, climate change may lead to increased control of some species if the phenology of the host has evolved to lessen impact by herbivores. Although not an example from biological control, warming is expected to advance flowering in anemone (*Anemone trullifolia* var. *linearis* (Brühl) Hand.-Mazz.) but delay emergence of noctuid moth larvae (*Melanchra pisi* L.). These phenological shifts between host and herbivore are expected to generate drastically more herbivory on plants (100-fold higher damage in experiments) during a time that they would normally be mostly free

from herbivore impacts (Liu et al. 2011). Similarly, experimental elevation of CO_2 in a field trial led to an advance in the phenology (earlier flowering, faster seed head development) of the prairie invader *Centaurea diffusa* Lam., but damage by the introduced agent *Larinus minutus* Gyllenhal was higher under elevated CO_2 (Reeves et al. 2015). Based on this work, the authors suggested that *C. diffusa* and *L. minutus* phenologies would be better matched in future climates. Although the potential for climate change to disrupt phenological matching of agents and hosts has not received much attention to date, examination of invertebrate biological control has revealed that earlier and warmer springs is likely to reduce efficacy in some systems because of increased phenological mismatch between agent and host (Evans et al. 2013).

Anthropogenic disturbances

Management of weeds is often difficult to coordinate across large areas and jurisdictions. Thus, efforts in one area focused on release and establishment of biological control agents may be negatively impacted by management activities (or lack thereof) by other agencies in the same or adjacent areas. For instance, there has been considerable interest in the compatibility of using herbicides with weed biological control to successfully suppress water hyacinth in Florida, USA (Center et al. 1999). Because coordination between weed biological control practitioners and herbicide applicators may not be possible, Center et al. (1999) examined whether the two technologies were passively compatible and determined that they should be used in coordination, in a way that maintains the weed below damage levels but allows persistence of the agents. Similarly, roadside weed management of spotted knapweed (*Centaurea stoebe* sens. lat.) in Arkansas has been examined to determine whether mowing practices could be timed to lessen the negative impact on the seed weevil, *L. minutus* (Ferguson 2018). It was determined that

mowing causes massive larval mortality of *L. minutus*, but that earlier mowing (before first bud formation) is more compatible with biological control because spring floral resources are required for early-season egg development in *L. minutus* (Ferguson 2018). Similar to Center et al. (1999), Ferguson (2018) also recommends providing non-mowed refuge areas adjacent to mowed areas to allow long-term local persistence. Although to our knowledge there is no examination of geographic variability in anthropogenic disturbances and its role in biological control efficacy, there is the potential to examine this in the future. Timing of weed management by mowing or herbicides will largely be dependent on geographic location, so comparisons between managed areas, their interactions with other biotic or abiotic factors, and the role in success of biological control are possible.

Many target weeds are problematic in areas near agriculture and may be subject to drift of insecticides used for agricultural purposes. For example, insecticide drift from citrus orchards was implicated in the failure to establish *Trichapion lativentre* (Beguin Billecocq) on *Sesbania punicea* (Cav.) Benth. in South Africa (Hoffmann and Moran 1995). In a survey of alligatorweed biological control by the alligatorweed flea beetle in the southeastern US, Cofrancesco (1988) also mentioned pesticide use in adjacent agricultural areas as potentially limiting effectiveness of the introduced agents. Only a few examples are found in the literature, but unintended drift of agricultural pesticides should be further examined for impacts on biological control successes. In particular, investigation into establishment of agents and control successes in proximity to agricultural pesticide use may provide insights into some control failures. Although less than 1% of all programs reviewed in the catalogue were thought to be impacted by anthropogenic disturbances such as pesticide application or mowing, it is possible that such disturbances are more important than realized but are not often identified.

DISCUSSION

Consequences of geographic variability

Incomplete geographic overlap between agent and host

The primary consequence of variability across an agent's range is the inability to accurately predict outcomes of introductions, at least early in release programs. As control programs mature, practitioners learn the types of habitats that support populations of the agents. Incomplete geographical overlap occurs when different factors act on agent and host to delineate their distributions, or the same factors are differentially limiting to agent and host plant. This generates a pattern in which the agent is much more localized than the host. Although biological control is often seen as a solution to the problem of "enemy-release" (Keane and Crawley 2002), when agent abundance is limited within the larger host distribution, enemy-free space may be locally or regionally maintained (Lu et al. 2013). Additionally, if enemy-release occurs as a gradient (due to corresponding climate or other limiting gradients on agents), then control of target weeds could be expected to follow a similar local or regional pattern. For example, abundance of the tamarisk biological control agent *D. carinulata* now reflects a latitudinal gradient in the western US because of rapid adaptive response to photoperiod and critical day length requirements for diapause induction (Bean et al. 2007, Bean et al. 2014). How this gradient in agent abundance affects control has not been quantified, but it is thought that agent efficacy should also correlate with the latitudinal gradient (Bean et al. 2014).

Evolutionary consequences of complete or partial enemy-release (e.g., increased competitive ability, reduced constitutive defenses) are frequently observed in introduced plant populations (Blossey and Notzold 1995, Maron et al. 2004, Zou et al. 2008, Bhattarai et al. 2017a, Lin et al. 2019). Adaptive effects of enemy-release may be observed as increased growth rates or altered defensive chemistry relative to native populations. In multiple introduced ranges

(Australia, New Zealand, North America), tansy ragwort was found to have evolved increased photosynthetic rate, reduced carbohydrate storage, and increased tolerance to generalist herbivory over native European populations (Lin et al. 2019). When incomplete geographical overlap occurs after introduction of biological control agents in the introduced range, these processes may continue in marginal and extra-marginal populations, leading to further divergence between introduced plant populations or between introduced and source populations. In one of the few reported examples of this type of post-biological control evolution in target weeds, purple loosestrife (*Lythrum salicaria* L.) in North America has evolved increased defense against herbivory in populations that have been historically subject to biological control versus those that have not been exposed to biological control agents (Stastny and Sargent 2017). Should agents be sourced for use in these areas without current biological control impacts, the relative effect of agents should be high because of lower levels of resistance in plant populations.

Current biogeographical practices for developing biological control agents

The process of biological control agent development has greatly matured since early introductions and now uses criteria that promote safety and cost-effectiveness. In particular, the selection process now includes, in addition to lengthy and phylogenetically-informed host-specificity testing, climate-matching coupled with molecular matching of target species to ensure potential agents will be preadapted to conditions where the host occurs (Goolsby et al. 2006, Van Driesche et al. 2009).

The foundation of biological control programs is in the exploration of areas within the native range of target plants to identify and prioritize damaging natural enemies (Goolsby et al. 2006). However, given the constraints on distributions of natural enemies and hosts, and that

abundances and associated impacts may vary along limiting gradients, it is useful to consider the location of surveys along such gradients. For example, observations of potential agents near their native range boundary may suggest they are unsuitable as control agents in certain portions of the introduced range. However, it may also be that multiple locally-adapted agent genotypes could be sourced in the native range for use in the introduced range, if the introduced range is large and environmentally variable. That limiting gradients are unknown during initial surveys is unavoidable and illustrates the importance of further investigations into native range biology of the agents and plants to understand the position of collection locations along limiting gradients.

Opportunities for research using biological control systems

Abundance distribution patterns and range margin dynamics

Invasion biologists conduct extensive examinations of large-scale plant invasions and their implications for management (e.g., Gaskin et al. 2013, Ordonez and Olff 2013, Cronin et al. 2015, Lu et al. 2018, Lu et al. 2019). Examination of species' distributions and the variables constraining them hold particular value for biological control programs because the geographical limits of an agent (and agent abundance within those limits) determines where and what magnitude of control may be expected to occur. A fruitful area of research might be to use distributional hypotheses such as the Abundant Center Distribution (ACD) to examine patterns of agent and target weed abundance across a large area. Sagarin and Gaines (2002) reviewed tests of the ACD and generated a list of testable hypotheses, many of which would be suitable for testing using biological control systems. Moreover, the following hypotheses (adapted from Table 1 *in* Sagarin and Gaines 2002, and others) would be of particular value for advancing range margin ecology and biological control:

1. Sites near the range edge of biological control agents will see gradual population changes as climate change shifts species' ranges.
2. Edge populations of biological control agents are genetically distinct and may promote local adaptation of control agents to marginal environments.
3. Gene flow into marginal populations will have negative impact on biocontrol success. In contrast to #2, gene flow from interior (central) to marginal (edge) populations may limit biological control agent adaptation to marginal environments through genetic swamping.
4. Gene flow into marginal biological control agent populations will be beneficial to counteract negative effects of inbreeding depression.
5. Edge population dynamics will be more variable than interior population dynamics. Because the climate will be more extreme at the edge (relative to agent's limits), and thus abiotic factors will be more important to biocontrol success, variability in abiotic factors may promote unstable agent-host interactions in marginal populations.
6. Extinctions in biological control agent populations are more likely at edges and therefore biological control will be less successful in marginal environments.
7. Outbreak dynamics will be, on average, more likely in interior populations. However, as the geographic area representing the range center shifts or expands, biological control agent outbreaks may be more common and occur in new places, leading to increased temporary control in marginal areas.
8. Competition will be more/less important at range edges. If multiple biological control agents are introduced or generalist herbivores are common, stress on agents corresponding to marginal environments coupled with competitive interactions may lessen biological control effect (competition more important). In contrast, competition in

marginal populations may be reduced because marginal populations of agents are expected to be small (competition less important).

9. A stochastic event at a range edge will have a greater impact than at a range center. Because a marginal environment is already at the extreme of the agent's niche limit, random pulses of stress or disturbance will have a greater relative effect on agent demography in marginal than in interior populations.

Tests of these hypotheses could help to understand the ways in which biological control agent abundance varies across geographic ranges and the potential for range expansion in the future. For instance, genetically distinct edge/marginal populations (hypothesis #2) (Pironon et al. 2017) may have greater adaptive potential to future climate change and other extreme events (Lesica and Allendorf 1995). However, whether marginal populations remain genetically isolated may depend on the agent, with strong-dispersing agents promoting gene flow from interior to marginal populations and limiting opportunities for local adaptation to marginal environments (hypothesis #3). On the other hand, in genetically depauperate marginal populations (such as those undergoing multiple genetic bottlenecks during agent development and introduction), gene flow from other areas may enhance adaptive potential and reduce negative effects of inbreeding depression (hypothesis #4) (Sexton et al. 2011). Because the basis of these hypotheses is that abundance will depend to one degree or another on environmental gradients and connectedness of agent and plant populations, a combined metapopulation and population growth-modelling approach may be useful to predict agent and host occurrence and abundance along environmental gradients (Gotelli and Kelley 1993) in marginal areas (Öckinger 2006). This may be particularly valuable to further explain and refine predictions about control successes in those areas under future climates.

Climate change will produce range shifts of organisms, with many species extending their ranges poleward (Hickling et al. 2006, Mason et al. 2015). Although a number of authors have considered the effects of a changing climate on limits of species distributions, biological control systems offer a relatively simple opportunity to study plant-herbivore interactions in which the range boundaries of species are potentially shifting at different rates (Chen et al. 2011). Additional to increasing mean temperatures, the effects of climate variability will likely provide the largest challenge to biological control practitioners when predicting agent ranges in the future because it is temperature extremes, rather than means, that best define many species range boundaries (Lynch et al. 2014, Ma et al. 2018). Perhaps greater mechanistic focus should be placed on biotic or abiotic variables when developing agents to better understand potential distributions once introduced.

Biological control systems are typically thought to be simple (i.e., few interacting species). For that reason, they can be good model systems for addressing basic ecological and evolutionary hypotheses surrounding plant invasions and trophic interactions. Additionally, implementation of biological control often occurs once the target plant has spread and reached damaging levels in the introduced range. When targeted weed populations occur across a large geographic area, variability in control success may be expected due to differential limitations on the weed and introduced biological control agents. Since effectiveness of biological control relies heavily on the successful establishment, population build-up, and subsequent impact on the target weed, factors that contribute to geographic variation in these items are important to understand. Here we presented a review of biotic and abiotic factors implicated in variability of weed biological control efficacy and found that although climate was most commonly reported, many studies had insufficient information to determine limitations. Also, although limiting

gradients (e.g., winter severity) are certainly present in many instances, there are few studies that explicitly address their effects on agents and subsequent control of the target weed. We recommend a biogeographical approach to weed biological control. Although biological control (and invasion biology) are inherently biogeographical fields of study (Wilson et al. 2009), and this approach is not new (Zalucki and Van Klinken 2006), explicit incorporation of geographical variability into biological control programs may increase the understanding of why agents perform better in some areas but not others, encourage more accurate modeling of species distributions and abundance in introduced areas, and contribute to the broader ecological and evolutionary literature.

CHAPTER 3.
GEOGRAPHIC AND GENETIC VARIATION IN SUSCEPTIBILITY OF *BUTOMUS UMBELLATUS* TO FOLIAR FUNGAL PATHOGENS[1]

INTRODUCTION

Investigations into large-scale patterns of plant invasions are important for understanding variable impacts by invaders in the introduced range and for predicting potential management outcomes (Gaskin et al. 2013, He and Rocchini 2013, Ordonez and Olff 2013, Cronin et al. 2015, Allen et al. 2017). Such spatial variation in invader impacts may be the result of differences in local-site characteristics (e.g., community composition or structure, soil type, resource availability), regional differences in climate or biotic limiting factors (e.g., novel predators or competitors; Wolfe et al. 2004, Schaffner et al. 2011, Stricker et al. 2016b), or genetically-based variation in key invader traits (e.g., dispersal, competitive ability, enemy resistance; Rejmanek and Richardson 1996, Richardson and Pyšek 2006, Maron and Vilà 2008, Van Kleunen et al. 2010b). In particular, geographic (e.g., latitudinal) differences in community resistance to invaders (i.e., biotic resistance) or susceptibility of the invader to novel enemies may be expected when the invaded range is very large (i.e., continental; Maron and Vilà 2008, Cronin et al. 2015). Biotic resistance thus represents a spatially variable ecosystem service through prevention of establishment and consequent negative impacts by plant invaders (Levine et al. 2004). Although examinations of its role in invasion success often take place at the local or regional scale, ignoring possible geographic variation in the strength of biotic resistance, a number of authors

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have taken a larger-scale approach (DeRivera et al. 2005, Parker et al. 2006, Freestone et al. 2013, Cronin et al. 2015, Allen et al. 2017, Castillo et al. 2018).

Geographic variation in biotic resistance or invader success may result from genetic variation within the invading plant species that is spatially heterogeneous. Spatial variability in propagule pressure, or founder or bottleneck processes during plant introduction and establishment (Sax et al. 2005) may lead to multiple introduced genotypes that do not interbreed or have limited gene flow between them. Cryptic invasions involving multiple genotypes have been reported in a number of instances (Saltonstall 2002, Mukherjee et al. 2012, Burrell et al. 2015, Tano et al. 2015, Morais and Reichard 2018). An example is the aquatic invasive plant, *Hydrilla verticillata* (L.f.) Royle which was introduced at least twice into the U.S during the 20th century, resulting in two widespread ecologically and genetically distinct haplotypes which vary in their response to introduced biological controls (Madeira et al. 2004, Grodowitz et al. 2010). Several studies have demonstrated that enemy release or biotic resistance can vary considerably among genotypes of the same plant species in the introduced range (Maron and Vilà 2008, Cronin et al. 2015, terHorst and Lau 2015, Allen et al. 2017). However, most studies examining these differences focused on generalist herbivores (e.g., Siemann and Rogers 2003, Cronin et al. 2015, Liu et al. 2018), specialist herbivores (e.g., Garcia-Rossi et al. 2003, Maron and Vilà 2008, Liu et al. 2018), or specialist pathogens (Burdon et al. 1981) in the introduced range, with limited examination of generalist pathogens (Maron and Vilà 2008). Additionally, there is evidence that intraspecific variation in chromosome number (i.e. ploidy) may generate patterns in which increased ploidy leads to broader environmental tolerances through an enhanced adaptive potential, creating a more invasive phenotype (Levin 1983, Pandit et al. 2011, Hahn et al. 2012, te Beest et al. 2012, Hao et al. 2013). For instance, increased chromosome numbers in the genus

Leucanthemum resulted in greater resistance to herbivory by a specialist insect (Stutz et al. 2016). However, historical interactions with herbivores and local adaptation may be more important than ploidy in other cases (Meyerson et al. 2016). The importance of ploidy in plant invasions for structuring associated herbivore communities and influencing herbivore performance has received some attention (Pandit et al. 2014), but still relatively little is known about the role of plant genetic variation in invasive plant - disease dynamics. For an invading species with multiple ploidy levels in the invaded range, higher chromosome number is expected to produce disease-resistant phenotypes if increased ploidy is associated with higher allelic diversity at, or increased expression of, immune genes (Oswald and Nuismer 2007, King et al. 2012).

An ideal study system for investigating geographic and genetic variation in pathogen susceptibility in a plant invasion is *Butomus umbellatus* L. (Butomaceae; flowering rush), an invasive wetland plant of Eurasian origin. In North America, *B. umbellatus* populations are either diploid or triploid (Kliber et al. 2005). Populations in the Northwest, upper Midwest, and far northeastern US constitutes the widespread triploid cytotype (genotype 1; G1), whereas a diploid cytotype (genotype 4; G4) occurs primarily in the Northeast and Great Lakes region (Lui et al. 2005). In total, seven AFLP genotypes (G1, G2, G3, G4, G5, G6, G9) have been documented thus far in North America (Gaskin, unpublished data). Other than G1 and G4, other introduced genotypes are exceedingly rare, many only identified from a single location. Although G1 and G4 plants have not been documented to co-occur at the same location, they are sympatric in the upper Midwest. As management tools are developed for *B. umbellatus* in the US, it is necessary to better understand the importance of genetic variability on factors that limit plant performance. Because biological control agents are under development currently in Europe, a better

understanding of the variability of plant response to natural enemies would increase likelihood of using future agents effectively, potentially by allowing managers to choose agents based on host genotype.

We conducted field and laboratory studies of the geographic and genetic variation in susceptibility of *B. umbellatus* to foliar fungal pathogens. Over three years, we surveyed disease incidence in populations of the two common genotypes, representing both cytotypes, across the US distribution and tested for differences in genotype resistance in a laboratory experiment. If a difference exists between genotypes, success of one invasive genotype over the other in areas where they geographically overlap may occur during future control operations. We tested the following hypotheses: 1) Latitudinal clines in disease exist for common *B. umbellatus* genotypes. 2) Triploid G1 would be more resistant to disease in both field and greenhouse studies due to increased ploidy. Because disease symptoms may be the result of infection by multiple agents, we tested whether 3) pathogen richness was greater on diploid G4 plants and differed spatially with latitude or climate. We predicted that, in both field and laboratory studies, genotype G1 would be significantly more resistant to disease than G4 due to higher ploidy.

MATERIALS AND METHODS

Study system

Butomus umbellatus is an introduced wetland monocot, first documented in North America in the Saint Lawrence River in the late 1800s (Knowlton 1923). The earliest U.S. populations were reported from River Rouge, MI in 1918 (Anderson et al. 1974) and subsequently throughout the Great Lakes region (Witmer 1964). Although present for nearly 100 years, spread of *B. umbellatus* has been limited mostly northward from the Great Lakes into

Canada, with the southernmost record in Colorado (Bargeron and Moorhead 2007). Infestations now persist across the northern tier of the U.S. and evidence for multiple introductions from separate source areas is strong (Anderson et al. 1974). Spread is primarily clonal, but reproductive ability varies between sexual (diploid plants) and vegetative (diploid and triploid plants) forms (Eckert et al. 2000, Eckert et al. 2003). Although populations in the native European range are thought to be mostly triploid, in North America the diploid G4 cytotype is most common (Kliber et al. 2005).

Field survey- disease

To examine whether there were geographic and genotype differences in frequency of disease, we surveyed 27 *B. umbellatus* populations (17 G1 and 10 G4 populations; Figure 3.1; Appendix A3.1) during mid-June to early September over three years (2014-2016). Sites were located by a variety of means, including internet database searches (e.g., www.eddmaps.org), consultation with state personnel (Minnesota Department of Natural Resources, Washington State Department of Ecology), and chance encounters during transit. Surveyed sites spanned approximately 9 degrees latitude (~1000 km). At each site, leaf tissues were collected from at least 10 plants separated by ~1 m for genotyping by amplified fragment length polymorphism polymerase chain reaction (AFLP-PCR). Ploidy determination was made by flow cytometry (e.g., Delaat et al. 1987, Bohanec 2003). Previous results confirmed that plants within sites were the same genotype (i.e., we have not detected multiple co-occurring genotypes in our sites; J. Gaskin, unpublished data). Sites were sampled between mid-July and early-September.

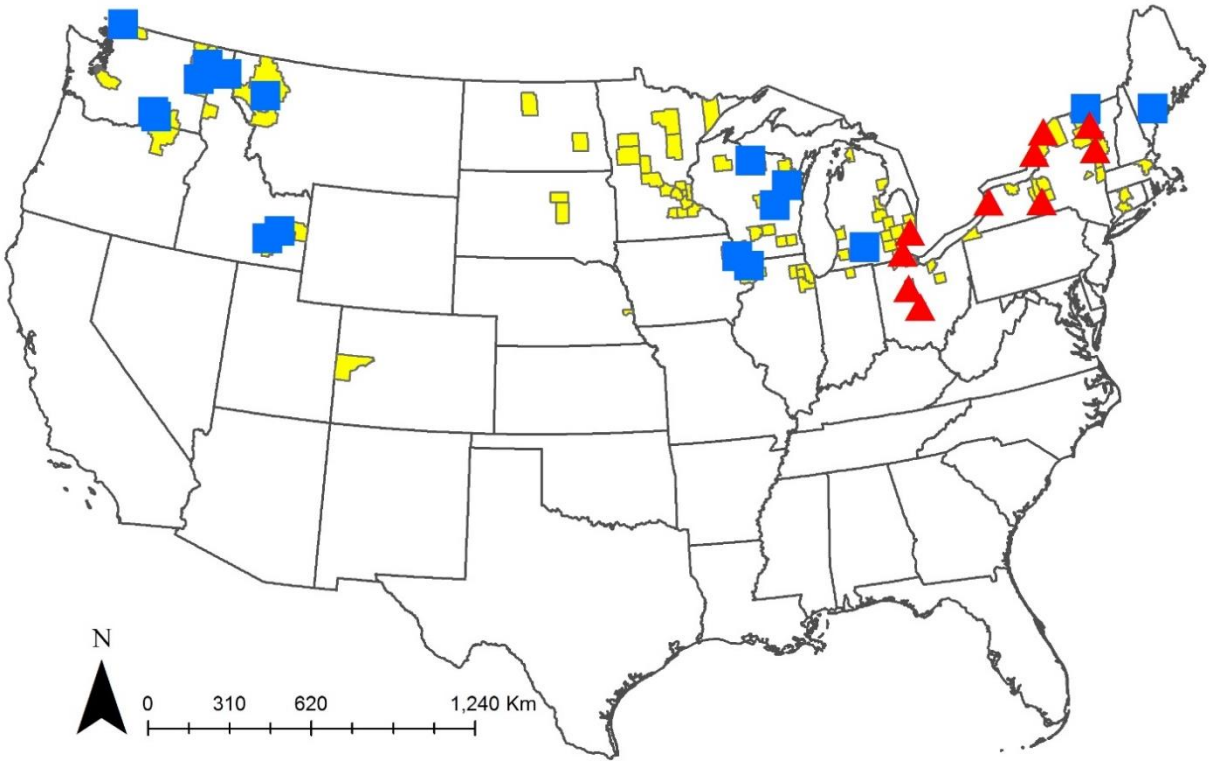

Figure 3.1. Map of *B. umbellatus* census sites. Blue squares represent sites with the triploid genotype G1 and red triangles are sites with the diploid genotype G4. Counties where B. umbellatus has been recorded are yellow (EDDMaps.org).

Sampling and examination protocols were as described in Harms and Shearer (2015). At each site, 20 whole *B. umbellatus* ramets were excavated for examination and damage assessment. Ramets were collected by hand on shore, by wading in shallow water, or from a boat. Care was taken to sample separate plants, though in some instances plants may have been connected underground. Within 12 hours of sampling, plants were examined with a hand lens and presence or absence of disease symptoms (leaf lesions, discoloration or distinct leaf spots) (Harms and Shearer 2015) was recorded. For each site, we determined the proportion of sampled plants that displayed disease symptoms.

Field survey- fungal richness

To determine whether fungal richness varied with *B. umbellatus* genotype or environment, during 2016, we collected diseased leaf material. At 18 sites (9 G1 and 9 G4), we excised approximately 5 cm leaf sections from five plants per site. Leaves were kept refrigerated and were processed in the laboratory within five days of collection. Sections of tissue were surface sterilized in 10% bleach for one minute then rinsed in sterile water. The sections were subsequently inserted into slits cut into Martin's Agar (Martin 1950) plates and incubated in the dark at room temperature (20-22 C^o) for one week. Fungal isolates that emerged from the tissues were transferred to Potato Dextrose Agar (PDA) and Corn Meal Agar (Difco, Detroit MI) slants for preservation. They were also plated onto PDA and Potato Carrot Agar (Dhingra and Sinclair 1995) for identification purposes. Isolates were identified using morphological characteristics and taxonomic literature (Ellis 1971, Domsch et al. 1980, Weir et al. 2012). Using literature reports, we categorized each fungal species as pathogenic or not. In some cases, the literature was ambiguous (i.e., a species may be a facultative pathogen). In cases where we suspected the species was not pathogenic, or only sometimes pathogenic, we categorized it as nonpathogenic. For some taxa, we were unable to obtain satisfactory taxonomic resolution (e.g., a number of Dematiaceous or Moniliaceous Ascomycetes) so made no determination on their pathogenicity. With categorization of isolates, pathogen richness was determined for each site and compared between genotypes.

Climatic environmental data

Climate data for surveyed locations were extracted from the first three principal components (PCs) of the 35 bioclimatic variables in the CliMond 1975H dataset (Kriticos et al.

2014). The three PCs differ in the influence of various climate variables, with PC1 (Bio36) being primarily a temperature variable, PC2 (Bio37) a wetness index, and PC3 (Bio38) a dryness index (Kriticos et al. 2014). We used these PCs to obtain climate information for each survey location and as potential explanatory variables in statistical model selection below.

Leaf infection experiment

From fungal species isolated during 2016, we experimentally tested whether G1 plants were more resistant to infection by fungal pathogens than G4 plants. Plants used in this experiment were field-collected in 2016 and propagated repeatedly at the Engineer Research and Development Center (ERDC), Vicksburg, MS until July 2017. Plants were initially grown outdoors in commercially-available topsoil supplemented with Osmocote® slow release fertilizer (15-9-2; Scotts Miracle-Gro, Marysville, OH). After a season of growth, main rhizomes or rhizome branches were split into ~3 cm pieces and planted into new topsoil. Diploid plants produce relatively little rhizome material so in addition to rhizome splitting, we planted the corm-like bulbils. This procedure was repeated 2 times over the course of one year to reduce maternal effects (Roach and Wulff 1987).

For each genotype, we used four replicate populations from our garden (Table 1), chosen because sufficient plant material was available for the experiment. Plants were grown in two shallow tanks in a greenhouse for six weeks before the experiment. Charcoal-filtered water was delivered from the local municipal water supply and maintained at 5 cm below the sediment surface prior to the experiment.

Table3.1. *Butomus umbellatus* populations used in this study.

Ploid	Genotype (G)	Population	Latitude	Longitude
Triploid	1	Rose Pond, ID	43.247	-112.315
Triploid	1	Yakima River, WA	46.379	-119.431
Triploid	1	Flathead Lake, MT	47.697	-114.071
Triploid	1	Pend Oreille River, ID	48.362	-117.285
Diploid	4	Kildeer Pond, OH	40.709	-83.369
Diploid	4	Point Rosa Marsh, MI	42.576	-82.805
Diploid	4	Unity Island, NY	42.934	-78.9084
Diploid	4	Oswegatchie River, NY	44.69	-75.495

To test for differences between genotypes in resistance to foliar pathogens, we conducted an excised-leaf assay. This type of assay has been validated in other pathogen-plant systems (including Colletotrichum sublineolum P. Henn. on sorghum and Alternaria solani (Ell. and Mart.) Jones and Grout. on potatoes; Bussey and Stevenson 1991, Pratt 1996) and comparisons with traditional greenhouse whole-plant assays are consistently similar (Prom et al. 2015). In addition to the excised leaf experiment, we conducted a smaller whole-plant experiment which generated similar results (Online Resource 1), but report only the excised-leaf experiment here. We inoculated leaves with one of three plant fungal pathogens, *Plectosphaerella cucumerina* Kleb., *Colletotrichum fioriniae* Marcelino & Gouli ex R.G. Shivas & Y.P. Tan, and the ubiquitous *Alternaria alternata* (Fr.) Keissl. These fungal species were chosen because they have previously been reported as plant pathogens (Uecker 1993, Agrios 2015). *Plectosphaerella cucumerina* was present in three G1 and seven G4 sites from the northeastern to northwestern USA during our surveys; *C. fioriniae* was identified from two G4 and a single G3 site in the northeastern and upper Midwestern US and *A. alternata* from all surveyed sites. Fungal species were isolated from G4 plants at Kildeer Pond, OH (*P. cucumerina* and *A. alternata*) and G3

plants in Springbrook Pond, IL (*C. fioriniae*), then cultured in bulk for this experiment using previously reported methods (Appendix B). A potential drawback of using pathogens isolated from G4 plants (*P. cucumerina* and *A. alternata*) is that they are adapted to that genotype. Colony forming units (CFUs) for all isolates were 1 x 10^5 CFUs. Leaf sections (10 cm) were cut from culture plants and randomly assigned to one of three pathogen treatments: *A. alternata*, *C. fioriniae*, or *P. cucumerina*. Leaf pieces were lightly abraded with 200 grit sandpaper then placed on water agar in petri dishes (six per treatment combination). Previously prepared inoculum (100 µl) was applied to the abraded leaf area. Petri dishes were covered and left on the benchtop at room temperature (~23 °C) for 48 hr. After 48 hr, petri dish lids were removed and leaf photographs were taken with a Nikon D60 digital camera. Photographs were imported into ImageJ image processing software (Rasband 2016) then lesion area (mm^2) and damage score were determined.

The damage score used here is similar to that previously applied by Shearer et al. (2011) and is a qualitative assessment of leaf condition on an ordinal scale (Table 2). We defined the damage scale so that levels of damage were approximately equally-spaced on the scale. The same observer (NEH) made all damage assessments. A higher damage rating represents lower resistance to infection. Examples of infected leaves assigned to various scores are provided in Online Resource 1.

Table 3.2. Damage rating applied to infected *B. umbellatus* leaves.

Damage Rating	Description
0	Green and healthy leaf, no signs of disease
1	Small lesion
2	Distinct larger lesion, local discoloration
3	Definite disease symptoms/ lesions, widespread discoloration
4	Entire leaf dead or collapsed

Statistical Analyses

Field survey- disease

To test whether there were latitudinal gradients in the proportion of infected *B. umbellatus* plants and whether they differed with plant genotype or climate, we used a generalized linear model with beta error distribution and log-link function. Proportion of diseased plants was the dependent variable in the model; genotype (G), latitude (L), the genotype by latitude (GxL) interaction, and each of the three bioclimatic PCs (PC1, PC2, PC3) were included as predictors. The interaction between genotype and latitude was included in the model because nonparallel gradients in species interactions can result from genotype-specific differences in disease or herbivore resistance across latitudes (e.g., Cronin et al. 2015). Although in some cases longitude is an appropriate spatial predictor variable, in this case, longitude and PC3 were highly correlated ($r = 0.91$), and so longitude was removed from the analysis. Sampling occurred over multiple years with nine sites out of 28 sampled during two years. To account for this, Year (Y) was included in the model as a random effect (Kwong et al. 2017b).

Additionally, we used Akaike information criterion adjusted for small sample size (AICc) to select the most informative model (Burnham and Anderson 2003). Candidate models were constructed from the full model (G, L, PC1, PC2, PC3, GxL) with the constraint that interaction term was included only if their main effects also were in the model. ΔAICc was calculated as the difference between the top model and all others. Models with $\Delta AICc \leq 2$ were considered to have substantial support (Burnham and Anderson 2003). Akaike weights are also reported, which represent the relative likelihood that the model is the best given the data and other candidate models. Finally, if the best-supported model contained an interaction term, separate linear models were performed for each genotype (Allen et al. 2017).

Field survey- fungal richness

Similar to overall disease frequency, we were interested in whether variation in pathogen richness could best be explained by genetic (genotype), spatial (latitude) or climatic differences among sites. Therefore, we used a general linear model with pathogen richness as the dependent variable and genotype, latitude, and genotype x latitude as main effects and bioclimatic PCs and latitude as covariates. Our pathogen survey was conducted over a single season (summer 2016), so Year was not included in the model. We used the same model selection procedure as outlined above to identify the best model. We surveyed nine populations each of G1 and G4 (Appendix 3) for fungi. In order to achieve normality and homogeneity of variances, pathogen richness values were natural log (+0.05) transformed prior to analysis.

Leaf infection experiment

We did not have enough common garden populations to provide a rigorous test for genetic-based latitudinal clines in disease. Therefore, in laboratory trials we tested only for genetic differences in disease resistance. We predicted that our results would support field observations that G4 plants are more susceptible to disease than G1 plants. As such, the former genotype was predicted to have greater lesion size and higher damage score than the latter genotype. To test for differences in lesion size (excised-leaf experiment) we used generalized linear models with normal distribution and log-link function. In models, genotype was a fixed effect and population was a random effect to account for the nesting of populations within a genotype (Bhattarai et al 2017). To test whether disease rating was higher in G4 plants, we used generalized linear mixed models with multinomial error distribution and cumulative logit link function (Gbur et al. 2012). In the excised-leaf disease rating model, population within genotype

was random and genotype was a fixed effect. Separate models were used for each pathogen species in both experiments. Additionally, because we used two pathogens (*A. alternata*, *P. cucumerina*) originally isolated from a diploid G4 population (Kildeer Pond) in our experiment, we conducted a comparison of damage rating and lesion size between diploid populations only. A difference in damage rating or lesion size between plants from Kildeer Pond and other diploid populations might suggest local adaptation in these fungal pathogens to *B. umbellatus* populations. As above, to test for differences in damage rating between diploid populations, we used a generalized linear model with multinomial error distribution and cumulative logit link function with population as a fixed effect. To test for differences in lesion size, we used generalized linear models with normal distribution and log-link function and population was a fixed effect. As above, separate models were used for each pathogen.

All statistical analyses were performed in Statistica version 12 (Statsoft Inc, Tulsa, Oklahoma) or SAS version 9.4 (SAS Institute, Cary, North Carolina).

RESULTS

Field surveys- disease incidence

Across the northern tier of the US, latitude, genotype, and climate (separate from latitude) influenced patterns of disease incidence in *B. umbellatus*. Specifically, variation in the proportion of plants with disease symptoms was equally explained by two top candidate models which included genotype, latitude, the genotype x latitude interaction and either the temperature (PC1; adj. $R^2 = 0.48$, AICc = -9.61, Akaike weight = 0.26; Table 3) or wetness (PC2; adj. $R^2 = 0.45$, AICc = -9.69, Akaike weight = 0.27) principal component. Regarding our first hypothesis that latitude would influence disease occurrence, the relationship was nonparallel between

genotypes (Figure 3.2; GxL: $F = 6.74$, $P = 0.02$). Separate models for each genotype detected a significant relationship between latitude and disease for G1 but not G4 populations (G1: $F = 6.31$, $P = 0.02$; G4: $F = 1.4$, $P = 0.27$). Our second hypothesis, that triploid G1 plants would display less disease incidence than G4 plants, was confirmed. The proportion of plants with disease symptoms was nearly double for G4 (0.75 ± 0.1, mean ± SE) than G1 (0.39 ± 0.05) plants after accounting for effects of latitude and climate in the top model (Figure 3.2). Bioclimatic variables were influential in five out of the top six models. In the top model, PC2 (wetness index) was significantly influential (df = 1, F = 5.44, $P = 0.03$). In general, disease incidence was positively correlated with PC2 (r = 0.25).

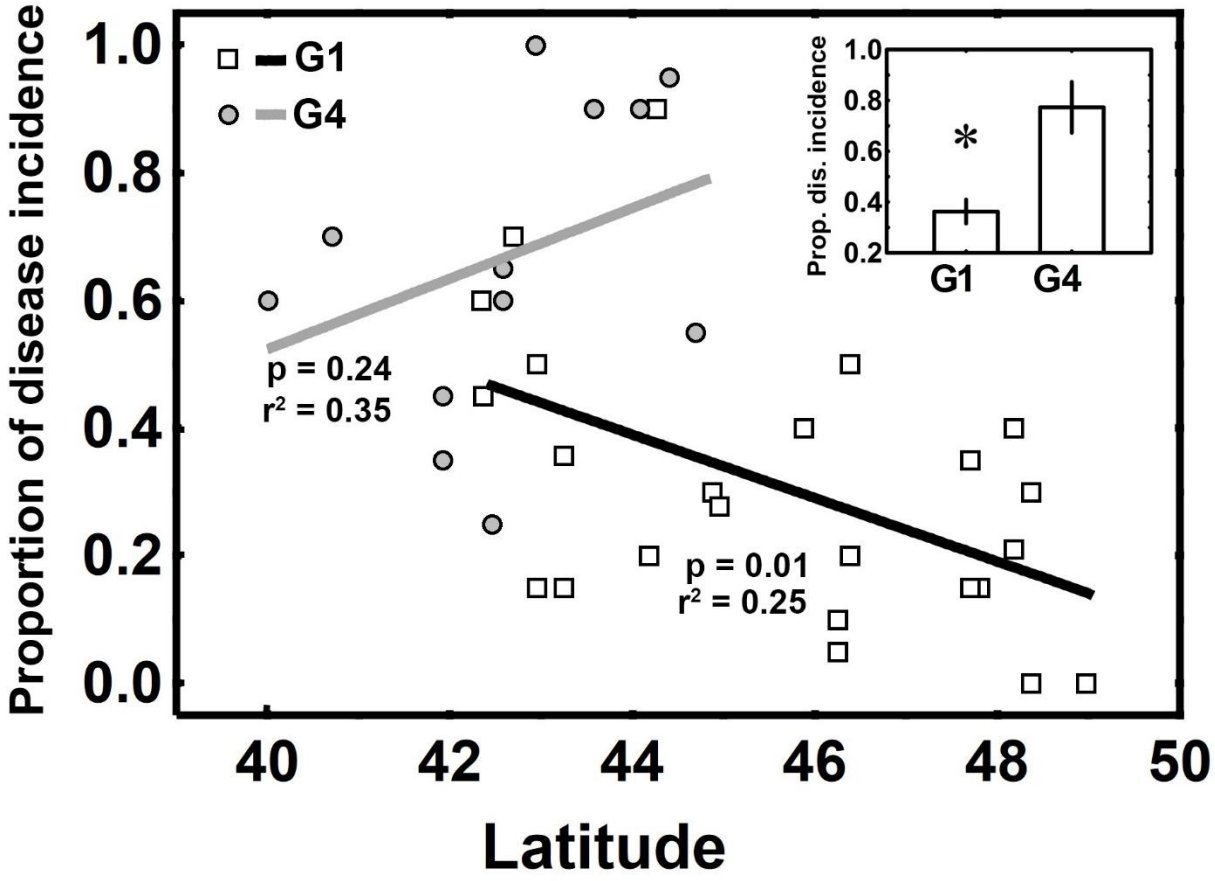

Figure 3.2. From field surveys for disease, the relationship between latitude and the proportion of plants with disease symptoms for the two common introduced *B. umbellatus* genotypes (based on the AICc-best model; Table 1). The solid black line (and square points) represents genotype G1, and the gray line (and gray dots) is genotype G4. The AICc-best model includes the interaction term (G x L). Genotype means (±SE) are displayed in the inset and statistically significant differences between means noted with an asterisk (df = 1, 27; F = 6.89, p = 0.01). Lines are fit by least-squares regression (G1: Proportion plants diseased = 2.58 – 0.050*Latitude; G4: Proportion plants diseased = -1.69 + 0.055*Latitude).

Field surveys- pathogen richness

We recovered 39 species of fungi from *B. umbellatus* during our surveys, including 20 that were deemed likely pathogenic. The AICc top model explained little variation in fungal richness among *B. umbellatus* populations and included only a single variable (Genotype) (Table 3; AICc = 69.49, Akaike weight = 0.21, Adj. R^2 = 0.009). G4 plants had, on average, 37% more

associated pathogen species than G1 plants (G4 pathogen richness: 3.33 ± 0.43 mean ± SE; G1 pathogen richness: 2.44 ± 0.43). In addition to the top model, seven other candidate models emerged as having substantial support (ΔAICc ≤ 2); five included climate variables, two included latitude, and six included plant genotype (range of AICc = 69.89 – 71.49, Akaike weight = 0.21 – 0.08). The inclusion of genotype in the majority of top models, and its large effect size, strongly support its importance in determining the number of pathogens infecting *B. umbellatus* in the USA.

Table 3.3. Top best-fit models for the proportion of diseased *B. umbellatus* plants collected during field surveys and *B. umbellatus*–associated pathogen richness, based on AICc selection procedure. G=Genotype, L=Latitude, PC1, PC2 and PC3 are bioclimatic principal components (see Methods).

Dependent variable	Model	AICc	ΔAICc	Likelihood	Akaike Wt	Adj. R^2
Proportion diseased plants	G, L, GxL, PC2	-9.69	0.00	1.00	0.27	0.45
	G, L, GxL, PC1	-9.61	0.08	0.96	0.26	0.48
	G, L, GxL, PC1, PC2	-8.29	1.40	0.50	0.14	0.47
	G, L, GxL, PC3, PC2	-7.97	1.72	0.42	0.12	0.44
	G, PC1	-7.86	1.82	0.40	0.11	0.43
	G, L, GxL,	-7.73	1.96	0.38	0.10	0.45
Pathogen richness	G	69.49	0.00	1.00	0.21	0.009
	G, PC1	69.89	0.40	0.82	0.17	-0.010
	G, PC2	70.15	0.66	0.72	0.15	-0.010
	L	70.26	0.77	0.68	0.14	0.001
	PC3	70.80	1.31	0.52	0.11	-0.020
	G , L	71.38	1.89	0.39	0.08	-0.050
	G , PC2, PC1	71.46	1.97	0.37	0.08	-0.070
	G, PC3	71.49	2.00	0.37	0.08	-0.060

Laboratory excised-leaf experiment

By multiple measures, G1 leaves were more susceptible to infection than G4 leaves in the excised-leaf experiment (Figure 3.3). Damage ratings assigned to G1 leaves were approximately 100%, 150%, and 45% higher than G4 when infected by *P. cucumerina* (df = 1, F = 10.95, P = 0.02), *C. fioriniae* (df = 1, F = 10.72, P = 0.02), and *A. alternata* (df = 1, F = 5.14, P = 0.06), respectively. Mean lesion size, although not significantly different, was 80% larger in G1 leaves

infected by *C. fioriniae* (df = 1, $F = 4.15$, $P = 0.09$), 24% for *P. cucumerina* (df = 1, $F = 2.57$, $P = 0.17$), and 7% for *A. alternata* (df = 1, $F = 0.16$, $P = 0.70$). Within diploid plants, there was no evidence of local adaptation for either *A. alternata* or *P. cucumerina* (Figure 3.3). Damage rating and lesion sizes were not significantly different between diploid populations (*A. alternata* damage rating: df = 3, $F = 0.42$, $P = 0.74$; lesion size: df = 3, $F = 0.2$, $P = 0.89$; *P. cucumerina* damage rating: df = 3, $F = 1.27$, $P = 0.31$; lesion size: df = 3, $F = 0.5$, $P = 0.69$).

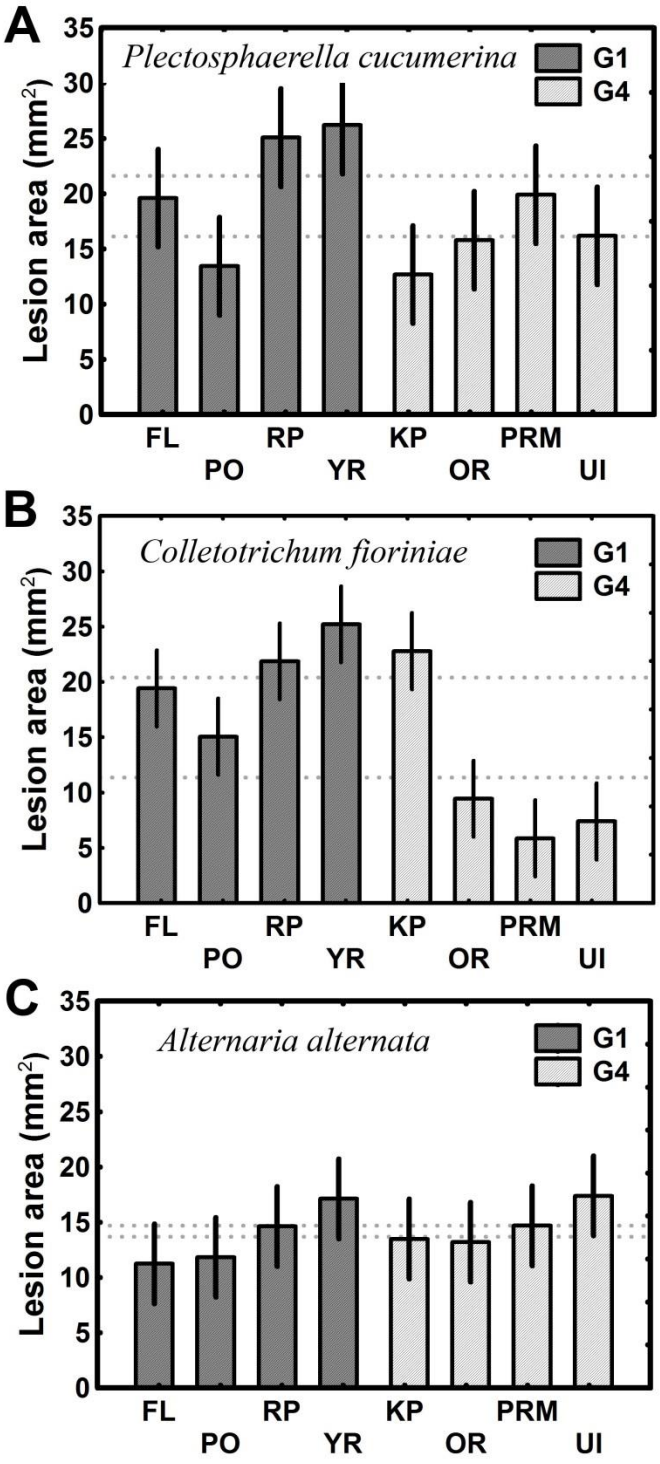

Figure 3.3. Mean (± SE) leaf lesion area for leaves of *B. umbellatus* infected by the generalist pathogens *Plectosphaerella cucumerina*, *Colletotrichum fioriniae*, and *Alternaria alternata*.Genotype means are indicated by dashed lines. Site abbreviations are as follows: FL=Flathead Lake, PO=Pend Oreille River, RP=Rose Pond, YR=Yakima River, KP=Kildeer Pond, OR=Oswegatchie River, PRM=Point Rosa Marsh, UI=Unity Island.

DISCUSSION

The two common introduced genotypes of *B. umbellatus* in North America differed in disease incidence during our two-year survey, with triploid G1 populations displaying 75% less disease symptoms in the field. Genotypes also displayed nonparallel clines in the proportion of plants with disease. Temperature (PC1) and moisture (PC2) climate variables were consistently selected as explanatory in top models, and are likely important in determining effects of pathogens on *B. umbellatus*. Spatially variable environmental stressors such as temperature or drought are known to be associated with changes in host resistance to disease or altered rates of pathogen development (Seherm and Coakley 2003). Additionally, accumulation of pathogen species by introduced plants may be explained by stress and physical characteristics of the plant, the diversity of invaded habitat, total area invaded, or time since invasion (Mitchell et al. 2010, Flory and Clay 2013). In our study, disease incidence increased in G1 plants at low latitudes, a pattern which may reflect stress associated with limiting environmental or biotic conditions at expanding range fronts (Hoffmann and Blows 1994, Hilker et al. 2005, Louthan et al. 2015). Differences in disease resistance between genotypes along latitudinal gradients may also reflect preadaptation by G4 plants to the range of environmental stressors experienced in North America. Although the native ranges of both genotypes are unknown, it is plausible that if G4 plants have a larger native distribution, they may demonstrate broader physiological plasticity in response to environments in the introduced range (Higgins and Richardson 2014, Schmidt et al. 2017), making the formation of clines related to disease in North America less likely for G4 plants.

Geographic and genetic variation in the effects of biotic resistance during plant invasions is most likely common, and support that biotic resistance is important in determining large-scale

patterns of invasion is increasing (Parker et al. 2006, Freestone et al. 2013, Cronin et al. 2015, Allen et al. 2017, Bhattarai et al. 2017b). For example, recent investigations of the grass *Phragmites australis* (Cav.) Trin. Ex Steud. have found nonparallel latitudinal gradients in foliar and stem-herbivore impacts between native and invasive haplotypes of *P. australis* in marshes of North America (Cronin et al. 2015). In this case, native populations exhibited a strong latitudinal cline in herbivory but invasive populations did not. Cronin et al. (2015) argued that the absence of a cline for the invasive haplotype was likely attributed to insufficient time for the invader to locally adapt to an environmental gradient correlated with latitude (e.g., climate). One implication from that work is that biological control agents, if introduced, would more strongly impact native haplotypes, especially at low latitudes where the difference in attack rates between the two haplotypes was highest (Cronin et al. 2015). In our study system, we detected nonparallel clines in disease incidence between diploid G4 and triploid G1 populations of *B. umbellatus*. *Butomus umbellatus* was introduced in North America at least twice, and probably more, during the last 150 years, with G4 plants first found in the St. Lawrence River in 1897 and G1 plants in Idaho by 1949 (Anderson et al. 1974). Although it seems unlikely a difference in residence time of ~50 years is enough to generate the latitudinal variation in disease resistance that we observed in G1 plants, the pattern may reflect a central-marginal gradient in which stress and susceptibility to infection increases at the invasion front/range margin (i.e., in lower latitudes; Hoffmann and Blows 1994, Louthan et al. 2015).

Genetic variation in disease susceptibility and implications for biological control

In contrast to the two-fold higher pathogen incidence on G4 versus G1 plants in the field, we found pathogenicity in the laboratory was higher for G1 plants. Reasons for these seemingly

contradictory findings are currently under investigation, but may reflect differences in residence time between introduced taxa and associated pathogen accumulation, variable developmental stage-resistance relationships, novel associations with pathogens, environmental variation across the invaded range, or some combination of the above. Escape from pathogens is likely to explain invasion success in some taxa (Torchin and Mitchell 2004), but the importance of pathogen escape for *B. umbellatus* is unknown because native range surveys for damaging pathogens are lacking. Separate from latitudinal patterns, the difference in pathogen accumulation and impact between introduced genotypes in the US may be due to differences in residence time between them (Mitchell et al. 2010). Thus, the older populations of G4 plants could be expected to have a larger pathogen pool associated with them, a pattern which was confirmed during our study. This may help explain why disease was more common on G4 plants during field surveys but not in laboratory experiments. Another possible explanation for contrasting field and laboratory results is that susceptibility to natural enemies varies during developmental stages and between genotypes. The importance of developmental susceptibility has been demonstrated in a number of plant systems and is actually widespread (Develey-Rivière and Galiana 2007). If disease susceptibility changes with age, but the rate of change differs between genotypes, then this could explain the pattern we observed.

Despite using pathogens isolated from multiple genotypes, consistent damage patterns were documented regarding pathogenicity to G1 and G4 plants. Local adaptation to host plant taxa by pathogens (Gandon and Van Zandt 1998, Croll and McDonald 2017), if occurring in populations of *B. umbellatus*, may have produced the opposite pattern than we observed in the laboratory and isolates should have performed better on their local hosts. We used strains of *P. cucumerina* and *A. alternata* isolated from G4 plants at Kildeer Pond, OH, one of the populations

used in our experiments. Damage to Kildeer Pond experimental plants was not significantly higher than damage to other G4 populations for either pathogen. Likewise, our conclusions based on observed genotypic differences in infection would remain the same if only taking into account *C. fioriniae*. Local adaptation by pathogens has been previously observed in other plant-pathogen systems and is indicated by increased performance of the pathogen on the local host over foreign hosts (Hokkanen and Pimentel 1989, Laine 2005, Laine 2007, Bowen et al. 2017).

Spatial variation in biotic interactions has clear importance to management of invasive plants using biological control agents. Currently, a number of insect herbivores and the rust fungus *Doassansia niesslii* De Toni (Exobasidiomycetes: Doassansiaceae) are under examination for their potential as biological control agents of *B. umbellatus* in North America. From research in Europe, there is an indication that *B. umbellatus* plants of different ploidy levels vary in their susceptibility to infection, although the range of plant populations used so far has been limited. In our study, we used generalist pathogen taxa encountered during domestic surveys in the US. Unfortunately, we do not know whether results of the current study using generalist pathogens will be transferrable to predict impacts of specialist biological control pathogens if approved for introduction in the US. Additionally, European test plants are genotypes which have not been found yet in the USA. To develop better predictive assays, a broader range of North American genotypes should be included in efficacy tests for prospective biological control agents.

It is now well known that both host- and agent-genotype effects on biological control success can be substantial and spatially variable (Boughton and Pemberton 2011, Mukwevho et al. 2017, Mukwevho et al. 2018). For example, biological control agents of *Hydrilla verticillata* vary in performance between dioecious and monoecious genotypes in the US (i.e., host-genotype effects), genotypes which occur mostly in separate geographic areas (i.e., monoecious hydrilla

has a northern US distribution and dioecious hydrilla has a southern distribution). This has generated interest in introducing agents that are better-adapted to specific host genotypes and led to additional overseas exploration for new agents (Grodowitz et al. 2010, Harms and Grodowitz 2011, Harms et al. 2017, Purcell et al. 2019). Likewise, cryptic species of *Diorhabda* beetles were introduced for control of saltcedars (*Tamarix* spp.) in the western USA, leading to variable control in introduced areas due to both climatic limitations on beetles and variable host-agent interactions between the beetles and several saltcedar species (DeLoach et al. 2007, Tracy and Robbins 2009). Similarly, unsuccessful biological control of giant salvinia (*Salvinia molesta* Mitchell) and waterhyacinth (*Eicchornia crassipes* (Mart.) Solms) in some southern US locations due to climate limitations has led to exploration for more cold-hardy (*Cyrtobagous salviniae* Calder and Sands; Russell et al. 2017) or heat-tolerant (*Megamelus scutellaris* Berg; Foley et al. 2016, Freedman and Harms 2017) agents. These examples highlight management programs in which spatially variable control has been attributed to genetic or climate limitations on agents. In the current study, we demonstrated that introduced *B. umbellatus* genotypes have different susceptibilities to foliar fungal pathogens and disease incidence varied with latitude for one but not the other genotype. This suggests that it may be necessary to consider biological control agents of *B. umbellatus* that are genotype, climatic, or latitude-specific.

CHAPTER 4.
VARIABILITY IN WEED BIOLOGICAL CONTROL: EFFECTS OF FOLIAR NITROGEN ON LARVAL DEVELOPMENT AND DISPERSAL OF THE ALLIGATORWEED FLEA BEETLE, *AGASICLES HYGROPHILA*[2]

INTRODUCTION

The importance of plant quality (e.g., leaf toughness, moisture, defensive chemistry) and nutrition (e.g., nitrogen, phosphorus, potassium) to herbivorous insects is well established (Scriber and Slansky Jr 1981, Awmack and Leather 2002). Herbivore physiology (e.g., development and fecundity), behavior (e.g., host choice and movement) and population dynamics (Denno and McClure 1983, Helms and Hunter 2005) are directly tied to local host plant conditions (Scriber and Slansky Jr 1981, Awmack and Leather 2002, Van Hezewijk et al. 2008) and may vary with phenological, herbivory (Hunter et al. 1996, Larsson et al. 2000), or stress-induced changes in host plants (Uyi et al. 2018). Weed biological control agents, because they are restricted to a single host species, are particularly vulnerable to changes in host quality, especially if the life stage that feeds on the plant is immobile or a poor disperser and cannot seek out higher-quality hosts. Variability in quality or nutrition may occur spatially, such as over soil moisture or fertilizer gradients, or even between parts of a single clone (Wheeler and Center 1996a, Spencer et al. 2005, Spencer et al. 2010). Changes in nutrition may also occur over a season or between years. For example, many plants show a decline in quality (nitrogen) with age

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(Scriber and Slansky Jr 1981), a pattern which may explain seasonality in some biological control successes (e.g., Coulson 1977, Spencer et al. 2010, Harms and Shearer 2017).

A clearer picture is emerging regarding the importance of nitrogen availability to weed biological control. Studies have shown that increased host nitrogen typically leads to improved control agent performance. For example, nitrogen amendment, through foliar application, was used to improve control efficacy of giant salvinia (*Salvinia molesta* D.S. Mitchell; Salviniaceae) in Australia and Papua New Guinea by enhancing establishment and population growth of *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) (Room and Thomas 1985b, Room et al. 1989). Other studies have shown that variable nitrogen levels in host plants can affect density-dependent processes such as development, mortality, or dispersal (Wilson et al. 2007). However, to our knowledge the only published examination of the interaction between host nitrogen and density-dependence in an external-feeding biological control agent found that enhanced nitrogen increased development rate but not survival of *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) on water hyacinth (*Eichhornia crassipes* (Mart.) Solms; Pontederiaceae) (Wilson et al. 2007). In other systems, higher nutrient availability in the environment may differentially improve plant performance over agents such that plant compensation to herbivory under elevated nutrient conditions ultimately limits control (Coetzee and Hill 2012). That increased nitrogen in host plants can improve agent performance has also been exploited in mass-rearing programs with the goal to produce large numbers of high quality biological control agents for release (Blossey and Hunt 1999, Wheeler 2001, Harms et al. 2009). Combined with an understanding of seasonal variability in reproductive status, insects reared on high nitrogen plants should demonstrate increased fecundity upon release, thus increasing likelihood of establishment (Blossey and Hunt 1999, Van Hezewijk et al. 2008).

Biological control agents are not only exposed to hosts of varying nutritional status during development, but to varying temperatures as well. Seasonal temperature patterns can be limiting to agents if developmental thresholds are surpassed. However, the ways in which ambient temperature and host nutrition interact to influence herbivore development and population dynamics are unknown for many systems but could be important, particularly near upper and lower thermal limits of the agent. Thus the interaction between nitrogen and ambient temperature may explain why control agents persist or go regionally extinct, and may reveal reasons for changes in seasonal patterns of control. Harms and Shearer (2017) suggested that seasonal patterns in alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb; Amaranthaceae) control may have been related to the interaction between seasonal variation in plant quality and ambient temperature. Varying temperature and nitrogen levels had variable effects on performance of the water hyacinth bug, *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae) with the largest reductions in fitness occurring at high temperatures and low nitrogen (Ismail et al. 2017).

Despite levels of control that have been achieved in the southeastern U.S., alligatorweed plants growing in terrestrial environments or in the northern region of its introduced range remain largely undamaged (Coulson 1977, Harms and Shearer 2017). Early investigations into variable control by *Agasicles hygrophila* Selman and Vogt (Coleoptera: Chrysomelidae) focused primarily on structural and nutritional differences between plants growing in terrestrial or aquatic environments and led to limited examination of phosphate deficiency effects on adult feeding (Maddox and Rhyne 1975, Coulson 1977). However, studies of effects of nitrogen on larval development are lacking. To understand the importance of variation in host nitrogen for biological control of alligatorweed, we combined field measurements of plant nitrogen with a

series of laboratory experiments. First, we collected and analyzed alligatorweed leaves from sites across Louisiana over four years to determine seasonal variation in foliar nitrogen. We then examined the effects of alligatorweed foliar nitrogen and temperature on *A. hygrophila* immature developmental rate and survival. Finally, we tested whether foliar nitrogen and larval density interact to affect larval development or dispersal from host plants. We first predicted that foliar nitrogen in alligatorweed at field sites would be highest in spring and early summer, coinciding with peak population growth of *A. hygrophila* (Harms and Shearer 2017). We then predicted that increasing nitrogen levels in host plants would increase development rates and survival of *A. hygrophila* and mediate negative effects of high temperatures on larvae during laboratory experiments. Additionally, we predicted that nitrogen levels would influence larval dispersal such that plants with higher foliar nitrogen would support more larvae. We report here the variation in alligatorweed nutritional conditions potentially encountered by biological control agents in the field and multiple ways that host nutrition may influence *A. hygrophila* performance. Finally, we discuss implications of our findings for regions with poor alligatorweed control.

MATERIALS AND METHODS

Study system

The alligatorweed flea beetle was first released in the southeastern United States in 1960s to control alligatorweed and has widely been considered the world's first successful aquatic weed biological control program (Coulson 1977, Buckingham 1996b). Initial releases of *A. hygrophila* were made in all southeastern US states, largely resulting in suppression of alligatorweed to the point where it was no longer considered a nuisance. Cofrancesco (1988)

conducted a survey of southeastern infestations nearly 20 years after original biological control introductions and found minimal levels of alligatorweed in coastal areas which largely coincided with presence of control agents. However, alligatorweed infestations can be extensive in areas outside the distribution of *A. hygrophila* (e.g., in Arkansas, Tennessee, Alabama), which is thought to be primarily limited by winter temperatures (Coulson 1977, Julien et al. 1995).

Experimental procedures

This work was conducted at the Department of Biological Sciences, Louisiana State University (LSU), Baton Rouge, LA and at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS. Plants for all experiments were collected from a roadside ditch in Baton Rouge, LA (N 30.35°, W 91.14°) in 2015 and propagated multiple times prior to experimentation to minimize maternal environmental effects. Plants were cultured hydroponically in either a greenhouse at LSU or indoor environmental growth chambers (27°C, 14:10 light:dark) at the ERDC. Regardless of location, plants were grown in a series of 20-L plastic buckets filled with 15-L full-strength (200 mg/L N) Hoagland's solution (Hoagland and Arnon 1950). Nutrients were exchanged every other week and reverse osmosis water was added periodically to maintain water levels in between nutrient exchanges.

For experiments, a modified Hoagland's nutrient solution stock was created with zero nitrogen and amended with NH_4NO_3 to reach desired nutrient levels, maintaining constant levels of other macro- and micronutrients (P: 32 mg/L; K: 170 mg/L; Fe: 3 mg/L; Ca: 100 mg/L; Mg: 30 mg/L; S: 93 mg/L; B: 0.5 mg/L; Mo: 0.01 mg/L; Mn: 0.5 mg/L; Zn: 0.05 mg/L; Cu: 0.02 mg/L; Cl: 177 mg/L). Three final nutrient solutions were created by this technique, a low-N (2 mg/L N), medium-N (20 mg/L N), and high-N (200 mg/L N) solution. Foliar nitrogen levels

generated by growing alligatorweed in these media were comparable to the range of foliar nitrogen in plants collected at field sites in Louisiana. Plants were cultured in appropriate nutrient media for at least eight weeks prior to beginning an experiment. Generally, nutrients were exchanged every two weeks for the first month of culture, then weekly prior to and during the experiment. Leaf samples were collected from cultures three times during the experiment (initial, mid-way, end of experiment) to verify foliar nitrogen levels differed among the treatment levels and were consistent over time. Nitrogen analysis was performed by the Soil and Plant Sciences laboratory at the Agricultural Chemistry Laboratory at LSU and confirmed that we were able to induce foliar nitrogen differences in our culture plants: Low= 2.37 ± 0.06 % DW N, Medium= 3.49 ± 0.16 % DW N, High= 6.43 ± 0.11 % DW N (ANOVA, df=2, $F = 251.33$, $P < 0.001$).

Variation in foliar nitrogen at alligatorweed field locations

Field sites in Louisiana were visited every 2-3 weeks during the majority of the growing season in 2015-2018. Eleven sites were located in Louisiana and one in Mississippi (Openwood pond). Sites consisted of ponds, rivers/ bayous, wildlife management area wetlands and lakes (Table 1). Approximately 10 g fresh alligatorweed leaves were collected, primarily from the third and fourth apical nodes, then combined for each site and each sample date. The sampling area within a site was limited to approximately 5 m^2 and the same area was sampled during each visit to minimize variation in FN stemming from variation in local conditions within a site. Leaves were dried in a forced-air oven at 60 °C then FN was determined as above for experimental plants.

Table 4.1. Sites where alligatorweed leaves were collected to determine foliar nitrogen concentrations.

Site	Lat	Long	Waterbody type	Years sampled
Choctaw landing	29.85	-90.68	River	2016, 2017, 2018
Chevreuil Bayou	29.91	-90.73	River	2016, 2017, 2018
Blind River	30.09	-90.78	River	2016, 2017, 2018
Maurepas WMA	30.15	-90.81	Swamp	2016, 2017, 2018
Blackwater Conservation Area	30.54	-91.09	Wetland	2016, 2017, 2018
Greenwood Community Park	30.57	-91.17	Pond	2016, 2017
Simmesport Pond	30.97	-91.81	Pond	2016, 2017, 2018
Spring Bayou WMA	31.14	-92.01	River	2016, 2017, 2018
Lake St. Joseph	32.08	-91.23	Lake	2016, 2017, 2018
Bayou Macon	32.09	-91.56	River	2016, 2017
Openwood Pond	32.40	-90.79	Pond	2016
Poverty Point Reservoir	32.53	-91.49	Reservoir / Lake	2016, 2017, 2018

Effects of foliar nitrogen and temperature on Agasicles hygrophila *larval development*

To determine the independent and interactive effects of foliar nitrogen and temperature on larval development and survival, a 3x3 factorial experiment was conducted. *Agasicles hygrophila* egg masses were field-collected from the Blind River, LA (N 30.0949°, W -90.7785°), incubated in Petri dishes sealed with parafilm at 23 °C and observed daily for hatching. Once sufficient neonates were hatched (within a 24hr period), the experiment was initiated. A single larva was placed in a 30 ml plastic cup on a leaf of varying foliar nitrogen (Low, Medium, High) then sealed with a plastic lid before placing at one of three temperatures (23, 26, 30° C). Temperatures were chosen to be within the optimal (23-25 °C) and upper (30 °C) range suitable for development of *A. hygrophila* (Stewart et al. 1999b) which corresponds to spring and summer temperatures in the southern US range. A total of 20 flea beetles per treatment combination was used. Temperatures and photoperiod (14:10 light:dark) were maintained within controlled-growth chambers until adult emergence. Cups were monitored

daily for insect survival, pupation, and adult emergence. Leaves were replaced every two days such that the beetles were never resource limited. Dates of pupation and adult emergence were recorded and used to determine development duration. Dead larvae and pupae were discarded and survival was determined for larvae, pupae, and overall. Additionally, we sexed and weighed newly-eclosed adults within 24 hours to determine whether nitrogen, temperature, or sex affected adult mass. In particular, since the adult stage is the long-distance dispersing stage, sex and size of the dispersing individual may affect spread rate and distance of *A. hygrophila* through flight strength or fecundity (Dingle et al. 1980, Berger et al. 2008).

Effects of foliar nitrogen and larval density on development and dispersal

Density-dependence in *A. hygrophila* development rate was determined for two foliar nitrogen levels (2, 200 mg/L N). Larval densities of 1, 5, or 20 larvae plant^{-1} were chosen to be representative of average and maximum values observed at field sites during the growing season (Harms and Shearer 2017). Beginning six weeks prior to the experiment, plants were cultured in appropriate nutrient conditions as described above. One week before the experiment began, approximate 10-cm-long plant fragments were collected from greenhouse cultures and placed individually in nutrient solution to root. Once roots were observed, plant fresh weight (g) was obtained and they were planted in 1000 ml tubs with 200 ml fine sand and 200 ml nutrient solution (as described above). Egg masses were collected three days before the experiment from the Blind River, LA, incubated as previously described, and observed for hatching. Neonates (<1 day old) were placed in experimental containers at an abundance of one, five, or twenty larvae per plant. Experimental containers were covered in fine mesh (~200 µm), and placed in a Conviron environmental growth chamber at 23 °C, a temperature determined during the first

experiment to produce high survival (see below). Photoperiod within chambers was set to 14:10 (light:dark). There were eight replicate experimental units per treatment combination.

The density-dependence experiment lasted seven days. Larvae were collected and separated into those that were on the plant and those that were off the plant (i.e., were collected from the sides or roof of the container), counted and removed from containers to be weighed. Proportion of larvae remaining on the plant was determined.

Statistical approach

To analyze variation in alligatorweed FN at field locations, a polynomial regression was fit for each year of the study to FN values for all study locations and dates. For the first experiment, we used two-way ANOVA to test for effects of temperature and nitrogen on *A. hygrophila* development time and adult biomass. To test for differences in survival, we used a generalized linear model with a binomial distribution of the response variable (survived or died) and logit link function (Bolker et al. 2009). Temperature (T; 23, 26, 30 °C), foliar nitrogen (FN; Low, Medium, High), and their interaction (TxFN) were independent variables in these models. Likewise, two-way ANOVAs were used for the experiment on the effects of conspecific density and FN on larval fresh mass and the proportion of dispersing larvae. Density (D; 1, 5, 20 larvae per plant), FN (low, high), and their interaction (D*FN) were independent variables. Proportion dispersing was arcsine square-root transformed (Gotelli and Ellison 2004) and larval fresh weight was *ln*-transformed to achieve normality and homogeneity of variances. For each ANOVA, post-hoc mean separation was determined by Tukey HSD test. Statistical tests were performed using SigmaPlot, version 12.3 (Systat Software, Inc., San Jose CA) or Statistica, version 12 (Stat Soft, Inc., Tulsa OK).

RESULTS

Variation in foliar nitrogen at alligatorweed field locations

Alligatorweed FN varied among sites, seasonally, and among years but seasonal patterns were consistent each year (Figure 3.1). Foliar nitrogen peaked during the spring, declined during the summer, and then increased slightly during the late summer/fall. On several occasions (e.g., Blind River, LA on March 21, 2018), FN values as high as 8% DW were recorded. Of the five largest FN values, all occurred from February to April. The amount of variation in FN between locations was considerable on some census dates. For example, during the first census of 2018, FN ranged from 3.7 to 7.9 % DW (Figure 3.1). However, there was no seasonal pattern in the level of variation observed. Although we did not sample sediment or water nutrients for this study, the differences in FN among sites were most likely related to local nutrient conditions.

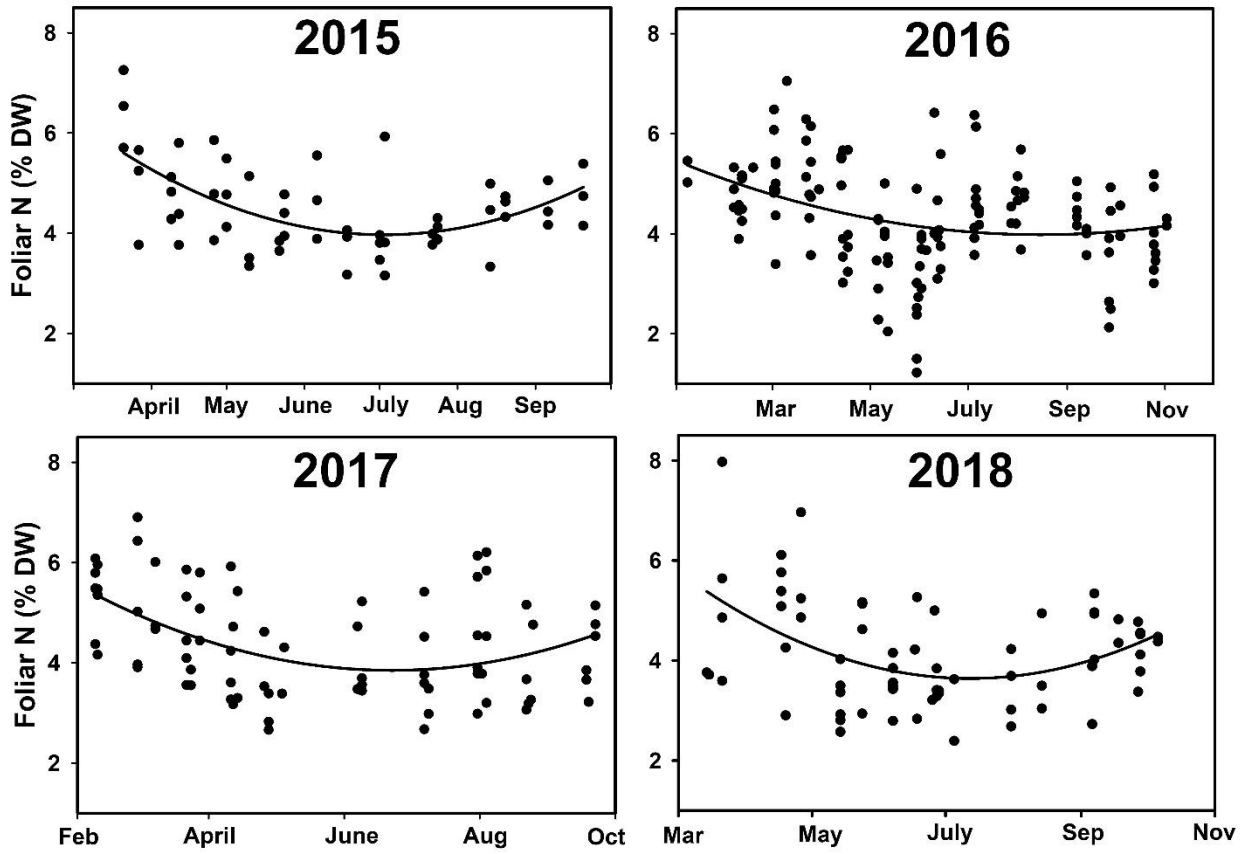


Figure 4.1. Seasonal variation in foliar nitrogen at alligatorweed field sites in Louisiana during 2015-2018. Solid lines are best-fit curves determined by nonlinear regression (2015: FN = 269,581 + -12.8x + 0.0002x2, P < 0.001, R2 = 0.33; 2016: FN = 51,938 + -10.4x + 0.0001x2, P = 0.002, R2 = 0.18; 2017: FN = 156,454 + -7.3x + 0.00008x2, P < 0.001, R2 = 0.21; 2018: FN = 225207+-10.40x+0.0001x2, P = 0.002, R2 = 0.18). Points are FN values for each site and date.

Effects of foliar nitrogen and temperature on Agasicles hygrophila *larval development*

Developmental duration of *A. hygrophila* ranged from 14 ± 0.3 days at high N and high temperature (30 °C) to 23 ± 0.5 days at low N and low temperature (23 °C), and was shortened by both increased FN and temperature (Figure 3.2; Table 2). Increasing FN from low to high decreased development duration by 16%, regardless of temperature. In the high FN treatment, developmental duration was shortened by 28% from 23 to 30 °C. Although there was no significant interaction among treatments detected for survival, survival increased by 40% from low (57%) to high (80%) FN and decreased by 44% with increasing temperatures from 23 °C

(85%) to 30 °C (49%) (Figure 3.2). Adult mass increased by 11% from low (0.0060 ± 0.0002 g) to high (0.0066 ± 0.0002 g) FN but decreased 15% from low (0.0068 ± 0.0002 g) to high (0.0058 ± 0.0003 g) temperature.

Table 4.2. Differences in various larval traits caused by a combination of temperature (T) and foliar nitrogen (FN) (left) or density and foliar nitrogen (right) on *Agasicles hygrophila* during two experiments.

Temperature and nitrogen experiment					**Density and nitrogen experiment**				
Adult mass	**Effect**	**df**	**F**	***P***	**Larval mass**	**Effect**	**df**	**F**	***P***
	T	2	6.76	0.002		D	2	9.90	<0.001
	FN	2	3.15	0.047		FN	1	9.00	0.005
	T*FN	4	1.49	0.21		D*FN	2	0.16	0.19
Developmental duration	**Effect**	**df**	**F**	***P***	**Dispersal**	**Effect**	**df**	**F**	***P***
	T	2	292.16	<0.001		D	1	1.03	0.32
	FN	2	77.36	<0.001		FN	1	0.22	0.64
	T*FN	4	0.99	0.47		D*FN	1	1.64	0.21
Survival	**Effect**	**df**	***z***	***P***					
	T	2	19.2	<0.001					
	FN	2	8.8	0.012					
	T*FN	4	2.5	0.64					

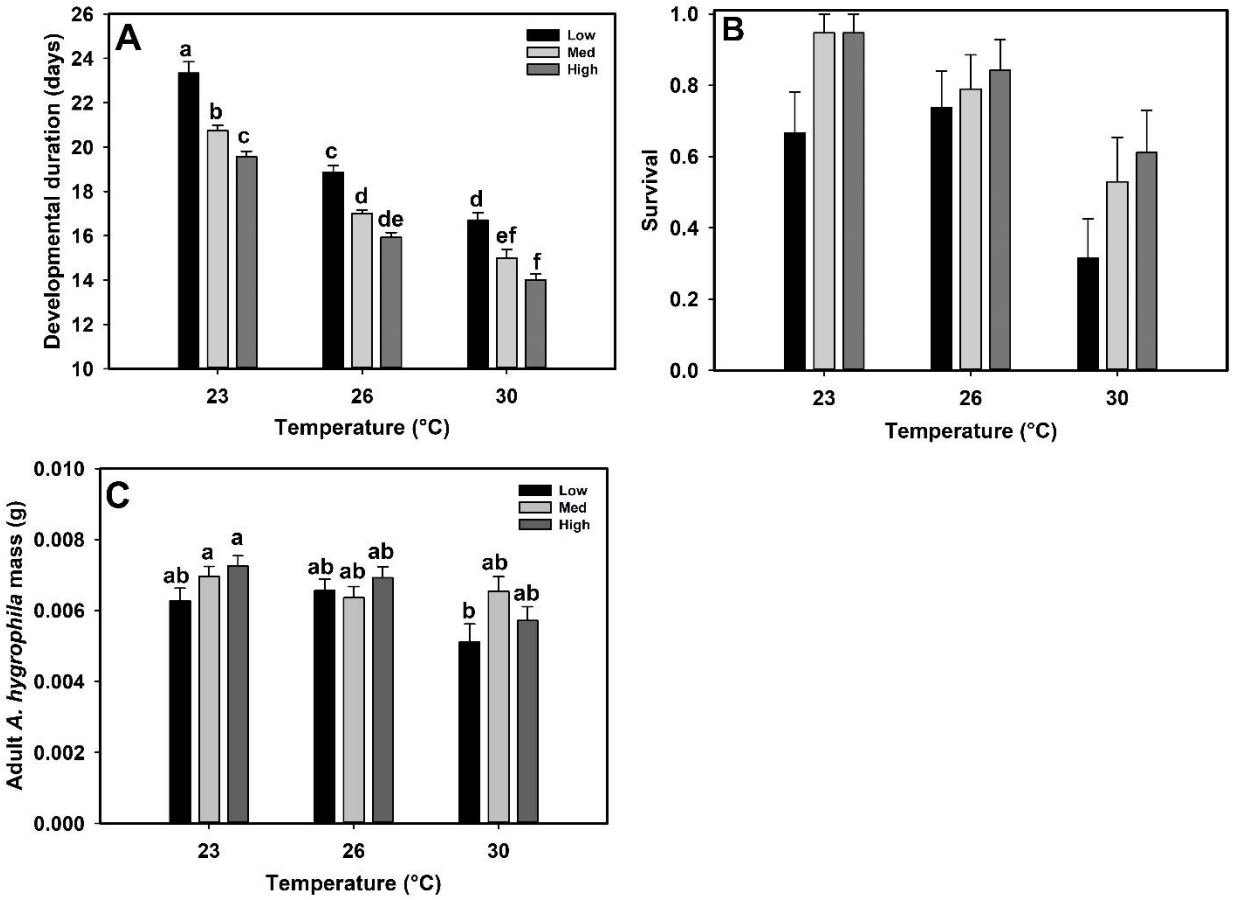


Figure 4.2. Mean (±SE) developmental duration from egg hatch to adult (A) survival (B), and adult mass (C) for *A. hygrophila* at combinations of foliar nitrogen and temperature. Low = 2 mg/L N, Med = 20 mg/L N, High = 200 mg/L N. Letters above error bars in (A) and (C) indicate Tukey's significantly different means for ANOVA tests.

Effects of foliar nitrogen and larval density on development

Larval dispersal was zero in the low density (1 larva per plant) treatments (Figure 3.3). As such, we statistically compared only the medium (5 larvae per plant) and high (20 larvae per plant) treatments. Excluding the low density treatment, dispersal was not significantly different between medium and high densities or FN (Table 2; Figure 3.2A), despite a two-fold increase in mean dispersal between high and low FN plants at intermediate density (0.51 ± 0.11 vs 0.23 ± 0.12). At high (20 larvae/ plant) larval density, dispersal was high (0.48 – 0.58 of larvae), regardless of nitrogen level. Differences in larval fresh mass were due to both density and foliar

nitrogen (Figure 4.3B). Increased foliar nitrogen in leaves led to 23% larger larvae, and larval biomass was highest (0.0084 ± 0.0005 g) at intermediate density. Interestingly, FN only had a significant effect on larval biomass at high densities; larval size was 64% larger in high (0.0068 ± 0.00065 g) than low (0.0041± 0.00065 g) FN.

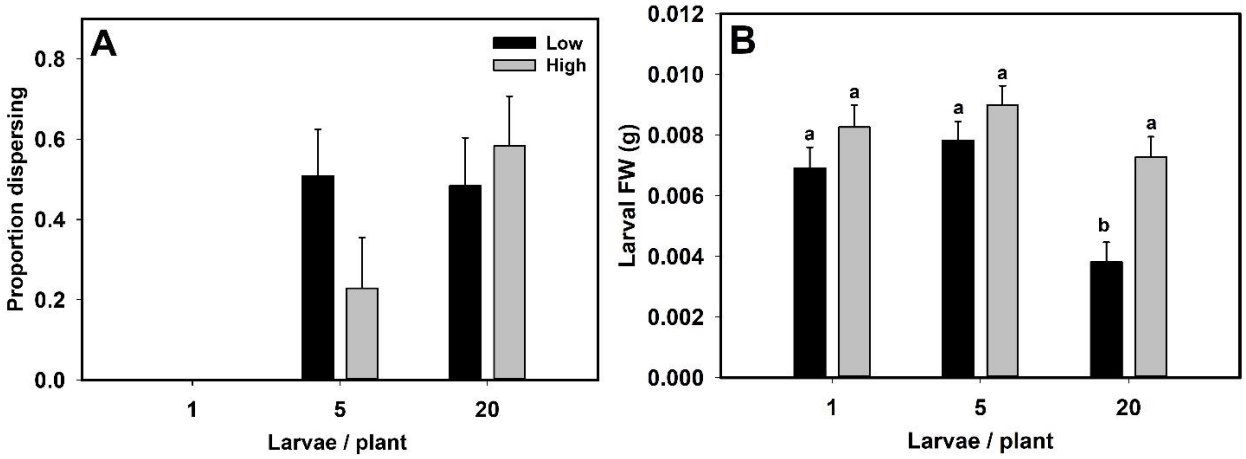


Figure 4.3. A) Mean (±SE) proportion of *A. hygrophila* larvae dispersed and B) larval fresh weight, relative to larval density and foliar nitrogen. The lowest larval density (one larva plant-1) is not visible in A) because there was zero dispersal from plants in all replicates. Letters above error bars indicate Tukey's significantly different means.

DISCUSSION

Among a growing list of studies for other targets of weed biological control (e.g., Room et al. 1989, Center and Dray Jr 2010, Coetzee and Hill 2012, Ismail et al. 2017), our study is the first to show that alligatorweed varies spatially and temporally in FN and that this variation has important fitness and life history consequences for its biological control agent, *A. hygrophila*. Plant nitrogen effects on insect herbivores can be significant, with positive effects on larval and adult physiology and behavior. This may explain variation in the population dynamics of some biological control agents in the field in relation to spatial or temporal (i.e., seasonal) variation in host plant quality (Mattson Jr 1980). For instance, *A. hygrophila* overwintering on host plants

(southern USA sites) generally follow the pattern of rapid population increase early in the year (i.e., spring) when temperatures are mild (Harms and Shearer 2017) and host plant nutrition is high (the current study). However, individuals that disperse from low to high latitude sites arrive during a time when temperature may be limiting (i.e., summer) or host plant quality is insufficient to support population growth (Harms and Shearer 2017), thus providing inadequate control in those areas. We found independent but not interactive effects of temperature and host nitrogen on developmental duration or survival, which is in contrast with other studies that have examined the effects of the interaction on biological control agents (e.g., Ismail et al. 2017). However, variability of the nitrogen effect on survival was ten times higher at low and high than medium temperature ($\sigma^2_{survival}$ = 0.02 at 23° and 30°C, $\sigma^2_{survival}$ = 0.002 at 26°C). We anticipated that negative effects of high temperature stress on immature development would be disproportionately reduced by increasing foliar nitrogen, but the positive effect of nitrogen was consistent across all temperatures (Figure 3.2). This could be in part due to the range of temperatures we chose for our experiment. If had we chosen a wider range of temperatures (i.e., those closer to developmental thresholds of *A. hygrophila*), we may have detected interactions between temperature and FN in development and survival. However, survival of larvae in the 30 °C, low FN treatment (30%) was similar to that reported by Stewart et al. (1999b) (20%) at the same temperature. During our study, the effect of FN on survival at 30 °C was significant by a factor of two (30% at low FN and 60% at high FN) and underlines the importance of including plant quality measurements in baseline biology studies of weed biological control agents. The range of FN we used for developmental studies was within the range observed at field sites during our surveys (Figure 3.1). In fact, FN in our high treatment was less than that recorded

from plants in the field, suggesting that the importance of FN for *A. hygrophila* development and survival, and ultimately population growth, may be greater in the field than we've estimated.

Local variability in nutrient available to host plants may lead to control in some parts of the site but not others. Although research into nutritional deficiencies has been conducted to address alligatorweed inter-plant variability in attack rate by *A. hygrophila*, past examinations focused on feeding attraction and adult consumption of plants, mostly ignoring importance of nitrogen to larvae (Maddox and McCready 1975, Maddox and Rhyne 1975). Levels of nitrogen are often higher in young, newly-developing leaves of many species (Mattson Jr 1980). Regrowth of alligatorweed following a defoliation event is likely to be high in nitrogen and nutritious for *A. hygrophila* populations. Regrowth tissues on alligatorweed plants treated with herbicides were attacked by *A. hygrophila* at higher rates than untreated plants, presumably due to high nutritional content of young leaves (Coulson 1977). This positive feedback may only support *A. hygrophila* populations for a limited time though, until either plants no longer have regenerative capacity due to depleted carbohydrate reserves or summer temperatures become limiting on agents. However, quality of alligatorweed regrowth following defoliation has received some attention with regards to the induction of increased chemical and physical defenses in response to feeding by *A. hygrophila* or other herbivores (Liu et al. 2018, Yu and Fan 2018). An examination of induced defensive responses to larval feeding, especially as they may be influenced by environmental heterogeneity (e.g., available nitrogen or ambient temperatures), would further enhance our understanding of the complex spatial and temporal interactions between agents and hosts.

Regional variability in nutrition/quality of plants may also be a factor influencing dispersal activity from low to high latitude sites since typical spring defoliation in low latitude sites may lead to temporarily reduced-quality plants. Larvae fed on high nitrogen plants accumulated more mass (experiment #2) and became larger adults (experiment #1). Adult insect size and flight ability are often correlated (Dingle et al. 1980, Kaufmann et al. 2013), but whether body size in *A. hygrophila* is related to long-distance dispersal ability has not been studied despite the well-known occurrence of annual long-distance dispersal into areas outside the overwintering zone of *A. hygrophila* (Coulson 1977, Buckingham 1996b, Harms and Shearer 2015). We did not measure dispersal in adult beetles, but it may be valuable to determine whether larval conditions (i.e., FN and conspecific density) contribute to the likelihood and distance of adult dispersal after emergence. If larger individuals made up the majority of dispersers, then collection and comparison of adults between low and high latitude sites soon after their arrival should yield body size differences between the two, with high latitude sites harboring larger individuals than low latitude sites. Although larvae are not the primary dispersal stage, we demonstrated the propensity for dispersal once larval density increases above one individual per plant, but the dispersal likelihood was reduced by half under moderate larval density, on high nitrogen plants. Thus, seasonal variation in foliar nitrogen of alligatorweed at field sites may lead to associated variation in density dependent dispersal of *A. hygrophila*, independent of other environmental variables such as temperature or predation.

Spatial and temporal variability in FN may generate inconsistent patterns of control, even in areas where biological control is thought likely to succeed. For example, control of the floating aquatic weed, giant salvinia (*Salvinia molesta*) has been shown to be highly contingent on nutritional quality of the plant when establishing biological control agents (Room and

Thomas 1985b). This may be particularly important to consider when introducing *A. hygrophila* into areas where they do not overwinter (e.g. Arkansas, Tennessee), and time their introduction to coincide with high plant quality. Incidentally, the time of year (spring; Figure 3.1) when plant quality is highest coincides with mild temperatures and may provide optimal chance of establishment and control in those areas. Future experiments could be designed to test the importance of nutrition and temperature on establishment of *A. hygrophila* by manipulating the timing of agent release at northern sites, documenting plant nitrogen, ambient temperatures, establishment success, and ultimately reduction in infestation level.

CHAPTER 5.
BIOLOGICAL CONTROL AGENT PHENOLOGY AND VARIABILITY, BUT NOT DENSITY, BEST EXPLAINS HOST DENSTIY ALONG A LATITUDINAL GRADIENT

INTRODUCTION

Latitudinal or elevational patterns of species abundance may reflect biotic (e.g., competition, predation) or abiotic factors (e.g., climate) that act to delimit their distributions (Calosi et al. 2010, Sirén and Morelli 2019). Individuals in populations at the margins of their geographic range (e.g., high latitude sites) may be periodically subject to environmental extremes (e.g., weather events) that meet or exceed their physiological tolerances (Sexton et al. 2009). Climate, and climate-driven weather events, are major contributors to defining range margins of many organisms, but ectotherms are especially vulnerable to climatic variation, a topic which is currently important for understanding the ecological and evolutionary implications of climate change (Bale et al. 2002). Variation in temperature extremes, for instance, may lead to shifts in species' distributions and abundances, generating spatial variation in the timing and type of species interactions and population dynamics of those species (Trân et al. 2007, Reeve 2017, Posledovich et al. 2018).

Interacting species may be particularly vulnerable to changing climate if their interactions are modified by increasing mean temperatures or increased climate variability and frequency of extreme weather events (Allan and Soden 2008, Fischer and Knutti 2015, Romero et al. 2018). One result of climate change may be unequal rates of range expansion/contraction between predators and prey or plants and herbivores, altering spatial patterns in the timing, frequency and magnitude of their interactions (Schweiger et al. 2008, Tylianakis et al. 2008, Blois et al. 2013, Schleuning et al. 2020). For example, future climate change is predicted to result in a large spatial mismatch between the distributions and abundances of the butterfly *Boloria titania* Esper

and its host plant *Polygonum bistorta* L. in Europe, but the degree of mismatch may depend on their individual abilities to track changing environmental conditions (Schweiger et al. 2008). Increased variability in biotic or abiotic factors that mediate interactions is expected to lead to more frequent pest outbreaks (Marini et al. 2012) and density-dependence among herbivores (e.g., resource-based competition), especially in years where effects of abiotic factors are attenuated (e.g., when warm winter temperatures allow higher overwintering survival) (Goodsman et al. 2018). For example, the altitudinal range of the European spruce bark beetle *Ips typographus* (L.) (Coleoptera: Curculionidae) is tied to annual variation in rainfall and temperature, with outbreaks in European forests expected to increase with future climate variation (Marini et al. 2012). A better understanding of interannual variation in herbivore distribution and abundance relative to climate is especially relevant to economically important agronomic or natural systems in which herbivorous pests cause crop or other financial loss (Battisti and Larsson 2015, Deutsch et al. 2018), or weed biological control programs in which monophagous herbivores are intentionally introduced to reduce the abundance of a pest plant species (e.g., Zalucki and Van Klinken 2006).

Weed biological control has a history of varied successes. Although some variation may be explained by the different impacts of climate on agent and target weed, which leads to spatial gradients in abundance of agents and hosts, explicit examination of this phenomenon is rare (Harms et al. in review). For example, although both the aquatic weed giant salvinia (*Salvinia molesta*) and its biological control agent the giant salvinia weevil (*Cyrtobagous salviniae* Calder and Sands; Coleoptera: Curculionidae) are limited by cold temperatures (Whiteman and Room 1991, Owens et al. 2004, Mukherjee et al. 2014), the geographic distribution of the weevil is considerably more restricted than its host (i.e., northernmost populations of *C. salviniae* in the

US occur at lower latitudes than *S. molesta*), requiring annual reintroduction of the weevil in higher latitude locations (Mukherjee et al. 2014). Despite its rarity, an explicit examination of these systems from range interior to margin of the agent may provide insight into the relative importance of biotic (i.e., plant quality) and abiotic factors (i.e., climate) on abundance and distribution of agents, and ultimately successful control (Harms et al., in review).

A number of testable hypotheses exist regarding expected abundance patterns of species across their geographic ranges (Sagarin and Gaines 2002), including several adapted specifically for relevance to biological control systems (Harms et al. in review). Predicted patterns often reflect greater abundance in interior relative to marginal areas but this has received mixed support (Sagarin and Gaines 2002, Dallas et al. 2017) and may depend on a combination of range size and latitude (i.e., Rapoport's rule; Stevens 1989, McLoughlin and Ferguson 2000, Gaston 2003). On the other hand, environmental, rather than geographic, gradients may be a better predictor of species abundance *and* correlate with geographic gradients (e.g., the correlation between latitude and temperature). Low biological control agent abundance might be expected in high-latitude marginal areas because climate variables there (e.g., winter temperature) are likely to be at or near the agent's physiological limits. As a result, stochastic events (e.g., extreme weather events) should have disproportionate negative effects on biological control agent vital rates in marginal relative to interior areas, ultimately leading to reduced control (i.e., increased weed abundance).

Here, we examine the role of biotic and abiotic factors on plant-herbivore interactions along a latitudinal gradient that spans much of the range of a biological control agent in the US. This is accomplished by focusing on biological control of *Alternanthera philoxeroides* (Mart.) Griseb. (alligatorweed) by the chrysomelid beetle *Agasicles hygrophila* Selman and Vogt

(alligatorweed flea beetle) in Louisiana, USA. This system is well-suited to test for patterns of climate variability and related host plant-herbivore densities and variability because, 1) the distributions (and latitudinal range limits) of agent and host are mostly known and it has been observed that host distribution extends much farther north in the US than the agent; 2) the agent's distributional limit occurs at lower latitudes than the host and, under these circumstances, we would expect abiotic factors (climate, winter severity) to be primarily responsible for shaping the northern distributional limit of the agent; and 3) because the approximate location of the range margin is known for *A. hygrophila*, studying populations there can be especially valuable for identifying the factors associated with shaping range limits, agent abundance, and ultimately successful control in those areas (Fourcade and Öckinger 2016). We tested the following hypotheses and predictions (Table 1): 1) mean densities of *A. hygrophila* (Hypothesis 1a) and *A. philoxeroides* (Hypothesis 1b) reflect climate-related latitudinal gradients (i.e., *A. hygrophila* decreases with latitude due to increasing climate limitations correlated with latitude; *A. philoxeroides* increases with latitude due to decreased control by *A. hygrophila* at higher latitudes); 2) local density of *A. hygrophila* (Hypothesis 2a), but not *A. philoxeroides* (Hypothesis 2b), will be more variable in higher relative to lower latitude populations due to occasional temperature exposure at or beyond thermal tolerances of *A. hygrophila* but not *A. philoxeroides*; 3) winter severity is primarily responsible for the timing of *A. hygrophila* activity (Hypothesis 3); 4) winter severity is the best predictor of *A. hygrophila* (Hypothesis 4a) and *A. philoxeroides* (Hypothesis 4b) density; and 5) mean *A. hygrophila* density, independent of climate, explains the most variation in *A. philoxeroides* density (Hypothesis 5). Although climate-related variability in biological control of weeds has received considerable attention

around the world, our study is unique in that we examine direct and indirect relationships between latitude-associated abiotic factors, an invasive plant, and its biological control agent.

Table 5.1. Hypotheses about the relationship between latitude and weather, biological control agent and host abundance.

	Hypothesis/Prediction	**Reasoning**
1a	Density of *A. hygrophila* reflects climate-related latitudinal gradients	*A. hygrophila* decreases with latitude due to increasing climate limitations correlated with latitude.
1b	Density of *A. philoxeroides* reflects climate-related latitudinal gradients	*A. philoxeroides* increases with latitude due to decreased control by *A. hygrophila* at higher latitudes.
2a	Populations of *A. hygrophila* will be more variable in marginal relative to interior habitats (i.e., with increasing latitude)	Due to occasional temperature exposure at or beyond thermal tolerances of *A. hygrophila*.
2b	Populations of *A. philoxeroides* will not be more variable in marginal relative to interior habitats (i.e., with increasing latitude)	Temperature exposure across the study area is not near thermal limits of *A. philoxeroides.*
3	Winter severity is primarily responsible for the timing of *A. hygrophila* activity	Cold winters will suppress overwintering abundance and delay detection of *A. hygrophila* activity until population increases sufficiently
4a	Winter severity is the best predictor of *A. hygrophila* abundance	Because overwintering will be related to winter severity, harsher winters will lead to slower spring and summer buildup of *A. hygrophila* populations
4b	Winter severity is the best predictor of *A. philoxeroides* density	Due to reduced *A. hygrophila* activity (H3) and smaller populations (H4a), *A. philoxeroides* density will be higher.
5	*A. hygrophila* density, independent of climate, explains the most variation in *A. philoxeroides* density	As biological control agent density increases, consumption and effects on *A. philoxeroides* will increase, leading to lower plant density.

MATERIALS AND METHODS

Study system

Alternanthera philoxeroides (Amaranthaceae) is a South American aquatic clonal plant, introduced into the US during the 20th century and now common in aquatic systems of the southeastern US (Buckingham 1996b), with disjunct populations in California (Walden et al. 2019). In the 1960s, biological control of *A. philoxeroides* was initiated, culminating with the release of *A. hygrophila* (Coleoptera: Chrysomelidae), *Amynothrips andersonii* O'Neill (alligatorweed thrips) (Thysanoptera: Phlaeothripidae), and *Arcola* (=*Vogtia*) *malloi* Pastrana (alligatorweed moth) (Lepidoptera: Crambidae) (Buckingham 1996b). Source populations for the original introductions of *A. hygrophila* in the US were from Ezeiza Lagoon, near Buenos Aires, Argentina (~34.5 °S; released in California and South Carolina in 1964) and areas near Montevidea, Uruguay (~ 35 °S; released in South Carolina in 1964). Although *A. philoxeroides* and its biological control agents are present throughout the southeastern US, the plant has a broader distribution than its control agents. Overwintering of *A. hygrophila* is limited to areas that remain warm during winter (>11.1°C) (Coulson 1977), and timing of *A. hygrophila* attack in the spring is thought to be related to the severity of the previous winter (Harms and Shearer 2017).

Alligatorweed and flea beetle density along a latitudinal gradient in Louisiana

From 2015 – 2018, we monitored biological control of *A. philoxeroides* along a latitudinal gradient (29.8 – 32.5 °N) in Louisiana, which encompasses most of the latitudinal range of *A. hygrophila* in North America (Figure 1). In 2015, we surveyed six sites (three southern Louisiana = range interior, three northern Louisiana = range margin) and in 2016 – 2018 we surveyed 9 – 12 sites spanning the range of *A. hygrophila* in LA (Appendix 1).

Locations were visited, on average, every three weeks beginning in February or March of each year through October or November. During 2015, visits ended in September because of site access issues due to flooding. During visits, plant density was estimated by placing a 1/10 m^2 PVC quadrat in four haphazardly chosen locations within alligatorweed infestations then counting all emergent *A. philoxeroides* stems. Mean plant density (number of *A. philoxeroides* stems per 1/10 m^2) and variability in plant density (coefficient of variation) was calculated for each site and year. Because timing of growth and abundance of plants varied among sites based on latitude and conditions each year, we use a single mean density estimate for annual *A. philoxeroides* abundance in each site. Additionally, leaves were collected on most sampling dates for foliar nitrogen determination as previously described by Harms and Cronin (2019b). In that study, we found that foliar nitrogen was important for *A. hygrophila* development and survival.

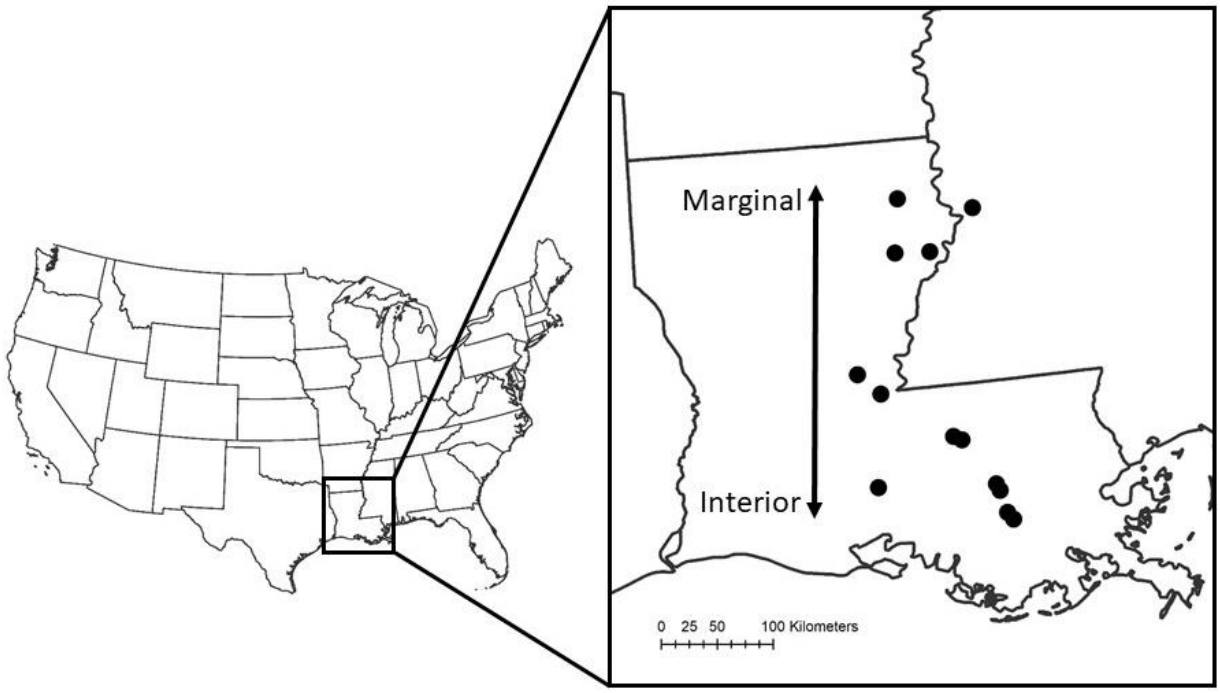


Figure 5.1. Locations in Louisiana, USA where biological control of *A. philoxeroides* was monitored over four years. Marginal and interior habitats for the biological control agent *Agasicles hygrophila* are labeled.

For each site visit, *A. hygrophila* density was estimated. 10-20 alligatorweed plants (Mean ± SE: 17.8 ± 0.2 stems) were collected, placed in plastic zip top bags, and examined within 24 hr. *Agasicles hygrophila* egg masses, larvae, pupae, and adults were counted. When entrance or exit holes were observed, stems were dissected to detect larvae, pupae, and adults. Total density of *A. hygrophila* is reported based on the sum of all life stages (except egg masses) per stem. In addition to mean herbivore abundance during the year, rapid defoliation events caused by biological control agent outbreak events may be important for successful biological control (DeClerck-Floate and Bourchier 2000). Therefore, in addition to mean density we used maximum biological control agent density in statistical models. Mean density for both agent and host was calculated first as the averaged sum of individuals per stem (or stems per m^2) on a particular sampling date and site, then averaged for each site and year. Maximum density of *A.*

hygrophila was determined from mean abundances for each site and sampling dates. Within-year variability in agent or host density was calculated for each site and year as the coefficient of variation from site visit data within that year.

Weather data

Although winter temperatures are thought to be the primary determinant of *A. hygrophila* density, other seasonal factors may contribute to *A. hygrophila* population dynamics across its range, particularly maximum summer temperatures and humidity (Jia et al. 2020). Therefore, we obtained additional weather data (winter minimum temperature, spring minimum/maximum/average temperature, spring precipitation, summer maximum temperature, summer precipitation) from 82 weather stations within the state of Louisiana for 2014-2019 from the National Oceanic Administration Agency (NOAA) National Centers for Environmental Information (NCEI) online climate database (ncdc.noaa.gov). Relevant weather variables were selected based on previous experience in this system or literature review. In particular, winter temperatures are thought to limit overwintering of *A. hygrophila*, spring weather may be important because that is the time of year when *A. hygrophila* first becomes active and is thought to exert the most control of its host (Coulson 1977, Vogt et al. 1992), and summer maximum temperatures likely limit activity of the agent through impacts on egg hatching and fecundity (Zhao et al. 2016). Average daily minimum temperatures were calculated from November 1 to March 1 each year. A winter severity index (WSI) was also calculated, equal to the number of days with minimum temperatures below freezing. Average spring temperatures were calculated as the daily minimum/maximum average, then averaged over the period March 1 until June 1. Maximum daily summer temperatures were the average maximum daily temperature between

June 1 and September 1 each year. Weather data were calculated for each weather station then we spatially interpolated study site-specific weather information by kriging in ArcMap v10.5 (ESRI, Redlands, California) (Kumar et al. 2007).

Reduction of weather variables to principal components

The eight weather variables (winter minimum daily temperature, winter severity index, spring minimum daily temperature, spring maximum daily temperature, spring average temperature, spring precipitation, summer maximum daily temperature, summer precipitation) were standardized, then reduced to three factor loadings (i.e., principal components; PC1-3; Figure 2) using PROC FACTOR in SAS version 9.4 (SAS Institute, Cary, North Caroline). The three PCs represent independent linear combinations of the weather variables and accounted for 86% of total variance present in the original variables. The retention of three variables was based on examination of eigenvalues and the scree diagram (Kriticos et al. 2014). Although PC3 had an eigenvalue less than one (0.79), it was retained because of the high loadings associated with precipitation that were not present in the first two PCs. Examination of PCs indicated that PC1 is positively correlated with winter and spring temperatures, PC2 is positively correlated with winter severity and summer maximum temperature (i.e., extreme temperatures), and PC3 is positively correlated with spring and summer precipitation (i.e., precipitation) (Figure 2). All three PCs were used in structural equation modeling described below.

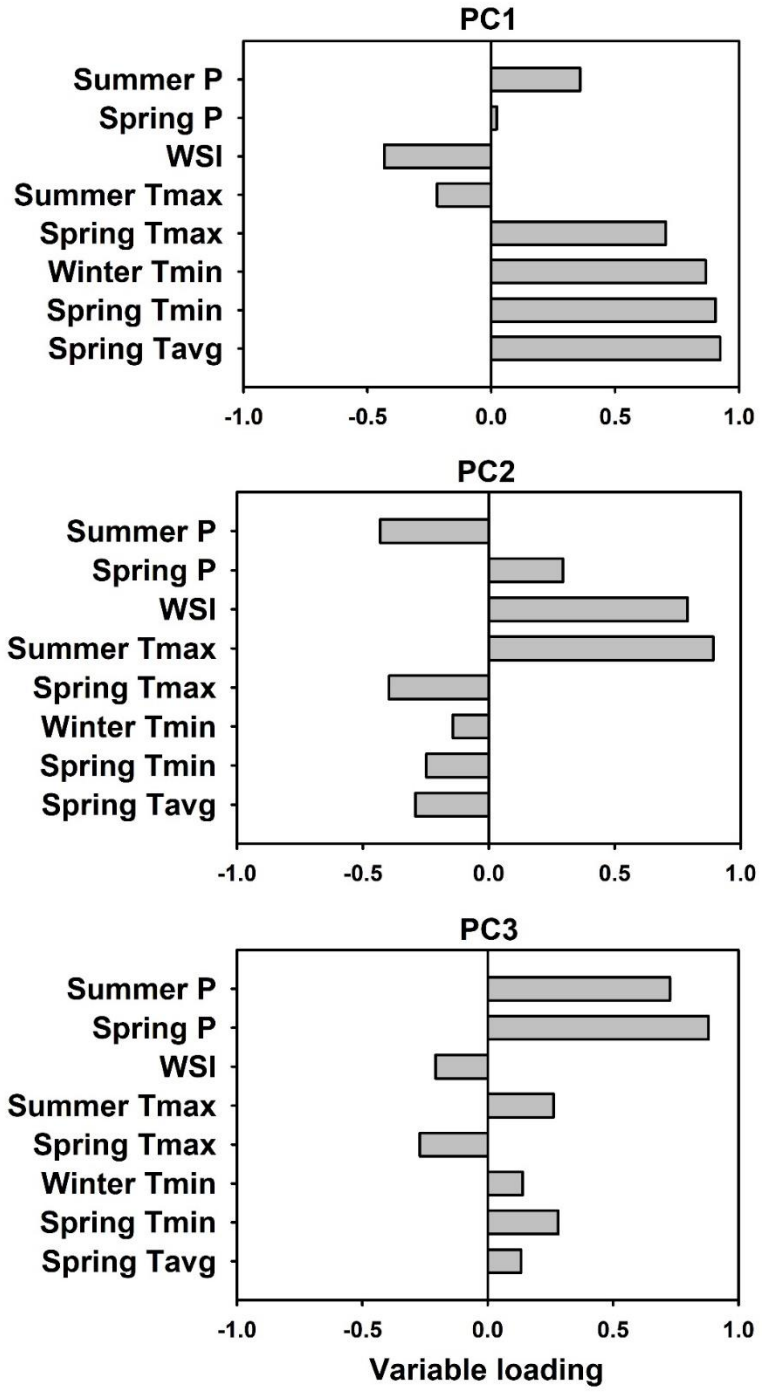


Figure 5.2. Results of factor analysis and weather variable loadings for each of three principal components. PC1 is largely positively correlated with winter and spring temperatures, PC2 is positively correlated with extreme seasonal temperatures (i.e., in winter and summer), and PC3 is positively correlated with precipitation in spring and summer. P = Precipitation, WSI = winter severity index, T_{max} = maximum daily temperature, T_{min} = minimum daily temperature, T_{avg} = average daily temperature.

Statistical Analyses

Relationship between latitude and agent or host density

We used a combination of techniques to determine whether alligatorweed biological control varied along a latitudinal gradient and to determine the relative importance of biotic (*A. hygrophila*) and abiotic (weather) factors on control. First, we used separate general linear models to examine effects of latitude on agent and host density (or variability). In these models, density (*A. hygrophila* mean or maximum density, *A. philoxeroides* mean density) was the dependent variable, latitude was a continuous predictor, year was a main effect, and the latitude x year interaction was included in the models. Distributions of density variables were normalized through natural log (+0.5) transformation before analysis. Transformation of coefficient of variation values was not necessary.

Direct and indirect effects of latitude and weather on agent and host density

Beyond the predicted bivariate relationships between agent or host density and latitude, we further explored direct and indirect effects of latitude and weather on these dependent variables using structural equation modeling (SEM). SEM, also known as 'modern path analysis', is a statistical approach to determine the direction and magnitude of relationships (including direct and indirect effects) between multiple associated variables, equivalent to a series of linear models (Grace et al. 2016). We used SEM to assess the importance of weather on biological control agent phenology (i.e., attack timing) and density and the dual importance of weather and biological control (i.e., agent abundance and variability) on host plant density. Based on our knowledge of the *A. philoxeroides* biological control system (through direct experience and based on literature review), we first generated a path diagram to depict the full

conceptual model including all measured or estimated weather, insect, and plant variables with direct and indirect interactions (Appendix 2). In the full model, covariance parameters were added between weather-related PCs and between *A. hygrophila* density and variability. Because maximum and not mean herbivore density may be more important to *A. philoxeroides* population density, we also constructed a second set of models using the same SEM approach but replaced mean *A. hygrophila* density with maximum *A. hygrophila* density, retaining all other connections and variables. The full model hypothesizes that all PCs are correlated with latitude, and that they directly influence *A. hygrophila* and *A. philoxeroides* densities. Specifically, PC1 and PC2 are likely to influence the timing of *A. hygrophila* activity because winter and spring (and extreme) temperatures have been previously reported as important (Stewart et al. 1999a, Guo et al. 2012). PC3 may be important if spring and summer precipitation leads to increased humidity or has a positive influence on plant quality, which is critical for larval survival and development (Wei et al. 2015). *Agasicles hygrophila* density should have a direct effect on *A. philoxeroides* density. Additionally, timing of *A. hygrophila* activity should have a strong indirect effect on *A. philoxeroides* because it has been suggested that timing of *A. hygrophila* attack, rather than absolute abundance was critical for control (Harms and Shearer 2017). Foliar nitrogen was predicted to positively relate to *A. hygrophila* density based on previous work (Harms and Cronin 2019b). Prior to SEM analysis, all variables except PCs were standardized to Z-scores (Gotelli and Ellison 2004). PCs were generated based on already-standardized variables.

Model fit and model selection were assessed several ways. First, to determine the best model that explained *A. philoxeroides* density from a subset of models that included the full models (with either mean or maximum *A. hygrophila* density), we used an iterative approach coupled with absolute and relative best-fit indices (Grace 2006). Data were fit using the

maximum likelihood estimation method. From the full models (one with mean *A. hygrophila* abundance and one with maximum *A. hygrophila* abundance), we examined results of Wald tests to determine which relationships did not contribute to the model (Kim 2014). Parameters with statistically insignificant univariate probabilities (i.e., $P > 0.05$) were removed from the model. We removed a single parameter at a time, reassessing parameter significance each time. From the models generated by variable removal (a total of 34 model iterations), we used Akaike Information Criterion adjusted for small sample size (AICc; Burnham and Anderson 2003) to select the most informative models from the set of full and partial model combinations (Grace 2006). ΔAICc was calculated as the difference between the top model and all others. Models with $\Delta AICc \leq 2$ were considered to have substantial support (Burnham and Anderson 2003). Akaike weights are also reported, which represent the relative likelihood that the model is the best given the data and other candidate models. Next, absolute model fit was assessed for the full model and models with $\Delta AICc \leq 2$ from the top model. This was done using chi-square lack-of-fit *P*-values, goodness-of-fit index adjusted for degrees of freedom (AGFI), and root mean square error of approximation (RMSEA) (Grace 2006). Good model fit is indicated by X^2 *P*-values > 0.05, AGFI ≥ 0.9, and RMSEA ≤ 0.08. SEM analysis and model fit parameters were determined in SAS using PROC CALIS.

RESULTS

Relationship between latitude and agent or host density

Over four years of field measurements to monitor biological control of *A. philoxeroides*, latitude was a significant predictor of biological control agent and host abundance. Mean (range: -0.40 ± 0.16 – 0.47 ± 0.15 ln[insects per stem]) and maximum (range: -0.02 ± 0.24 – 1.57 ± 0.22 ln[insects per stem]) densities of *A. hygrophila* and mean (range: 5.03 ± 0.72 – 5.80 ± 0.27

ln[stems per m^2]) densities of *A. philoxeroides* did not vary significantly among years. Additionally, the year x latitude interaction was statistically insignificant. Agent annual mean density and maximum density decreased with latitude (Figure 3A, B; in support of Hypothesis 1a) and within-year variability (coefficient of variation) of *A. hygrophila* mean density increased with latitude (Figure 3C; in support of Hypothesis 2a). Plant density increased with latitude (Figure 3D; in support of Hypothesis 1b), but plant variability did not (Figure 3E; in support of Hypothesis 2b). Overall, plant and insect densities were correlated (*A. philoxeroides* mean density – *A. hygrophila* mean density, r = -0.51; *A. philoxeroides* mean density – *A. hygrophila* maximum density, r = -0.45).

Table 5.2. Results for mixed effects models to examine the importance of latitude and year on *A. philoxeroides* and *A. hygrophila* density and variability across Louisiana.

	A. philoxeroides density			*A. philoxeroides* CV			Maximum *A. hygrophila* density			Mean *A. hygrophila* density			*A. hygrophila* CV		
Effect	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*
Latitude	1	13.53	**<0.001**	1	2.00	0.17	1	8.60	**0.006**	1	10.54	**0.002**	1	13.75	**0.001**
Year	3	0.26	0.85	3	0.53	0.67	3	1.66	0.20	3	0.71	0.56	3	0.27	0.84
Latitude x Year	3	0.22	0.88	3	0.56	0.65	3	1.90	0.15	3	0.78	0.51	3	0.27	0.85

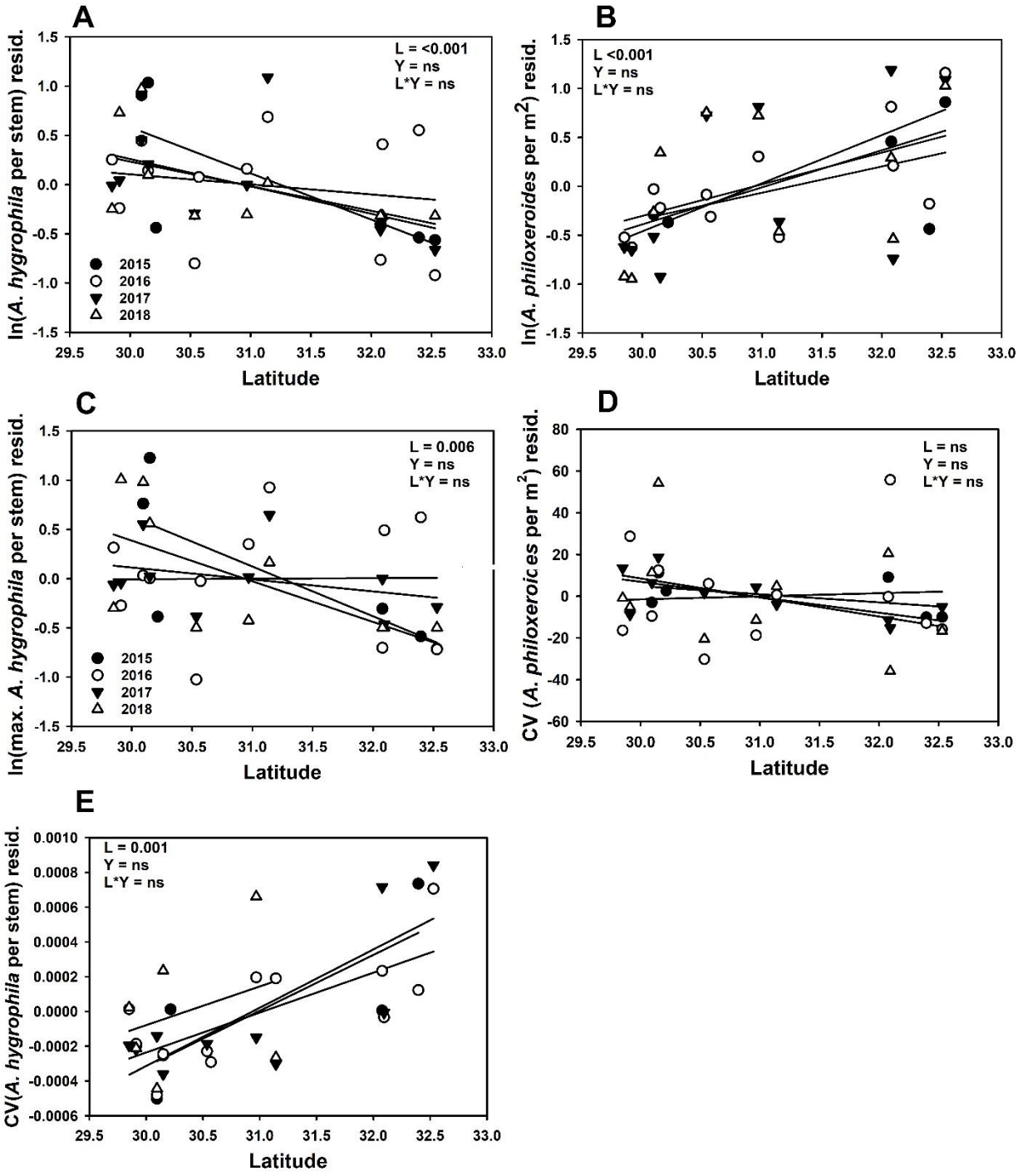


Figure 5.3. The relationships between latitude and the alligatorweed biological control agent, *Agasicles hygrophila* mean (a) and maximum (c) density, and variability (e), and *A. philoxeroides* density (b) and variability (d) in Louisiana. To emphasize the effect of latitude independent of year, a mixed effects model was conducted without latitude. The residuals from this analysis are plotted against latitude and best-fit lines based on least squares regression for each year are displayed. Separate lines are reported for each of the four years of this study.

Biotic and abiotic predictors of alligatorweed density

Alligatorweed density in the northern range of its primary biological control agent was explained by three equally likely models based on AICc (Table 1, Appendix 2). Based on a combination of AICc and other fit metrics, we selected a best model (Figure 4) that largely demonstrated the direct effects of weather on biological control agent but not plants (Table 2). The top model explained a substantial part of the total variance for *A. hygrophila* mean density ($R^2 = 0.28$) and variability ($R^2 = 0.49$), the date of first biological control activity ($R^2 = 0.59$) and *A. philoxeroides* mean density ($R^2 = 0.43$). In contrast to the analysis above, latitude did not have a direct effect on either agent or plant density, but was influential through indirect effects on both agent (-0.24) and plant density (0.34) as mediated by weather variables (Table 2). Weather variables had little to no direct effect on *A. philoxeroides* density over the study region and only the direct path between PC3 and plant density (0.18) was retained in the final model. Specifically, winter severity was not directly important for *A. philoxeroides* (rejection of Hypothesis 4b) or *A. hygrophila* (rejection of Hypothesis 4a) densities, except indirectly through mediation of attack timing (see below). In contrast, *A. philoxeroides* density had a strong positive relationship to variability in *A. hygrophila* density (direct effect: 0.66) and attack timing (indirect effect: 0.32), but not *A. hygrophila* mean density (rejection of Hypothesis 5) so this path was removed from the final model. Latitude was indirectly related to variation in *A. hygrophila* density (indirect effect; -0.24) and variability (indirect effect; 0.51), and *A. philoxeroides* density (indirect effect; 0.34).

Table 5.3. Model rank and fit indices for a subset (best and full models) of model combinations. The top model is highlighted and was chosen based on a combination of model selection (AICc), absolute fit (χ^2) and relative-fit (GFI, AGFI) indices. The first column denotes whether the model had a mean (Mean) or maximum (Max) density variable for *A. hygrophila* (*Ah*). AICc = Akaike Information Criterion corrected for small samples size, ΔAICc = difference between AICc of the model and AICc of the top model, RMSEA = root mean square error approximation, GFI = Goodness-of-fit index, AFGI = Sample-size adjusted goodness-of-fit index, χ^2 *P*= Chi-square probability.

***Ah* Mean/Max**	**AICc**	**ΔAICc**	**Likelihood**	**Akaike Wt.**	**RMSEA**	**GFI**	**AGFI**	**χ^2**
Mean	65.93	0.00	1.00	0.52	0.085	0.88	0.73	0.23
Max	67.18	1.25	0.53	0.28	0.10	0.87	0.72	0.15
Mean	67.85	1.92	0.38	0.20	0.079	0.89	0.73	0.26
Max-Full	162.95	97.02	0.00	0.00	0.17	0.91	0.53	0.043
Mean-Full	163.26	97.33	0.00	0.00	0.17	0.90	0.52	0.039

Table 5.4. Estimates and significance terms (P-values) for direct and indirect effects in the selected best model. PC1-3 are principal components of weather variables, *Ah = A. hygrophila*, *Ap = A. philoxeroides*, CV = Coefficient of variation.

	Path			**Estimate**	***P***
Direct effects	Latitude	===>	PC1	-0.81	<.0001
	Latitude	===>	PC2	0.3	0.06
	PC1	===>	Date of first *Ah* activity	-0.35	0.002
	PC1	===>	CV *Ah* density	-0.36	0.003
	PC2	===>	Date of first *Ah* activity	0.58	<.0001
	PC3	===>	Date of first *Ah* activity	-0.35	0.002
	CV *Ah* density	===>	Mean *Ap* density	0.66	<.0001
	Date of first *Ah* activity	===>	Mean *Ah* density	-0.53	<.0001
	Date of first *Ah* activity	===>	CV *Ah* density	0.49	<.0001
Indirect effects	Latitude	===>	Mean *Ap* density	0.34	<0.001
	Latitude	===>	CV *Ah* density	0.51	<0.001
	Latitude	===>	Mean *Ah* density	-0.24	0.01
	Date of first *Ah* activity	===>	Mean *Ap* density	0.32	0.001

Latitude-related weather variables are clearly important to *A. hygrophila* performance in marginal areas. All three weather PCs were influential for timing of *A. hygrophila* activity (PC1:

-0.35; PC2: 0.58; PC3: -0.35). In particular, winter and summer severity (PC2) had the strongest effect on activity timing, which supports our hypothesis about the importance of winter on subsequent activity and population growth of *A. hygrophila* (Hypothesis 3). Mean density of *A. hygrophila* was negatively related to date of first activity (direct effect: -0.53) and latitude. Variability of *A. hygrophila* density was negatively related to winter and spring temperatures (PC1: -0.36) and positively related to timing of activity (0.49). In agreement with the bivariate analysis, variability of A. *hygrophila* density was also positively related to latitude (indirect effect: 0.51; Hypothesis 2a). Contrary to our prediction, foliar nitrogen was not an important variable in the analysis and was not retained in the top models.

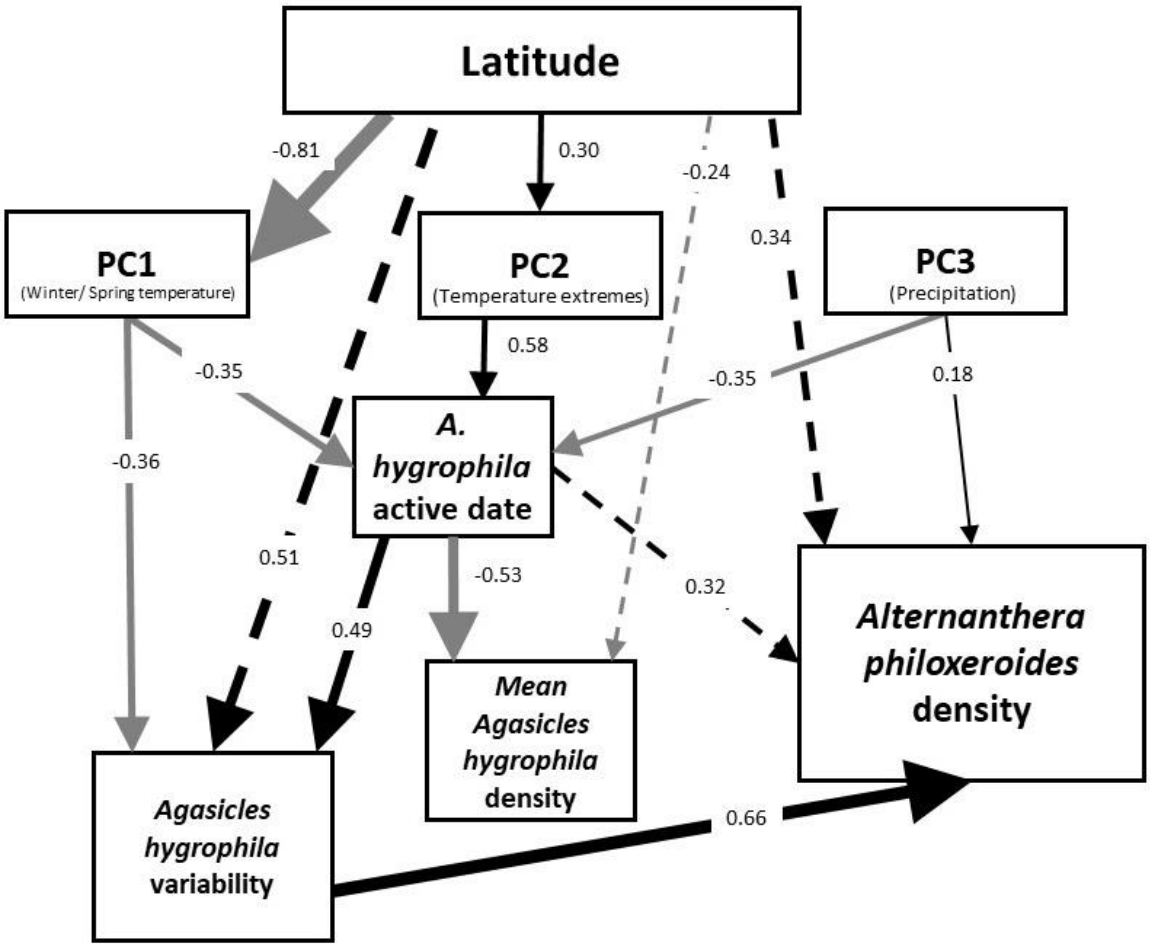


Figure 5.4. Best-fit model (Table 1) to explain *A. philoxeroides* density in relation to biological control agent abundance, variability and timing, and weather. Solid lines are significant direct effects and dashed lines are indirect effects between variables. PC1-3 are described in methods section above. Standardized path coefficients are given for each path. For clarity, positive paths are shown in black and negative paths are shown in gray.

DISCUSSION

For tightly-interacting species (e.g. specialized biological control agent and host plant), the relative importance of factors that shape abundance patterns and range margins of each species may be spatially heterogeneous (Schweiger et al. 2008, Schweiger et al. 2012). Studying organisms across their geographic range, with an explicit inclusion of marginal areas, allows for the identification and measurement of these factors and provides an opportunity to make predictions about future interactions with climate change (Fourcade and Öckinger 2016). Over

four years of intensive sampling in Louisiana, we discovered that the density of an invasive aquatic weed, alligatorweed, was largely determined by biotic factors such as phenology and variability of its biological control agent, whereas agent density was determined primarily by latitudinally-correlated abiotic factors (e.g., winter and spring temperatures, winter severity and summer temperature maxima) through their influence on the timing of agent activity.

Altered timing of herbivore-plant interactions is a critical prediction of the ecological effects of climate change (Bale et al. 2002, Forrest 2016, Ju et al. 2017, Posledovich et al. 2018, Renner and Zohner 2018, Davies 2019, Schleuning et al. 2020). Warmer winter temperatures at high latitudes may lead to range expansion mediated by increased winter survival in herbivores and their hosts. In cases where there is already some degree of phenological mismatch between herbivore and host, small changes in the timing of the interaction could be dire for herbivore populations (Singer and Parmesan 2010). For example, a pair of lepidopteran species were shown to frequently suffer high mortality because of mismatches in the seasonal timing of their early egg hatch (*Operophtera brumata* L.; Lepidoptera: Nymphalidae) or late eclosion (*Euphydras editha bayensis* Boisduval; Lepidoptera: Nymphalidae) with the presence of the suitable life stage of their host plants (*Quercus robur* for *O. brumata*, *Plantago* or *Castilleja* for *E. editha*). Further increases in asynchrony between lepidopteran and plant life histories (e.g., as a result of climate change) will likely lead to more frequent population extinctions of the herbivore (Singer and Parmesan 2010). On the other hand, warming temperatures may not disrupt plant-herbivore relationships, but only advance their timing within the year (Sparks and Yates 1997, Ju et al. 2017). Phenology of *Corythucha ciliata* (Say) (Hemiptera: Tingidae) and its host plant *Platanus x acerifolia* (Platanaceae) (London plane) both responded similarly to experimental warming, with an advance of post-overwintering activities for *C. ciliata* and leaf

expansion for *P.* x *acerifolia* in spring during the study. Although phenological synchrony between herbivores and hosts may be maintained for the near future, a plastic response (i.e., earlier activity) by *C. ciliata* to warming is thought to increase the likelihood of future outbreaks by increasing insect population size early in the year (Ju et al. 2015, Ju et al. 2017). Whether or not individual herbivore-host systems will be drastically altered may be related to the relative importance of climate variables on each of the interacting species, which itself depends on location within the ranges of the species (Trân et al. 2007).

It is now well understood that factors responsible for limiting a population at high latitudes (or elevations) may not be as important as at low latitudes (or elevations) (Dvorský et al. 2017, Sirén and Morelli 2019). For instance, the stress-trade-off hypothesis suggests a general rule that abiotic stressors (e.g. temperature) limit species distributions in harsh environments (i.e., high latitudes) and that biotic interactions (e.g., predation, competition) have a larger influence in benign environments (i.e., low latitudes) (Louthan et al. 2015, LaManna et al. 2017, Roslin et al. 2017, Sirén and Morelli 2019). Although evidence in support of this hypothesis is mixed (e.g., Anderegg and HilleRisLambers 2019), it is clear that different variables can contribute to species density in different parts of the geographic range. For example, in the forest pest *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytinae) (the southern pine beetle), winter severity explained a sizeable portion of the variation associated with population dynamics in northern but not southern locations (Trân et al. 2007). In the same study, it was demonstrated that supercooling points (a metric of cold-hardiness) of a northern *D. frontalis* population was significantly lower than a southern population, apparently the result of an adaptive response to more consistently low temperatures in high latitudes. Our study was conducted across a large portion of the latitudinal range of *A. hygrophila* in the US, and included its northern range limit,

but we did not survey a large enough area to determine which factors were responsible for the southern range limits of *A. hygrophila* (*or* northern range limits of *A. philoxeroides)*. However, our findings suggest that the system is inherently climate-limited in that variables such as winter and spring temperatures, and winter and summer temperature extremes, influenced the timing, variability, and density of *A. hygrophila*, which in turn influenced alligatorweed density.

With global climate change, there is an expected increase in mean temperatures and variability of seasonal conditions (e.g., more frequent extreme events), with complex impacts on performance of organisms (Easterling et al. 2000a, Vasseur et al. 2014, Matthews et al. 2016, Büntgen et al. 2020). Given that we found temperature (PCs 1, 2) to be important for predicting the timing of flea beetle activity, warming climate should lead to earlier timing of flea beetle activity in high latitudes and associated decreased abundance of *A. philoxeroides*. However, if climate variability increases, as is predicted (Easterling et al. 2000b), then negative impacts of increasingly-frequent extreme weather events (i.e., climate variation) may outweigh the benefits of consistently warmer mean winter and spring temperatures (Kingsolver et al. 2013, Vasseur et al. 2014). Additionally, the relative importance of climate variability on performance of a species may reflect the latitudinal origin of that species (Shah et al. 2017). Across a number of taxonomic groups, higher latitude species typically have broader thermal tolerances (i.e., higher survival and performance across a greater range of temperatures) than low-latitude species (termed the climatic variability hypothesis; Addo-Bediako et al. 2000), but this range in temperatures is almost always skewed toward low temperatures (i.e., upper thermal limits do not appreciably change with latitude whereas lower thermal limits do change). Because *A. hygrophila* is a tropical/ subtropical species, it may not possess the thermal adaptations to survive increased climatic variation in areas at the range margin but additional releases in the US

of *A. hygrophila* from different parts of the native range may provide the genetic variation needed to promote adaptation to climate variation in high latitude infestations. Although releases of putatively more cold-hardy *A. hygrophila* have been previously made in North and South Carolina, occurrence of *A. hygrophila* one year after releases could only be confirmed at 10% of sites and follow-up since then has not occurred in order to determine whether establishment was successful (Buckingham and Boucias 1982a). Additional research is needed to better predict how differences in temperature means and variability will impact future biological control of *A. philoxeroides* given current or future biological control agents.

This work represents an examination of the direct and indirect effects of latitude, weather, and biological control (i.e., agent phenology, density and variability) on target weed density across a latitudinal range of a biological control agent. In both bivariate and multivariate analyses, a latitudinal pattern in agent and plant density emerged and was consistent with theory and other observations of density patterns across environmental gradients (Watkinson 1985, Kikvidze et al. 2005, Miller et al. 2009). That *A. philoxeroides* density increased with latitude suggests that the benefit of reduced biological control at high latitudes outweighs potential limitations from weather. This study also highlights the value of biological control and the importance of agent phenology for explaining abundance of the host plant. Although it has been suggested previously for this system (Harms and Shearer 2017), agent phenology can be an important but overlooked component of a biological control program. In future climates and with associated variability in weather events, systems like this one may experience increasingly variable control efficacy due to changes in timing of agent activity and abundance. In these programs, consideration of new agents sourced from climatically-similar areas of the native range may be warranted. Additionally, the results of this study may assist other programs in

which variable control is observed, especially where agent and host geographic distributions are not fully-overlapping and limiting environmental gradients are suspected.

CHAPTER 6.
DISCUSSION

In this dissertation, I examined biogeographical variation in biological control of invasive plants. I used literature and a database review to explore causes and consequences of geographic variability in biological control, field and laboratory studies to determine pathogen susceptibility in flowering rush cytotypes, field and laboratory studies to elucidate the importance of foliar nitrogen on alligatorweed biological control, and a field study to determine the relative importance of climate-related weather variables and biological control on alligatorweed density across the range of its control agent. In combination, these studies demonstrate the importance of considering broad-scale spatial variation in plant-insect interactions when implementing management to mitigate the negative impacts of invasive plants in recipient systems.

To date, there has been no comprehensive examination of weed biological control programs to determine the ubiquity of spatially variable control outcomes. In chapter 2, I reviewed the World Catalogue of Agents and Their Target Weeds (WCATW) to estimate the causes and consequences of geographic variation in biological control successes. I found that the most common factors associated with variability included temperature (14% of variable programs, 5% of all programs), precipitation (18% of variable programs, 7% of all programs) and predation (10% of variable programs, 4% of all programs). Nearly half of all programs in which success was spatially variable were without clear explanations. I gave examples of limiting biotic and abiotic factors, programs in which these were likely important for generating spatially variable control outcomes, then provided potential ecological and evolutionary consequences of the variability. Where biotic or abiotic factors occur along gradients, spatial patterns of control may be expected to reflect those gradients, but this has rarely been directly tested and data to examine this may not exist due to the geographic scope of the studies

necessary to do so. Gradients in agent presence or abundance may then have evolutionary consequences such as adaptive responses in plant defense-growth relationships or phenology. Future research objectives should include explicit consideration of spatial variability in biotic and abiotic factors that constrain agent and plant performance. Conducting studies on agent or plant performance and control success along environmental gradients may provide valuable insights into the relative importance of limiting factors on agents and host plants, how their importance varies spatially, and whether biological control implementation can adapt to this variation through sourcing additional agents or using existing agents more strategically (e.g., through optimizing rearing/release/establishment strategies).

Prior to introduction of biological control agents, studies to document and compare spatial variation in baseline natural enemy levels (i.e., biotic resistance) can be used to predict relative impacts of future agents, particularly when the invader has a large geographic distribution and includes multiple populations of unique lineages (Cronin et al. 2015). Although past studies of this type have mainly involved the examination of herbivory (Maron and Vilà 2001, Garcia-Rossi et al. 2003), no large-scale investigations into potential for biotic resistance due to generalist pathogen/disease differences in invader lineages has occurred. In chapter 3, I studied US flowering rush populations to determine whether the two introduced cytotypes (diploids and triploids) differed in evidence of biotic resistance due to generalist foliar fungal pathogens and whether biotic resistance varied along latitudinal or climate gradients. I found that triploid plants displayed 75% less disease than diploid plants during field surveys but only triploid plants displayed a latitudinal gradient in which disease more than doubled from high to low latitudes, possibly due to increased stress near the southern expanding range margin of triploid plants (Hilker et al. 2005). In a follow-up laboratory excised-leaf experiment, I found

that leaves from diploid plants were overall less susceptible (i.e., lower damage ratings and smaller leaf lesions) than triploid plants to infection from three generalist fungal pathogens. The seemingly contradictory results between the field and laboratory may be due to climatic differences between areas that limit the regional pool of pathogens or their effect on plant lineage susceptibility to infection (i.e., a genotype x environment interaction). My results demonstrate that two widespread *B. umbellatus* lineages exhibit different susceptibility to pathogens, that susceptibility may depend on local conditions, and that effectiveness of pathogen biological controls, if introduced, may similarly vary latitudinally by lineage. This type of study is important for better predicting variability in control once biological control agents are introduced. However, it is also important to test future agents because they may have genotype-specific searching or feeding adaptations that reduce the relative importance of plant defenses which may provide protection primarily against generalist herbivores (Liu et al. 2018).

A critical component of biological control performance that is often overlooked is that of variation in host quality (Room and Thomas 1985a, Steinger and Müller-Schärer 1992, Wheeler and Center 1996b, 1997, Hinz and Müller-Schärer 2000, Coetzee and Hill 2012, Uyi et al. 2016, Nachtrieb et al. 2019). It is well known that host quality (i.e., foliar nitrogen, plant defensive chemistry) can fluctuate seasonally or with variation in soil conditions. However, many investigations into variation in host quality and implications for biological control are limited in scope or scale, making direct connections with long-term field performance of agents difficult. In chapter 4, I used a combination of field measurements and laboratory experiments to determine the range of foliar N that larvae of the biological control agent, alligatorweed flea beetle are exposed to in the field and its importance to larval development and dispersal. There was strong seasonality of foliar N in field sites with peak levels (4 – 8 % dry weight nitrogen; DW N)

recorded early during each year, declining during summer, and slightly increasing again in the fall. In the laboratory, foliar N and rearing temperature had strong independent effects on larval development rate, larval size, survival to adult, and adult size. I demonstrated that increasing nitrogen in leaf tissues shortens larval *A. hygrophila* developmental time and increases survival to adulthood, regardless of exposure temperature during development. Foliar nitrogen may have important effects on biological control of alligatorweed, particularly as a result of seasonal variation in temperature and plant nutrition at field sites, and could contribute to observed variation in *A. hygrophila* efficacy in the field as has been demonstrated in other programs. For example, establishment of biological control agents and subsequent control of giant salvinia (*Salvinia molesta*) has been shown to be related to nutritional quality of the plant and its importance for the biological control agent, *Cyrtobagous salviniae* (Room and Thomas 1985b, Nachtrieb et al. 2019). For alligatorweed biological control, levels of plant nutrients (and amendment, if necessary) may be important to consider when introducing *A. hygrophila,* particularly in areas where they do not overwinter (e.g. Arkansas, Tennessee). Although clearly important for release and establishment in some cases, other phases of biological control implementation (e.g., foreign exploration, host-range testing) may be especially sensitive to variation in host quality and should take into account plant nutrition relative to needs of the agent (Room and Thomas 1985a, Harms and Cronin 2019a). For example, during foreign exploration, studies into seasonal variation in plant quality at survey sites may provide insights into spatial and temporal variation in herbivore species occurrence and abundance. In pre-release quarantine studies, high-quality test plants are necessary for accurate host-range testing, but how variation in quality related to tissue nitrogen affects results of these studies has not been assessed, though might provide a valuable direction for future research.

Spatial variability in biological control can result from incomplete geographic overlap between the agent and host plant caused by biotic (e.g., disease, competition, predation) or abiotic (e.g., temperature, precipitation) factors (Chapter 2). The geographic ranges of alligatorweed and its biological control agent *Agasicles hygrophila* incompletely overlap in the southeastern US, producing spatial heterogeneity in control efficacy that may be related to environmental (i.e., climate) gradients (Vogt et al. 1992, Harms and Shearer 2017). In chapter 5, I investigated the biotic and abiotic factors contributing to alligatorweed abundance across the majority of the latitudinal range of *A. hygrophila* in the US. I explicitly included marginal populations of *A. hygrophila* in this work because in those areas organisms are likely to periodically experience extreme environmental conditions relative to their physiological limits (Sexton et al. 2009). Therefore, those locations are well-suited to identify the types and magnitude of factors responsible for shaping the species' geographic distribution (Fourcade and Öckinger 2016). I used structural equation modelling to analyze four years of data collected in Louisiana field sites to determine direct and indirect effects of latitude and weather on the agent and host. I found that mean *A. hygrophila* density decreased approximately 56% from low to high latitude sites, maximum *A. hygrophila* density decreased approximately 37% from low to high latitude sites, *A. hygrophila* variability nearly doubled with latitude, and alligatorweed density was 2.5 times higher at high versus low latitude sites but variability in alligatorweed density was similar across latitudes. Winter severity and summer maximum temperatures were positively correlated to *A. hygrophila* phenology- a colder winter and hotter summer led to later activity of *A. hygrophila* in the study area which, in turn, influenced alligatorweed abundance. The major finding of this work was that a combination of weather and climate-related variation in biological control agent activity and population variability, but not density, contributes to the

spatial heterogeneity observed in target weed populations. This study has implications not just for the alligatorweed biological control system, but for other systems that may be affected by climate change. If the timing or magnitude of species interactions is modified, then outbreaks of plant or insect pests could become more frequent and severe (Ju et al. 2015, Ju et al. 2017). In the case of alligatorweed biological control, it is unclear how the complex effects of warmer mean winter temperatures (allowing greater overwintering survival of *A. hygrophila*) will interact with seasonal extremes (i.e., more frequent severe winter minimum or summer maximum temperatures leading to decreased survival of *A. hygrophila* during those periods) to shape biological control in these areas. For biological control programs overall, consideration of new agents sourced from climatically-similar areas of the native range may be worth pursuing if performance of current agents declines in the future. Alternatively, it may be worth exploring areas in the native range with increased climate variability to select agents that are pre-adapted to variable, but not necessarily mean, temperatures likely to be experienced in the introduced range.

Despite the long history of successful weed biological control, there are instances when agents fail to establish, or establish but fail to build up sufficient densities to suppress the target plant (Chapter 2). Perhaps more commonly, agents establish and provide control in some areas but not others. It is important to understand and explain spatial variability in biological control outcomes in order to predict the success of future programs. In this dissertation, I used a combination of experimental approaches and plant invader systems to investigate spatial variability in plant-natural enemy interactions with implications for biological control. This biogeographic approach is necessary to identify patterns that often elude local release and monitoring programs due to scale (i.e., geographic, temporal) limitations. I found that spatial variation in the interaction between natural enemy and plant invader was likely ubiquitous

among biological control systems (Chapter 2), present in both flowering rush and alligatorweed systems, that it could be due to genetic structure in plant populations (Chapter 3), seasonal or geographic variability in host quality (Chapter 4), or climate and its complex effects on agent (i.e., phenology, population variability) and host (indirectly through effects on agents) (Chapter 5). These results will support future biological control of weeds, where studies in the native or introduced range can be tailored toward better understanding the interplay between invader genetics, biotic and abiotic limiting factors, and geographic distributions (and abundance) of invader and control agent.

APPENDIX A. SUPPLEMENTARY MATERIAL FOR CHAPTER 2.

Table A.1. Biological control agents, their target weeds, and proposed explanations for variable control impacts.

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Acacia cyclops	A. Cunn. ex G. Don	*Melanterius servulus*	Pascoe	Republic of South Africa	dispersal, competition with *Dasineura*
Acacia cyclops	A. Cunn. ex G. Don	*Dasineura dielsi*	Rübsaamen	Republic of South Africa	parasitism, time of flowering (climate)
Acacia dealbata	Link	*Melanterius maculatus*	Lea	Republic of South Africa	?
Acacia decurrens	(Wendl.) Willd.	*Melanterius maculatus*	Lea	Republic of South Africa	?
Acacia mearnsii	De Wild.	*Melanterius maculatus*	Lea	Republic of South Africa	unknown
Acacia pycnantha	Benth.	*Melanterius maculatus*	Lea	Republic of South Africa	?
Acacia saligna	(Labill.) H. L. Wendl.	*Melanterius compactus*	Lea	Republic of South Africa	?
Acanthocereus tetragonus	(L.) Hummelinck	*Hypogeococcus festerianus*	(Lizer y Trelles)	Australia	predation
Ageratina adenophora	(Spreng.) R. M. King & H. Rob.	*Procecidochares utilis*	Stone	Hawaii USA	parasitism, elevational climate (moisture)
Ageratina adenophora	(Spreng.) R. M. King & H. Rob.	*Oidaematophorus beneficus*	Yano & Heppner	Hawaii USA	?
Ageratina adenophora	(Spreng.) R. M. King & H. Rob.	*Procecidochares utilis*	Stone	Thailand	?
Ageratina riparia	(Regel) R. M. King & H. Rob.	*Oidaematophorus beneficus*	Yano & Heppner	Hawaii USA	parasitism
Ageratina riparia	(Regel) R. M. King & H. Rob.	*Procecidochares alani*	Steyskal	Hawaii USA	parasitism
Alternanthera philoxeroides	(Mart.) Griseb.	*Agasicles hygrophila*	Selman & Vogt	New Zealand	climate (temperature, precipitation)
Alternanthera philoxeroides	(Mart.) Griseb.	*Agasicles hygrophila*	Selman & Vogt	People's Republic of China	climate (temperature, precipitation)

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Alternanthera philoxeroides	(Mart.) Griseb.	*Agasicles hygrophila*	Selman & Vogt	United States of America	climate (temperature, precipitation)
Alternanthera philoxeroides	(Mart.) Griseb.	*Arcola malloi*	(Pastrana)	Australia	climate (temperature, precipitation)
Alternanthera philoxeroides	(Mart.) Griseb.	*Arcola malloi*	(Pastrana)	New Zealand	climate (temperature, precipitation)
Alternanthera philoxeroides	(Mart.) Griseb.	*Arcola malloi*	(Pastrana)	United States of America	climate (temperature, precipitation), competition with other agents
Alternanthera philoxeroides	(Mart.) Griseb.	*Agasicles hygrophila*	Selman & Vogt	Australia	habitat, climate (temperature, precipitation)
Alternanthera philoxeroides	(Mart.) Griseb.	*Amynothrips andersoni*	O'Neill	United States of America	predation, competition with other agents?
Alternanthera philoxeroides	(Mart.) Griseb.	*Agasicles hygrophila*	Selman & Vogt	Puerto Rico	?
Ambrosia artemisiifolia	L.	*Ponometia candefacta*	(Hübner)	Russia	?
Ambrosia artemisiifolia	L.	*Stobaera concinna*	(Stål)	Australia	?
Ambrosia artemisiifolia	L.	*Zygogramma suturalis*	(Fabricius)	Russia	?
Asparagus asparagoides	(L.) Druce	*Puccinia myrsiphylli*	(Thüm.) Wint.	Australia	climate (precipitation/wet years)
Asparagus asparagoides	(L.) Druce	*Tribe Erythroneurini*	undescribed	Australia	parasitism
Baccharis halimifolia	L.	*Bucculatrix ivella*	Busck	Australia	?
Baccharis halimifolia	L.	*Trirhabda bacharidis*	(Weber)	Australia	evolution of phenology?
Baccharis halimifolia	L.	*Megacyllene mellyi*	(Chevrolat)	Australia	habitat (soil nutrients effect on host defenses)

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Baccharis halimifolia	L.	*Puccinia evadens*	Harkn.	Australia	local habitat (shade, temperature), climate (precipitation)
Baccharis halimifolia	L.	*Rhopalomyia californica*	Felt	Australia	parasitism, climate (prefer cooler, wetter)
Baccharis halimifolia	L.	*Aristotelia ivae*	Busck	Australia	?
Caesalpinia decapetala	(Roth) Alston	*Sulcobruchus subsuturalis*	(Pic)	Republic of South Africa	?
Calluna vulgaris	(L.) Hull	*Lochmaea suturalis*	(Thomson)	New Zealand	climate (winter) limitations associated with genetic bottlenecks
Carduus acanthoides	L.	*Trichosirocalus horridus*	(Panzer)	United States of America	?
Carduus acanthoides	L.	*Trichosirocalus horridus*	(Panzer)	Canada	parasitism by native wasp, competition with thrips
Carduus acanthoides	L.	*Rhinocyllus conicus*	(Frölich)	Canada	phenological asynchrony; longer flower period and early activity by weevil
Carduus acanthoides	L.	*Urophora solstitialis*	(L.)	Canada	?
Carduus nutans	L. subsp. *nutans*	*Trichosirocalus horridus*	(Panzer)	New Zealand	?
Carduus nutans	L. subsp. *nutans*	*Urophora solstitialis*	(L.)	New Zealand	competition with Rhinocyllus conicus, phenological asynchrony with host
Carduus nutans	L.	*Trichosirocalus horridus*	(Panzer)	United States of America	disease by Nosema
Carduus nutans	L.	*Rhinocyllus conicus*	(Frölich)	United States of America	parasitism

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Carduus nutans	L.	*Trichosirocalus horridus*	(Panzer)	Canada	parasitism by native wasp, competition with thrips
Carduus nutans	L.	*Rhinocyllus conicus*	(Frölich)	Canada	?
Carduus nutans	L. subsp. *nutans*	*Rhinocyllus conicus*	(Frölich)	Australia	?
Carduus pycnocephalus	L.	*Trichosirocalus horridus*	(Panzer)	United States of America	genetic incompatibility with host (dif preferred host)
Carduus pycnocephalus	L.	*Cheilosia grossa*	(Fallén)	United States of America	?
Carduus pycnocephalus	L.	*Rhinocyllus conicus*	(Frölich)	New Zealand	?
Carduus pycnocephalus	L.	*Rhinocyllus conicus*	(Frölich)	United States of America	?
Carduus tenuiflorus	Curtis	*Rhinocyllus conicus*	(Frölich)	United States of America	climate?
Carduus tenuiflorus	Curtis	*Trichosirocalus horridus*	(Panzer)	United States of America	genetic incompatibility with host (dif preferred host)
Carduus tenuiflorus	Curtis	*Cheilosia grossa*	(Fallén)	United States of America	?
Carduus tenuiflorus	Curtis	*Rhinocyllus conicus*	(Frölich)	New Zealand	?
Centaurea diffusa	Lam.	*Agapeta zoegana*	(L.)	United States of America	?
Centaurea diffusa	Lam.	*Agapeta zoegana*	(L.)	Canada	Climate
Centaurea diffusa	Lam.	*Cyphocleonus achates*	(Fåhraeus)	United States of America	climate (precipitation * effect on host)
Centaurea diffusa	Lam.	*Larinus obtusus*	Gyllenhal	Canada	climate (precipitation)
Centaurea diffusa	Lam.	*Sphenoptera jugoslavica*	Obenberger	Canada	climate (precipitation)
Centaurea diffusa	Lam.	*Cyphocleonus achates*	(Fåhraeus)	Canada	climate (temperature), habitat (soil, shade)

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Centaurea diffusa	Lam.	*Metzneria paucipunctella*	Zeller	United States of America	?
Centaurea diffusa	Lam.	*Pelochrista medullana*	(Staudinger)	United States of America	?
Centaurea jacea	L. nothosubsp. *pratensis* (W.D.J. Koch) Čelak.	*Larinus minutus*	Gyllenhal	United States of America	?
Centaurea jacea	L. nothosubsp. *pratensis* (W.D.J. Koch) Čelak.	*Metzneria paucipunctella*	Zeller	United States of America	?
Centaurea solstitialis	L.	*Puccinia jaceae*	var. solstitialis Savile	United States of America	habitat (moisture, shade), climate (temperature, precipitation)
Centaurea stoebe	L. sens. lat.	*Agapeta zoegana*	(L.)	Canada	Climate
Centaurea stoebe	L. sens. lat.	*Larinus obtusus*	Gyllenhal	United States of America	climate (precipitation)
Centaurea stoebe	L. sens. lat.	*Sphenoptera jugoslavica*	Obenberger	Canada	climate (temperature, precipitation * effect on host?)
Centaurea stoebe	L. sens. lat.	*Cyphocleonus achates*	(Fåhraeus)	United States of America	climate (temperature, precipitation,*impact is on host making damage more severe), habitat
Centaurea stoebe	L. sens. lat.	*Terellia virens*	(Loew)	United States of America	competition with other agents
Centaurea stoebe	L. sens. lat.	*Larinus minutus*	Gyllenhal	United States of America	predation, habitat, climate (may be working on plant susceptibilty to agent)
Centaurea stoebe	L. sens. lat.	*Larinus minutus*	Gyllenhal	Canada	?
Centaurea stoebe	L. sens. lat.	*Pelochrista medullana*	(Staudinger)	United States of America	?
Centaurea stoebe	L. sens. lat.	*Sphenoptera jugoslavica*	Obenberger	United States of America	?

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Centaurea virgata	Lam. subsp. *squarrosa* (Boiss.) Gugler	*Larinus minutus*	Gyllenhal	United States of America	competition with other agents
Centaurea virgata	Lam. subsp. *squarrosa* (Boiss.) Gugler	*Bangasternus fausti*	(Reitter)	United States of America	competition?
Centaurea virgata	Lam. subsp. *squarrosa* (Boiss.) Gugler	*Sphenoptera jugoslavica*	Obenberger	United States of America	?
Cereus jamacaru	DC. subsp. *jamacaru*	*Nealcidion cereicola*	(Fisher)	Republic of South Africa	?
Chondrilla juncea	L.	*Bradyrrhoa gilveolella*	(Treitschke)	United States of America	?
Chondrilla juncea	L.	*Aceria chondrillae*	(Canestrini)	Australia	genetic incompatibility with host, predation by Typhlodromus pyri, climate (winter temps)
Chondrilla juncea	L.	*Aceria chondrillae*	(Canestrini)	United States of America	genetic incompatibility with host, predation by Typhlodromus pyri, climate (winter temps)
Chondrilla juncea	L.	*Puccinia chondrillina*	Bubák & Syd.	United States of America	habitat (moisture, shade), genetic incompatibility with host
Chondrilla juncea	L.	*Cystiphora schmidti*	(Rübsaamen)	Australia	parasitism by native *Tetrastichus* sp.
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Actinote thalia*	pyrrha Fabricius	Indonesia	?
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Actinote thalia*	thalia Keifer	Indonesia	?
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Cecidochares connexa*	Macquart	India	Climate (precipitation), parasitism and predation locally

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Cecidochares connexa*	Macquart	Indonesia	Climate (precipitation), parasitism and predation locally
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Cecidochares connexa*	Macquart	Papua New Guinea	Climate (precipitation), parasitism and predation locally
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Cecidochares connexa*	Macquart	Timor Leste	Climate (precipitation), parasitism and predation locally
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Pareuchaetes pseudoinsulata*	Rego Barros	Papua New Guinea	climate (seasonal precipitation)
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Calycomyza eupatorivora*	Spencer	Republic of South Africa	habitat (shade)
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Pareuchaetes pseudoinsulata*	Rego Barros	Indonesia	parasitism
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Actinote anteas*	(Doubleday)	Indonesia	predation
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Pareuchaetes pseudoinsulata*	Rego Barros	Malaysia	predation
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Pareuchaetes pseudoinsulata*	Rego Barros	India	predation, climate, disease
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Pareuchaetes insulata*	(Walker)	Republic of South Africa	?
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Pareuchaetes pseudoinsulata*	Rego Barros	Federated States of Micronesia	?
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Pareuchaetes pseudoinsulata*	Rego Barros	Ghana	?
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Pareuchaetes pseudoinsulata*	Rego Barros	Northern Mariana Islands	?
Chromolaena odorata	(L.) R. M. King & H. Rob.	*Pareuchaetes pseudoinsulata*	Rego Barros	Sri Lanka	?
Chrysanthemoides monilifera	(L.) Norl. subsp. *rotundata* (DC.) Norl.	*Cassida sp.*	3	Australia	?

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Chrysanthemoides monilifera	(L.) Norl. subsp. *rotundata* (DC.) Norl.	*Tortrix sp.*		Australia	predation
Cirsium arvense	(L.) Scop.	*Rhinocyllus conicus*	(Frölich)	New Zealand	?
Cirsium vulgare	(Savi) Ten.	*Urophora stylata*	(Fabricius)	Australia	?
Cirsium vulgare	(Savi) Ten.	*Urophora stylata*	(Fabricius)	United States of America	?
Cirsium vulgare	(Savi) Ten.	*Cheilosia grossa*	(Fallén)	United States of America	?
Cirsium vulgare	(Savi) Ten.	*Rhinocyllus conicus*	(Frölich)	Republic of South Africa	?
Cirsium vulgare	(Savi) Ten.	*Rhinocyllus conicus*	(Frölich)	United States of America	?
Cirsium vulgare	(Savi) Ten.	*Trichosirocalus horridus*	(Panzer)	United States of America	?
Clidemia hirta	(L.) D. Don	*Colletotrichum clidemiae*	B. Weir & P.R. Johnst.	Hawaii USA	climate (temperature, precipitation)
Clidemia hirta	(L.) D. Don	*Liothrips urichi*	Karny	Palau	habitat (shade)
Clidemia hirta	(L.) D. Don	*Liothrips urichi*	Karny	Hawaii USA	habitat, predation by native ants and pirate bugs
Clidemia hirta	(L.) D. Don	*Antiblemma acclinalis*	Hübner	Hawaii USA	parasitism
Clidemia hirta	(L.) D. Don	*Ategumia matutinalis*	(Guenée)	Hawaii USA	parasitism
Coccinia grandis	(L.) Voigt	*Acythopeus cocciniae*	O'Brien & Pakaluk	Northern Mariana Islands	parasitism
Coccinia grandis	(L.) Voigt	*Acythopeus cocciniae*	O'Brien & Pakaluk	Hawaii USA	parasitism possibly by *Eupelmus* prob. *cushmani,* herbicide management
Convolvulus arvensis	L.	*Aceria malherbae*	Nuzzaci	United States of America	genetic incompatibility with host, climate stresses on host plant

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Convolvulus arvensis	L.	*Tyta luctuosa*	(Denis & Schiffermüller)	United States of America	?
Cordia curassavica	(Jacq.) Roem. & Schult.	*Eurytoma attiva*	Burks	Sri Lanka	?
Cordia curassavica	(Jacq.) Roem. & Schult.	*Metrogaleruca obscura*	(Degeer)	Sri Lanka	?
Cryptostegia grandiflora	R. Br.	*Maravalia cryptostegiae*	(Cummins) Ono	Australia	climate (moisture), genetic incompatibility, initially,
Cylindropuntia fulgida	(Engelm.) F.M. Knuth var. *fulgida*	*Dactylopius tomentosus*	(Lamark)	Republic of South Africa	genetic incompatibility with host plant
Cynoglossum officinale	L.	*Longitarsus quadriguttatus*	(Pontoppidan)	Canada	?
Cyperus rotundus	L.	*Bactra venosana*	(Zeller)	Hawaii USA	parasitism
Cytisus scoparius	(L.) Link	*Bruchidius villosus*	(Fabricius)	Australia	?
Cytisus scoparius	(L.) Link	*Bruchidius villosus*	(Fabricius)	New Zealand	?
Cytisus scoparius	(L.) Link	*Leucoptera spartifoliella*	(Hübner)	Australia	?
Dolichandra unguis-cati	(L.) L. G. Lohmann	*Charidotis auroguttata*	Boheman	Republic of South Africa	predation
Echium plantagineum	L.	*Longitarsus echii*	(Koch)	Australia	climate (precipitation)
Echium plantagineum	L.	*Mogulones larvatus*	(Schultze)	Australia	climate (precipitation)
Echium plantagineum	L.	*Dialectica scalariella*	(Zeller)	Australia	climate, phenological asynchrony with host
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	India	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Mexico	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Benin	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Egypt	?

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	India	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Mexico	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Nigeria	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Australia	climate (temperature)
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	People's Republic of China	climate (temperature)
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Rwanda	climate (temperature)
Eichhornia crassipes	(Mart.) Solms	*Eccritotarsus catarinensis*	(Carvalho)	Republic of South Africa	climate (temperature), habitat
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Republic of South Africa	climate (temperature), habitat (flooding, water nutrients)
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	People's Republic of China	habitat (eutrophication, pollution), climate (temperature)
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Republic of South Africa	habitat (eutrophication, pollution), climate (temperature)
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Sri Lanka	habitat (eutrophication, pollution, wind/wave action)
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Australia	habitat (flooding)
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Egypt	habitat (water pollution?)
Eichhornia crassipes	(Mart.) Solms	*Niphograpta albiguttalis*	(Warren)	Republic of South Africa	parasitism
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Indonesia	predation
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Indonesia	predation

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Benin	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Ghana	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Kenya	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Malaysia	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Nigeria	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Rwanda	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	South Sudan	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Sudan	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina bruchi*	Hustache	Tanzania	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Ghana	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Kenya	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Malaysia	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Tanzania	?
Eichhornia crassipes	(Mart.) Solms	*Neochetina eichhorniae*	Warner	Vanuatu	?
Eichhornia crassipes	(Mart.) Solms	*Niphograpta albiguttalis*	(Warren)	Malaysia	?
Eichhornia crassipes	(Mart.) Solms	*Niphograpta albiguttalis*	(Warren)	South Sudan	?
Eichhornia crassipes	(Mart.) Solms	*Niphograpta albiguttalis*	(Warren)	Sudan	?
Eichhornia crassipes	(Mart.) Solms	*Niphograpta albiguttalis*	(Warren)	United States of America	?

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Elephantopus mollis	Kunth	*Tetraeuaresta obscuriventris*	(Loew)	Hawaii USA	?
Emex australis	Steinh.	*Perapion antiquum*	(Gyllenhal)	Hawaii USA	?
Emex spinosa	(L.) Campd.	*Perapion antiquum*	(Gyllenhal)	Hawaii USA	?
Euphorbia cyparissias	L.	*Aphthona lacertosa*	Rosenhauer	United States of America	?
Euphorbia cyparissias	L.	*Aphthona czwalinai*	(Weise)	United States of America	climate (seasonal temperatures)
Euphorbia cyparissias	L.	*Aphthona nigriscutis*	Foudras	Canada	habitat (moisture)
Euphorbia cyparissias	L.	*Aphthona nigriscutis*	Foudras	United States of America	habitat (moisture)
Euphorbia cyparissias	L.	*Aphthona cyparissiae*	(Koch)	Canada	habitat (shade, moisture) * effects on host resistance?
Euphorbia cyparissias	L.	*Aphthona cyparissiae*	(Koch)	United States of America	habitat (shade, moisture) * effects on host resistance?
Euphorbia cyparissias	L.	*Aphthona flava*	Guillebeau	United States of America	habitat, climate, competition
Euphorbia cyparissias	L.	*Hyles euphorbiae*	(L.)	United States of America	predation
Euphorbia cyparissias	L.	*Spurgia capitigena*	(Bremi)	Canada	?
Euphorbia esula	L.	*Aphthona lacertosa*	Rosenhauer	United States of America	?
Euphorbia esula	L.	*Aphthona czwalinai*	(Weise)	Canada	climate (seasonal temperatures)
Euphorbia esula	L.	*Aphthona czwalinai*	(Weise)	United States of America	climate (seasonal temperatures)
Euphorbia esula	L.	*Lobesia euphorbiana*	(Freyer)	Canada	climate (temperature), habitat (plant quality)
Euphorbia esula	L.	*Aphthona lacertosa*	Rosenhauer	Canada	habitat (moisture)

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Euphorbia esula	L.	*Aphthona nigriscutis*	Foudras	United States of America	habitat (soil type, moisture)
Euphorbia esula	L.	*Aphthona cyparissiae*	(Koch)	Canada	habitat, climate, competition
Euphorbia esula	L.	*Aphthona flava*	Guillebeau	United States of America	habitat, climate, competition
Euphorbia esula	L.	*Aphthona nigriscutis*	Foudras	Canada	habitat, climate, competition
Euphorbia esula	L.	*Aphthona flava*	Guillebeau	Canada	habitat, climate (temperature, precipitation)
Euphorbia esula	L.	*Hyles euphorbiae*	(L.)	United States of America	predation, disease
Euphorbia esula	L.	*Spurgia capitigena*	(Bremi)	Canada	?
Euphorbia esula	L.	*Spurgia esulae*	Gagné	Canada	?
Hakea gibbosa	(Sm.) Cav.	*Erytenna consputa*	Pascoe	Republic of South Africa	?
Hakea sericea	Schrad. & J.C. Wendl.	*Erytenna consputa*	Pascoe	Republic of South Africa	genetic incompatibility with host plant
Hakea sericea	Schrad. & J.C. Wendl.	*Aphanasium australe*	(Boisduval)	Republic of South Africa	?
Hakea sericea	Schrad. & J.C. Wendl.	*Cydmaea binotata*	Lea	Republic of South Africa	?
Harrisia martinii	(Labour.) Britton	*Nealcidion cereicola*	(Fisher)	Republic of South Africa	?
Heliotropium amplexicaule	Vahl	*Deuterocampta quadrijuga*	(Stäl)	Australia	?
Hydrilla verticillata	(L. f.) Royle	*Hydrellia pakistanae*	Deonier	United States of America	parasitism, climate
Hypericum perforatum	L.	*Aplocera plagiata*	(L.)	Canada	?
Hypericum perforatum	L.	*Aplocera plagiata*	(L.)	United States of America	climate

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Hypericum perforatum	L.	*Aphis chloris*	Koch	Australia	climate (precipitation) competition with Chrysolina, predation
Hypericum perforatum	L.	*Aphis chloris*	Koch	Canada	climate, possible competition with *Chrysolina* spp.
Hypericum perforatum	L.	*Chrysolina hyperici*	(Forster)	Canada	climate, possible competition with other introduced agents
Hypericum perforatum	L.	*Chrysolina hyperici*	(Forster)	New Zealand	climate, possible competition with other introduced agents
Hypericum perforatum	L.	*Chrysolina hyperici*	(Forster)	United States of America	climate, possible competition with other introduced agents
Hypericum perforatum	L.	*Chrysolina quadrigemina*	(Suffrian)	Canada	climate, possible competition with other introduced agents
Hypericum perforatum	L.	*Chrysolina hyperici*	(Forster)	Australia	competition with Chrysolina quadrigemina
Hypericum perforatum	L.	*Agrilus hyperici*	(Creutzer)	United States of America	competition with Chrysolina quadrigemina; attacks plants in shade avoided by Chrysolina
Hypericum perforatum	L.	*Aculus hyperici*	(Liro)	Australia	genetic incompatibility with host
Hypericum perforatum	L.	*Zeuxidiplosis giardi*	(Kieffer)	United States of America	habitat (moisture), parasitized
Jacobaea vulgaris	Gaertn.	*Tyria jacobaeae*	(L.)	New Zealand	?
Jacobaea vulgaris	Gaertn.	*Cochylis atricapitana*	(Stephens)	Canada	climate
Jacobaea vulgaris	Gaertn.	*Longitarsus jacobaeae*	(Waterhouse)	Canada	climate (temperature, precipitation)

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Jacobaea vulgaris	Gaertn.	*Longitarsus flavicornis*	(Stephens)	Australia	climate (temperature, precipitation), habitat (moisture)
Jacobaea vulgaris	Gaertn.	*Tyria jacobaeae*	(L.)	United States of America	predation, parasitism
Jacobaea vulgaris	Gaertn.	*Botanophila jacobaeae*	(Hardy)	New Zealand	?
Jacobaea vulgaris	Gaertn.	*Longitarsus jacobaeae*	(Waterhouse)	Australia	?
Jacobaea vulgaris	Gaertn.	*Longitarsus jacobaeae*	(Waterhouse)	United States of America	?
Lantana camara	L. sens. lat.	*Eutreta xanthochaeta*	Aldrich	Hawaii USA	?
Lantana camara	L. sens. lat.	*Uroplata girardi*	Pic	Hawaii USA	?
Lantana camara	L. sens. lat.	*Aconophora compressa*	Walker	Australia	Climate (peaks in winter and spring, limited by hot summer)
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Papua New Guinea	climate (precipitation *effect on host?)
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	St Helena	climate (precipitation *effect on host?)
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	New Caledonia	climate (precipitation *effect on weed?)
Lantana camara	L. sens. lat.	*Plagiohammus spinipennis*	(Thomson)	Hawaii USA	climate (precipitation *may be effect on host resistance)
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Kenya	climate (precipitation seasonal)
Lantana camara	L. sens. lat.	*Leptobyrsa decora*	Drake	Hawaii USA	climate (precipitation)
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Federated States of Micronesia	climate (precipitation)
Lantana camara	L. sens. lat.	*Hypena laceratalis*	Walker	Mauritius	climate (precipitation); possibly parasitism; genetic incompatibility with host

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Hawaii USA	climate (precipitation, temperature)
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Republic of South Africa	climate (precipitation, temperature)
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Guam	climate (precipitation, temperature), habitat (shade)
Lantana camara	L. sens. lat.	*Salbia haemorrhoidalis*	Guenée	Republic of South Africa	climate (temperature, precipitation), parasitism, genetic incompatibility with host
Lantana camara	L. sens. lat.	*Leptobyrsa decora*	Drake	Australia	climate (temperature, precipitation), predation
Lantana camara	L. sens. lat.	*Falconia intermedia*	(Distant)	Australia	climate, genetic incompatibility with host
Lantana camara	L. sens. lat.	*Ophiomyia camarae*	Spencer	Republic of South Africa	climate, genetic incompatibility with host
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Australia	genetic incompatibility with host
Lantana camara	L. sens. lat.	*Uroplata girardi*	Pic	Republic of South Africa	genetic incompatibility with host
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Republic of South Africa	genetic incompatibility with host, climate (precipitation)
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Northern Mariana Islands	habitat (shade)
Lantana camara	L. sens. lat.	*Uroplata girardi*	Pic	Fiji	habitat (shade)
Lantana camara	L. sens. lat.	*Uroplata girardi*	Pic	Niue	habitat (shade)
Lantana camara	L. sens. lat.	*Uroplata girardi*	Pic	Solomon Islands	habitat (shade)
Lantana camara	L. sens. lat.	*Octotoma scabripennis*	Guérin-Méneville	Hawaii USA	habitat (shade), climate (precipitation, temperature)

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Lantana camara	L. sens. lat.	*Neogalea sunia*	(Guenée)	Hawaii USA	parasitism, climate (precipitation)
Lantana camara	L. sens. lat.	*Falconia intermedia*	(Distant)	Republic of South Africa	predation, climate, genetic incompatibility with host, host-evolution of resistance
Lantana camara	L. sens. lat.	*Octotoma scabripennis*	Guérin-Méneville	Ghana	predation, parasistism
Lantana camara	L. sens. lat.	*Octotoma scabripennis*	Guérin-Méneville	Ghana	predation, parasistism
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Fiji	predation, seasonal weather
Lantana camara	L. sens. lat.	*Lantanophaga pusillidactyla*	(Walker)	Palau	?
Lantana camara	L. sens. lat.	*Octotoma championi*	Baly	Australia	?
Lantana camara	L. sens. lat.	*Orthezia insignis*	Browne	Hawaii USA	?
Lantana camara	L. sens. lat.	*Salbia haemorrhoidalis*	Guenée	Mauritius	?
Lantana camara	L. sens. lat.	*Septoria sp.*		Hawaii USA	?
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Palau	?
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Samoa	?
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Solomon Islands	?
Lantana camara	L. sens. lat.	*Teleonemia scrupulosa*	Stål	Vanuatu	?
Lantana camara	L. sens. lat.	*Uroplata fulvopustulata*	Baly	Australia	?
Lantana camara	L. sens. lat.	*Uroplata girardi*	Pic	Samoa	?
Linaria dalmatica	(L.) Mill. subsp. *dalmatica* (L.) Mill.	*Calophasia lunula*	(Hufnagel)	United States of America	climate (temperature)

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Linaria dalmatica	(L.) Mill. subsp. *dalmatica* (L.) Mill.	*Calophasia lunula*	(Hufnagel)	Canada	climate (temperature), parasitism
Linaria dalmatica	(L.) Mill. subsp. *dalmatica* (L.) Mill.	*Brachypterolus pulicarius*	(L.)	United States of America	genetic incompatibility with hosts
Linaria vulgaris	Mill.	*Calophasia lunula*	(Hufnagel)	Canada	climate (temperature), parasitism
Linaria vulgaris	Mill.	*Mecinus janthinus*	Germar	United States of America	?
Linaria vulgaris	Mill.	*Rhinusa linariae*	(Panzer)	Canada	?
Lythrum salicaria	L.	*Nanophyes marmoratus*	(Goeze)	United States of America	competition with other agents
Lythrum salicaria	L.	*Galerucella calmariensis*	(L.)	United States of America	predation, habitat (moisture; require dry overwintering sites)
Lythrum salicaria	L.	*Galerucella pusilla*	(Duftschmidt)	United States of America	predation, habitat (moisture; require dry overwintering sites)
Lythrum salicaria	L.	*Galerucella pusilla*	(Duftschmidt)	Canada	?
Marrubium vulgare	L.	*Wheeleria spilodactylus*	(Curtis)	Australia	climate (precipitation)
Marrubium vulgare	L.	*Chamaesphecia mysiniformis*	Rambur	Australia	predation
Melaleuca quinquenervia	(Cav.) S. T. Blake	*Oxyops vitiosa*	Pascoe	United States of America	habitat (soil moisture)
Melaleuca quinquenervia	(Cav.) S. T. Blake	*Boreioglycaspis melaleucae*	Moore	United States of America	predation, climate
Miconia calvescens	DC.	*Colletotrichum gloeosporioides*	(Penz.) Penz. & Sacc. f. sp. miconiae Killgore	French Polynesia	climate (temperature, precipitation/humidity)
Miconia calvescens	DC.	*Colletotrichum gloeosporioides*	(Penz.) Penz. & Sacc. f. sp. miconiae	Hawaii USA	habitat (moisture, wind)

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
			Killgore & L. Sugiyama		
Mikania micrantha	Kunth	*Actinote thalia*	pyrrha Fabricius	Indonesia	?
Mikania micrantha	Kunth	*Actinote anteas*	(Doubleday)	Indonesia	predation
Mimosa diplotricha	C. Wright	*Heteropsylla spinulosa*	Muddiman, Hodkinson & Hollis	Vanuatu	climate (precipitation)
Mimosa diplotricha	C. Wright	*Heteropsylla spinulosa*	Muddiman, Hodkinson & Hollis	Papua New Guinea	climate (precipitation); *maybe effect on host resistance to psyllid
Mimosa pigra	L.	*Acanthoscelides puniceus*	Johnson	Australia	?
Mimosa pigra	L.	*Acanthoscelides puniceus*	Johnson	Malaysia	?
Mimosa pigra	L.	*Carmenta mimosa*	Eichlin & Passoa	Australia	?
Mimosa pigra	L.	*Malacorhinus irregularis*	Jacoby	Australia	?
Mimosa pigra	L.	*Macaria pallidata*	(Warren)	Australia	climate (precipitation; seasonal)
Mimosa pigra	L.	*Chalcodermus serripes*	Fåhraeus	Australia	?
Mimosa pigra	L.	*Chlamisus mimosae*	Karren	Australia	?
Mimosa pigra	L.	*Leuciris fimbriaria*	(Stoll)	Australia	?
Onopordum spp.		*Eublemma amoena*	(Hübner)	Australia	?
Onopordum spp.		*Trichosirocalus briesei*	Alonso-Zarazaga & Sanchez-Ruiz	Australia	?
Opuntia aurantiaca	Lindl.	*Cactoblastis cactorum*	(Berg)	Australia	?

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Opuntia aurantiaca	Lindl.	*Tucumania tapiacola*	Dyar	Australia	?
Opuntia aurantiaca	Lindl.	*Dactylopius austrinus*	De Lotto	Republic of South Africa	Climate (may be working on plant susceptibilty to agent)
Opuntia engelmannii	Salm-Dyck ex Engelm.	*Dactylopius opuntiae*	(Cockerell)	Republic of South Africa	predation by
Opuntia ficus-indica	(L.) Mill.	*Cactoblastis cactorum*	(Berg)	Hawaii USA	?
Opuntia ficus-indica	(L.) Mill.	*Dactylopius opuntiae*	(Cockerell)	Hawaii USA	?
Opuntia ficus-indica	(L.) Mill.	*Lagocheirus funestus*	Thomson	Hawaii USA	?
Opuntia ficus-indica	(L.) Mill.	*Lagocheirus funestus*	Thomson	Republic of South Africa	?
Opuntia ficus-indica	(L.) Mill.	*Metamasius spinolae*	(Gyllenhal)	Republic of South Africa	?
Opuntia humifusa	(Raf.) Raf.	*Dactylopius opuntiae*	(Cockerell)	Republic of South Africa	?
Opuntia spp.		*Cactoblastis cactorum*	(Berg)	Cayman Islands	?
Opuntia spp.		*Dactylopius ceylonicus*	(Green)	Kenya	?
Opuntia spp.		*Dactylopius ceylonicus*	(Green)	Tanzania	?
Opuntia spp.		*Dactylopius opuntiae*	(Cockerell)	Kenya	?
Opuntia spp.		*Dactylopius opuntiae*	(Cockerell)	Tanzania	?
Opuntia streptacantha	Lem.	*Moneilema blapsides*	(Newman) subsp. ulkei Horn	Australia	?
Opuntia streptacantha	Lem.	*Lagocheirus funestus*	Thomson	Australia	predation by vertebrates
Opuntia stricta	(Haw.) Haw.	*Dactylopius opuntiae*	(Cockerell)	Australia	climate (effects on host plant)

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Opuntia stricta	(Haw.) Haw.	*Dactylopius opuntiae*	(Cockerell)	Republic of South Africa	climate (precipitation)
Opuntia stricta	(Haw.) Haw.	*Cactoblastis cactorum*	(Berg)	Australia	climate (temperature)
Opuntia stricta	(Haw.) Haw.	*Dactylopius opuntiae*	(Cockerell)	Republic of South Africa	genetic incompatibility with host plant
Opuntia stricta	(Haw.) Haw.	*Cactoblastis cactorum*	(Berg)	New Caledonia	?
Opuntia tomentosa	Salm-Dyck	*Moneilema blapsides*	(Newman) subsp. ulkei Horn	Australia	?
Opuntia tomentosa	Salm-Dyck	*Dactylopius opuntiae*	(Cockerell)	Australia	habitat
Opuntia tomentosa	Salm-Dyck	*Cactoblastis cactorum*	(Berg)	Australia	habitat/ local variation in plant developmental state
Opuntia tomentosa	Salm-Dyck	*Lagocheirus funestus*	Thomson	Australia	?
Opuntia triacantha	(Willd.) Sweet	*Cactoblastis cactorum*	(Berg)	Cayman Islands	?
Paraserianthes lophantha	(Willd.) Nielsen	*Melanterius servulus*	Pascoe	Republic of South Africa	?
Parthenium hysterophorus	L.	*Carmenta sp.*	nr ithacae (Beutenmüller)	Australia	?
Parthenium hysterophorus	L.	*Zygogramma bicolorata*	Pallister	India	?
Parthenium hysterophorus	L.	*Puccinia abrupta*	var. partheniicola	Australia	climate (moisture, temperature), spatial and temporally variable
Parthenium hysterophorus	L.	*Puccinia abrupta*	Dietel & Holw. var. partheniicola (H.S. Jacks.) Parmelee	Australia	climate (precipitation)
Parthenium hysterophorus	L.	*Smicronyx lutulentus*	Dietz	Australia	climate (precipitation)

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Parthenium hysterophorus	L.	*Zygogramma bicolorata*	Pallister	Australia	climate (precipitation)
Parthenium hysterophorus	L.	*Listronotus setosipennis*	(Hustache)	Australia	climate (precipitation), habitat (soil type)
Parthenium hysterophorus	L.	*Puccinia xanthii*	Schwein. var. parthenii-hysterophorae Seier, H.C. Evans & Á.	Australia	climate (temperature, precipitation)
Parthenium hysterophorus	L.	*Conotrachelus albocinereus*	Fiedler	Australia	?
Parthenium hysterophorus	L.	*Platphalonidia mystica*	(Razowski & Becker)	Australia	?
Passiflora tarminiana	Coppens & V. E. Barney	*Septoria passiflorae*	Sydenham	Hawaii USA	habitat (wind, moisture)
Pereskia aculeata	Mill.	*Phenrica guerini*	Bechyné	Republic of South Africa	?
Persicaria perfoliata	(L.) H. Gross	*Rhinoncomimus latipes*	Korotyaev	United States of America	climate (temperature, precipitation)
Pilosella officinarum	Vaill.	*Aulacidea subterminalis*	Niblett	New Zealand	climate (precipitation)
Pilosella officinarum	Vaill.	*Macrolabis pilosellae*	(Binnie)	New Zealand	climate (precipitation)
Pistia stratiotes	L.	*Neohydronomus affinis*	Hustache	Australia	climate (temperature)
Pistia stratiotes	L.	*Neohydronomus affinis*	Hustache	United States of America	climate (temperature)?
Pistia stratiotes	L.	*Neohydronomus affinis*	Hustache	Papua New Guinea	habitat (flooding)
Pistia stratiotes	L.	*Neohydronomus affinis*	Hustache	Botswana	?
Pistia stratiotes	L.	*Neohydronomus affinis*	Hustache	Ghana	?
Pistia stratiotes	L.	*Neohydronomus affinis*	Hustache	Kenya	?

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Pistia stratiotes	L.	*Neohydronomus affinis*	Hustache	Republic of Congo	?
Pistia stratiotes	L.	*Neohydronomus affinis*	Hustache	Senegal	?
Pistia stratiotes	L.	*Neohydronomus affinis*	Hustache	Zambia	?
Prosopis spp.		*Evippe sp.*	#1	Australia	?
Rhaponticum repens	(L.) Hidalgo	*Subanguina picridis*	(Kirjanova) Brzeski	United States of America	climate (precipitation)
Rubus argutus	Link	*Schreckensteinia festaliella*	Hübner	Hawaii USA	habitat (shade *effect on host?)
Rubus argutus	Link	*Croesia zimmermani*	Clarke	Hawaii USA	parasitism
Rubus fruticosus	L. agg.	*Phragmidium violaceum*	(Schultz) G. Winter	Australia	climate (temperature, precipitation)
Salsola tragus	L.	*Coleophora klimeschiella*	Toll	United States of America	predation, parasitism
Salsola tragus	L.	*Coleophora parthenica*	Meyrick	United States of America	predation, parasitism, phenological asynchrony with host
Salvia aethiopis	L.	*Phrydiuchus tau*	Warner	United States of America	?
Salvinia molesta	D.S. Mitch.	*Cyrtobagous salviniae*	Calder & Sands	United States of America	climate (precipitation, temperature)
Salvinia molesta	D.S. Mitch.	*Cyrtobagous singularis*	Hustache	Botswana	genetic incompatibility with host plant
Salvinia molesta	D.S. Mitch.	*Cyrtobagous salviniae*	Calder & Sands	Ghana	?
Salvinia molesta	D.S. Mitch.	*Cyrtobagous salviniae*	Calder & Sands	Kenya	?
Salvinia molesta	D.S. Mitch.	*Cyrtobagous salviniae*	Calder & Sands	Malaysia	?
Salvinia molesta	D.S. Mitch.	*Cyrtobagous singularis*	Hustache	Zambia	?
Salvinia molesta	D.S. Mitch.	*Paulinia acuminata*	(De Geer)	Zimbabwe	?

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Sida acuta	Burm. f.	*Calligrapha pantherina*	Stål	Australia	climate (precipitation)
Sida rhombifolia	L.	*Calligrapha pantherina*	Stål	Australia	climate
Silene vulgaris	(Moench) Garcke	*Cassida azurea*	Fabricius	Canada	?
Solanum elaeagnifolium	Cav.	*Leptinotarsa defecta*	(Stål)	Republic of South Africa	?
Solanum mauritianum	Scop.	*Gargaphia decoris*	Drake	Republic of South Africa	predation
Solanum sisymbriifolium	Lam.	*Gratiana spadicea*	(Klug)	Republic of South Africa	parasitism, predation, climate (precipitation), phenological asynchrony with host
Solanum viarum	Dunal	*Gratiana boliviana*	Spaeth	United States of America	climate (temperature), habitat (shade)
Tamarix spp.		*Diorhabda elongata*	(Brullé)	United States of America	predation
Tamarix spp.		*Diorhabda sublineata*	(Lucas)	United States of America	predation
Tamarix spp.		*Diorhabda carinulata*	(Desbrochers)	United States of America	predation, habitat (flooding)
Tribulus cistoides	L.	*Microlarinus lypriformis*	(Wollaston)	Hawaii USA	?
Tribulus cistoides	L.	*Microlarinus lypriformis*	(Wollaston)	Papua New Guinea	?
Tribulus terrestris	L.	*Microlarinus lypriformis*	(Wollaston)	Hawaii USA	?
Tribulus terrestris	L.	*Microlarinus lareynii*	(Jacquelin du Val)	United States of America	climate (winter temperature), egg parasitism, predation
Tribulus terrestris	L.	*Microlarinus lypriformis*	(Wollaston)	United States of America	climate (winter temperature), egg parasitism, predation
Ulex europaeus	L.	*Sericothrips staphylinus*	Haliday	Hawaii USA	anthropogenic (management-burning)

Weed Name Full		Agent Name Full		Country of Release	Limiting Factors
Ulex europaeus	L.	*Tetranychus lintearius*	Dufour	Chile	climate (precipitation), predation
Ulex europaeus	L.	*Tetranychus lintearius*	Dufour	United States of America	climate (temperature *effect on host)
Ulex europaeus	L.	*Tetranychus lintearius*	Dufour	St Helena	predation
Vachellia nilotica	subsp. *indica* (Benth.) Kyal. & Boatwr	*Chiasmia assimilis*	(Warren)	Australia	Climate (precipitation)

APPENDIX B. SUPPLEMENTARY MATERIAL FOR CHAPTER 3.

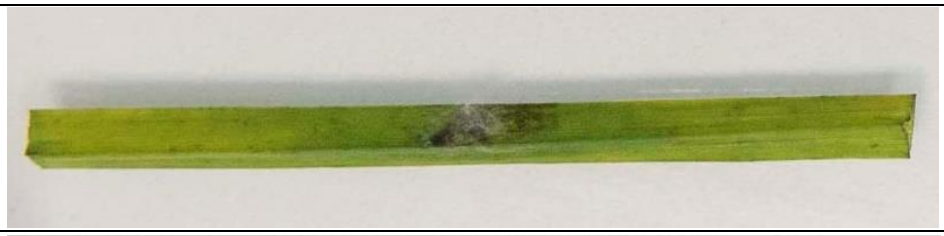
Damage rating	Representative leaf of *Butomus umbellatus*
0	
1	
2	
3	
4	

Figure B.1. Examples of damage ratings assigned to infected *B. umbellatus* leaves during the excised-leaf laboratory experiment.

METHODS AND RESULTS FROM GREENHOUSE WHOLE-PLANT INFECTION EXPERIMENT

We experimentally tested whether whole G1 and G4 plants varied in resistance to infection by fungal pathogens. For each genotype, we used four replicate populations from our garden (Table 1), and from each population we planted six replicate plants in 1 gallon nursery pots filled with fine sand and 6 g fertilizer. Populations were chosen because sufficient plant material was available for the experiment. Diploid plants produce little rhizome biomass but invest heavily in viable bulbils, often producing hundreds of vegetative propagules per plant (Eckert et al. 2000, Lui et al. 2005). Diploid plants also reproduce sexually with viable seed production observed in all populations studied by Brown and Eckert (2005). In contrast, triploid plants almost never flower and typically produce few, if any, bulbils, but invest substantially in underground rhizomes (Lui et al. 2005). Because of the life-history differences between ploids, it was not possible to perfectly standardize initial conditions in the experiment. However, despite differences in biology, we standardized wet biomass of our plantings for each population and genotype. Plants were randomly assigned treatments and randomly placed within one of two shallow water baths in a greenhouse. Replicates were divided equally between water baths and baths were treated as blocks for analysis. Plants were grown in two shallow tanks in a greenhouse for 6 wk before inoculating with fungi. Water level was maintained at 5 cm below the sediment surface for the entire experiment. Water was delivered from the local municipal water supply and charcoal-filtered.

Table B.1 Flowering rush populations used in this study.

Ploid	Genotype	Population	Latitude	Longitude
Triploid	1	Flathead Lake, MT	47.697	-114.071
Triploid	1	Pend Oreille River, ID	48.362	-117.285
Triploid	1	Rose Pond, ID	43.247	-112.315
Triploid	1	Yakima River, WA	46.379	-119.431
Diploid	4	Point Rosa Marsh, MI	42.576	-82.805
Diploid	4	Kildeer Pond, OH	40.709	-83.369
Diploid	4	Unity Island, NY	42.934	-78.9084
Diploid	4	Oswegatchie River, NY	44.690	-75.495

We inoculated plants with one of two plant fungal pathogens, *Plectosphaerella cucumerina* Kleb. and *Colletotrichum fioriniae* Marcelino & Gouli ex R.G. Shivas & Y.P. Tan. These fungal species were chosen because they have previously reported as plant pathogens (Uecker 1993, Agrios 2015). *P. cucumerina* was present in three G1 and seven G4 sites from the northeastern to northwestern US during our surveys, and *C. fiorinae* was identified from two G4 and a single G3 site in the northeastern and upper Midwestern US. Fungal species were isolated from diploid G4 plants at Kildeer Pond, OH (*P. cucumerina*) and G3 plants in Springbrook Pond, IL (*C. fioriniae*), then cultured in bulk for this experiment using previously reported methods. Isolates were retrieved from storage and plated onto petri dishes containing Potato Dextrose Agar (PDA) (Difco Inc. Detroit MI). The cultures were allowed to grow 2 wk at room temperature (21-22 ˚C). One plate each of the *P. cucumerina* and *C. fioriniae* cultures were cut into small pieces (1 x 1 mm) and placed in 250 ml flasks containing 100 mL of Richard's V-8 broth (10 g; KNO_3, 10 g; $CaCO_3$ 3 g;V-8 juice (Campbells, Camden, NJ), 200 mL; H_2O, 800 mL). Flasks were placed on a platform shaker (New Brunswick, Edison, NJ) set at 200 rpm and

manually swirled daily to prevent fungal buildup along the sides. After 7 d incubation, the contents of each flask were combined in a blender and ground 20 seconds to homogenize the culture. Colony forming units (CFUs) were determined for both isolates as follows: 1 mL aliquots were added to 9-mL sterile water blanks and sequentially diluted to 1×10^6. Dilutions of 1×10^6 and 1×10^5 were plated onto Martin's agar (Martin 1950) (three plates per dilution), allowed to grow until distinct colonies were evident on the plate, and enumerated. It was determined that CFUs for both species were 1×10^6. To inoculate, two leaves from each plant were lightly abraded with 200 grit sandpaper, then 1mL of inoculum was applied to each of two cotton balls which were attached to separate leaves with Parafilm (Bemis NA, Neenah, WI). The same pathogen was applied to both leaves on each plant. Plants were misted three times daily (early morning, mid-day, late afternoon) to maintain high humidity during the experiment. We allowed the experiment to continue 96 hr, at which point damage assessments were made.

We assessed pathogen damage to plants two ways. First we assessed the leaves that had inoculum applied. Cotton plugs were removed from the leaf and a numeric damage score was applied to the entire leaf. The damage score we used here is similar to those used previously by Shearer et al. (2011) and is a qualitative assessment of leaf condition on an ordinal scale (Table 1). We defined the damage scale so that levels of damage were approximately equally-spaced on the scale. The same observer (JFS) made all damage assessments. A higher damage rating represents lower resistance to infection. We also assessed pathogen damage to the entire plant. To do this we counted the number of leaves that appeared diseased or discolored and divided it by the total number of leaves per plant to calculate the proportion of diseased leaves (percent damage).

Statistics

To test for differences in percent damage (the whole-plant experiment), we used generalized linear models with normal distribution and log-link function. Genotype was a fixed effect and population was a random effect to account for the nesting of populations within a genotype (Bhattarai et al 2017). To test whether disease rating was higher in G4 plants, we used generalized linear mixed models with multinomial error distribution and cumulative logit link function (Gbur et al. 2012). Our experimental design included subsamples (two infected leaves per plant), so the model consisted of disease rating as an ordinal dependent variable, replicates within population and population within genotype as random effects, and genotype as a fixed effect. Statistical analyses were performed in SAS version 9.4 (SAS Institute, Cary, North Carolina).

Results of whole plant experiment

Resistance to infection differed between *B. umbellatus* genotypes (Figure S1). Percent damage measured 2 wk after inoculation was greater for G1 plants infected with *P. cucumerina* ($F = 10.44$, $P = 0.02$) or *C. fioriniae* ($F = 16.65$, $P = 0.007$) than G4 plants. Additionally, overall damage scores were higher for G1 plants infected with *C. fioriniae* ($F = 5.85$, $P = 0.05$). Lesion length ($F = 9.62$, $P = 0.02$) was greater for G1 plants infected by *C. fioriniae* only. Damage by *P. cucumerina* was rapid and severe, so we were unable to assign a disease rating or measure lesions on leaves infected by that species.

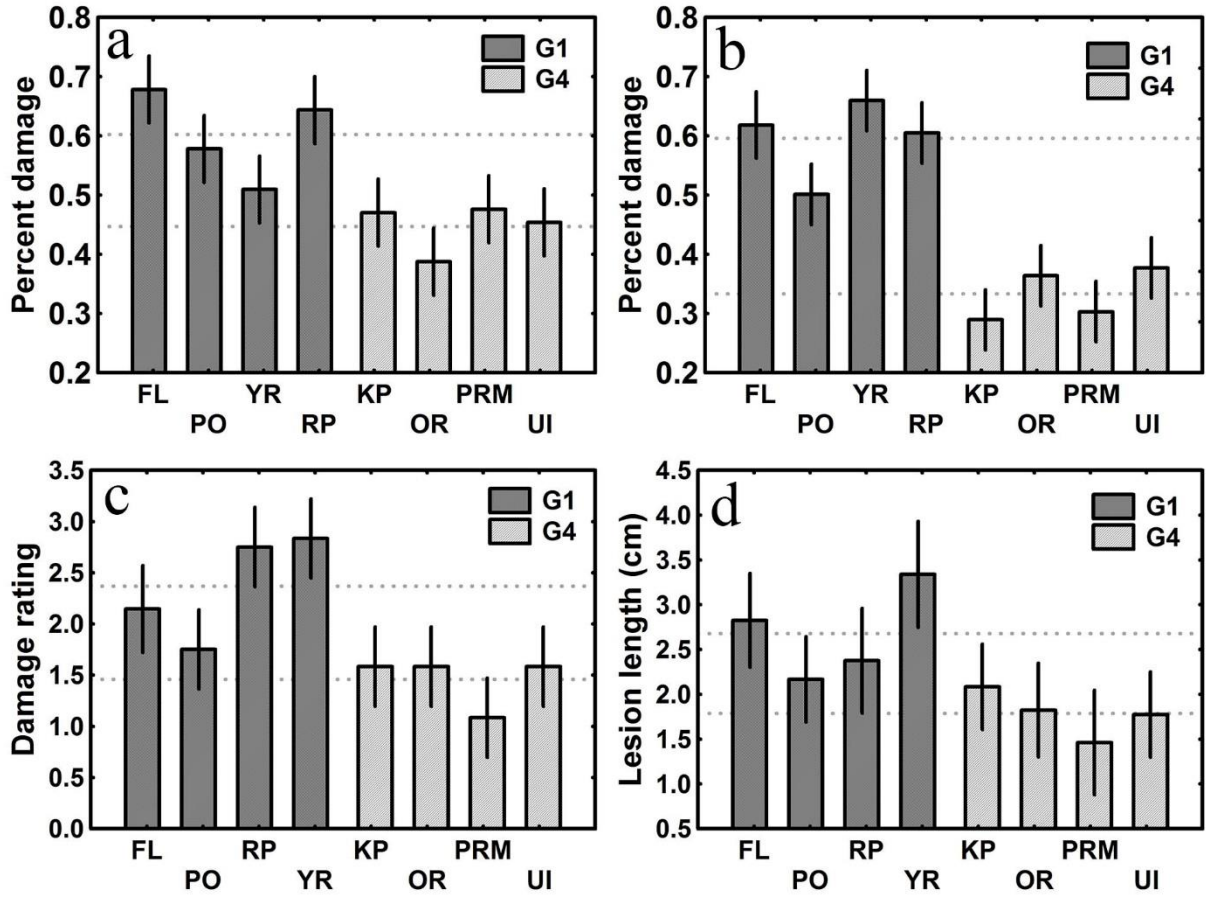


Figure B.2. Variation among *B. umbellatus* populations in the level of pathogen damage. Mean ± SE percent damage between plants infected by a) *Plectosphaerella cucumerina* and b) *Colletotrichum fioriniae*. Mean ± SE c) leaf disease rating and d) lesion for populations of *B. umbellatus* infected by *C. fioriniae.* Results of leaf damage and lesion size for *P. cucumerina* not shown because leaves were killed and ratings were all maximum values. Dotted grey lines represent genotype means (n=4). Site abbreviations are as follows: FL=Flathead Lake, PO=Pend Oreille River, RP=Rose Pond, YR=Yakima River, KP=Kildeer Pond, OR=Oswegatchie River, PRM=Point Rosa Marsh, UI=Unity Island.

Table B.2. *B. umbellatus* sites and site characteristics included in this study.

Site City, State	Waterbody Type	Infestation Degree, distribution	Latitude (DD)	Longitude (DD)
Cannon Lake, MN	Lake	Minor, Scattered	44.25321702	-93.39383704
Forest Lake, MN	Lake	Moderate, Scattered	45.27235201	-92.93743302
Lake Kawaguesaga, WI	Lake	Minor, Scattered	45.87592296	-89.72762503
Oconto Falls, WI	River	Severe, Continuous	44.87569101	-88.14696698
Village Park, WI	Pond	Moderate, continuous	44.25831296	-88.86423502
Bertrom Lake, WI	Lake/wetland	Minor, Scattered	42.68927903	-90.90530497
MS River, IL	Wetland	Severe, Continuous	42.34311397	-90.40898097
Springbrook Pond, IL	Pond	Moderate, Scattered	41.72989	-88.20536898
Reynolds Lake, MI	Lake	Minor, Scattered	42.20040604	-85.99434497
Lanes Lake, MI	Lake	Minor, Continuous	42.36236301	-84.98800898
Point Rosa Marsh, MI	Wetland	Moderate, Scattered	42.57596802	-82.80540599
Maceday Lake, MI	Wetland	Minor, Scattered	42.68141798	-83.42954404
Sterling State Park, MI	Lake	Minor, Scattered	41.91988301	-83.334766
Killdeer Pond 33, OH	Pond	Moderate, continuous	40.70950602	-83.36879804
Olentangy River, OH	Pond	Minor, Scattered	40.00414297	-83.02281101
Cayuga Lake, NY	Lake	Minor, Scattered	42.459836	-76.50407298
Long Pond , NY	Pond	Minor, Continuous	43.28727099	-77.70673896
Unity Island, NY	Pond	Moderate, Continuous	42.93367404	-78.908135
Three Mile Bay, NY	Lake	Moderate, Continuous	44.08110796	-76.19583897
Oswegatchie River, NY	River		44.68993404	-75.494589
Shelburne Bay, VT	Lake	Moderate, Continuous	44.39885703	-73.23522597
Sabattus Creek, ME	River	Minor, Continuous	44.17574996	-70.09783602
East Bay Wildlife Management Area, NY	Pond	Moderate, Scattered	43.57381901	-73.372701
Missisquoi River, VT	River	Minor, Scattered	44.95127801	-73.16210903
Silver Lake, WA			48.967217	-122.069717
Yakima River, WA	River	Moderate, Scattered	46.3794	-119.430733
Columbia River, WA	River	Moderate, Scattered	46.246367	-119.223183
Lake Spokane, WA		Minor, Scattered	47.801167	-117.555167
Pend Oreille River, WA	River	Minor, Continuous	48.361767	-117.284633
Lake Pend Oreille, ID	Lake	Severe, Continuous	48.179883	-116.23395
Flathead Lake, MT	Lake	Severe, Continuous	47.69655	-114.070517
Aberdeen Canal, ID	Canal	Minor, Continuous	42.950733	-112.829033
Rose Pond, ID	Pond	Moderate, continuous	43.247033	-112.31545

APPENDIX C. SUPPLEMENTARY MATERIAL FOR CHAPTER 5.

Table C.1. Locations where alligatorweed biological control was monitored for this study.

Location	Latitude	Longitude	Waterbody type	Years sampled
Choctaw Boat Ramp, LA	29.850	-90.679	River	2016, 2017, 2018
Bayou Chevruil, LA	29.912	-90.729	River	2016, 2017, 2018
Blind River, LA	30.095	-90.779	River	2015, 2016, 2017, 2018
Marepaus Wildlife Management Area, LA	30.150	-90.807	Swamp	2015, 2016, 2017, 2018
Martin Lake, LA	30.215	-91.900	Lake	2015
Blackwater Conservation Area, LA	30.535	-91.089	Wetland	2016, 2017, 2018
Greenwood Community Park, LA	30.570	-91.167	Pond	2016
Simmesport Pond, LA	30.969	-91.808	Pond	2016, 2017, 2018
Spring Bayou, LA	31.142	-92.009	River	2016, 2017, 2018
Lake Saint Joseph, LA	32.077	-91.233	Lake	2015, 2016, 2017, 2018
Bayou Macon, LA	32.094	-91.564	River	2016, 2017, 2018
Openwood Pond, MS	32.396	-90.794	Pond	2015, 2016
Poverty Point Reservoir, LA	32.529	-91.495	Reservoir / Lake	2015, 2016, 2017, 2018

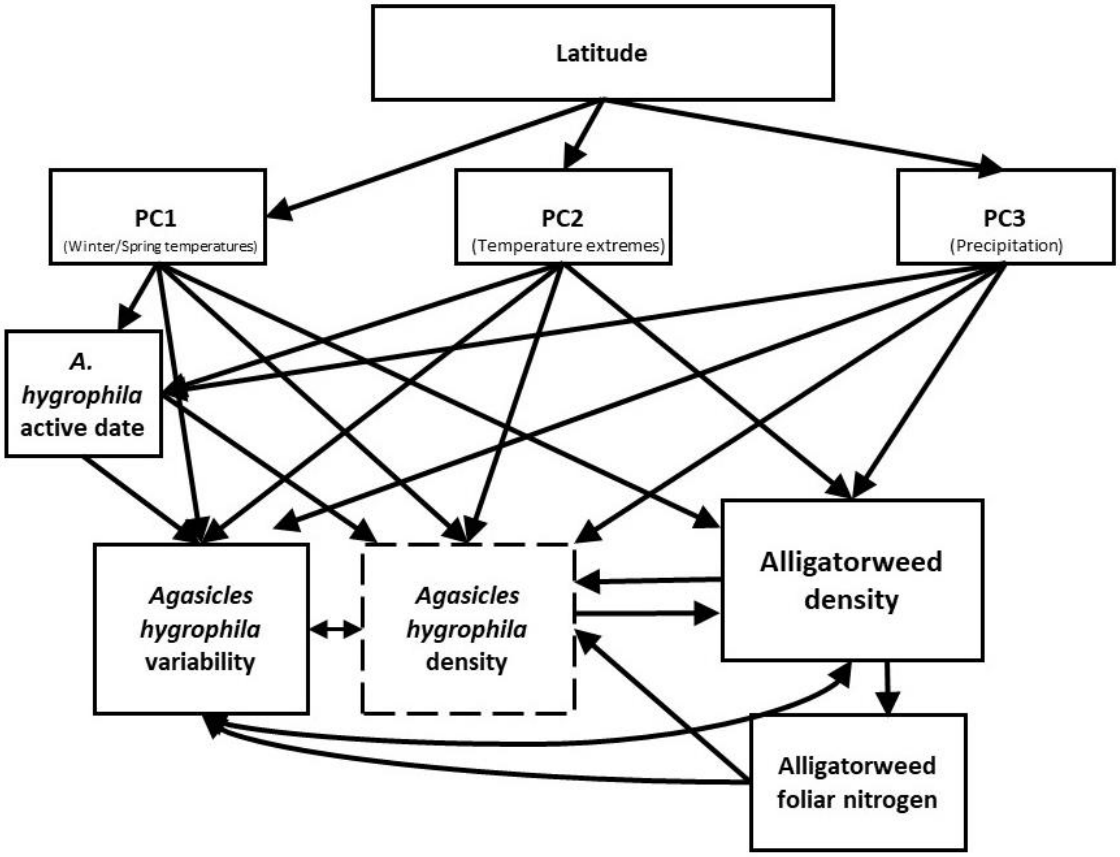


Figure C.1. Conceptual model of factors determining *A. philoxeroides* abundance in a biological control system. The dashed box around the *A. hygrophila* density variable is to denote that two separate conceptual models were considered- one in which mean density was the *A. hygrophila* density variable and one in which maximum density was the *A. hygrophila* density variable. The conceptual model was used as the full model for subsequent selection and examination of parameter significance. Indirect effects are not shown in this diagram.

Table C.2. Model rank and fit indices for a subset (best and full models) of model combinations for *A. philoxeroides* and biological control. Model 1 is presented in the text, full models are presented in Appendix 2 and models 2-3 are below. N = number of observations, K = number of estimated parameters, AIC = Akaike Information Criterion, AICc = AIC corrected for small sample size, ΔAICc = difference between AICc of the model and AICc of the top model, RMSEA = Root Mean Square Error Approximation, GFI = Goodness-of-fit index, AGFI = sample size corrected GFI, χ^2 *P* = Chi-square probability.

Model	**N**	**K**	**AIC**	**ΔAIC**	**AICc**	**ΔAICc**	**Likelihood**	**Akaike Wt.**	**RMSEA**	**GFI**	**AGFI**	**χ^2**
1-Mean	39	9	59.72	0.00	65.93	0.00	1.00	0.52	0.0853	0.8818	0.7341	0.233
2-Max	39	9	60.97	1.25	67.18	1.25	0.53	0.28	0.1048	0.8671	0.7187	0.1501
3-Mean	39	10	59.99	0.27	67.85	1.92	0.38	0.20	0.0789	0.8893	0.7343	0.2632
Full-Max	39	23	89.35	29.63	162.95	97.02	0.00	0.00	0.1702	0.9053	0.5265	0.0435
Full-Mean	39	23	89.66	29.94	163.26	97.33	0.00	0.00	0.17	0.9039	0.5193	0.0394

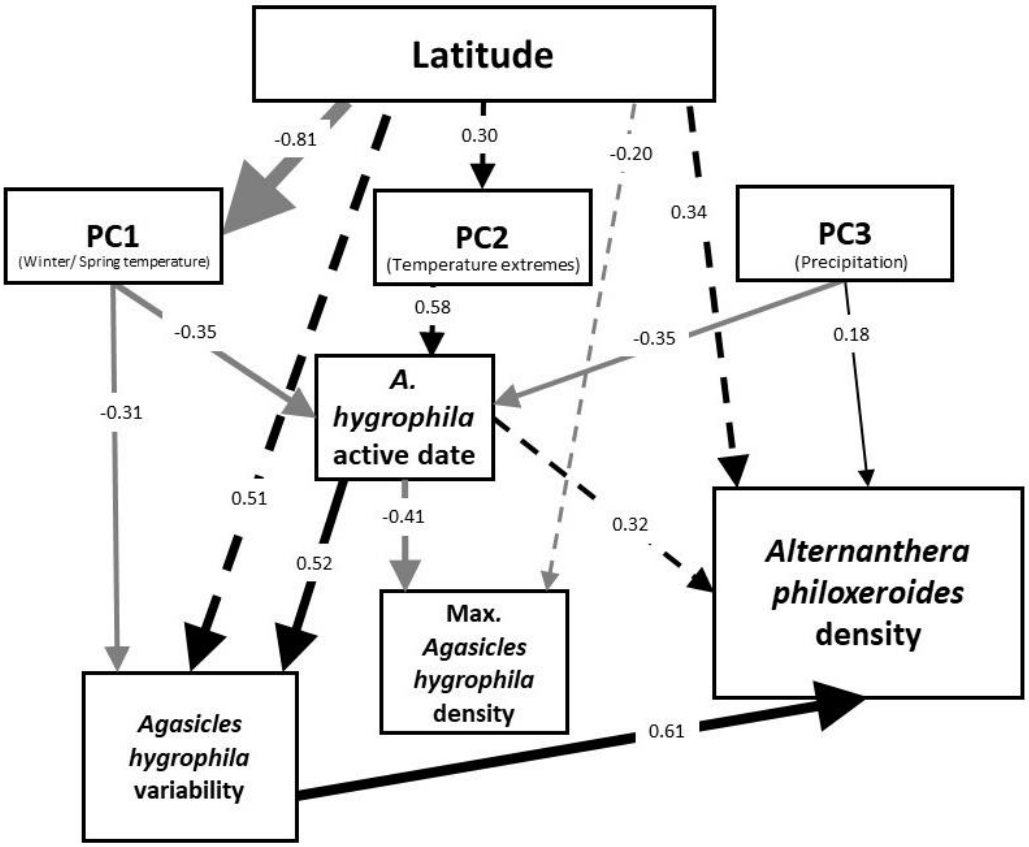


Figure C.2. Model 2 with maximum *A. hygrophila* density as the biological control agent abundance variable.

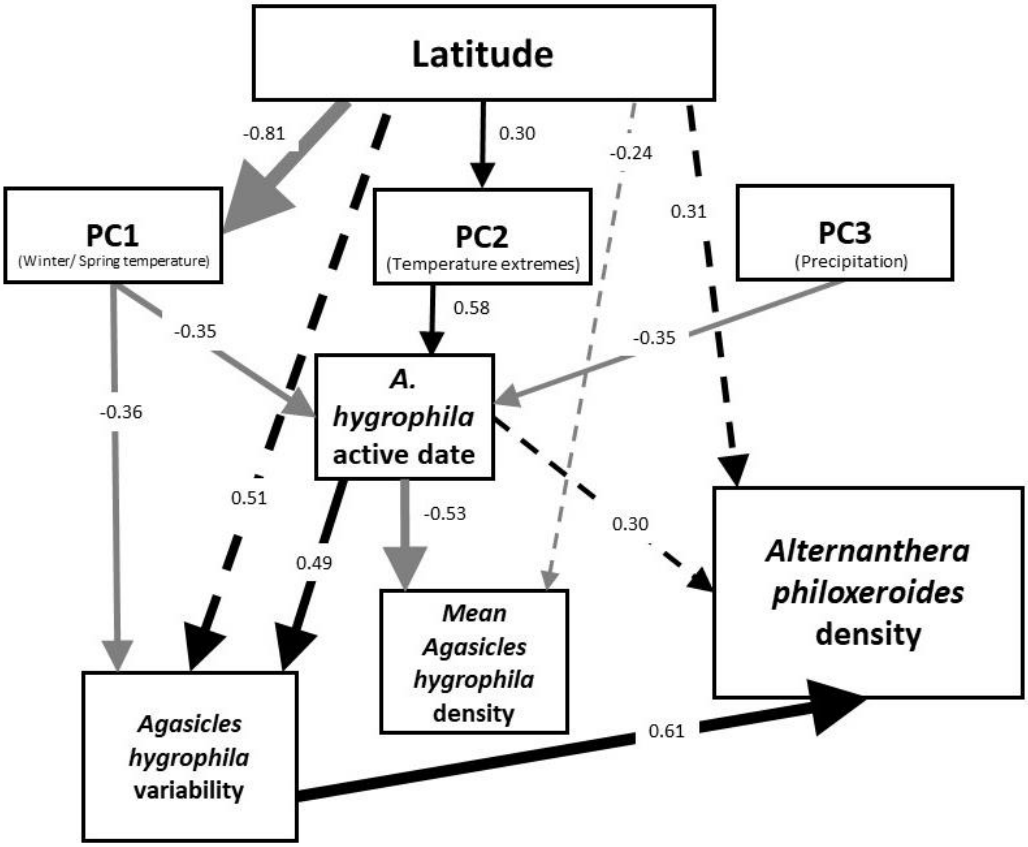


Figure C.3. Model 3 with mean *A. hygrophila* density as the biological control agent abundance variable.

APPENDIX D. COPYRIGHT INFORMATION.

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VITA

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