

BIOGEOGRAPHICAL APPROACHES FOR STUDYING SPECIES INVASION

A Dissertation

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ABSTRACT

Many of the most notorious biological invasions occur at continent-wide or global scales but studies investigating mechanisms enhancing species invasions are often conducted at small spatial scales. Moreover, the contribution of mechanisms facilitating invasion might also vary across a geographical space. I used biogeographical approaches to explore the mechanisms enhancing invasion of introduced genotypes of *Phragmites australis* along the coastal wetlands of North America. I tested the hypotheses that large-scale disturbance events, such as hurricanes and tropical storms, enhance the invasion success of introduced *P. australis* in North America. The growth rate of *P. australis* patches was strongly and positively related to the frequency of hurricane events along the coastal wetlands of the United States. Hurricane frequency alone explained 81% of the variation in the growth rate of *P. australis* patches over this broad geographical range.

I also examined the evolution of latitudinal gradients in native and invasive genotypes of *P. australis* in relation to plant-herbivore interactions in North America. Common garden experiments revealed that the native and invasive genotypes of *P. australis* have evolved latitudinal clines on traits associated with herbivory, specifically for aphid *Hyalopterus pruni*. For a chewing herbivore, *Spodoptera frugiperda*, only native genotypes exhibited latitudinal clines. The existence of non-parallel latitudinal gradients between native and invasive genotypes creates spatial heterogeneity in the importance of herbivory on *P. australis* invasion and suggests the greater susceptibility of high-latitude communities. These latitudinal and genotypic variations on plant defenses, palatability, and herbivory suggest a possibility of an asymmetric apparent competition between native and invasive genotypes. A replicated field study in four sites along the Atlantic Coast showed that invasive genotypes could suppress the fitness of native genotypes

by herbivore-mediated apparent competition. Moreover, the intensity of apparent competition declined with increasing latitude. These biogeographical studies suggest that multiple processes might be contributing to the spread of the introduced genotypes of *P. australis* in North America and that the importance of these processes might vary along an environmental gradient. These studies have broader implications for understanding species invasions at the continental scale and for managing natural habitats that are threatened by plant invasions.

CHAPTER 1 INTRODUCTION

Biological invasions constitute one of the major environmental problems today. They are widespread, have substantial negative impacts on the structure and function of ecological communities (D'Antonio and Vitousek 1992, Mack et al. 2000, Vila et al. 2011) and incur substantial economic cost (Pimentel et al. 2000, Pimentel et al. 2005). Although the ecological and economic impacts of biological invasions are well understood, the mechanisms enhancing species invasions are not fully explored.

In this dissertation, I used biogeographical approaches to study how large-scale disturbances and direct and indirect biotic interactions influence plant invasions across a large spatial scale. Disturbance events are shown to enhance the spread of an invasive species by increasing nutrient concentration in the habitat and reducing competition among the resident species hence opening spaces for species invasions (Hobbs and Huenneke 1992, D'Antonio 1999, Davis et al. 2000, *but see* Moles et al. 2012). Natural and anthropogenic disturbances at local or landscape levels support this hypothesis. However, the role of large-scale disturbances, such as hurricanes and storms, has not been evaluated on species invasion (Diez et al. 2012). The relevance of the research question is highlighted by climatological predictions suggesting an increase in frequency and/or intensity of storm activities with global climate change (Goldenberg et al. 2001, Emanuel 2005, Webster et al. 2005, Bender et al. 2010, Knutson et al. 2010, *but see* Pielke et al. 2005).

Environmental gradients in species interactions can also have important implications on the invasion success of introduced species across a large spatial scale (Bezemer et al. 2014, Cronin et al. 2014). In plant species, latitudinal gradients in plant-herbivore interactions are predicted to evolve in response to herbivory (Schemske et al. 2009). However, a novel invader

that has shown a rapid spread across the continent may not exhibit a gradient because of insufficient time to evolve one. The existence of non-parallel latitudinal gradients on herbivory between native and invasive species may create important spatial heterogeneity in the importance of biotic resistance or susceptibility (Bezemer et al. 2014, Cronin et al. 2014). Although spatial heterogeneity on the importance of biotic resistance/susceptibility has been documented in the field (Cronin et al. 2014), the genetic basis of this heterogeneity has not been explored.

Similarly, indirect biotic interactions can also play an important role in species invasions. Apparent competition, a reciprocal negative interaction between two species via shared natural enemies, is common in nature and has been shown to have strong influence on community structure and dynamics (Holt 1977, Holt and Kotler 1987, Connell 1990, Bonsall and Hassell 1997, Chaneton and Bonsall 2000, Morris et al. 2004, Cronin 2007). Introduced species have been shown to increase invasion success by increasing enemy pressure on co-occurring native species by habitat- or food-mediated mechanisms (Borer et al. 2007, Orrock et al. 2008, Orrock et al. 2010, Orrock and Witter 2010, Enge et al. 2013, Orrock et al. 2014). Because geographical variation in biotic interactions is common in nature (Schemske et al. 2009), similar gradients in the strength of apparent competition between interacting plant species can be expected. However, no study has ever examined it.

In my dissertation, I used a biogeographic approach to examine the importance of hurricane and tropical storm activities on the spread of invasive species. Then, I studied latitudinal variation in direct (plant-herbivore interactions) and indirect (apparent competition) species interactions, and evaluated their importance on plant invasion. I used native and introduced genotypes of common reed, *Phragmites australis*, as a model system to address these questions. *P. australis* is one of the most successful invaders in the wetlands of North America

(Saltonstall 2002, Guo et al. 2013). It has been invading aggressively inland freshwater and brackish coastal marshes of North America, producing large monotypic stands. The invasion by this species is followed by a series of ecological alterations in the wetland ecosystem. It replaces native plants (Silliman and Bertness 2004, Minchinton et al. 2006), alters the diversity and composition of associated faunal communities (Angradi et al. 2001), and changes ecosystem processes such as nutrient cycling (Bart and Hartman 2002, Windham and Ehrenfeld 2003) and hydrological regimes (Windham and Lathrop 1999). Spread of this species has been shown to correlate to natural and anthropogenic disturbances including alterations of habitat characteristics and coastal development (Bertness et al. 2002, Silliman and Bertness 2004, Bart et al. 2006, King et al. 2007, Chambers et al. 2008, Holdredge et al. 2010). However, the importance of large-scale disturbances and biotic interactions on its spread has not been evaluated.

In Chapter 2, I examined the importance of hurricane and storm activities on the spread of *P. australis* in the wetlands along the Gulf and Atlantic Coasts of the United States. I used historical aerial imagery to determine the growth rate of invasive *P. australis* patches. Information about hurricanes and storms passing through the study areas was extracted from International Best Track Archive for Climate Stewardship (IBTrACS, v03r04 WMO) for the North American Basin. I hypothesized that the frequency and intensity of hurricane events would enhance the growth rate of *P. australis* patches.

In Chapter 3, I performed a series of common garden experiments to examine whether native and invasive genotypes of *P. australis* exhibit different genetically based latitudinal gradients in defenses and palatability to their herbivores in North America. Native and invasive populations of *P. australis* were collected across an 18° latitudinal range in North America and reared in replicate northern (41.49° latitude) and southern (30.35°) common gardens. Using two

generalist herbivores, the mealy plum aphid *Hyalopterus pruni* and the fall armyworm *Spodoptera frugiperda*, genotypic and latitudinal variation in plant defense, nutritional and palatability traits of *P. australis* were evaluated. Because the plants collected across a wide geographic range were grown under similar environmental conditions, variation observed in herbivory traits is likely to have strong genetic influence. Moreover, variation in the expression of a trait for a population in different common gardens would represent the phenotypic plasticity of that trait.

In Chapter 4, I performed a replicated field experiment in four wetlands along the Atlantic Coast to examine whether apparent competition occurs between native and invasive genotypes of *P. australis* and whether the strength of this interaction varies with latitude. I set up cross-transplantation experiments with co-occurring native and invasive patches in the same wetland at each site and evaluated herbivore damage for all major feeding guilds on experimental plants. I evaluated if native plants received greater herbivore damage when they were moved to the invasive patch and vice versa. More damage on native plants growing in an invasive patch would indicate the occurrence of apparent competition. Finally, I examined if the intensity of interaction varied with latitude.

In Chapter 5, I outline the major findings of my experiments and discuss their implications for invasion biology.

CHAPTER 2

HURRICANE ACTIVITY AND THE LARGE -SCALE PATTERN OF SPREAD OF AN INVASIVE PLANT SPECIES*

INTRODUCTION

Biological invaders are widespread and increasing in number in marine, freshwater and terrestrial ecosystems worldwide (Ruiz et al. 1997, Pyšek and Hulme 2011), and because their occurrence is often linked to climate change, the rise in invasions is expected to continue into the future (Dukes and Mooney 1999, Stachowicz et al. 2002, Cheung et al. 2009, Walther et al. 2009, Bradley et al. 2010). Moreover, successful invaders can have dire consequences for the persistence of native species, food-web structure, ecosystem functioning (Mack et al. 2000, Vila et al. 2011), and, ultimately, the economy (Pimentel et al. 2005). Mechanisms promoting establishment and spread of invasive species in particular habitats (local scale) have been well studied and include possession of traits that facilitate establishment and invasion (e.g., strong dispersal ability, high reproductive rate, superior competitive ability) and release from natural enemies (Lodge 1993, Sakai et al. 2001).

Alterations of habitat characteristics by natural and anthropogenic disturbances, or change in disturbance regimes, are quite often associated with invasion success (Davis et al. 2000, Pyšek et al. 2010, Moles et al. 2012). Disturbances benefit invasive species by reducing competition with resident species and increasing resource availability (Davis et al. 2000, Diez et al. 2012). Large-scale disturbance events such as hurricanes, cyclones and typhoons have long been associated with the establishment and spread of invasive species (Censky et al. 1998, Bellingham

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et al. 2005). However, to date, few studies have considered whether the history of such extreme disturbance events has influenced invasion success at local scales (Bradley et al. 2010, Diez et al. 2012), and no studies have addressed whether these types of disturbances affect the patterns of establishment and spread at regional or continental spatial scales. For example, in the northern hemisphere historical patterns of spread of invasive species may be greater in the south where hurricanes are more frequent and intense than in the north. As such, range expansion and spread of an invader may be driven by disturbance regimes. The relevance of studying hurricane effects on the establishment and spread of invasive species is magnified by the expectation that hurricane activity, particularly high-intensity hurricanes, may increase with global climate change (Emanuel 2005, Bender et al. 2010, Knutson et al. 2010).

We studied the effect of storm and hurricane activities on the growth of patches of common reed, *Phragmites australis*, in the coastal wetlands of the eastern United States of America. Indigenous and/or introduced haplotypes (based on a microsatellite analysis of chloroplast DNA) of *P. australis* are found on all continents except for Antarctica, and in some cases the introduced haplotypes are recognized as aggressive invaders (Saltonstall 2002, Guo et al. 2013). Historically, *P. australis* has been an uncommon species of the wetlands of North America for millennia (Niering 1977). In the past 150 years, an introduced Eurasian haplotype has spread rapidly in both coastal and inland marsh ecosystems of North America, particularly near the Atlantic Coast (Saltonstall 2002). An additional haplotype that originated in Africa and is present in all of the Gulf Coast states (Gulf-Coast haplotype) (Lambertini et al. 2012) is also spreading locally and expanding its range to the southwestern US (Meyerson et al. 2010, Williams et al. 2012). It is unclear whether this haplotype's appearance into the Gulf Coast region was facilitated by human activities or the result of a natural range expansion from Central and South

America. Other non-native haplotypes of *P. australis* are present in North America, but they appear to have very restricted distributions (particularly, within the Mississippi River Delta) (Lambertini et al. 2012). Marshes that have been invaded by *P. australis* have been characterized by the loss of native plant species, reduced diversity and altered composition of associated faunal communities, and changed ecosystem processes such as nutrient cycling and hydrological regimes (Windham and Ehrenfeld 2003, Minchinton et al. 2006, Dibble et al. 2013).

In spite of the serious ecological and economic impacts of *P. australis* invasion, almost nothing is known about the factors responsible for the continent-scale patterns of spread of these invasive haplotypes in North America. Using historical aerial images (spanning 5-27 years), we determined the growth rate of *P. australis* patches within each of 13 marsh sites (9 inhabited by the Eurasian and 4 inhabited by the Gulf-Coast haplotype) distributed along the Gulf and Atlantic Coasts of the US (Figure 2.1, Table 2.1). For each site, we estimated wind speeds of all storms, counting only those that qualified as a tropical or subtropical storm (maximum sustained wind speeds of 65 - 119 km/h) or hurricane (≥ 119 km/h). By dividing storms into these two wind-speed categories, we were able to test the *a priori* prediction that growth rates of *P. australis* patches were more strongly related to the frequency of more intense storms.

MATERIALS AND METHODS

Study sites and estimation of growth rate

We selected 13 freshwater-to-brackish marshes distributed along the Gulf and Atlantic Coasts of the US that were occupied by one of two non-indigenous haplotypes of *P. australis* that exhibit both aggressive patterns of local spread and range expansion (Saltonstall 2002, Meyerson et al. 2010, Lambertini et al. 2012, Williams et al. 2012) (Figure 2.1, Table 2.1). Because patches of native haplotypes are difficult to distinguish from the background marsh

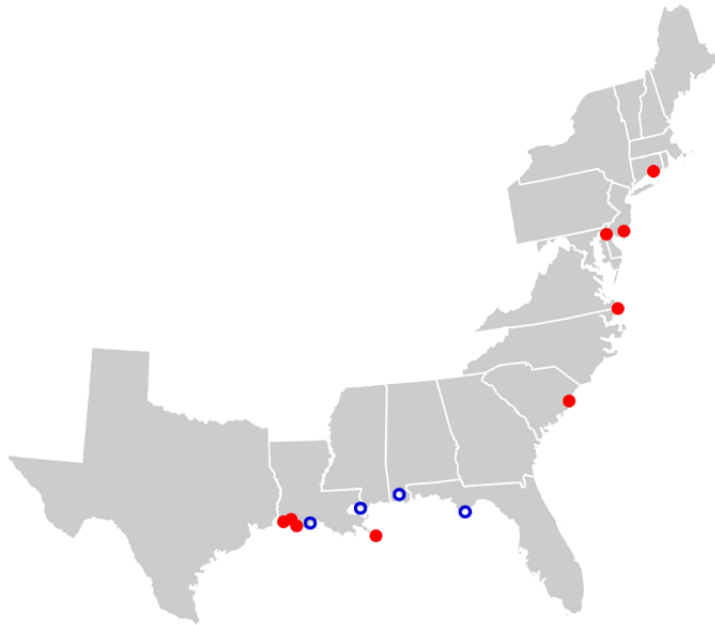


Figure 2.1. Location of study sites along the Gulf and Atlantic Coasts of the United States. Filled and open symbols represent sites occupied by Eurasian and Gulf-Coast haplotypes of *P. australis* respectively.

vegetation in historical aerial images (JT Cronin, GP Bhattarai, WJ Allen, LA Meyerson unpublished data), the native haplotype was excluded from this study. We selected relatively undisturbed open marsh habitats where *P. australis* was at an early stage of invasion and had the potential to grow. Eight of our sites were located in protected areas (wildlife refuges, management areas and state parks) but all 13 sites were relatively undisturbed during the study period. All sites along the Atlantic coast and four sites in Louisiana were occupied by an introduced Eurasian haplotype. The remaining four sites (one in Florida, one in Alabama, and two in Louisiana) were occupied by a non-native Gulf-Coast haplotype.

P. australis patches were identified initially based on morphological characters and, then, confirmed by an analysis of the chloroplast DNA (Saltonstall 2002). Marsh sites were ≤ 25 km from the ocean or gulf and could potentially flood from storm surge. Most of the sites were tidal

Table 2.1. Description of the study sites and duration of the study.

Site	State	Long	Lat	Area (km ²)	Period of imagery	Imagery dates	Haplo-type*	No. patches	Initial patch area (m ²) (mean ± SE)	Number of	
										Tropical storms	Hurricanes
Pettipaug Yacht Club	CT	-72.38	41.37	2.21	1991-2010	1991, 1994, 1997, 2005, 2008, 2010	<i>M</i>	12	3686.20 ± 2328.97	3	1
Estell Manor Park	NJ	-74.72	39.40	3.89	1991-2011	1991, 1995, 2002, 2006, 2007, 2008, 2010, 2011	<i>M</i>	6	1299.26 ± 714.16	4	0
Appoquinimink River	DL	-75.67	39.43	1.00	1989-2009	1989, 1997, 2006, 2009	<i>M</i>	16	3116.01 ± 1780.68	4	0
Mackay Island NWR	NC	-75.94	36.51	17.48	1993-2010	1993, 1998, 2005, 2006, 2008, 2009, 2010	<i>M</i>	6	1468.97 ± 389.20	10	1
Georgetown	SC	-79.26	33.37	12.10	1990-2011	1990, 1994, 1999, 2005, 2006, 2009, 2011	<i>M</i>	7	592.79 ± 206.12	12	2
Apalachicola Bay	FL	-84.97	29.72	8.48	1984-2010	1984, 1995, 1999, 2004, 2007, 2010	<i>I</i>	7	2343.99 ± 1008.56	8	2
Mobile Bay	AL	-87.95	30.66	15.68	2006-2011	2003, 2006, 2009, 2011	<i>I</i>	8	276.99 ± 123.20	3	1
Delta NWR	LA	-89.19	29.13	20.03	1983-2010	1983, 1994, 1998, 2004, 2005, 2007, 2009, 2010	<i>M</i>	7	271.40 ± 128.35	10	5
Big Branch Marsh NWR	LA	-89.82	30.25	1.00	1998-2010	1988, 1998, 2004, 2005, 2007, 2009, 2010	<i>I</i>	2	960.61 ± 354.20	6	1

(Table 2.1. continued)

Site	State	Long	Lat	Area (km ²)	Period of imagery	Imagery dates	Haplo-type*	No. patches	Initial patch area (m ²) (mean ± SE)	Number of	
										Tropical storms	Hurricanes
Intracoastal City	LA	-92.20	29.78	25.00	1998-2010	1994, 1998, 2003, 2005, 2010	<i>I</i>	6	1806.27 ± 820.15	2	2
Rockefeller WR	LA	-92.83	29.68	25.00	1988-2010	1988, 1994, 1998, 2003, 2005, 2008, 2009, 2010	<i>M</i>	8	1317.67 ± 279.94	7	2
Cameron Prairie NWR	LA	-93.08	29.95	8.62	2003-2010	1998, 2003, 2005, 2007, 2008, 2009, 2010	<i>M</i>	16	387.88 ± 98.11	5	2
Sabine NWR	LA	-93.44	29.86	4.01	1998-2010	1994, 1998, 2003, 2005, 2007, 2009, 2010	<i>M</i>	5	272.65 ± 87.71	5	2

Notes: Long = Longitude, Lat = Latitude, NWR = National Wildlife Refuge, WR = Wildlife Refuge.

*Haplotype designations are from Saltonstall (2002). *M* represents invasive European haplotype and *I* represents Gulf-Coast haplotype.

but the sites along the Gulf Coast experience smaller tides in comparison to those on the Atlantic Coast. These sites were separated from each other by at least 40 km and none shared the same drainage system.

Within each site, we selected a 1 - 25 km² area within the interior of the marsh that contained discrete *P. australis* patches (Table 2.1). Patches within this area were unconstrained by any physical barriers to expansion (e.g., roads, bodies of water, agricultural lands, marsh edges). These dense and usually circular patches of *P. australis* were readily identifiable in aerial images (color, color-infrared and black-and-white images) because of their distinct color and texture against the background marsh vegetation (Rice et al. 2000, Rosso et al. 2008).

Twenty to thirty *P. australis* patches were identified in the most recent set of aerial images available for each site, and digitized in ArcMAP 10.1 (ESRI, Redlands, CA). The existence of those patches was verified during field visits to the sites. Patches were then followed backward in time through a series of aerial images to the early 1980s or until they were no longer visible on the images. Only those patches which were present in the oldest set of imagery were considered in this study. The number of focal patches within each site averaged 8.15 ± 1.14 (mean \pm SE; range: 2-16, Table 2.1). Annual growth rate per patch was determined as the proportional change in area per year: $\ln [(final\ patch\ area/initial\ patch\ area)]/number\ of\ years$ (Rice et al. 2000).

Clonal growth is expected to be the primary means of *P. australis* patch expansion (Amsberry et al. 2000) but we cannot rule out the contribution of sexual reproduction (Kettenring et al. 2011).

For each marsh site, an average growth rate was computed from the collection of focal patches.

Hurricane and tropical storm frequency

We used wind speed as an indicator of the strength of the storm as a disturbance to *P. australis*. Data on other disturbances associated with tropical storms and hurricanes (e.g., storm

surge, change in salinity, nutrient levels, deposition of silt and wrack) are mostly unavailable. However, it is likely for coastal marshes that wind speed is correlated with these other variables. Information about hurricane and tropical storm (tropical and sub-tropical) tracks and maximum wind speeds along those tracks were collected from the International Best Track Archive for Climate Stewardship (IBTrACS, v03r04 WMO) for the North American Basin <http://www.ncdc.noaa.gov/oa/ibtracs/index.php?name=ibtracs-data>. Using ArcMAP 10.1, storm tracks passing within a radius of 200 km around each study site during the study period were extracted. The maximum wind speed of each storm in the study site was estimated using the Rankine combined vortex approximation model (Holland et al. 2010). First, the minimum distance between the center of the study site and storm track was determined for each storm. Second, because the radius of maximum winds for a hurricane is estimated to be 48 km (Hsu and Yan 1998), if the storm passed within this distance of the study-site center, the maximum sustained wind speed was considered the wind speed experienced at the site. For the storms more than 48 km from the site center, maximum sustained wind speed for that site was estimated as

$$v = v_m \left(\frac{r}{r_{v_m}} \right)^x$$

where v is wind speed at the site, v_m is the maximum wind speed, r is the distance between the site and hurricane path, r_{v_m} is the radius of maximum winds, and x is the scaling parameter (Holland et al. 2010). We used $x = 0.7$ as recommended by Hsu and Babin (Hsu and Babin 2005).

All storm events with wind speeds ≥ 35 knots (64.9 km/hr), the minimum for categorization of a tropical storm based on the Saffir-Simpson hurricane wind scale http://www.nhc.noaa.gov/pdf/sshws_table.pdf, were included in the analysis. For each site,

storms were categorized as either tropical or sub-tropical storms (35- 64 knots, or 64.9 – 118.5 km/hr) or hurricanes (above 64 knots or 118.6 km/hr) based on a popular convention. A total of 79 tropical and sub-tropical storms and 21 hurricanes (average wind speed = 99.47 km/hr, SE = 3.11, range = 65-231.5 km/hr) passed through our sites during the study period. Annual frequencies of tropical storms and hurricanes were determined for each site.

Climate data

One of the objectives of this study was to evaluate whether *P. australis* growth rates were influenced more by large-scale storm events than by local climatic conditions. To this end, the following climate data for each site were obtained from the BIOCLIM database (Hijmans et al. 2005): annual mean temperature, isothermality (mean of monthly [maximum temperature – minimum temperature]/annual temperature range), temperature seasonality (standard deviation of weekly mean temperatures), maximum temperature of warmest month, minimum temperature of coldest month, temperature annual range, annual precipitation, precipitation seasonality (standard deviation of weekly mean precipitation estimates expressed as the percentage of mean of those estimates), precipitation of wettest quarter, and precipitation of driest quarter. A principal component analysis was run to reduce the dimensionality of climatic data. The first two principal components, which explained 94.8% and 5.01% variability of the climatic data respectively, were used in our model-selection procedure.

Model selection

We examined the effects of latitude, initial patch size, climatic variables (PC1 and PC2), frequency of tropical storms, and frequency of hurricanes on growth rate of *P. australis* patches. Using general linear models in Systat 12 (Systat Inc., Chicago, IL), we developed statistical models using all combinations of latitude (x_1), initial patch size (x_2), PC1 (x_3), PC2 (x_4),

frequencies of tropical storms (x_5) and hurricanes (x_6). The best model was selected using corrected Akaike weights (Burnham and Anderson 2002) (Appendix A). The time interval over which *P. australis* growth was measured for each site ($P = 0.11$) and intensity of hurricanes (sum total of hurricane categories [1-5; Saffir-Simpson scale]) ($P = 0.71$) did not have a significant effect on growth rates of patches. Therefore, we did not include them in analysis. Examination of the standardized residuals in our best model showed that one of the data points was an outlier (Intracoastal City, LA). Removal of that point in the analysis improved the fit of the model to the data ($F_{2,9} = 36.53$, $P = 0.001$, $R^2 = 0.89$). Because we have no reason to conclude that this data point is spurious, we retained it in our analysis.

RESULTS/DISCUSSION

Average annual growth rate of *P. australis* patches within a site varied from 6.3% to 35.3% among our sites. The best-fit model for explaining the variation in *P. australis* growth rates among sites included only hurricane frequency (Appendix A; *P. australis* growth rate = $a[\text{hurricane frequency}] + b[\text{hurricane frequency}]^2 + k$; Akaike weights = 0.61, Evidence ratio = 4.72, Normalized evidence ratio = 0.83). The growth rates of *P. australis* patches in semi-protected coastal marshes of the US (Eurasian and Gulf-Coast haplotypes combined) increased significantly, but nonlinearly, with hurricane frequency (Figure 2.2). Eighty-one percent of the variation in *P. australis* growth rate was explained by just this one abiotic factor. Interestingly, the occurrence of lower-intensity storms did not contribute in an appreciable way to the growth of *P. australis* patches (Appendix A). Hurricane frequency was greatest in the south and decreased with increasing latitude ($P = 0.004$) but storm frequency was independent of latitude ($P = 0.16$, Figure 2.3). Despite these latitudinal patterns, latitude was uncorrelated with *P.*

australis growth rates ($P = 0.20$, Figure 2.4). Growth rates of *P. australis* patches were also independent of the climatic variables (Appendix A).

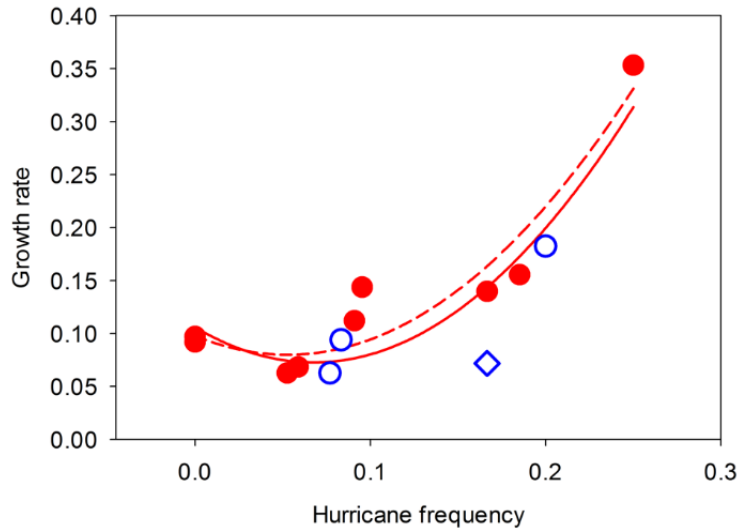


Figure 2.2. Effect of hurricane frequency on *P. australis* growth. Annual growth rate (proportional change in \ln area) of *P. australis* patches as a function of hurricane frequency in the coastal marshes of the United States. Filled and open symbols represent sites occupied by Eurasian and Gulf-Coast haplotypes respectively. Solid curve is the best-fit model representing all sites ($F_{2,10} = 21.66$, $P < 0.001$, $R^2 = 0.81$). The diamond-shaped symbol was identified as an outlier based on the examination of standardized residuals. The relationship was still significant when it was removed from the analysis ($P < 0.001$, $R^2 = 0.89$). The dotted curve represents the best-fit model for only the sites occupied by the Eurasian haplotype ($F_{2,6} = 26.87$, $P = 0.001$, $R = 0.90$).

Although it would have been desirable to statistically test whether the growth rates of the Eurasian and Gulf-Coast haplotypes responded differently to hurricane frequency, we did not have sufficient replication for the Gulf-Coast haplotype to allow for this comparison. However, we were able to compare the best-fit model for the growth rate of *P. australis* with and without sites representing the Gulf-Coast haplotype. Exclusion of sites with the Gulf-Coast haplotype did not alter the results ($F_{2,6} = 26.87$, $P = 0.001$, $R^2 = 0.90$, Figure 2.2). Moreover, a comparison of the growth rates of sites occupied by Gulf-Coast haplotype with those of sites occupied by

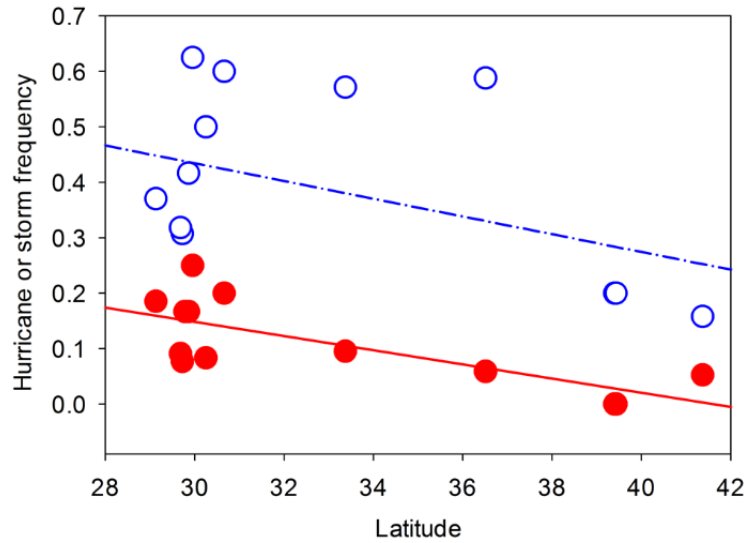


Figure 2.3. Relationship between the tropical storm and hurricane frequencies (number per year) and latitude. Filled and open symbols represent hurricane and tropical storm frequencies respectively. Lines for each storm category are fit by separate least-squares regression analyses (Tropical storms: $R^2 = 0.16$, $P = 0.16$; Hurricanes: $R^2 = 0.55$, $P = 0.004$).

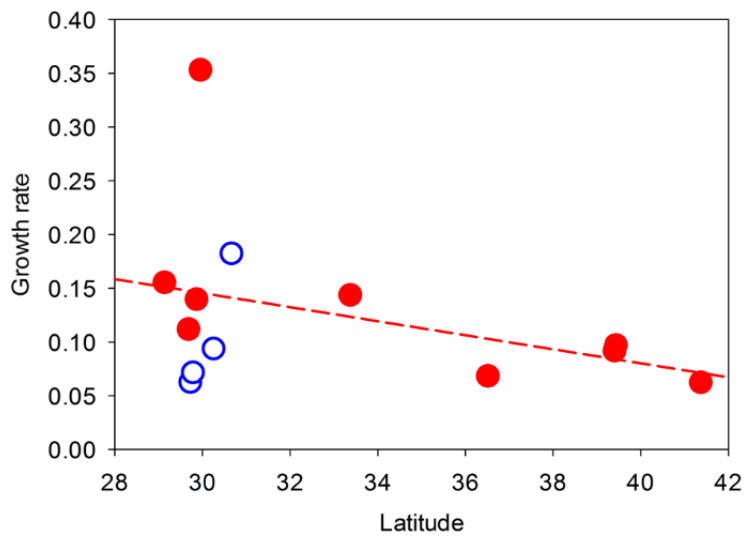


Figure 2.4. Effect of latitude on *P. australis* growth. Annual growth rate (proportional change in \ln area) of *P. australis* patches as a function of latitude in the coastal marshes of the United States. Filled and open symbols represent sites occupied by Eurasian and Gulf-Coast haplotypes respectively. Line is fit by least-squares regression (both haplotypes combined; $R^2 = 0.14$, $P = 0.20$).

Eurasian haplotype indicates that growth rates were not significantly different (Eurasian: 0.13 ± 0.03 [mean \pm SE]; Gulf-Coast: 0.10 ± 0.03 [mean \pm SE]; $t_{11} = 0.69$, $P = 0.50$). These results suggest that the Gulf-Coast haplotype is not only spreading rapidly in marshes along the Gulf Coast of the US but also is responding to disturbance events similarly to the well-known Eurasian invader.

The strong positive correlation between hurricane frequency and the patch growth rate of the Eurasian and Gulf-Coast haplotypes reveals the importance of large-scale disturbances on biological invasions. Severe destruction of natural vegetation accompanied with drastic changes in habitat characteristics including hydrology, salinity, and geomorphology (Chabreck and Palmisano 1973, Michener et al. 1997, Turner et al. 2006) should create room for the spread of an invasive plant (Diez et al. 2012). *P. australis* is one of the early species to recover after a major hurricane (Chabreck and Palmisano 1973). An extensive underground system of rhizomes and roots representing over two-thirds of the total biomass of *P. australis* may enable this species to survive catastrophic disturbances and re-sprout much earlier than the native vegetation. In coastal-area marshes, storm surge brought about by hurricanes often results in temporary flooding and elevated salinity (Chabreck and Palmisano 1973). Increased salinity in freshwater and brackish marshes may on its own, or in combination with the damage from winds, inhibit the recovery of native species. In the case of the Eurasian and Gulf-Coast haplotypes of *P. australis*, which have been shown to tolerate mesohaline levels of salinity (Vasquez et al. 2005, Howard and Rafferty 2006), storm surge may greatly increase their competitive advantage over native species. Alternatively, excessive rainfall during hurricanes which could account for up to 40% of total annual precipitation in a site may reduce salinity in hypersaline marshes (Michener et al. 1997, Paerl et al. 2001) allowing for establishment and growth of *P. australis* in these

environments. Increased concentration of organic matter in the wetland following a hurricane event (Chabreck and Palmisano 1973, Blood et al. 1991, Michener et al. 1997) may also benefit the growth of plant species like *P. australis* that are amongst the first species to recover from a hurricane. The fact that we found no effect of tropical storm frequency on *P. australis* growth rates suggests that these lower wind-speed storms may not sufficiently free up resources, or alter salinity and nutrient levels, to an extent that favors increased growth of *P. australis*. Although we could not estimate the emergence rate of new stands from aerial images (because patches appeared and merged too quickly), it is likely the case that increased hurricane activity also caused an increase in the proliferation of new *P. australis* patches.

The invasion of the Eurasian haplotype of *P. australis* in the Atlantic Coast of the US has been attributed to increased anthropogenic disturbance and nutrient enrichment following coastal development (Bertness et al. 2002, Silliman and Bertness 2004, Bart et al. 2006, King et al. 2007, Chambers et al. 2008, Holdredge et al. 2010). Construction of highway networks has also been linked to the spread of the introduced haplotype in Canada (Lelong et al. 2007). In our study, we specifically selected sites from marshes that were relatively undisturbed by humans to minimize the effects of anthropogenic disturbances on the expansion of patches. Our study provides compelling evidence that large-scale disturbance events can be of overriding importance in the spread of *P. australis* in semi-protected areas in the coastal regions. Hurricane frequency over the past several decades explained over 80% of the variation in the growth rates of *P. australis* patches across the Gulf and Atlantic Coasts of the US. In this case, *P. australis* growth rates were greater along the Gulf and southern Atlantic Coast where hurricanes occurred more frequently. This geographic pattern in growth rates appears to be driven by factors associated with hurricanes, not other climatic or environmental variables associated with latitude

because growth rates were unrelated to latitude (Figure 2.4) and climatic variables in our statistical models (Appendix A). However, the contribution of specific components of hurricane disturbance (e.g., storm surge, nutrient fluxes, changes in salinity) on the growth rate of *P. australis* has yet to be evaluated.

A high priority in the future should be a comparison of the growth rates of native and the European exotic haplotypes in response to large-scale disturbance events. Native haplotypes are found in coastal marshes from North Carolina to Canada (Saltonstall 2002, JT Cronin, GP Bhattarai, WJ Allen, LA Meyerson unpublished data) and are present in four of our study sites (North Carolina, Delaware, New Jersey and Connecticut). In general, patches of native haplotypes are rare (in terms of area of coverage) and are thought to be disappearing, in part, due to the invasion of exotic *P. australis* (Saltonstall 2002, Meyerson et al. 2010). Because native haplotypes are reported to be less tolerant to disturbances and salinity levels (Vasquez et al. 2005), we would expect that they may respond less positively, or even negatively, to hurricane events. With higher resolution color and infrared imagery, hyperspectral imagery, and LIDAR (Rice et al. 2000, Rosso et al. 2008), it should be possible in the future to map the growth and spread of native haplotypes over time.

Our study suggests that in semi-protected areas like national wildlife refuges and preserves, where introduced *P. australis* has invaded, the outlook is dire. Left unchecked, nonnative haplotypes grow very rapidly. The end result is likely to resemble areas like the Chesapeake Bay and the New Jersey Meadowlands which are now dominated by *P. australis*. In protected areas where chemical control may not be an option, management of *P. australis* poses a great challenge. The management of this species through biological control does not appear promising, as most herbivores prefer and perform better on the rare native haplotypes (Lambert and

Casagrande 2007, GP Bhattarai, WJ Allen, LA Meyerson, JT Cronin unpublished data).

Biological control using fungal pathogens is under consideration (Shearer and Harms 2012) but this approach is likely also to be limited by the need for within-species specificity. Mechanical removal during the early stages of invasion has been employed (Hazelton et al. 2014) but those methods are costly, labor intensive, and potentially damaging to the hydrology of the system and neighboring plants (Martin and Blossey 2013, Hazelton et al. 2014). Unfortunately, this may be the only option available to managers of these sensitive lands. In areas where *P. australis* is just beginning to arrive, managers must respond immediately to the threat.

Many of our most notable species invasions have occurred or are occurring at continent-wide scales. To date, studies of these biological invasions have rarely considered the possibility that large-scale phenomena may underlie geographic variation in invader establishment and spread. Recent studies on the effects of global climate change on biological invasions (Stachowicz et al. 2002, Cheung et al. 2009) are an important step in the right direction but clearly more attention needs to be paid to other large-scale climatic disturbances (e.g., storms, droughts) and their effects on all types of invasive species, not just plants.

Understanding the role of hurricanes and storms in biological invasions is particularly relevant in the current context of global climate change. Sea surface temperature has been shown to strongly relate to tropical storm and hurricane activity (Emanuel 2007) suggesting a recent increase in storm counts and their destructiveness (Emanuel 2005, Knutson et al. 2010). Although still a very contentious issue, some climatic models predict an increase in the intensity and frequency of storms in the future (Bender et al. 2010, Knutson et al. 2010 *but see* Pielke et al. 2005). This does not bode well for the susceptibility of coastal ecosystems to the future establishment and spread of invasive species.

CHAPTER 3 FORMATION OF LATITUDINAL GRADIENTS IN INVASIVE PLANT-HERBIVORE INTERACTIONS

INTRODUCTION

One of the most obvious biogeographical patterns in ecology is the increase in species richness as latitude decreases (Rosenzweig 1995, Hillebrand 2004, Brown 2014). Over the years, much interest also has focused on latitudinal gradients in consumer-resource interactions (e.g., MacArthur 1972, Coley and Aide 1991, Pennings et al. 2001, Pennings et al. 2009, Schemske et al. 2009, Cronin et al. 2014). For plant-herbivore interactions, herbivore damage increases towards lower latitudes (Hay and Fenical 1988, Coley and Aide 1991, Bolser and Hay 1996, Pennings et al. 2001, *but see* Andrew and Hughes 2005, Adams and Zhang 2009, Pennings et al. 2009, Moles et al. 2011a, Moles et al. 2011b, Kozlov et al. 2014), which should select for increased defenses or reduced palatability in plant species at lower than higher latitudes (Coley and Aide 1991, Schemske et al. 2009).

Recent spread of an invasive species across a broad geographical scale in the introduced range is likely to be followed by evolutionary changes in response to novel environmental conditions and biotic interactions. A number of studies have documented that invasive species have rapidly evolved (< 100 years) in response to an environmental gradient in their introduced range. For example, invasive species have evolved distinct clines in growth and fitness related traits with latitude (Huey et al. 2000, Maron et al. 2004) and altitude (Alexander et al. 2009) that parallel the clines for the same species in their native range. Although the evolution of latitudinal clines in plant defenses or palatability to herbivores is expected to occur with invasive species, no study has ever examined whether or how quickly latitudinal clines have formed in traits related to plant-herbivore interactions.

The evolution of latitudinal gradients in defenses and/or palatability traits in an invasive species may have important implications for invasion success. For example, native plants are expected to exhibit latitudinal gradients in defense and palatability traits (Schemske et al 2009). But an invasive species may not exhibit a parallel gradient because it hasn't had enough time for one to evolve. These non-parallel gradients between native and invasive species, particularly early in the invasion process, may create large scale heterogeneity in biotic resistance or susceptibility across a broad spatial scale (Bezemer et al. 2014, Cronin et al. 2014). As a result, at some latitudes, invasive plants may have lower levels of defenses and receive greater herbivore damage than co-occurring native plants (i.e., biotic resistance), whereas at other latitudes, we may find the opposite pattern (i.e., biotic susceptibility) (Levine et al. 2004). With sufficient time, we would expect the invasive species to evolve latitudinal clines in defense traits that parallel the clines for co-occurring native species.

We conducted a series of common garden experiments to examine whether latitudinal gradients in defense and palatability traits are evident in native genotypes of *Phragmites australis* from an 18° transect in eastern North America, and whether similar gradients have formed in invasive genotypes of *P. australis*. The continent-wide invasion by introduced European genotypes of *P. australis* in North America has been underway for about the past 150 years (Saltonstall 2002). Co-occurrence of native and introduced genotypes of this species across a wide latitudinal range along the Atlantic Coast of North America allows for strong phylogenetically controlled comparisons across latitudes (Cronin et al. 2014), overcoming many of the flaws reported in previous studies of latitudinal gradients in species interactions (Pennings et al. 2001, Moles et al. 2011a). Recently, Cronin et al. (2014) conducted field surveys along the Atlantic and Gulf Coasts of North America and found that plant defenses, nutritional traits, and

herbivore damage varied linearly with latitude. However, it was unknown from this study whether those gradients were environmental or genetically based. To assess whether latitude influenced results, the experiments were conducted in replicate common gardens, one at 30.35° (Louisiana State University) and the other at 41.49° (University of Rhode Island) latitude. We quantified plant defense levels (leaf toughness, total phenolics) and nutritional condition (percent carbon, percent nitrogen, C:N ratio, water content), and conducted experiments to assess palatability to two generalist herbivores (the mealy-plum aphid, *Hyalopterus pruni*, and the fall armyworm, *Spodoptera frugiperda*). We tested the following hypotheses. 1) Owing to its history of aggressive spread (Chambers et al. 1999, Saltonstall 2002, Meyerson et al. 2010, Bhattarai and Cronin 2014), we hypothesized that invasive genotypes were better defended against herbivory, had reduced palatability to herbivores, and had lower nutritional condition than native genotypes. Support for this hypothesis would suggest that native communities have low biotic resistance to invasion by European *P. australis*. 2) As suggested by field study, we hypothesize that plant defenses should increase and palatability to herbivores decrease with decreasing latitude. Because the study was conducted in a common-garden environment, any latitudinal patterns found would be genetically based. 3) We test the hypothesis that because the invasive genotypes have had about 150 years for them to adapt to their novel environment, they are less likely to have evolved latitudinal gradients in their interactions with herbivores than the native genotypes. If the relationship between a plant-herbivore trait and latitude differs between native and invasive genotypes, it would indicate large-scale heterogeneity in plant-herbivore interactions that could have important implications for invasion success. Finally, 4) by comparing plant defense, nutritional and palatability traits between gardens, we test whether invasive genotypes are more plastic than native genotypes.

METHODS

Study system

Phragmites australis is a tall and robust perennial wetland grass with a worldwide distribution (Marks et al. 1994, Clevering and Lissner 1999, Lambertini et al. 2006). It produces annual shoots and perennial rhizomes and often forms monospecific stands in coastal and freshwater marshes. It has been an uncommon species of wetland communities in North America for millennia (Niering 1977) but it has exhibited dramatic and rapid spread over the past ~150 years, particularly in the mid-Atlantic region of North America (Chambers et al. 1999). The rapid spread is attributed to the introduction of an invasive Eurasian genotype (haplotype *M*) in the mid-1800s (Chambers et al. 1999, Saltonstall 2002). Additional introduced genotypes have been discovered from the Atlantic and Gulf Coast regions of North America (Lambertini et al. 2012, Meyerson and Cronin 2013). These genotypes have been spreading rapidly in the freshwater and brackish marsh ecosystems of North America (Saltonstall 2002, Meyerson et al. 2009, Meyerson et al. 2010, Lambertini et al. 2012, Bhattarai and Cronin 2014). At least 14 genotypes of native *P. australis* have been distributed throughout North America (Saltonstall 2002, Meadows and Saltonstall 2007) which have been reported to be threatened by the spread of invasive genotypes (Meyerson et al. 2010). Phylogenetic analysis revealed that native and introduced genotypes belong to two different clades often regarded as separate subspecies (Saltonstall and Hauber 2007).

Biogeographical patterns of herbivory on native and invasive genotypes of *P. australis* in North America were recently studied by Cronin et al. (2014). Among the most common and important herbivores of *P. australis* in North America are several introduced species (Tewksbury et al. 2002), including mealy plum aphid *Hyalopterus pruni* (Aphididae; Lozier et al. 2009) and

the gall flies of genus *Lipara* (Chloropidae: Lambert et al. 2007). Native *P. australis* genotypes receive substantially greater herbivore damage than the invasive genotypes (Lambert and Casagrande 2007, Park and Blossey 2008, Cronin et al. 2014, Allen et al. in review). Moreover, native genotypes exhibit latitudinal gradients in herbivore damage for three major feeding guilds (sucking, stem-feeding [galling], and chewing) but introduced genotypes show a gradient only for sucking herbivores (Cronin et al. 2014). Infestation rates for galling and chewing herbivores decreased with latitude whereas for sucking herbivores, it increased with latitude (Cronin et al. 2014). These results suggest that both native and invasive *P. australis* genotypes may have evolved latitudinal clines in response to herbivore pressure, although the case is much stronger for the native genotypes.

Common gardens

We established replicate common gardens at Louisiana State University, Baton Rouge, LA (LSU: 30.35°, -91.14°) and University of Rhode Island, Kingston, RI (URI: 41.49°, -71.54°). Because the two common gardens are separated by 11° latitude and are subjected to very different climates, we were able to quantify phenotypic plasticity in plant traits that may influence plant-herbivore interactions (Maron et al. 2004, Colautti et al. 2009). Also, because we obtained aphids (*see below*) from local source populations, it is possible that these herbivores may be adapted to plants from the same climate. Consequently, for aphids, different gradients may be evident in each garden (*see Pennings et al. 2001*).

In each garden, *P. australis* populations were initiated with rhizome materials excavated from patches of native (10 populations) and invasive (18 populations) genotypes from multiple sites along the Gulf and Atlantic Coasts of North America (Appendix B). Rhizome materials were planted in Metromix[®] soil in 19-liter nursery pots and maintained in outdoor plastic pools

filled with fresh water. Plants were propagated vegetatively to get at least 32 pots per population. To reduce the possibility of maternal effects, plants were grown for at least one year in the common garden before any experiments were performed. Plants were fertilized with Mega Green organic fertilizer upon detection of leaf yellowing and sprayed with Safer[®] insecticidal soap (Woodstream Corp., Lititz, PA) to protect them from unwanted herbivores. Safer soap was used because it has a very short (< 2 week) residual time on the plants.

Plant palatability

Caging experiments were performed to assess the palatability of *P. australis* populations to herbivores from two feeding guilds: the mealy-plum aphid (*H. pruni*, Aphididae) and the fall armyworm (*Spodoptera frugiperda*, Noctuidae). *H. pruni* is an invasive pest of dried plum (*Prunus domestica*) that was introduced from Europe and uses *P. australis* as a secondary host plant (Lozier et al. 2009). *H. pruni* is one of the most widespread herbivores of *P. australis* throughout North America (Cronin et al. 2014) which can produce massive outbreaks and cause severe damage to *P. australis* stands. First recorded in California in 1881 (Smith 1936) it was probably introduced to North America after the introduction of invasive *P. australis* (Lozier et al. 2009). Consequently, the native and invasive genotypes of *P. australis* in North America most likely had an equivalent amount of time to adapt to aphid herbivory and evolve gradients. Therefore, we may expect parallel latitudinal gradients for traits associated with aphid herbivory between native and invasive *P. australis*.

S. frugiperda is native to North America and is a serious pest of cereal crops (Sparks 1979, Meagher et al. 2004). It is known to feed on many grass species including *P. australis* (Sparks 1979, Meagher et al. 2004, GP Bhattarai personal observation). Native genotypes of *P. australis* that potentially have been experiencing damage from these herbivores, are expected to

exhibit gradients with respect to traits associated with the interaction with fall armyworms, whereas such a gradient may not exist for the invasive genotypes due to their relatively short history of interaction.

Palatability to aphids. To assess the palatability of *P. australis* populations to aphids, we caged aphids on plants and measured colony growth rate as an index of palatability. The source of aphids was a naturally occurring stand of *P. australis* within 80 km of each common garden. For ethical reasons and to minimize the genetic variation among aphids within each garden, we used a single source population of aphids for each garden. Cages for the aphid experiment were constructed using five cm lengths of clear acrylic tubing (2.8 cm in diameter, 0.1 cm in thickness) that were inserted over the *P. australis* leaves. The ends of the tubes were sealed with closed-cell foam plugs that had a small fine-screen window cut into their centers for air circulation (*see* photo, Appendix B). Aphid colonies were initiated with two adult aphids caged on the youngest fully open leaf on a randomly selected stem from each pot. Aphids reproduce parthenogenetically and produce a colony within a few days. After 10 d, leaves with aphid colonies were collected, transported on ice to the laboratory, and stored in a freezer at -20° C. With a suitable host, aphid colonies can increase in size by 100-fold in 10 d without any evidence of leaf deterioration or resource depletion due to intraspecific competition (GP Bhattarai personal observation). Aphids per colony were enumerated and then dried at 40°C for two days. Dry mass of each colony was determined using a Mettler microbalance (0.1 mg precision). Because aphid mass was strongly correlated with aphid colony size ($r = 0.878$, $P < 0.0001$) we used only colony size in further analyses. Survivorship of aphids was determined as the proportion of cages per *P. australis* source population that had a viable aphid colony. Survivorship and colony size were considered as measurements of plant palatability to the

aphids. The aphid experiment was conducted at LSU from April 13-23, 2012 with nine introduced and five native populations (20 cages per population), and repeated at URI from June 17-27, 2012 with 12 introduced and six native populations (8-12 cages per population). The experiments were performed at different times in each garden so that plants were in comparable developmental stages at each location. Further information about the populations used in the aphid experiment (and fall armyworm, *see below*) are provided in Appendix B.

Palatability to fall armyworms. For the fall armyworm, we also performed a caging experiment and measured the performance of individual larvae feeding on plants from each source population. For both gardens, fall armyworms were obtained as eggs from the same source (Benzon Research Inc., Carlisle, PA) and larvae were reared in the lab on artificial diet provided by Southland Products (Lake Village, AR). At the fourth instar stage, the mass of each larva was determined, and those larvae within 20-50 mg were selected for the experiment. Sleeve cages (45×60 cm² or 60×75cm² in size) were built using insect netting. The cage was inserted over the upper portion of a stem (containing 5-10 leaves) and enclosed around the stem at the bottom using a cable tie. A single caterpillar was released into each cage through a hole cut open in the top of the cage. The hole was subsequently stapled closed. A photograph of the cage is provided in Appendix B. Within each pot, a single stem was selected at random for a cage. The experiment was terminated after 8 d, before any caged plants had all available leaf material consumed by the armyworm. Each larva was collected, transported on ice to the laboratory and its fresh mass was determined using a Mettler microbalance. Larval mass gain was calculated as proportional change in fresh biomass during the experiment (final mass [mg]/initial mass [mg]). We excluded from the analyses all cases in which the larva died before the termination of the

trial. Survivorship of larvae was determined for each population as the proportion of cages with a live larva at the end of the experiment.

We took photographs of all the remaining leaves inside the cage to quantify leaf area consumed by each larva. Using ImageJ (Rasband 2014), we quantified the remaining leaf area (cm^2) for each plant and estimated pre-consumption leaf area by extrapolation. Leaf area consumed by each larva was estimated (pre-consumption area – remaining area). The amount of leaf area consumed by larvae could be indicative of plant defense levels (e.g., Coley 1986). However, herbivores may also consume more to compensate for the lower quality of leaf tissues (Mattson 1980). Finally, we determined biomass conversion efficiency of larva (change in larval mass per unit area of leaf consumed) as a palatability measure. Leaf-area measurements for plants with dead or missing larvae were excluded from the analyses. These four variables (caterpillar mass gain and survivorship, leaf area consumed, and biomass conversion efficiency) were used as the measurements of plant palatability to chewing herbivores. Nine native and 13 invasive populations (20 plants per population) were used in the experiment at LSU, and six native and eight invasive populations (7-10 plants per population) were used at URI.

Plant defense and nutritional traits

Plant characteristics related to defense and nutritional quality were measured concurrently with the aphid experiment and from the same pots as those with cages. Water content of leaves has been shown to have a positive relationship with population growth rate of aphids (e.g., Johnson 2008, *but see* Woods et al. 2012) and lepidopteran larvae (Scriber and Feeny 1979). Water content of leaves was estimated as the proportion of water per unit fresh biomass of three newly opened leaves collected from each pot ($n=10$ per population). For nutrient analysis (percent carbon, percent nitrogen and C:N ratio), the top three leaves were

collected from a single plant per pot (n = 5 pots per population). Leaves were lyophilized and ground to a fine powder in the laboratory. Leaf nutritional levels were assayed using an elemental analyzer at Brown University Environmental Chemistry Facilities (<http://www.brown.edu/Research/Evchem/facilities/>). Herbivores often prefer and perform better on plants with higher % nitrogen (Mattson 1980, Agrawal 2004). Also, carbon content of leaves has been shown to influence herbivore performance (Agrawal 2004, Cronin et al. 2014).

Leaf toughness (force [kg] required to push a blunt steel rod [4.8 mm in diameter] through the leaf) and total phenolics concentration were our measures of plant defenses against herbivores. As a member of the Poaceae, *P. australis* defenses are likely limited to structural defenses and phenolics (Tscharntke and Greiler 1995). In our field surveys (Cronin et al. 2014), leaf toughness and phenolics were negatively related to leaf-area consumed by chewing herbivores and aphid density, respectively. Using a penetrometer (Itin Scale Co., Inc., Brooklyn, NY), toughness was measured for the fully open uppermost leaf from a randomly selected stem per pot. Leaf toughness was also measured for the top-most leaf inside each cage of the fall armyworm experiment in 2013. Total phenolics (nM/g of dried leaf tissue) were estimated using a modified version of the Folin-Ciocalteu method (Waterman and Mole 1994, Cronin et al. 2014).

Statistical methods

We tested whether putative defense (leaf toughness, total phenolics), nutritional (water content, % nitrogen, % carbon, C:N ratio) and plant palatability (aphid colony size, aphid survivorship, fall armyworm larval mass gain, larval survivorship, leaf area chewed and biomass conversion efficiency) traits varied between gardens, genotypes and across the latitude. Garden (LSU, URI), genotype (native, invasive), and latitude were treated as fixed effects, and source

populations were nested within genotypes. For aphid colony size, we used a generalized mixed effect model (GLMM) with Laplace estimation method and Poisson distribution of errors (Bolker et al. 2009). Observation-level random effect was added to the model to account for overdispersion of the data (Bolker et al. 2009). Survivorship of aphids and larvae were analyzed using general linear model. All other variables were evaluated using linear mixed effect models (LMM). For toughness, we had two years of data and, therefore, year (2012, 2013), was also included in the model as a repeated measure. For each dependent variable, a total of nine candidate models were developed using fixed effects (garden, genotype and latitude), and all possible combinations of two- and/or three-way interactions among them. The best model for each response variable was selected based on Akaike Information Criteria corrected for finite sample size (Burnham and Anderson 2013). Goodness of fit of each mixed effects model was reported as marginal ($R^2_{GLMM_m}$, hereafter R^2_m , variance explained by fixed effects) and conditional R^2 ($R^2_{GLMM_c}$, hereafter R^2_c , variance explained by the entire model) that are comparable in interpretation to the coefficient of determination, R^2 , for linear models (Nakagawa and Schielzeth 2013). All analyses were run in R 3.1.2 (R Development Core Team 2014) using lme4 and MuMIn packages.

RESULTS

Defense and nutritional traits

Our results provide strong evidence for genetically based latitudinal gradients in defense and nutritional traits in the native and invasive genotypes of *P. australis* in North America. Leaf toughness ($P < 0.0001$), water content ($P = 0.001$) and percent carbon ($P < 0.001$) decreased linearly with increasing latitude (Figure 3.1, Appendix B). On average, toughness, water content, and percent carbon decreased by 51%, 32% and 3%, respectively, between our southernmost and

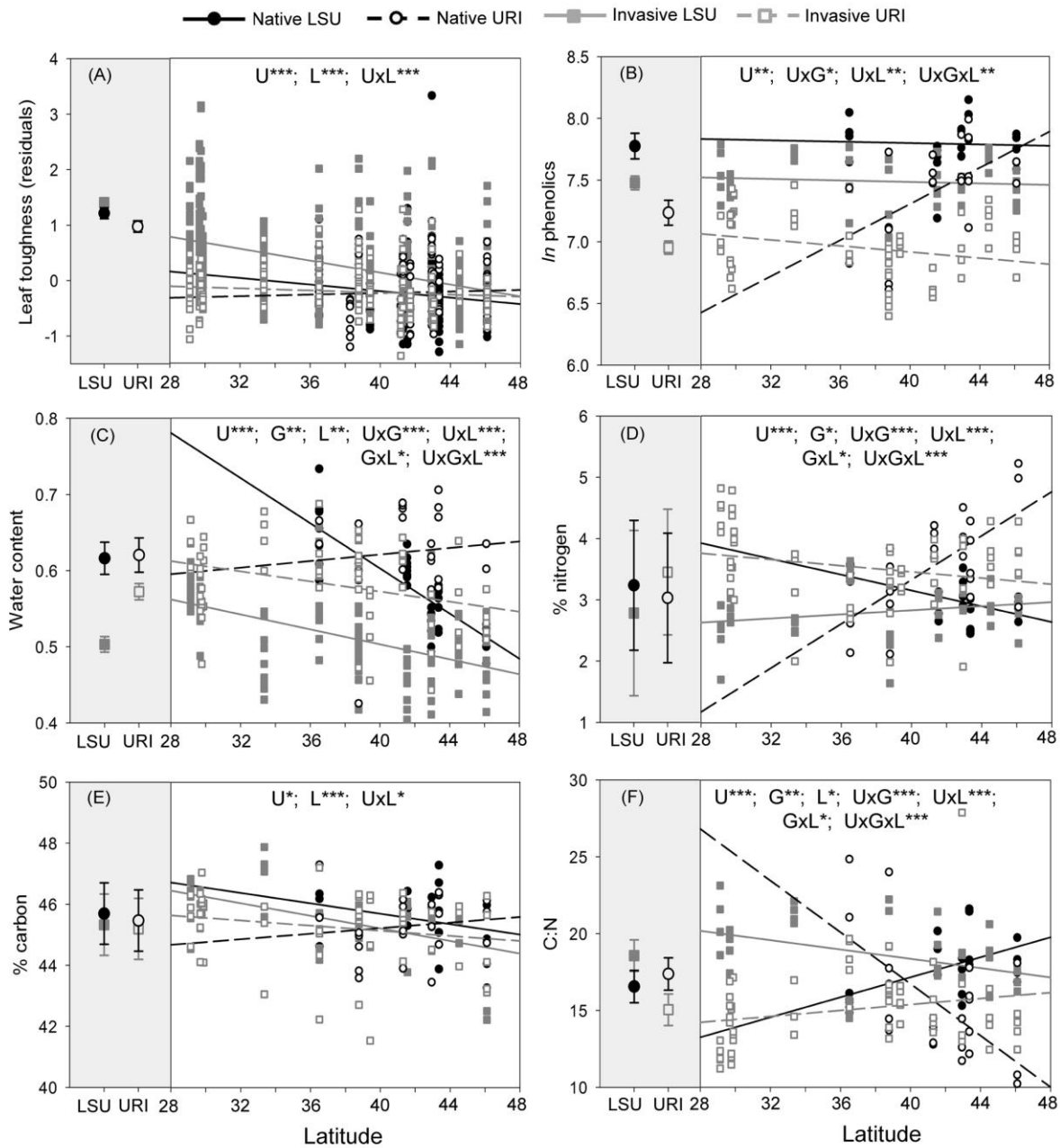


Figure 3.1. Effects of genotype (G), latitude (L) and garden (U) on defense and nutritional traits of native and invasive *P. australis* grown in common gardens at Louisiana State University and University of Rhode Island. Symbols in the shaded part show LS means (\pm SE) for different genotypes in different gardens. Relationship between plant trait and latitude are shown by best-fit lines when latitude or one of the interactions with latitude was significant in the best model. Only significant effects are shown in the figure and probability values are represented by asterisk (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

northernmost populations. C:N ratio was positively related to latitude with a 12% higher value for northernmost populations compared to the southernmost populations ($P = 0.042$, Figure 3.1F).

Plant defense and nutrient traits differed between native and invasive genotypes of *P. australis*. Water content was 8% higher in native than invasive genotypes ($P = 0.004$, Figure 3.1C). Native plants had 34% more total phenolics than the invasive plants, the difference was not significant; although, a significant three-way interaction between garden, genotype, and latitude ($P = 0.009$, $R^2_m = 0.634$, $R^2_c = 0.751$, Figure 3.1B), may have obscured this effect.

Plants reared at URI garden were more nutritious and less defended than those in LSU (Figure 3.1, Appendix B). Plants reared at URI had 4% more leaf water content ($P = 0.004$) and 0.23% more nitrogen ($P = 0.019$) than those at LSU, whereas plants at LSU produced 133% tougher leaves ($P < 0.0001$), and had 1.05 % more carbon, 96% more total phenolics ($P = 0.003$) and 8% greater value for C:N ratio ($P < 0.0001$) than those at URI (Figure 3.1, Appendix B). We found evidence of genetically based variation in plasticity for all of these defense and nutritional traits as suggested by a significant interaction between garden and latitude or garden, genotype and latitude (Appendix B).

Palatability to herbivores

In general, *P. australis* palatability to herbivores was greater for native than invasive genotypes, and for aphids was affected by the latitude of origin of the plants (Figure 3.2, Appendix B). Aphid colony size was 11 times larger on native than invasive plants ($P < 0.0001$) and negatively related to latitude ($P < 0.0001$, $R^2_m = 0.449$, $R^2_c = 0.482$, Figure 3.2A). Also, aphid survivorship was 35% higher on native plants ($P < 0.0001$) and declined with increasing latitude ($P = 0.003$, $R^2 = 0.569$, Figure 3.2B).

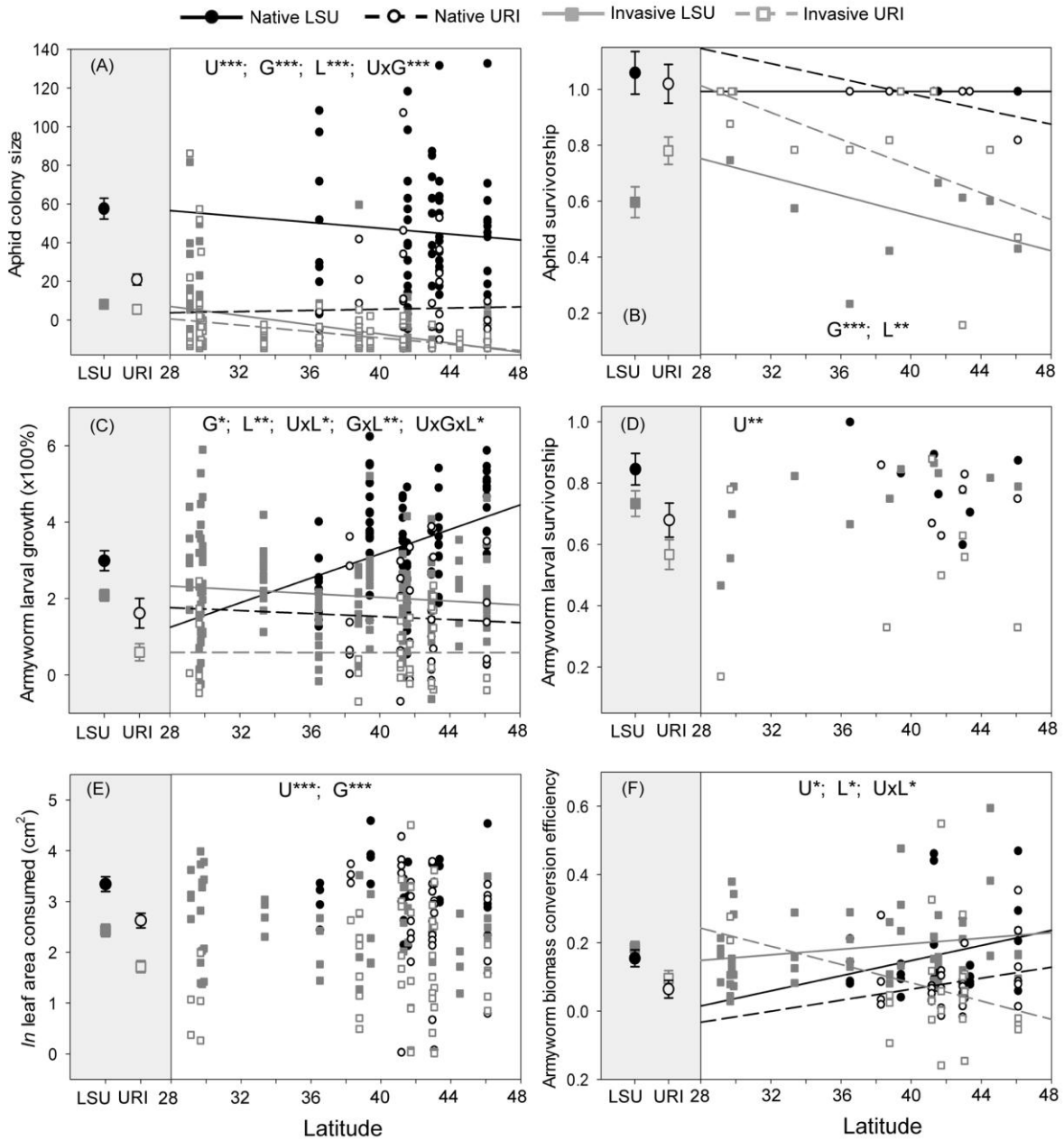


Figure 3.2. Effects of genotype (G), latitude (L) and garden (U) on palatability traits on native and invasive *P. australis* grown in common gardens at Louisiana State University and University of Rhode Island. Symbols in the shaded part show LS means (\pm SE) for different genotypes in different gardens. Relationship between plant trait and latitude are shown by best-fit lines when latitude or one of the interactions with latitude was significant in the best model. Only significant effects are shown in the figure and probability values are represented by asterisk (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

For the fall armyworm, larval survivorship was 11% higher on native plants but the relationship was only marginally significant ($P = 0.07$, Figure 3.2D). Larvae consumed 254% more leaf area of native plants than the invasive plants ($P < 0.0001$, $R^2_m = 0.268$, Figure 3.2E, Appendix B). Growth rate of larvae was 176% higher on native plants than on invasive ($P = 0.016$, Figure 3.2C). For native plants, growth rate of larvae feeding on northernmost plants was three times greater than those feeding on southernmost plants ($P = 0.012$, $R^2_m = 0.40$) but it was not related to latitude for invasives (Figure 3.2C). Although larvae showed 29% greater mass conversion efficiency on invasive plants, it was only marginally significant ($P = 0.052$). Conversion efficiency was 58% greater for the larvae feeding on northernmost plants in comparison to those feeding on southernmost plants ($P = 0.019$, Figure 3.2F)

In spite of the higher defenses and lower nutrition, plants grown at LSU were more palatable to herbivores than those at URI (Figure 3.2, Appendix B). Colony size of aphid was more than two times greater at LSU (12.55 ± 1.44) than at URI (5.64 ± 1.15 ; $P < 0.0001$). Moreover, garden \times genotype \times latitude interaction was significant in the model for growth rate of larva ($P = 0.041$, $R^2_m = 0.392$, $R^2_c = 0.456$). Larval survivorship was 16% higher ($P = 0.006$) at LSU (0.79 ± 0.04) than at URI (0.62 ± 0.04). Larvae consumed two times more leaf area (Figure 3.2E, Appendix B) and showed 113% greater conversion efficiency in LSU (0.17 ± 0.02) than in URI (0.08 ± 0.02 ; Figure 3.2F).

DISCUSSION

Latitudinal variation on plant defense and nutritional traits

Our study is the first to demonstrate genetically based latitudinal gradients for traits related to plant-herbivore interactions involving an invasive species. Moreover, it is likely that these clines evolved in the invasive genotypes in about 150 years. Although the invasive plants

had less total phenolics, they were less palatable to herbivores and received lower herbivore damage than the native plants. In addition to exhibiting genetic-based latitudinal clines in defenses, nutritional condition and palatability to herbivores, native and invasive genotypes also exhibited substantial phenotypic plasticity in these traits. Moreover, genetic variation for plasticity was indicated by statistically significant garden-by-genotype or garden-by-latitude interactions for nine of the 12 traits evaluated. Variation in plant defenses and palatability between different genotypes and across the latitudinal range of invasion likely generate substantial spatial heterogeneity in biotic resistance/susceptibility that can have important implications for the establishment and spread of invasive genotypes and species.

Geographical variation in the intensity of biotic interactions is expected to select for clinal evolution in species (Coley and Aide 1991, Salgado and Pennings 2005, Schemske et al. 2009) and because such clines should be adaptive we may expect parallel clines between similar species in a region. Evaluation of traits associated with *P. australis* – herbivore interactions in common garden experiments provided support to these predictions. For both native and invasive genotypes, northern populations of *P. australis* were less palatable for the aphid, *H. pruni*. Latitudinal gradients in aphid herbivory could be explained by the fact that aphid abundance on *P. australis* increases with increasing latitude in North America (Cronin et al. 2014). *H. pruni* is one of the most prevalent herbivores of *P. australis* in Europe and North America (Tschamtko 1989, Lambert and Casagrande 2007, Cronin et al. 2014) and can produce massive population outbreaks on suitable host reaching an average of > 4800 aphids per stem (Cronin et al. 2014). These outbreaks cause substantial damage to plants which include yellowing and early senescence of aboveground plant parts. At northern latitudes, strong selection pressure by these herbivores could have resulted in higher resistance or lower palatability.

Because *H. pruni* was introduced to North America not long after the introduction of invasive genotypes of *P. australis* (Lozier et al. 2009) both native and invasive genotypes likely had an equivalent amount of time to adapt to aphids. The existence of parallel gradients between native and invasive genotypes demonstrates that both genotypes showed similar evolutionary responses to these aphids. A few studies to date have reported the formation of clines along an environmental gradient (latitudinal or elevational) by an invasive species or genotype that parallels the clines in the native range (Huey et al. 2000, Maron et al. 2004, Alexander et al. 2009). However, our experimental study is the first to provide a time frame for the formation of latitudinal clines in traits associated with species interactions (palatability and defenses), and to do so for genotypes (or species) that have been subjected to the same environmental conditions. Most previous studies (e.g., Huey et al. 2000, Maron et al. 2004) involved cross-continental comparisons between native and invasive species and it was a possibility that within the same latitude, these species were exposed to similar environmental conditions. In contrast, our native and invasive genotypes are found in the same marshes along the East and Gulf Coasts and experience the same abiotic and biotic conditions.

Although our experiment shows the existence of latitudinal clines involving traits related to aphid herbivory for both genotypes of *P. australis*, the mechanisms behind the evolution of these clines are not clear. One possible explanation for the clines is that geographically widespread aphids (and perhaps in combination with other selective agents) selected for the evolution of latitudinal clines in *P. australis* defense and palatability traits. The existence of parallel gradients for the native genotypes suggests that this is the most likely scenario. A less likely alternative is that invasive *P. australis* arrived in North America with its cline intact. For this to occur, multiple introduction events would have to have taken place in which the sources

and destinations of *P. australis* were the same. Although multiple introduction of European genotypes to North America have been reported (Lambertini et al. 2012, Meyerson and Cronin 2013), those genotypes have shown a restricted distribution in the region where they were introduced and are not responsible for the continent wide invasion of *P. australis*. Therefore, we believe that the first possibility (i.e., *de novo* clinal formation in the introduced range) is the most parsimonious explanation.

A non-parallel latitudinal gradient between native and invasive genotypes of *P. australis* was found for one measure of palatability to the native herbivore, the fall armyworm. Growth rate of larvae was positively related to latitude for native genotypes but was independent of latitude for invasive genotype. Leaf area consumed did not vary with latitude for either genotype but greater biomass conversion efficiency of larvae on the plants from higher latitude indicates that northern populations of native *P. australis* are more palatable to fall armyworm than southern populations. These patterns are consistent with the results presented by Cronin et al. (2014) that herbivory from the whole guild of chewing insects decreased with latitude for native plants but did not depend on latitude for invasive plants. Because of these similar findings from field and common garden studies we may expect genetically based gradients in native *P. australis* for the whole guild of chewing herbivores. However, the contrasting patterns observed for palatability traits to aphids and fall armyworms suggest that latitudinal gradients may vary with herbivore guilds and depend on the degree of specialization (Anstett et al. 2014).

Parallel latitudinal gradients between native and invasive genotypes were evident for leaf toughness, water content and %C. Each of these traits was negatively correlated with latitude. Although, our results with leaf toughness are consistent with the prediction that longer lifespan of leaves in the areas with longer growing season should favor tougher leaves (Coley and Aide

1991, Salgado and Pennings 2005), there was no latitudinal variation in leaf toughness in *P. australis* in nature (Cronin et al. 2014). In nature, *P. australis* populations exhibited increased %N with latitude in North America (Cronin et al. 2014) but no clear latitudinal pattern was found in the common gardens. The latitudinal patterns could have been obscured by the significant two- and three-way interactions between garden, genotype and latitude. C:N ratio was positively related to latitude. A similar trend was found by Moles et al. (2011b) for plants in the Northern Hemisphere. Although phenolics concentration decreased with latitude in *P. australis* genotypes in the field (Cronin et al. 2014), we did not find a latitudinal pattern in the gardens. The inconsistent patterns observed between common gardens and field studies suggest a greater influence of environmental variables on these chemical traits.

Herbivory between native and invasive genotypes

Enemy release, i.e., reduced pressure by natural enemies in the introduced range (Elton 1958, Keane and Crawley 2002), has been considered as one of the most prominent hypotheses explaining invasion success of introduced species. Although biogeographic studies comparing enemy pressure between native and invaded ranges support this hypothesis (e.g., Mitchell and Power 2003, Liu and Stiling 2006), comparisons between invasive species and co-occurring native species have yielded mixed results (Colautti et al. 2004, Chun et al. 2010). Comparisons of palatability between native and invasive genotypes of *P. australis* in common gardens provide support for the hypothesis that invasive species are under reduced enemy pressure in their invaded range. Invasive genotypes of *P. australis* had aphid colonies that were 91% smaller, and had fall armyworms that consumed 74% less leaf material and exhibited 54% lower growth rates. These results are consistent with the recent findings by Cronin et al. (2014) that herbivory for all major feeding guilds (leaf chewing, sucking, and stem feeding) of herbivores across a broad

(19°) latitudinal range in North America was greater on native than invasive *P. australis* genotypes. These findings also provide strong evidence for the biotic susceptibility of North American communities for the invasion of introduced *P. australis* genotypes.

Moreover, gradients in herbivory along a broad geographic range between native and invasive genotypes may create spatial heterogeneity in the importance of biotic susceptibility or resistance (Bezemer et al. 2014). Parallel latitudinal gradients for aphid herbivory with substantially lower damage on invasive genotypes (Figure 3.2A) suggests biotic susceptibility across the entire 18° latitudinal range studied in this study (Bezemer et al. 2014, Cronin et al. 2014). In the case of the fall armyworm, herbivory increased with latitude for native genotypes but did not vary with latitude for the invasive genotype (Figure 3.2C). Spatial heterogeneity created by non-parallel gradients on fall armyworm herbivory may render higher latitudes more susceptible to the spread of invasive genotypes (Cronin et al. 2014).

Phenotypic plasticity in defense and palatability traits

Defense and palatability traits evaluated in this study displayed a substantial amount of plasticity under different environmental conditions (i.e., gardens). We used identical rearing conditions in each garden so that any differences between them are likely due to climate. Plants grown in the southern garden at LSU showed higher levels of defenses (toughness and total phenolics), % carbon and C:N ratio than those in northern garden at URI. Plants in northern garden contained more % nitrogen and moisture content. These significant garden effects showed phenotypic plasticity on the measured plant traits. Moreover, AICc best models for ten of the twelve traits evaluated included interaction between garden and genotype or latitude. Four traits (phenolics, water content, % nitrogen, and C:N ratio) showed significant garden-by-genotype interactions suggesting that the genotypic variations on plasticity are genetically

determined (Pigliucci 2001, Richards et al. 2006). Similarly, statistically significant garden-by-latitude interactions for a total of eight traits indicated the existence of genetically based clines on plasticity (Pigliucci 2001, Richards et al. 2006, Davidson et al. 2011).

Our study shows that invasive genotypes of *P. australis* were more plastic than the native genotypes. By increasing niche breadth, phenotypic plasticity could facilitate colonization and spread of a species across a wide range of environmental conditions (Richards et al. 2006, Davidson et al. 2011). Plasticity in plant defense and palatability traits are likely to enhance the fitness of invasive genotypes in the environments with varying herbivore pressure, and therefore, contribute on invasion success (Rejmánek 2000, Richards et al. 2006, Davidson et al. 2011). Moreover, different levels of plasticity exhibited by native and invasive genotypes of *P. australis* in North America may also be important in understanding heterogeneity in invasion success across the continent. Finally, greater plasticity is likely to contribute towards the performance of invasive genotype in the novel environments created by changing climatic conditions.

CHAPTER 4
LATITUDINAL VARIATION IN APPARENT COMPETITION BETWEEN NATIVE
AND INVASIVE GENOTYPES OF *PHRAGMITES AUSTRALIS*

INTRODUCTION

Indirect biotic interactions are common in nature and have been shown to strongly influence the structure and dynamics of ecological communities (Wootton 1994, Walsh 2013). Apparent competition, in which species within the same trophic level are negatively impacted through the action of shared natural enemies, is widely regarded as one of the most common forms of indirect interactions (Holt 1977, Connell 1990, Holt and Lawton 1993). Both theory (Holt 1977, Holt and Kotler 1987) and empirical data provide compelling evidence that apparent competition is as important as competition in structuring ecological communities (Futuyma and Wasserman 1980, Bonsall and Hassell 1997, Chaneton and Bonsall 2000, Morris et al. 2004, Cronin 2007). The importance of apparent competition in facilitating species invasions has mostly been unexplored (White et al. 2006; *but see* Borer et al. 2007, Enge et al. 2013).

An introduced species may enhance its invasion success by suppressing the fitness of co-occurring native competitors by apparent competition. A few studies have evaluated the role of apparent competition in the spread of invasive plants. For example, apparent competition mediated by viral pathogens has been suggested to drive the invasion of California grasslands by competitively inferior introduced annual grasses (Seabloom et al. 2003, Borer et al. 2007). Refuge-mediated apparent competition has recently been proposed as one of the mechanisms for plant invasions (Orrock et al. 2010). The taller and denser structures of invasive plants are hypothesized to increase the abundance of natural enemies by providing refuge that ultimately causes greater negative impacts on the fitness of neighboring native plants (Connell 1990, Orrock et al. 2010). Enge et al. (2013) experimentally demonstrated that refuge-mediated

apparent competition was solely responsible for the invasion of otherwise less chemically defended red algae, *Bonnemaisonia hamifera*, along the Atlantic Coast of Europe. Reduced performance of native plant species, due to increased seed predation by mammalian herbivores when a refuge was provided by invasive plants, has been reported in several different systems (Meiners 2007, Orrock et al. 2008, Dangremond et al. 2010, Orrock and Witter 2010, Orrock et al. 2014). Owing to refuge mediated apparent competition, the spread of an invasive plant species may be enhanced (Orrock et al. 2010). Similarly, competitively superior introduced species could also enhance their invasion success by increasing enemy pressure on native competitors by refuge- and/or food-mediated mechanisms of apparent competition (Caccia et al. 2006, Orrock et al. 2010).

Empirical studies suggest that the strength of species interactions should generally decrease with increasing latitude (Schemske et al. 2009), and although it has never been investigated before, it should also extend to indirect species interaction such as apparent competition. Evidence for latitudinal gradients in species interactions has been mixed (Schemske et al. 2009, Moles et al. 2011a). However, latitudinal gradients in herbivore pressure appear to be a widespread phenomenon in nature (e.g., Pennings et al. 2001, Pennings et al. 2009, Schemske et al. 2009, Anstett et al. 2014, Cronin et al. 2014). This latitudinal variation in herbivory may underlie latitudinal variation in apparent competition. Also, invasive species may not have had sufficient time to evolve latitudinal gradients in traits associated with local herbivore pressure (e.g., defense traits) that parallels the gradients for native plant species (Bezemer et al. 2014, Cronin et al. 2014, Bhattarai et al. in review). These non-parallel gradients in herbivory may create spatial variation in the strength of apparent competition between native

and invasive species. The importance of apparent competition between native and invasive species across the latitudinal range of an invasion has never been examined.

We examined whether apparent competition occurred between native and invasive genotypes of the wetland grass *Phragmites australis*, and whether the intensity of apparent competition varied with latitude. The continent-wide spread of invasive genotypes of *P. australis* into North America wetlands containing native genotypes of *P. australis* (Chambers et al. 1999, Saltonstall 2002) represents an ideal system to study biotic interactions between native and invasive species (Cronin et al. 2014). A recent study on herbivory on *P. australis* genotypes across a wide latitudinal range in North America revealed latitudinal gradients in plant defenses, nutritional condition, and herbivory (Cronin et al. 2014). Common garden experiments confirmed the genetic basis of these gradients (Bhattarai et al. in review). In the field and common gardens, native genotypes suffered substantially greater herbivore damage than invasive genotypes (Cronin et al. 2014, Allen et al. in review, Bhattarai et al. in review). These results suggest the possibility that invasive genotypes of *P. australis* enhance invasion success by indirectly reducing the fitness of native genotypes through apparent competition.

To examine whether apparent competition occurs between native and invasive genotypes of *P. australis*, we conducted replicated field experiments at four coastal wetlands located across 7° latitude (900 km) range along the Atlantic Coast of the United States. Plants were cross-transplanted between sympatric native and invasive patches and herbivory by different feeding guilds of insects was quantified. We tested the following hypotheses. (1) Native plants would experience increased herbivory due to apparent competition with invasive plants. (2) Because of the greater abundance of herbivores in more palatable native patches, invasive plants would suffer increased herbivory when they were transplanted in native patches. (3) The strength of

apparent competition would be greater for native than invasive *P. australis*. Support for this latter hypothesis would suggest the existence of asymmetrical apparent competition favoring the invasive genotypes. (4) Finally, owing to the greater difference in herbivory between native and invasive plants in lower latitudes (Cronin et al. 2014), we hypothesized that the intensity of apparent competition acting on the native genotypes would be greatest in the south and decrease with increasing latitude.

METHODS

Study system

Phragmites australis is a perennial emergent wetland grass that occurs on all continents except for Antarctica (Marks et al. 1994, Clevering and Lissner 1999, Lambertini et al. 2006). It produces annual stems that grow 2-5 m in height and often produce monospecific stands in the wetland habitats. Historically, it has been an uncommon species in the freshwater and brackish marshes of North America (Niering et al. 1977). An invasive Eurasian genotype (haplotype *M*) of this species was introduced to North America in mid-1800s and has subsequently spread throughout North America (Chambers et al. 1999, Saltonstall 2002). Additional introduced genotypes have been discovered from Atlantic and Gulf coast regions (Lambertini et al. 2012, Meyerson and Cronin 2013). At least 14 native genotypes of *P. australis* are distributed across North America and overlap in distribution with the invasive genotypes (Saltonstall 2002, Meadows and Saltonstall 2007). Molecular studies show that native and invasive genotypes belong to different clades (Saltonstall and Hauber 2007).

A diverse assemblage of arthropod herbivores, representing a wide range of feeding guilds, has been reported to feed on *P. australis* (Tewksbury et al. 2002, Lambert and Casagrande 2007, Lambert et al. 2007, Park and Blossey 2008, Cronin et al. 2014). Some of the

most prominent herbivores of *P. australis* in North America include introduced species such as the mealy plum aphid, *Hyalopterus pruni* (Aphididae; Lozier et al. 2009) and gall forming flies in the genus *Lipara* (Chloropidae; Tewksbury et al. 2002). *H. pruni* is the most widespread herbivore of *P. australis* throughout North America (Lozier et al. 2009, Cronin et al. 2014). Numbers per stem can reach an average of > 4,800 and native patches average about 70% more individuals than invasive patches (Cronin et al. 2014). Although actual fitness costs have not been quantified for aphids, they often show severe population outbreaks and cause substantial damage to the plants (GP Bhattarai personal observation).

At least three *Lipara* spp. are common in the northeastern region of the US (Tewksbury et al. 2002, Lambert et al. 2007, Cronin et al. 2014, Allen et al. in review). Incidence of stem-feeders on patches of the native genotypes averaged 33%, three times higher than on patches of the invasive genotypes. Incidence of galling and boring herbivores (stem-feeders, collectively) represents a measurement of the fitness costs to the plants because stems occupied by these herbivores fail to produce flowers 100% of the time and also experience substantial reduction in stem growth (Lambert et al. 2007, Allen et al. in review).

Damage from chewing herbivores is common on both genotypes of *P. australis*, with the percentage of stems with chewing damage averaging 24% and 20% for patches of native and invasive genotypes, respectively (Cronin et al. unpublished data). However, the actual proportion of leaf area lost to chewers was <1% (Cronin et al. 2014). Similarly, leaf miners (*Dicranoctetes sacchaella*; Lepidoptera: Elachistidae) are generally uncommon but have been observed to reach >50% infested stems (Cronin et al. unpublished data). Even though tissue loss from chewers and leaf miners is low, feeding is more common on upper leaves that likely contribute more to photosynthesis.

Moreover, herbivore pressure varies linearly with latitude for stem-feeding, leaf-chewing and sap-sucking (aphids) herbivores on native genotypes but only for sucking herbivores for invasive genotypes (Cronin et al. 2014). Common garden experiments revealed genetically determined latitudinal gradients on plant defense and palatability traits on both genotypes of *P. australis* in North America (Bhattarai et al. in review).

Field experiment

Our experiments were conducted in Rachel Carson National Wildlife Refuge, Maine (43.298°, -70.579°), Murkwood Conservation Area, Massachusetts (41.741°, -70.434°), Choptank Wetlands, Maryland (38.773°, -75.975°), and Mackay Island National Wildlife Refuge, North Carolina (36.513°, -75.952°). Early in the growing season (late April, 2013 for the NC and MD sites; mid May 2013 for the MA and ME sites), we set up a cross-transplantation experiment at each site. Within each site (and wetland), a pair of native and invasive patches (> 40 m in diameter) was selected that were located < 1 km apart (contiguous in MD; 20 m in MA; 85 m in ME; 980 m in NC). In each patch, we established a 30-m long transect. A pair of rhizome clumps were excavated at every three m along the transect. Each clump of rhizome was transferred to a 19-L nursery pot (filling the pot completely). One of the pots from each pair was returned to its transect of origin and the second pot was transported to the transect of the other patch. As a result each transect contained 10 pairs of potted native and invasive plants, with each pair spaced 3 m apart along the transect. Pots were sunk flush to the ground (in the holes created by excavating plant material) and all stems were clipped to their base. Therefore, all growth was from new shoots, thus eliminating pre-experimental herbivore damage.

Our cross-transplanted plants were left in the field through to the end of summer to accumulate herbivores and their damage. In early September, 2013, we returned to the field sites

to quantify herbivory from each of the main feeding guilds on experimental plants and the patches within which they were embedded. Every stem within a pot was examined for the incidence of internal stem feeders, easily identifiable by the presence of a swelling near the apical portion of the stem or dead apical leaves. Presence of an unknown species of stem borer is also indicated by a tattered apical tip.

Next, leaves of all potted plants were thoroughly inspected for signs of folivory. We recorded the proportion of leaves per plant with chewing damage, presence of a leaf miner, and the presence of an aphid colony. For aphids, total density per plant was also enumerated. Finally, we also recorded the number of stems per pot and height of a randomly selected stem for each pot.

Statistical analysis

We tested for differences in herbivore damage (proportion of stems with stem-feeders [gallers and borers combined], proportion of leaves with mining damage, chewing damage, and aphids present, and number of total aphids per plant) between native and invasive plants growing in different patches. For each response variable, a separate general linear model was run with genotype of the patch (native, invasive [=patch genotype effect]), genotype of the potted plant (native, invasive [= plant genotype effect]) and patch-by-plant genotype interaction treated as fixed effects. Latitude, stem density, plant height and number of leaves were included in the model as covariates. When patch-by-plant interactions were significant, pairwise comparisons were performed using Bonferroni correction to α to control for an inflated type I error. From this set of analyses, apparent competition was considered to be occurring if herbivory from a particular species or guild was significantly greater for potted plants grown in the patch of the other genotype than in a patch of its own genotype. It is conceivable that herbivory could be less

when the plant is grown with the other genotype, resulting in apparent mutualism (e.g., Abrams and Matsuda 1996). All analyses were run in Systat 12 (Systat Inc, Chicago, IL).

We also quantified the strength or intensity of the indirect interaction (i.e., apparent competition or apparent mutualism) so that we could evaluate whether it varies among sites or with respect to latitude. For each site, feeding guild and *P. australis* genotype, we calculated the strength of indirect interaction as the mean herbivory in the patch of the different genotype (“DIFF”) – mean herbivory in the patch of its own genotype (“SAME”). 95% confidence intervals were estimated by bootstrapping the data for each patch type, calculating the difference between the new bootstrapped means ($\text{DIFF}_x - \text{SAME}_x$; where x = bootstrap repetition number), and repeating the process 1000 times. Sites differed significantly in strength of the indirect interaction if 95% CIs did not overlap.

RESULTS

We found strong evidence for asymmetrical apparent competition between native and invasive genotypes of *P. australis* along the Atlantic Coast of North America. In general, native plants placed in an invasive patch suffered greater herbivory than when placed in a patch of their own genotype (Figure 4.1, Appendix C). Incidence of stem-feeders was 43% higher when native plants were placed in an invasive patch relative to a native patch ($F_{1,143} = 13.36$, $P < 0.001$, followed by pairwise comparisons, $P = 0.005$, Figure 4.1A). Similarly, the frequency of leaf miners increased by 191% when native plants were moved to the invasive patch, but the effect was only marginally significant ($F_{1,143} = 6.52$, $P = 0.012$, followed by pairwise comparisons, $P = 0.053$, Figure 4.1B). In comparison, proportion of chewed leaves increased by 12% on native plants moved to invasive patch but the difference was not significant ($P = 1.00$, Figure 4.1C).

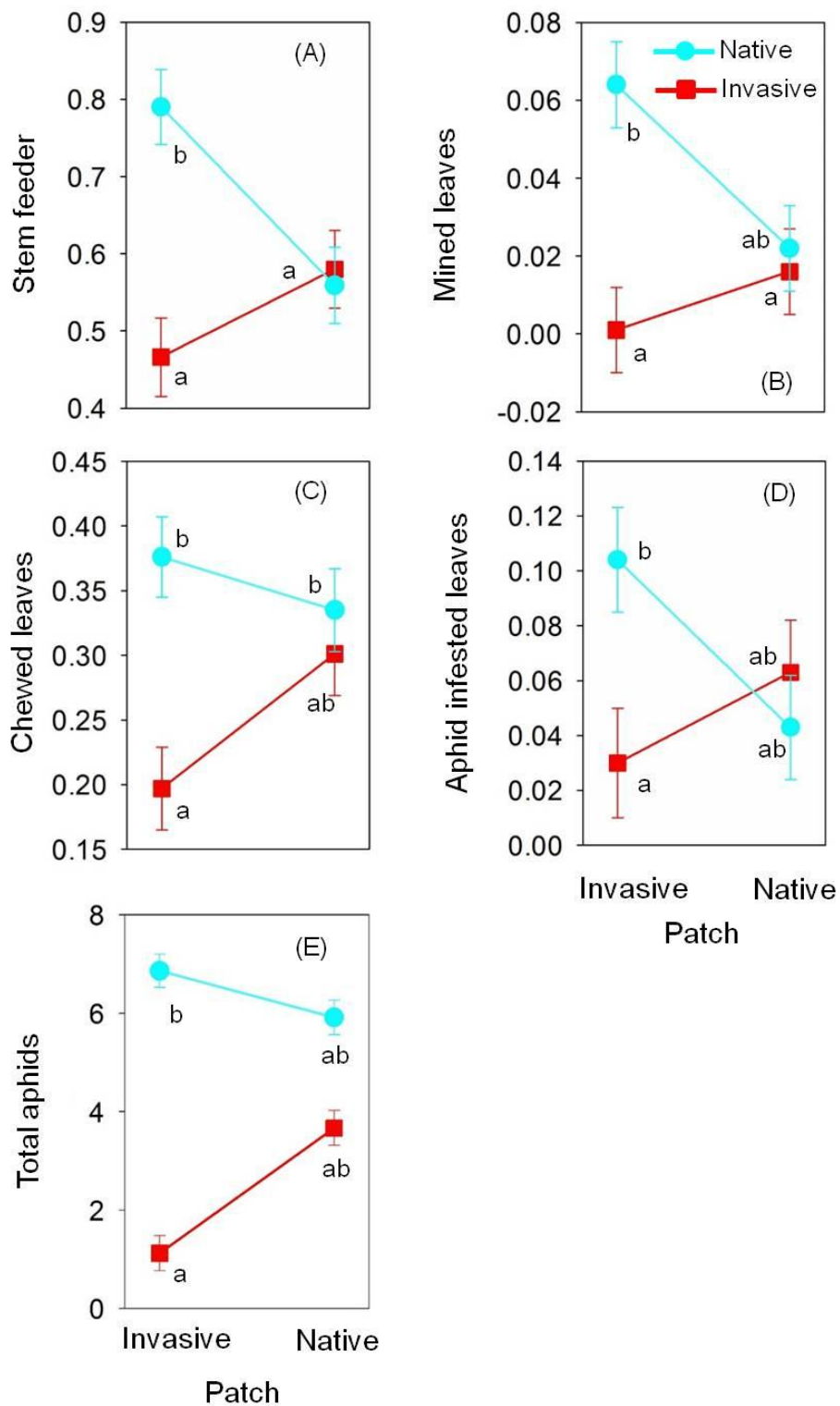


Figure 4.1. Herbivory on native and invasive *Phragmites australis* plants growing in different patches. LS means (\pm SE) for herbivory for each feeding guild has been shown. Symbols with different letters are significantly different from each other.

Although not significant, native plants received 142% and 16% increase in the proportion of aphid infested leaves ($P = 0.15$, Figure 4.1D) and total aphids ($P = 1.0$, Figure 4.1E), respectively when moved to the invasive patch.

Although pressure from all herbivore guilds was greater for invasive plants placed in a native as compared to an invasive patch, the differences were not significant (Figure 4.1) for invasive genotypes. Invasive plants experienced 27%, 1500%, and 53% increase in the incidence of stem feeders ($P = 0.52$, Figure 4.1A), leaf miners ($P = 1.00$, Figure 4.1B), and chewed leaves ($P = 0.17$, Figure 4.1C), respectively, when they were growing in the native patch. Similarly, proportion of leaves with aphids ($P = 1.00$, Figure 4.1D) and total number of aphids ($P = 0.47$, Figure 4.1E) increased by 110% and 103%, respectively.

Regardless of patch genotype, native plants received greater herbivore damage than the invasive plants. After controlling for the effects of patch genotype, native plants suffered 26%, 438% and 43% greater incidence of stem-feeders ($F_{1,143} = 7.74$, $P = 0.006$, Figure 4.1A), proportion of leaves occupied by leaf miners ($F_{1,143} = 9.14$, $P = 0.003$, Figure 4.1B) and proportion of leaves with chewing damage ($F_{1,143} = 10.91$, $P = 0.001$, Figure 4.1C), respectively, than the invasive plants. Although number of aphids per plant was low in this experiment, aphid density was 196% higher on native plants than on invasive ($F_{1,143} = 7.71$, $P = 0.006$, Figure 4.1E). Proportion of aphid infested leaves was 55% greater on native plants than on invasive but the difference was not significant ($P = 0.48$, Figure 4.1D).

Examination of apparent competition between native and invasive plants within each site for different feeding guilds indicated significant spatial variation in the strength of indirect interactions (Figure 4.2A-E). For stem-feeders on native plants, the strength of the indirect interaction was positive (i.e., native plants suffer greater herbivory in the invasive patches than

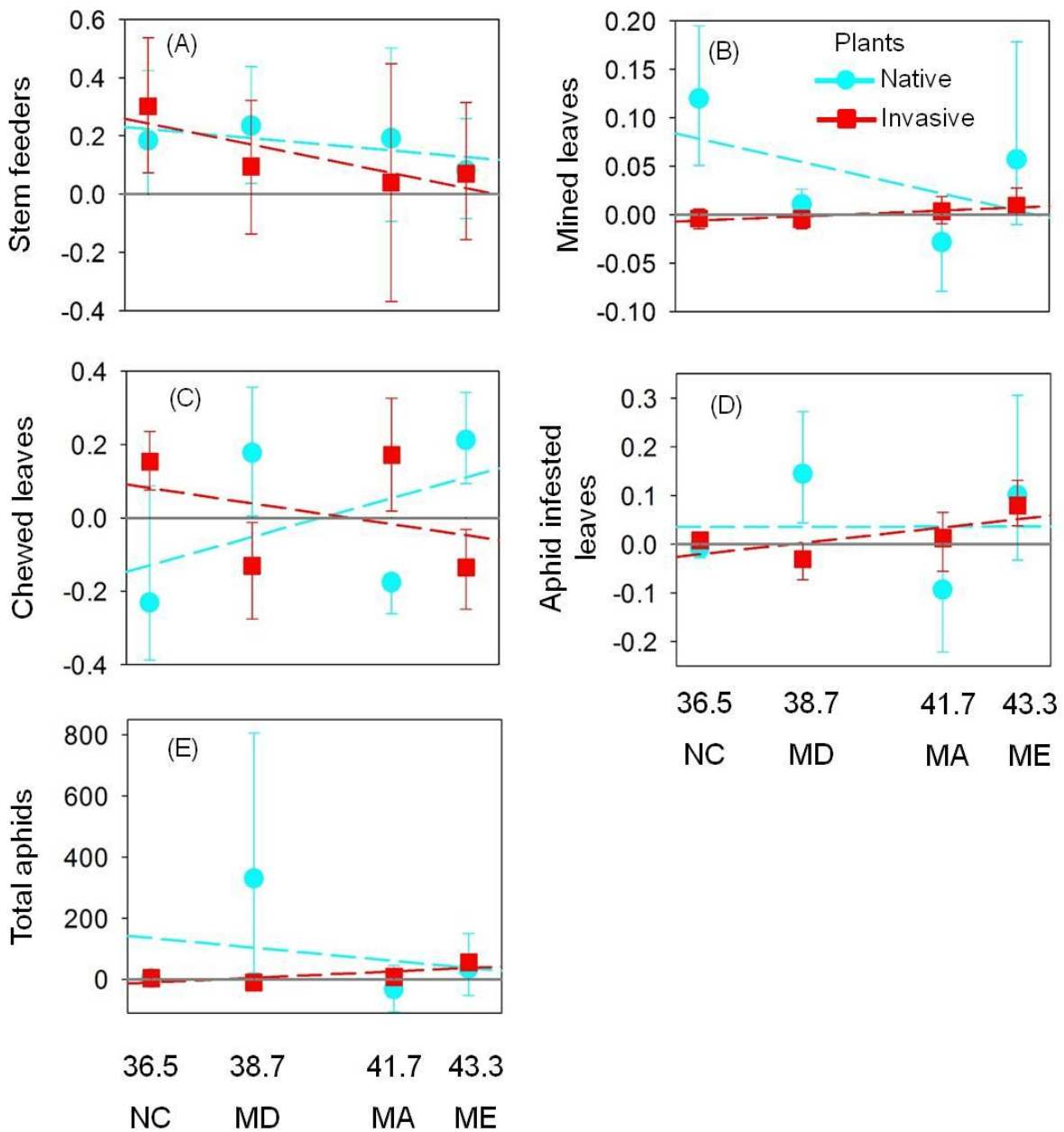


Figure 4.2. Spatial variation on the intensity of apparent competition between native and invasive genotypes of *P. australis*. X-axis represents the latitude of the sites and abbreviation for the state (ME = Maine, MA = Massachusetts, MD = Maryland, NC = North Carolina). Symbols for each genotype represent effect size (herbivory in different patch – herbivory in own patch). Error bars were for these effect sizes were determined by bootstrapping data for 1000 times. The interactions were significant when error bars did not include 0. Sites differed significantly when error bars did not overlap.

in the native patches), indicating apparent competition. Also, southern sites (NC and MD) had stronger apparent competition than northern sites (MA and ME) (Figure 4.2A). Apparent competition via shared internal stem feeders was evident only in NC site for invasive plants (Figure 4.2A). In the case of mining herbivore, the indirect interaction was positive for native plants in NC and ME, indicating apparent competition, but negative in MA, indicating apparent mutualism. Native plants received substantially greater herbivore damage in the southernmost sites in NC (Figure 4.2B). Indirect interaction mediated by leaf miners was almost non-existent for invasive plants across all sites.

Damage by chewing herbivores showed remarkable variability on the nature of indirect interactions (Figure 4.2C). For native plants, the interaction was positive for ME and MD but negative for MA and NC. In contrast, invasive plants in ME and MD had apparent mutualism but apparent competition in MA and NC. All the interactions, except for native plants in NC, were significantly different from 0. For aphid infested leaves, native plants experienced apparent competition in MD and ME but apparent mutualism in MA (Figure 4.2D). Apparent competition in MD for native plants was significant. For invasive plants, the interaction was significant and positive in ME. Finally, native plants in MD and invasive plants in ME experienced significant apparent competition mediated by total aphids (Figure 4.2E).

DISCUSSION

Results from our experiment suggest that apparent competition mediated by stem-feeding and leaf mining herbivores is one of the mechanisms that may inhibit the fitness of native genotypes and enhance the fitness and spread of invasive genotypes of *P. australis* along the Atlantic Coast of the United States. The indirect interaction was asymmetrical with substantially greater herbivory on the native plants when associated with invasive plants than when associated

with other native plants. The invasive plants did tend to suffer more herbivory when associated with native as compared to invasive patches but the difference was relatively small. Our study is the first to demonstrate that the strength of apparent competition between closely related native and invasive genotypes of a species can vary across the broad range of an invasion. However, we only found weak support for the prediction that the strength of indirect species interactions decreases with increasing latitude.

As predicted by theory (Connell 1990, Orrock et al. 2010), apparent competition between native and invasive plants mediated by herbivores appears to be a common phenomenon in natural ecosystems (Borer et al. 2007, Enge et al. 2013). Two out of four feeding guilds examined in this experiment, stem gallers and leaf miners, provided support for this expectation. Invasive genotypes of *P. australis* indirectly negatively affected native genotypes of *P. australis* via shared stem-feeders, specifically by *Lipara* spp. Almost 80% of the stems in native plants experienced *Lipara* infestation when they were growing in an invasive patch (Figure 4.1A). Individuals of the invasive genotype also received more herbivore damage due to apparent competition with individuals of the native genotype, but the relationship was not statistically significant. Consistent with previous studies (Lambert et al. 2007, Park and Blossey 2008, Cronin et al. 2014, Allen et al. in review), incidence of *Lipara* infestation was greater in native patches than in invasive patches across all four sites (GP Bhattarai personal observation). Therefore, it is reasonable that invasive plants growing in native patches suffer from greater herbivory because of higher abundance of herbivores in the native patches. In the case of native plants, the infestation rates could increase in invasive patches because of higher nutritional quality or lower defense levels of native plants. Although invasive plants of *P. australis* may not be the preferred host, invasive patches appear to maintain high abundance of *Lipara* by

providing habitat and food resources which ultimately cause greater harm to the more preferred host plants. Because *Lipara* infestation occurs early in the growing season when there was minimum competition between the transplanted plants and the natural plants in the patch for nutrients and light, these patterns are less likely to be driven by resource competition. It has been predicted that the interacting species or genotype that is less vulnerable to the enemy but still can maintain high abundance of natural enemies should exclude the more vulnerable competitor by apparent competition (Orrock et al. 2010). Taller and denser stands of invasive genotype might be maintaining the high abundance of these herbivores by providing safer refuge from their natural enemies but receiving relatively less damage from these herbivores probably because of lower plant quality. Moreover, infestation levels by stem-feeders have been shown to decline with increasing stem density (Cronin et al. 2014), suggesting that native patches, that typically have lower stem densities than invasive patches, may predispose them to higher infestation levels of stem galls. However, we accounted for stem density in our statistical models.

Similar asymmetric patterns were exhibited by leaf miners. In the southernmost site, where they were most abundant, they appear to have strong negative impact on the native plants. Chewing herbivores showed substantial variation in the strength and direction of indirect interactions between sites and genotypes. However, these variations appear to be driven by the difference in background herbivore load between the native and invasive patches within a site. Similar type of variability was shown by aphids in all sites except NC. Native plants receive greater herbivory from leaf chewers and aphids than invasives along the Atlantic Coast of North America (Cronin et al. 2014). In controlled common garden experiments, native plants of *P. australis* were found to suffer from 254% greater chewing damage and also sustain 11 times larger aphid colonies than the invasive plants (Bhattarai et al. in review).

These remarkably strong asymmetrical effects caused by herbivores are likely to make strong contributions to competitive superiority of the invasive genotype. Although direct measurements of fitness costs associated with herbivory have not been documented for *P. australis* genotypes, damage in these feeding guilds are likely to have strong fitness cost. Stems infested by *Lipara* fail to produce flowers 100% of the times and also suffer 55% reduction on stem growth (Lambert et al. 2007, Allen et al. in review). These observations make a strong case for the reduced fitness of plants caused by *Lipara* infestation through both sexual and asexual (clonal growth) methods of reproduction. With ~80% of the stems unable to produce flowers and compromised biomass production, native plants experience severe fitness cost due to apparent competition with the invasive genotype. Aphids have been reported to produce massive outbreaks on suitable hosts (mostly, on native *P. australis*) and result into yellowing and death of aboveground parts (GP Bhattarai personal observation).

Geographical variations in the strength of apparent competition between native and invasive species might have important implications for invasion success. In spite of the increased interest in latitudinal gradients on biotic interactions (e.g., Schemske et al. 2009) spatial variation in the intensity of indirect interactions was never been examined. The strength of this indirect interaction appears to be greater towards the lower latitudes (Figure 4.2A,B). Moreover, these results are consistent with our expectation based on latitudinal variation in herbivory on native and invasive genotypes of *P. australis* along the Atlantic Coast of North America (Cronin et al. 2014). Incidence of stem-feeders on native genotypes decreased with latitude but it did not vary with latitude on invasive genotypes (Cronin et al. 2014). Spatial variation created by these non-parallel latitudinal gradients on herbivore pressure and plant quality might be the reason for the geographical variation on the strength of apparent competition between native and invasive

genotypes. Variation in the strength of apparent competition is of great relevance to invasion biology because it may help explain the spread rate of invasive species across a broad spatial scale. Within the distributional range of *Lipara* gall flies (between NC to ME, Allen et al. in review), these results suggest a faster expansion of invasive genotype towards lower latitudes.

Our study strongly suggests apparent competition might be enhancing the spread of invasive genotype of *P. australis* in the Atlantic Coast of the US. The massive patches of invasive genotype are likely to support huge densities of herbivores which are likely to spill over to co-occurring native plants resulting into increased herbivory. Although native patches are found to increase herbivory on the invasive genotype, they are likely to have inconsequential impacts because of small patch size. In addition to apparent competition, the invasive genotypes of *P. australis* have been shown to be superior competitors than native genotypes for resources (e.g., Mozdzer and Zieman 2010) and also suffer from lower herbivore damage (e.g., Cronin et al. 2014). In this way, several mechanisms may work in concert to facilitate the invasion success of the introduced genotype in North America. Future studies should focus on partitioning the contributions of these direct and indirect processes in species invasion (e.g., Orrock et al. 2014).

Understanding the actual effect of direct and indirect processes will also be critical in the management of native genotypes of *P. australis* in North America. Apparent competition has been shown to influence the persistence of co-occurring species in different ecosystems (Bartholomew 1970, Bonsall and Hassell 1997, Morris et al. 2004, Cronin 2007). Recently, invasive plants have been shown to inhibit native plant performance by apparent competition (Sessions and Kelly 2002, Meiners 2007, Orrock et al. 2008, Dangremond et al. 2010, Orrock and Witter 2010). Native genotypes of *P. australis* have been reported to decline following the spread of invasive genotypes (Meyerson et al. 2010). Strong negative impact of apparent

competition primarily mediated by *Lipara* spp. could be one of the causes of the decline of native populations. If apparent competition is the main mechanism behind the decline than controlling herbivore pressure might be helpful in the management of native genotypes.

CHAPTER 5 CONCLUSIONS

In this dissertation, I used biogeographical approaches to study mechanisms enhancing plant invasions and biotic interactions between native and invasive genotypes. My experiments highlight the importance of biogeographical approaches in understanding biotic invasions and subsequent species interactions in the invaded region. Because many of the most successful invaders show continent-wide invasions it is necessary to evaluate the causes and consequences of species invasion at a similar scale.

My dissertation contributes towards our understanding of the mechanisms that facilitate species invasion at large spatial scale. First, my observations provide support to the hypothesis that hurricane activities enhance plant invasion (Chapter 2). Using aerial imagery and GIS techniques, I quantified the growth rate of *P. australis* patches and analyzed the effects of frequency and intensity of hurricanes and tropical storms on it. Of the greatest novelty and significance, I found that hurricane frequency strongly enhances the growth rate of *P. australis* patches along the Atlantic and Gulf Coasts of the United States. Small storms (<119 km/hr) do not have an appreciable contribution in this process. Explaining over 80% variation on the growth rate of patches hurricane activities appear to be of overriding importance in the spread of invasive genotypes along the coastal region of the US. These results are particularly relevant in the context of current global climatic change which has predicted to increase the frequency (Bender et al. 2010) and/or intensity (Goldenberg et al. 2001, Emanuel 2005, Knutson et al. 2010) of hurricanes and tropical storms.

In the second part of my dissertation, I studied the genetic basis for the spatial variation on biotic interactions. Native and invasive genotypes of *P. australis* have been shown to exhibit latitudinal variation in defense and nutritional traits, and herbivory along the Atlantic Coast of

North America (Cronin et al. 2014). Common garden experiments showed the genetic basis of these gradients for both genotypes (Chapter 3). Both native and invasive genotypes showed latitudinal clines in palatability to herbivory by an introduced aphid *Hyalopterus pruni*. *H. pruni* was also introduced from Europe in 1800s, most likely following the introduction of invasive genotype of *P. australis* (Lozier et al. 2009). Therefore, both the native and invasive genotypes of *P. australis* most likely had equivalent amounts of time to evolve latitudinal clines in response to aphid herbivory. The existence of parallel latitudinal gradients between native and invasive genotypes provides support to this idea. This is the first study to provide a time frame for the evolution of latitudinal clines in traits related to herbivory. Moreover, both genotypes showed substantial phenotypic plasticity in traits related to herbivory and that the plasticity were genetically determined, and therefore, should be adaptive.

In Chapter 4, I performed field experiments to explore the occurrence of apparent competition between native and invasive genotypes of *P. australis* and latitudinal variation in its interaction strength. Genotypic and geographical variations in plant defenses and nutritional quality, and herbivore pressure suggest the possibility of apparent competition between native and invasive genotypes. My experiments support the hypothesis that apparent competition occurs between native and invasive genotypes with disproportionately greater fitness cost to the native genotypes. These results strongly suggest that herbivore mediated apparent competition might be one of the mechanisms enhancing the spread of invasive genotype. Moreover, the strength of apparent competition was stronger in lower latitudes.

Although my dissertation suggests the importance of various factors (namely, hurricanes, herbivory, and apparent competition) on the invasion success of introduced genotypes, the actual contribution of each of these processes on invasion has not been documented. Besides, the

underlying mechanisms generating these large spatial scale patterns have to be identified. For example, hurricane events result in severe destruction of natural vegetation and alteration of habitat characteristics including hydrology, salinity, nutrient concentrations etc. Future researches should evaluate the contribution of these different aspects of hurricane and tropical storm events on plant invasion. Identification of the actual mechanism facilitating invasion is critical for the effective management of invasive species and wetland ecosystems along the coasts. Similarly, future studies should also explore whether storm events facilitate the dispersal of *P. australis* propagules in the coastal areas.

In spite of the strong genotypic and geographical variations in plant defense and palatability traits, and herbivory in *P. australis*, mechanisms generating these patterns are not fully understood. Our measurement of physical defense (toughness) did not differ between genotypes. Total phenolic concentration was greater on native plants. But herbivore performance was substantially greater on native plants. These results show the need for more detailed chemical analyses in understanding the genotypic and geographical variations on herbivory. Future work should also explore the other aspects of herbivory traits including resistance, tolerance and induced defenses between native and invasive genotypes across a wide spatial scale.

Finally, these findings have strong implications for the management of coastal marsh ecosystems of North America. These results might be helpful in predicting *P. australis* invasion in North America. Because hurricanes are common in southern latitudes, we may expect faster growth rate of *P. australis* patches in the lower latitudes. This pattern could also be strengthened by a greater susceptibility of the low latitude communities to invasion (Cronin et al. 2014, Chapter 3). Apparent competition that was found to be stronger in southern latitudes might also

contribute. However, the role of apparent competition mediated by *Lipara* spp. is limited to the distribution range of the herbivore (NC to ME; Allen et al. in review). In contrast to these results, there was no support for the faster growth rate of *P. australis* patches in the lower latitudes (Chapter 2). Therefore, it is important to quantify the contribution of different biotic and abiotic processes in *P. australis* invasion in the wetlands of North America by performing field experiments across a broad spatial scale.

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APPENDIX A. SUPPLEMENTARY MATERIAL FOR CHAPTER 2

MODEL SELECTION USING AICC PROCEDURE

Table A.1. Comparison of models estimating the effects of latitude (x_1), patch size (x_2), PC1 (x_3), PC2 (x_4), tropical storm frequency (x_5), hurricane frequency (x_6) on mean patch growth rate (y).

Model	Number of Parameters	AICc	$w_i(\text{AICc})$
$y = k$	1	-25.150	0.001
$y = ax_1 + k$	2	-23.665	0.000
$y = ax_2 + k$	2	-27.238	0.002
$y = ax_3 + k$	2	-22.746	0.000
$y = ax_4 + k$	2	-22.550	0.000
$y = ax_5 + k$	2	-27.329	0.002
$y = ax_6 + k$	2	-31.584	0.014
$y = ax_1 + bx_2 + k$	3	-22.915	0.000
$y = ax_1 + bx_3 + k$	3	-19.836	0.000
$y = ax_1 + bx_4 + k$	3	-20.890	0.000
$y = ax_1 + bx_5 + k$	3	-23.428	0.000
$y = ax_1 + bx_6 + k$	3	-29.093	0.004
$y = ax_2 + bx_3 + k$	3	-22.936	0.000
$y = ax_2 + bx_4 + k$	3	-23.754	0.000
$y = ax_2 + bx_5 + k$	3	-24.216	0.000
$y = ax_2 + bx_6 + k$	3	-27.825	0.002
$y = ax_3 + bx_5 + k$	3	-23.108	0.000
$y = ax_3 + bx_6 + k$	3	-33.057	0.029
$y = ax_4 + bx_5 + k$	3	-23.902	0.000
$y = ax_4 + bx_6 + k$	3	-27.924	0.002
$y = ax_3 + bx_4 + k$	3	-19.355	0.000
$y = ax_3 + bx_4 + cx_5 + k$	4	-18.471	0.000
$y = ax_3 + bx_4 + cx_6 + k$	4	-27.831	0.002
$y = ax_5 + bx_6 + k$	3	-29.307	0.005
$y = ax_1 + bx_2 + cx_3 + k$	4	-17.385	0.000
$y = ax_1 + bx_2 + cx_4 + k$	4	-18.208	0.000
$y = ax_2 + bx_3 + cx_4 + k$	4	-18.200	0.000
$y = ax_1 + bx_2 + cx_5 + k$	4	-18.645	0.000
$y = ax_1 + bx_2 + cx_6 + k$	4	-25.577	0.001
$y = ax_1 + bx_5 + cx_6 + k$	4	-26.258	0.001
$y = ax_3 + bx_5 + cx_6 + k$	4	-30.374	0.008
$y = ax_2 + bx_3 + cx_5 + k$	4	-18.666	0.000
$y = ax_2 + bx_4 + cx_5 + k$	4	-19.536	0.000
$y = ax_2 + bx_3 + cx_6 + k$	4	-28.818	0.004
$y = ax_2 + bx_4 + cx_6 + k$	4	-22.967	0.000

(Table A.1. continued)

Model	Number of Parameters	AICc	$w_i(\text{AICc})$
$y = ax_2 + bx_5 + cx_6 + k$	4	-23.739	0.000
$y = ax_4 + bx_5 + cx_6 + k$	4	-24.507	0.000
$y = ax_1 + bx_2 + cx_3 + dx_5 + k$	5	-11.388	0.000
$y = ax_1 + bx_2 + cx_3 + dx_6 + k$	5	-21.806	0.000
$y = ax_1 + bx_2 + cx_4 + dx_5 + k$	5	-12.200	0.000
$y = ax_1 + bx_2 + cx_4 + dx_6 + k$	5	-18.185	0.000
$y = ax_2 + bx_3 + cx_4 + dx_5 + k$	5	-12.118	0.000
$y = ax_2 + bx_3 + cx_4 + dx_6 + k$	5	-21.766	0.000
$y = ax_3 + bx_4 + cx_5 + dx_6 + k$	5	-23.377	0.000
$y = ax_5 + bx_5^2 + k$	3	-23.903	0.000
$y = ax_1 + bx_5 + cx_5^2 + k$	4	-20.569	0.000
$y = ax_2 + bx_5 + cx_5^2 + k$	4	-21.663	0.000
$y = ax_3 + bx_5 + cx_5^2 + k$	4	-18.863	0.000
$y = ax_4 + bx_5 + cx_5^2 + k$	4	-18.801	0.000
$y = ax_3 + bx_4 + cx_5 + dx_5^2 + k$	5	-11.828	0.000
$y = ax_6 + bx_6^2 + k^*$	3	-39.113	0.608**
$y = ax_1 + bx_6 + cx_6^2 + k$	4	-33.797	0.043
$y = ax_2 + bx_6 + cx_6^2 + k$	4	-36.009	0.129
$y = ax_3 + bx_6 + cx_6^2 + k$	4	-33.654	0.040
$y = ax_4 + bx_6 + cx_6^2 + k$	4	-33.543	0.038
$y = ax_3 + bx_4 + cx_6 + dx_6^2 + k$	5	-26.229	0.001
$y = ax_5 + bx_5^2 + cx_6 + dx_6^2 + k$	5	-34.173	0.051
$y = ax_1 + bx_2 + cx_5 + dx_5^2 + k$	5	-14.914	0.000
$y = ax_1 + bx_2 + cx_6 + dx_6^2 + k$	5	-28.585	0.003
$y = ax_2 + bx_3 + cx_5 + dx_5^2 + k$	5	-14.243	0.000
$y = ax_2 + bx_3 + cx_6 + dx_6^2 + k$	5	-28.748	0.003
$y = ax_2 + bx_4 + cx_5 + dx_5^2 + k$	5	-14.316	0.000
$y = ax_2 + bx_4 + cx_6 + dx_6^2 + k$	5	-28.580	0.003
$y = ax_2 + bx_3 + cx_5 + dx_6 + k$	5	-23.015	0.000
$y = ax_2 + bx_4 + cx_5 + dx_6 + k$	5	-17.081	0.000
$y = ax_1 + bx_2 + cx_3 + dx_4 + ex_5 + k$	6	-2.920	0.000
$y = ax_1 + bx_2 + cx_3 + dx_4 + ex_6 + k$	6	-12.722	0.000
$y = ax_1 + bx_2 + cx_3 + dx_5 + ex_6 + k$	6	-13.533	0.000
$y = ax_1 + bx_2 + cx_4 + dx_5 + ex_6 + k$	6	-8.889	0.000
$y = ax_2 + bx_3 + cx_4 + dx_5 + ex_6 + k$	6	-13.046	0.000
$y = ax_1 + bx_2 + cx_3 + dx_5 + ex_5^2 + k$	6	-7.064	0.000
$y = ax_1 + bx_2 + cx_3 + dx_6 + ex_6^2 + k$	6	-18.530	0.000
$y = ax_1 + bx_2 + cx_4 + dx_5 + ex_5^2 + k$	6	-4.728	0.000
$y = ax_1 + bx_2 + cx_4 + dx_6 + ex_6^2 + k$	6	-18.185	0.000

(Table A.1. continued)

Model	Number of Parameters	AICc	$w_i(\text{AICc})$
$y = ax_2 + bx_3 + cx_4 + dx_5 + ex_5^2 + k$	6	-3.951	0.000
$y = ax_2 + bx_3 + cx_4 + dx_6 + ex_6^2 + k$	6	-18.350	0.000
$y = ax_2 + bx_5 + cx_5^2 + dx_6 + ex_6^2 + k$	6	-23.886	0.000
$y = ax_1 + bx_5 + cx_5^2 + dx_6 + ex_6^2 + k$	6	-23.790	0.000
$y = ax_3 + bx_5 + cx_5^2 + dx_6 + ex_6^2 + k$	6	-23.914	0.000
$y = ax_4 + bx_5 + cx_5^2 + dx_6 + ex_6^2 + k$	6	-23.819	0.000
$y = ax_3 + bx_4 + cx_5 + dx_5^2 + ex_6 + fx_6^2 + k$	7	-8.386	0.000
$y = ax_1 + bx_2 + cx_3 + dx_4 + ex_5 + fx_5^2 + k$	7	6.826	0.000
$y = ax_1 + bx_2 + cx_3 + dx_4 + ex_6 + fx_6^2 + k$	7	-3.055	0.000
$y = ax_1 + bx_2 + cx_3 + dx_4 + ex_5 + fx_6 + k$	7	-0.061	0.000
$y = ax_1 + bx_2 + cx_3 + dx_4 + ex_5 + fx_5^2 + gx_6 + hx_6^2 + k$	9	69.006	0.000

*Best model based on AICc weights.

** Evidence ratio of the best model = 4.72, normalized evidence ratio = 0.82.

APPENDIX B. SUPPLEMENTARY MATERIAL FOR CHAPTER 3

***PHRAGMITES AUSTRALIS* POPULATIONS USED IN THE COMMON GARDEN EXPERIMENTS**

Table B.1. List of *Phragmites australis* populations used in the aphid and the fall armyworm experiments in the common gardens at Louisiana State University and University of Rhode Island.

Site of collection	Population	Latitude	Longitude	Haplotype*	Experiments	
					Aphid	FAW
Moncton, New Brunswick	NBM	46.10	-64.80	<i>M</i>	LSU, URI	LSU, URI
Moncton, New Brunswick	NBS	46.10	-64.80	<i>S</i>	LSU, URI	LSU, URI
Bath, Maine	BSCM	44.51	-70.35	<i>M</i>	LSU, URI	LSU
Rachel Carson NWR, Wells, Maine	RCN	43.36	-70.48	<i>F</i>	LSU, URI	LSU
Great Bay, Stratham, New Hampshire	GBM	43.05	-70.90	<i>M</i>		URI
Great Bay, Stratham, New Hampshire	GBN	43.05	-70.90	<i>E</i>		URI
Montezuma NWR, New York	NYM	42.94	-76.70	<i>M</i>	LSU, URI	LSU, URI
Montezuma NWR, New York	NYE	42.94	-76.70	<i>E</i>	LSU, URI	LSU, URI
Jacob's Point, Warren, Rhode Island	JPM	41.71	-71.29	<i>M</i>		URI
Jacob's Point, Warren, Rhode Island	JPN	41.71	-71.29	<i>E</i>		URI
Falmouth, Massachusetts	FPM	41.55	-70.60	<i>M</i>	LSU	LSU
Falmouth, Massachusetts	FPN	41.55	-70.60	<i>F</i>	LSU	LSU
Essex, Connecticut	CTM	41.30	-72.35	<i>M</i>	URI	LSU
Essex, Connecticut	CTN	41.30	-72.35	<i>F</i>	URI	LSU
Block Island, Rhode Island	BIM	41.18	-71.57	<i>M</i>		URI
Block Island, Rhode Island	BIN	41.18	-71.57	<i>AB</i>		URI
Estell Manor State Park, New Jersey	NJM	39.41	-74.73	<i>M</i>	URI	LSU
Estell Manor State Park, New Jersey	NJN	39.41	-74.73	<i>F</i>		LSU
Choptank Wetlands, Maryland	TCM	38.77	-75.95	<i>M</i>	LSU, URI	LSU, URI
Choptank Wetlands, Maryland	MDN	38.77	-75.95	<i>F</i>	URI	
Wimico Creek, Allen, Maryland	WCN	38.28	-75.69	<i>Z</i>		URI
Mackay Island NWR, North Carolina	NCM	36.51	-75.95	<i>M</i>	LSU, URI	LSU
Mackay Island NWR, North Carolina	NCN	36.51	-75.95	<i>F</i>	LSU, URI	LSU

(Table B.1. continued)

Site of collection	Population	Latitude	Longitude	Haplotype*	Experiments	
					Aphid	FAW
Georgetown, South Carolina	SCM	33.35	-79.26	<i>M</i>	LSU, URI	LSU
Creole, Louisiana	CRM	29.88	-93.07	<i>MI</i>	URI	LSU
East Cameron, Louisiana	ECM	29.77	-93.29	<i>MI</i>	URI	LSU
Rockefeller WR, Louisiana	RRM	29.68	-92.81	<i>MI</i>	LSU, URI	LSU, URI
Pass A Loutre State WMA, Louisiana	PLM	29.13	-89.23	<i>MI</i>	LSU, URI	LSU, URI

*Haplotype designations are from Saltonstall (2002) and Lambertini et al. (2012). *M* and *MI* are invasive European genotype. All others genotypes are native to North America.

PHOTOGRAPHS OF CAGES USED IN THE COMMON GARDEN EXPERIMENTS



Figure B.1. Cage used in the aphid experiment. (Photo credit: GP Bhattarai)



Figure B.2. Cage used in the fall armyworm experiment. (Photo credit: GP Bhattarai)

AICC BEST MODELS FOR PLANT DEFENSE, NUTRITIONAL AND PALATABILITY TRAITS

Table B.2. AICc best models for the effects of genotype (G), latitude (L), garden (U), and all possible two- and three-way interactions on each *P. australis* plant defense, nutritional and palatability traits. Aphid colony size was analyzed using a generalized linear mixed effect model (GLMM) with Poisson family. GLMM used Wald's z-statistics. Aphid and fall armyworm larval survivorship were analyzed using general linear models. All other variables were analyzed using Linear Mixed Effect Models (LMM).

Dependent variable	Effects	Estimate	SE	t/z	P	AIC _c	AIC _c Wt	R ² _m	R ² _c
<i>Plant defense traits</i>									
Leaf toughness	I	3.25	0.45	7.23	< 0.0001	1362.19	0.33	0.23	0.44
	U	-2.20	0.38	-5.72	< 0.0001				
	G	0.19	0.12	1.61	0.108				
	L	-0.05	0.01	-4.95	< 0.0001				
	U×G	-0.17	0.12	-1.46	0.145				
	U×L	0.05	0.01	5.78	< 0.0001				
Phenolics	I	7.61	0.92	8.30	< 0.0001	-15.68	0.52	0.63	0.75
	U	-2.59	0.86	-3.02	0.003				
	G	0.23	0.97	0.24	0.810				
	L	0.00	0.02	0.19	0.848				
	U×G	2.17	0.90	2.41	0.016				
	U×L	0.05	0.02	2.63	0.009				
	G×L	-0.01	0.02	-0.58	0.559				
	U×G×L	-0.06	0.02	-2.60	0.009				
<i>Plant nutritional traits</i>									
Leaf water content	I	1.15	0.17	6.68	< 0.0001	-737.45	0.94	0.45	0.55
	U	-0.71	0.20	-3.60	< 0.001				
	G	-0.52	0.18	-2.88	0.004				
	L	-0.01	0.00	-3.37	0.001				
	U×G	0.80	0.21	3.90	< 0.0001				

(Table B.2. continued)

Dependent variable	Effects	Estimate	SE	t/z	P	AIC _c	AIC _c Wt	R ² _m	R ² _c
	U×L	0.02	0.00	3.99	< 0.0001				
	G×L	0.01	0.00	2.43	0.015				
	U×G×L	-0.02	0.00	-3.91	< 0.0001				
Nitrogen	I	2.00	0.48	4.17	< 0.0001	-102.62	1.00	0.38	0.41
	U	-2.96	0.67	-4.40	< 0.0001				
	G	-1.20	0.51	-2.34	0.019				
	L	-0.02	0.01	-1.87	0.061				
	U×G	3.64	0.70	5.18	< 0.0001				
	U×L	0.07	0.02	4.67	< 0.0001				
	G×L	0.03	0.01	2.21	0.027				
	U×G×L	-0.09	0.02	-5.14	< 0.0001				
Carbon	I	3.91	0.03	148.06	< 0.0001	-677.95	0.36	0.12	0.22
	U	-0.06	0.03	-2.20	0.028				
	G	-0.01	0.01	-1.19	0.233				
	L	0.00	0.00	-3.54	< 0.001				
	U×L	0.00	0.00	2.07	0.039				
C:N ratio	I	2.06	0.40	5.15	< 0.0001	-133.53	1.00	0.45	0.46
	U	2.80	0.59	4.73	< 0.0001				
	G	1.26	0.43	2.96	0.003				
	L	0.02	0.01	2.03	0.042				
	U×G	-3.66	0.62	-5.89	< 0.0001				
	U×L	-0.07	0.01	-5.04	< 0.0001				
	G×L	-0.03	0.01	-2.89	0.004				
	U×G×L	0.09	0.01	5.88	< 0.0001				

(Table B.2. continued)

Dependent variable	Effects	Estimate	SE	t/z	P	AIC _c	AIC _c Wt	R ² _m	R ² _c
<i>Plant palatability traits</i>									
Aphid colony size*	I	7.65	0.90	8.52	< 0.0001	2341.40	0.44	0.45	0.48
	U	-1.67	0.27	-6.19	< 0.0001				
	G	-3.26	0.28	-11.55	< 0.0001				
	L	-0.09	0.02	-4.34	< 0.0001				
	U×G	1.74	0.34	5.19	< 0.0001				
Aphid survivorship**	I	1.77	0.25	7.10	< 0.0001	-14.38	0.32	0.57	
	U	-0.04	0.10	-0.39	0.699				
	G	-0.46	0.10	-4.86	< 0.0001				
	L	-0.02	0.01	-3.23	0.003				
	U×G	0.22	0.12	1.80	0.083				
Armyworm larval massgain	I	-3.77	2.61	-1.45	0.148	969.31	0.27	0.39	0.46
	U	7.25	4.27	1.70	0.089				
	G	6.60	2.74	2.41	0.016				
	L	0.17	0.06	2.76	0.006				
	U×G	-8.70	4.53	-1.92	0.055				
	U×L	-0.22	0.10	-2.20	0.028				
	G×L	-0.19	0.07	-2.88	0.004				
	U×G×L	0.22	0.11	2.04	0.041				
Armyworm larval survivorship**	I	0.61	0.23	2.63	0.013	-21.35	0.26	0.32	
	U	-0.17	0.06	-2.99	0.006				
	G	-0.11	0.06	-1.88	0.070				
	L	0.01	0.01	1.09	0.283				

(Table B.2. continued)

Dependent variable	Effects	Estimate	SE	t/z	P	AIC _c	AIC _c Wt	R ² _m	R ² _c
Armyworm leaf area chewed	I	3.95	0.64	6.15	< 0.0001	460.18	0.23	0.27	0.27
	U	-0.72	0.15	-4.87	< 0.0001				
	G	-0.91	0.15	-5.98	< 0.0001				
	L	-0.02	0.02	-0.98	0.326				
Armyworm larval mass conversion efficiency	I	-0.48	0.28	-1.70	0.089	-192.38	0.25	0.21	0.25
	U	0.45	0.23	1.99	0.047				
	G	0.56	0.29	1.94	0.052				
	L	0.02	0.01	2.34	0.019				
	U×L	-0.01	0.01	-2.47	0.013				
	G×L	-0.01	0.01	-1.91	0.057				

Notes: I = Intercept. Nutrient concentrations (% carbon, % nitrogen, C:N ratio and phenolics) and leaf area chewed (cm²) were *ln* transformed.

APPENDIX C. SUPPLEMENTARY MATERIAL FOR CHAPTER 4

GENERAL LINEAR MODELS FOR HERBIVORY MEASUREMENTS

Table C.1. General linear models for the effects of patch, plant, and patch×plant interaction on herbivory measurements. Latitude, stem density, number of leaves and plant height were treated as covariates.

Response variable	Effects	$F_{1,143}$	P
Stem feeders	Latitude	1.867	0.174
	Stems	6.464	0.012
	Leaves	9.278	0.003
	Height	1.003	0.318
	Patch	1.147	0.286
	Plant	7.737	0.006
	Patch×Plant	13.357	<0.0001
Mined leaves	Latitude	1.726	0.191
	Stems	0.049	0.826
	Leaves	2.094	0.150
	Height	2.362	0.127
	Patch	1.280	0.260
	Plant	9.138	0.003
	Patch×Plant	6.516	0.012
Chewed leaves	Latitude	13.304	<0.0001
	Stems	3.556	0.061
	Leaves	29.148	<0.0001
	Height	8.537	0.004
	Patch	0.899	0.345
	Plant	10.913	0.001
	Patch×Plant	5.218	0.024
Aphid infested leaves	Latitude	8.045	0.005
	Stems	3.838	0.052
	Leaves	12.766	<0.0001
	Height	0.010	0.919
	Patch	0.494	0.483
	Plant	1.820	0.179
	Patch×Plant	6.010	0.015
Total aphids	Latitude	11.493	0.001
	Stems	0.905	0.343
	Leaves	0.007	0.932

(Table C.1. continued)

Response variable	Effects	$F_{1,143}$	P
	Height	0.223	0.638
	Patch	1.119	0.292
	Plant	7.709	0.006
	Patch×Plant	2.320	0.130

Notes: Number of stems and leaves per plant, stem height (cm), and total aphids were *ln* transformed.

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VITA

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