MR. WARWICK J ALLEN (Orcid ID : 0000-0002-1859-1668)

Article type : Research Papers

Intraspecific and biogeographic variation in foliar fungal communities and pathogen damage of native and invasive *Phragmites australis*

Warwick J. Allen,[1,2,3†]* Aaron E. DeVries[4], Nicholas J. Bologna[1], Wesley A. Bickford[4,5], Kurt P. Kowalski[4,6], Laura A. Meyerson[7], James T. Cronin[1]

[1] Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803, United States.

[2] The Bio-Protection Research Centre, Lincoln University, Lincoln 7647, New Zealand.

[3†] The Bio-Protection Research Centre, School of Biological Sciences, University of Canterbury, Christchurch 8140, New Zealand.

[4] U.S. Geological Survey, Great Lakes Science Center, Ann Arbor, Michigan 48105, United States.

[5] Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan 48109, United States.

[6] School for Environment and Sustainability, University of Michigan, Ann Arbor, Michigan 48109, United States.

[7] Natural Resources Science, University of Rhode Island, Kingston, Rhode Island 02881, United States.

*Author for correspondence: email – warwick.j.allen@gmail.com

Running title: latitude, fungi, and plant invasions

ABSTRACT

Aim Recent research has highlighted that the relationship between species interactions and latitude can differ between native and invasive plant taxa, generating biogeographical heterogeneity in community resistance to plant invasions. In the first study with foliar pathogens, we tested whether co-occurring native and invasive lineages of common reed (*Phragmites australis*) exhibit nonparallel latitudinal gradients in foliar fungi communities, pathogen susceptibility and damage, and whether these biogeographic patterns can influence invasion success.

Location North America.

Time period 2015-2017.

Major taxa studied Perennial grass *Phragmites australis*.

Methods We surveyed 35 *P. australis* field populations, spanning 17° latitude and comprising four phylogeographic lineages, including one endemic to North America and one invasive from Europe. For each population, we quantified percent leaf pathogen damage and cultured fungi from diseased leaves, that we identified using molecular tools. To assess whether latitudinal gradients in pathogen damage were genetically-based, we inoculated plants from 73 populations with four putative pathogens in a complementary common garden experiment, and measured *P. australis* susceptibility (i.e., diseased leaf area).

Results We isolated 84 foliar fungi taxa. *Phragmites australis* lineage influenced fungal community composition but not diversity. Despite the invasive European *P. australis* lineage being the least susceptible to three of the four pathogens tested in the common garden experiment, pathogen damage in the field was similar between native and invasive lineages, providing no evidence that release from foliar pathogens contributes to invasion success. Genetically-based latitudinal gradients in pathogen susceptibility observed in the common garden were isolate-specific and obscured by local environmental conditions in the field, where pathogen damage was threefold higher for northern than southern populations, regardless of lineage.

Main conclusions Our results highlight that host plant lineage and genetically-based biogeographic gradients strongly influence foliar fungi communities and pathogen susceptibility, but do not translate to pathogen damage patterns observed in the field.

KEYWORDS: biotic resistance, diversity, endophytes, enemy release, genotype, invasive plant, latitudinal gradients, native plant, plant-fungi interactions, *Phragmites australis*

INTRODUCTION

Biological invasions threaten biodiversity and ecosystem function on a global scale (Mack et al., 2000). The success of invasive plants is increasingly being recognised as strongly influenced by their associated fungi, acting through a variety of direct and indirect interactions involving plant antagonists (i.e., pathogens) and mutualists (i.e., endophytes and mycorrhiza) (Dickie et al., 2017). For example, invasive plants may be successful because they escape fungal pathogens (the enemy release hypothesis: Elton, 1958; Keane & Crawley, 2002; Mitchell & Power, 2003), engage in diverse and strong direct interactions with fungal mutualists (the enhanced mutualism hypothesis: Reinhart & Callaway, 2006), or interact indirectly with other plants through spillover, spillback, and soil legacies (Power & Mitchell, 2004; Mangla, Inderjit & Callaway, 2008; Allen, Meyerson, Flick & Cronin, 2018). Conversely, pathogens can also have strong negative impacts on invaders (biotic resistance: Elton, 1958; Flory, Alba, Clay, Holt & Goss, 2018), while other studies have found no differences between native and invasive plants in their interactions with fungal pathogens and mutualists (van Kleunen & Fischer, 2009; Bunn, Ramsey & Lekberg, 2015). Whether invasive plants gain a systematic advantage over native species through their interactions with fungi remains an open question.

Studies of invasion hypotheses invoking biotic interactions have generally focused on small spatial scales, ignoring the possibility that the strength of these interactions could vary over broader geographic scales. Biogeographic variation in how species interactions influence invasion success may exist because the diversity, specialisation, and strength of species interactions are all expected to increase closer to the equator (the latitudinal diversity gradient: Hillebrand, 2004; Kinlock et al., 2018; and biotic interactions hypothesis: Dobzhansky, 1950; Schemske, Mittelbach, Cornell, Sobel & Roy, 2009; Coley & Kursar, 2014). However, empirical evidence of latitudinal gradients in the specialisation and strength of biotic interactions has been highly variable (Moles, Bonser, Poore, Wallis & Foley, 2011; Ollerton, 2012; Anstett, Nunes, Baskett & Kotanen, 2016; Moles & Ollerton, 2016), highlighting the need for an improved understanding of the mechanisms underlying these mixed results. For example, because of their different ecological and evolutionary histories, native and invasive taxa may be expected to experience nonparallel latitudinal gradients in specialisation or species interaction strength, which could generate biogeographical heterogeneity in the strength of enemy release or biotic resistance. To date, this hypothesis has been tested with varying levels of support for a range of direct and indirect plant-herbivore interactions (Cronin, Bhattarai, Allen & Meyerson, 2015; Allen et al., 2017; Bhattarai et al., 2017; Bhattarai, Meyerson & Cronin, 2017; Lu et al., 2019) but has not been

examined for plant-microbe interactions. The one exception is the recent study by Lu, He, Ding and Siemann (2018), who found that native and invasive *Alternanthera* species in China differed in their response to soil-borne enemies from along a latitudinal gradient, influencing the performance of a shared flea beetle herbivore (*Agasicles hygrophila*) and resulting in stronger enemy release at lower latitudes.

For fungi, evidence for latitudinal diversity gradients has been mixed, with the direction and magnitude of relationships often depending on the fungi functional group. For example, pathogen, saprotroph, and endophyte diversity appear to increase towards the equator (Wellman, 1968; Terhonen et al., 2011; Tedersoo et al., 2014; but see Millberg, Boberg & Stenlid, 2015), whereas results for mycorrhiza and various combinations of functional groups are more inconclusive (Öpik et al., 2010; Tedersoo et al., 2014; Kinlock et al., 2018). However, results have often been confounded by changes in host plant species richness and identity, and phylogenetically-controlled tests using a single host plant species over a broad latitudinal gradient are rare (Terhonen et al., 2011; Millberg et al., 2015, Lu et al., 2018). Fewer studies have examined latitudinal variation in fungal pathogen damage or plant susceptibility to pathogens but both positive and negative gradients have been reported (Björkman, 1963; Burdon, Oates & Marshall, 1983; Hamilton et al., 2013). Finally, for studies that are conducted in the field, local environmental conditions may alter the expression of genetically-based latitudinal gradients in plant resistance or susceptibility to natural enemies. It is therefore important that field surveys of pathogen abundance, diversity, or composition be paired with controlled greenhouse or common garden experiments (e.g., Bhattarai et al., 2017). To date, this combined approach has not been taken with pathogens of invasive species.

The widespread co-occurrence of multiple native and introduced phylogeographic lineages of *Phragmites australis* (Cav.) Trin. ex Steudel (common reed, hereafter *Phragmites*) in North America presents an ideal system to examine how latitudinal variation in plant-fungal interactions can influence invasion success (Cronin et al., 2015; Meyerson, Cronin & Pyšek, 2016). By minimising phylogenetic and environmental differences, the multiple *Phragmites* lineages allow for robust comparison of the interactions of foliar fungi with native and introduced plant taxa (*Phragmites*) along a biogeographic gradient (see also Lu et al., 2018). Using a field survey and complementary common garden experiment, we tested the following predictions (also summarised in Fig. 1): (1) introduced *Phragmites* lineages have lower foliar fungi diversity, fewer fungal pathogens, and suffer less pathogen damage than the native lineage (i.e., the enemy release hypothesis); (2) foliar fungal

communities, pathogen damage and *Phragmites* susceptibility to pathogens vary along a latitudinal gradient; (3) native and invasive *Phragmites* lineages exhibit nonparallel latitudinal gradients in foliar pathogen damage and susceptibility; and (4) latitudinal gradients observed in the field are repeated in a common garden experiment (i.e., are genetically-based).

MATERIALS AND METHODS

Study organisms

Phragmites australis is a tall perennial grass with a cosmopolitan distribution and is considered a model organism for studying plant invasions (Meyerson et al., 2016). Multiple phylogeographic lineages of *Phragmites* co-occur in North America (Saltonstall, 2002; Lambertini et al., 2012; Meyerson, Lambertini, McCormick & Whigham, 2012). The native lineage in North America is endemic and widespread, but an invasive lineage of *Phragmites* from Europe (also known as haplotype M, but hereafter referred to as the European lineage) has rapidly spread in North America since first appearing in herbarium records 150 years ago (Saltonstall, 2002; Meyerson et al., 2012). This European lineage forms large, dense, monocultures that provide ecosystem services such as erosion protection and carbon storage, but also negatively impact hydrology, native biodiversity, and ecosystem function (reviewed by Meyerson, Saltonstall & Chambers, 2009). A closely related lineage known as Delta, originating from the Mediterranean (Lambertini et al., 2012), has only been reported from the Mississippi River Delta in Louisiana (Hauber, Saltonstall, White & Hood, 2011; Knight et al., 2018). A fourth lineage, known as Gulf, is widely distributed along the Gulf of Mexico and west to California (Lambertini et al., 2012; Meyerson et al., 2012), is likely a recent arrival from Mexico or Central America (Colin & Eguiarte, 2016), and is spreading (Bhattarai & Cronin, 2014).

The diversity and function of the *Phragmites* microbiome has been investigated (see Kowalski et al., 2015 for review). Distinct oomycete, archaea, and bacteria communities have been reported from rhizosphere soil of native and European *Phragmites* lineages in North America (Nelson & Karp, 2013; Crocker, Karp & Nelson, 2015; Yarwood, Baldwin, Gonzalez Mateu & Buyer, 2016; Bowen et al., 2017), although no differences were detected for fungal, bacterial, and oomycete root endophyte communities in the Great Lakes Region (Bickford, Goldberg, Kowalski & Zak, 2018). However, *Phragmites* foliar fungi communities (i.e., pathogens and non-symptomatic endophytes) and their impacts have yet to be formally compared among native and invasive lineages (but see Shearer & Harms, 2012

for a local-scale survey in New York). Almost nothing has been reported to date regarding the ecology of the Gulf or Delta lineages (but see Bowen et al., 2017; Allen et al., 2018). Thus, this study provides one of the first comparisons involving more than just the two most common North American *Phragmites* lineages and is the first to examine differences in foliar fungi communities of *Phragmites* lineages in North America.

Finally, latitudinal gradients in plant nutritional condition, structural and chemical defenses, palatability to herbivores, herbivore damage, apparent competition strength, and tolerance to leaf tissue damage have previously been described for *Phragmites*, and often differ between the native and invasive lineage (Cronin et al., 2015; Allen et al., 2017; Bhattarai et al., 2017; Bhattarai, Meyerson & Cronin, 2017). However, this is the first study to compare latitudinal gradients in foliar fungi community structure and plant-pathogen interactions between any native and invasive taxa.

Field survey of pathogen damage and fungal communities

To examine interactions between *Phragmites* and foliar fungi, we surveyed 35 *Phragmites* populations (10 native, 10 European, 10 Gulf, 5 Delta) along the east coast of the United States from South Florida (26.6°) to Maine (44.0°) and along the Gulf Coast of Louisiana (see Appendix S1 in Supporting Information). East Coast populations represent where the invasive Eurasian lineage first appeared in herbarium records around 150 years ago. Populations of different lineages often occurred in the same watershed (same population location in Appendix S1) but were rarely intermixed. Populations in Louisiana were included because of the co-occurrence of two additional non-native lineages of *Phragmites*, but were not considered for latitudinal analyses because of their limited geographical range that did not overlap with the native and European lineage. Leaf material from each population was collected for later determination of lineage (based on chloroplast DNA) using the methods of Saltonstall (2002) with modifications outlined in Kulmatiski, Beard, Meyerson, Gibson and Mock (2010). The Delta lineage was identified based on morphology and flowering phenology (Hauber et al., 2011). Sampling was conducted in the late summer of 2015, from July 28 to September 12, from south to north along the latitudinal gradient of native and European populations (all were flowering at time of sampling), followed by the Gulf and Delta populations. Although populations were visited on only one occasion, *Phragmites* leaves persist throughout the growing season, meaning that our sampling design should estimate cumulative foliar pathogen damage and minimise phenologocial variation.

The proportion of stems with diseased leaves (i.e., presence of spots, lesions, discolouration, or chlorosis) was estimated by walking transects from the edge to interior of each population, examining the closest stem every metre for pathogen damage, until a total of 50 stems was reached. For 10 randomly selected stems that had pathogen damage, the number of leaves with and without pathogen damage were counted to determine the proportion of leaves damaged. Finally, damage severity of pathogenic fungi was quantified by photographing and estimating the percentage of area from 20 independent, randomly selected diseased leaves that have symptoms of pathogen damage per population using ImageJ software (Rasband, 2018). Overall percent leaf tissue area with disease symptoms for each population was calculated as the proportion of stems with pathogen damage × proportion of leaves per stem with pathogen damage × percent of leaf area with disease symptoms.

For culturing and identification of putative fungal pathogens and other endophytic fungi, we used the same 20 diseased leaves that were photographed to estimate pathogen damage. The leaves were stored in an ice chest, transported back to Louisiana State University, and processed the following day. Leaves were surface sterilised by sequential immersion and agitation in 95% ethanol, 5% sodium hypochlorite solution, and 70% ethanol (U'Ren et al., 2014). Using a sterilised hole punch (2 minutes in 5% sodium hypochlorite solution), tissue samples 3 mm in diameter were taken from the margins of symptomatic tissue and plated on water agar (17 g agar, 1 L H_2O) for isolation of emergent fungi. Plates were stored in an environmental chamber (21°C, 50% relative humidity, 16:8 h light-dark) and checked every 2-3 days for fungal growth. Emergent fungi were isolated and grown as pure cultures on potato dextrose agar (PDA; 5 g agar, 7.6 g PDA, 1 L H_2O), with leaf tissue samples regularly yielding multiple fungal isolates. Each isolate was transferred to a corn meal agar (CMA; 6 g agar, 8.5 g CMA, 1 L H_2O) slant for long-term storage at 4°C.

PDA cultures were photographed and identified to morphospecies based on colour, growth characteristics, and spore morphology, following Lacap, Hyde and Liew (2003). Morphospecies confirmation was conducted using DNA barcodes, detailed in Appendix S2. Finally, to investigate differences in prevalence of potential pathogens among lineages, we obtained putative function(s) (pathogen, saprophyte, endophyte, epiphyte) for each identified taxa based on the FUNGuild database (Nguyen et al., 2016) (Appendix S3) and used these data to calculate relative pathogen abundance (i.e., the proportion of isolates per population that were identified as potential pathogens) for each *Phragmites* population.

Common garden pathogen susceptibility experiment

A complementary common garden experiment was conducted in 2017 at Louisiana State University (30.36° N, 91.14° W) to assess whether *Phragmites* susceptibility to individual isolates of potential pathogens varies among lineages and with latitude of origin. The open-air common garden included 73 *Phragmites* populations (20 native, 27 European, 19 Gulf, and 7 Delta) from throughout North America, ranging in latitude from 26.1° to 47.4° (2, 245 km) (Appendix S4). Plants were grown from rhizome fragments in 75 litre pots with a sand substrate and regularly fertilised with Osmocote® (9 month, slow-release 15-9-12 NPK, The Scotts Miracle-Gro Company®, Marysville, Ohio) and Ironite® (Pennington®, Madison, Georgia). Plants were grown in the common garden for at least 2 years prior to being potted for the experiment to control for source population maternal effects, and panicles were removed before reaching maturity to prevent sexual reproduction and crossing among populations. For every pot in the garden (some *Phragmites* populations were planted in more than one pot), we inoculated plants with four different potential pathogens obtained from our field survey (one stem per pathogen per pot), selected because they were isolated from all four lineages, associated with diseased leaf tissue, and were identified as putative pathogens (Appendix S4). They were identified as *Stagonospora* sp. (isolated from the Gulf lineage in Florida), *Cladosporium* sp. (one isolate each from the Delta and Gulf lineages in Louisiana), and *Alternaria alternata* (from the native lineage in Maine).

To create spore solutions for inoculation, pure sporulated cultures of each isolate were flooded with 10 mL of 0.05% Tween-20 and mycelia scraped using a sterile metal spatula to release the spores. The resulting suspension was centrifuged for 5 minutes at 2000 x g and 25°C and then filtered through cheese cloth to remove mycelia. Spore concentration was quantified using a hemocytometer and adjusted to a final concentration of 1×10^5 spores per mL. A healthy *Phragmites* leaf, third or fourth from the top of the stem, was chosen for inoculation. Leaves were prepared by surface sterilisation with 75% ethanol, before a small piece of sanding sponge was twice turned through 180 degrees on the leaf adaxial surface around 1.5 cm from the leaf base, to create an abraded area of approximately 0.5 cm^2. Inoculations were performed on April 6-7, 2017, using a method modified from Li et al. (2014). To perform each inoculation, 0.2 mL of spore solution (or deionised water for the control) was pipetted onto a cotton ball. The treated part of the cotton ball was placed on the abraded leaf surface and secured with tape. The cotton balls were wetted twice per day with deionised water and fungi allowed to grow for 6 days, when infected leaves were removed and photographed for determination of total damaged area using ImageJ.

Data analysis

Field survey data were subjected to a two-stage analysis. First, pathogen damage (percent leaf tissue area with disease symptoms) and foliar fungi diversity metrics (isolate abundance, rarefied richness, Shannon diversity, and relative pathogen abundance) were compared among the four *Phragmites* lineages (native, European, Gulf, and Delta) using ANOVA and post-hoc Tukey tests (Bonferroni-corrected where appropriate). Fungal taxa richness was rarefied to six isolates, the lowest number of fungi isolated from a population. Latitude was not included as a covariate in these models because populations of the Gulf and Delta lineages had very narrow latitudinal ranges relative to the native and European *Phragmites* lineages (3.7°). The second stage of analyses involved testing for the presence of latitudinal gradients in pathogen damage and foliar fungi diversity for just the native and European *Phragmites* lineages. Due to the number of potential explanatory variables arising from testing for both linear and non-linear latitudinal gradients and their interactions with *Phragmites* lineage, we used the Akaike Information Criterion corrected for small sample size (AICc) to select best-fitting mixed-effect models from a set of candidate models for each response variable (Burnham & Anderson, 2010). The full model included the explanatory variables of *Phragmites* lineage, a linear and nonlinear (latitude^2) term for latitude, and their interactions with lineage (five total variables). Candidate models were based on subsets of the full model, using all possible combinations of the explanatory variables, but with the restriction that main effects must also be present in models containing interactions. We ranked candidate models from lowest to highest AICc and models with ΔAICc (= $AICc_i$ - $AICc_{min}$) of two or less were deemed to have substantial support (Burnham & Anderson, 2010). When multiple supported models were identified, the best-fitting model was used for post-hoc analyses, along with plausible models that contained any additional variables, so that each influential variable was subjected to post-hoc tests (these are the results that we report below).

Differences in the community composition of foliar fungi were analysed using a similar two-stage approach. A principal co-ordinates analysis was performed on Bray-Curtis dissimilarities calculated from fungal taxa abundances and community composition compared among *Phragmites* lineages and population latitude using PERMANOVA with 999 permutations. We drilled down into significant interaction terms using post-hoc pairwise PERMANOVAs to compare lineages. To examine differences in beta diversity (i.e., similarity of populations within lineage) among lineages and satisfy PERMANOVA

assumptions, we tested for multivariate homogeneity of dispersion. Like the richness and diversity metrics, we excluded the Gulf and Delta lineages for analyses of latitudinal gradients in community composition. We also calculated pairwise geographic distance between all populations within each lineage and used linear regression to test whether populations closer to one another had more similar fungal communities. Singleton taxa do not provide information on community similarity and were removed for these analyses.

For each inoculated leaf in the common garden experiment, we computed the leaf area with pathogen damage, which was then *ln*-transformed to satisfy normality of residuals. We analysed each putative pathogen isolate separately, using the same two-stage data analysis approach as described previously for the field survey. First, we used a linear mixed model with population included as a random effect (retained in all models) to test whether the damaged area differed among *Phragmites* lineages. Second, we used model selection to assess whether genetically-based latitudinal gradients in susceptibility existed for any of the four pathogens. As before, we used a linear mixed model with *Phragmites* lineage, latitude, and latitude^2 and their interactions considered in the full model, with population treated as a random effect. All analyses were conducted with R version 3.6.1 (R Development Core Team, 2019), using the vegan (Oksanen et al., 2019), lme4 (Bates et al., 2019), MuMIn (Bartoń, 2019), and esmeans (Lenth, 2019) packages. Analysis code is available upon request from the corresponding author.

RESULTS

Pathogen damage increased with latitude in the field

Percent leaf tissue area with disease symptoms was 3.3 ± 0.5% (mean ± S.E.) and ranged from 0.4% to 9.5% among populations. Pathogen damage did not differ among *Phragmites* lineages in the field ($F_{3,31} = 0.17$, $P = 0.916$). Model selection identified two plausible models (ΔAICc ≤ 2) with equal support (AICc weight = 0.5) to explain variation in pathogen damage, containing latitude and latitude^2, respectively (Appendix S5). Foliar pathogen damage was almost four times higher at the northern than southern end of our 993 km sampling distribution ($F_{1,18} = 5.74$, $P = 0.028$, $R^2 = 0.24$), regardless of *Phragmites* lineage (Fig. 2).

Fungi communities differed among Phragmites *lineages but did not significantly change with population latitude*

A total of 717 fungal isolates were obtained from the field survey and used for analyses. These cultures were separated based on molecular analyses or original morphospecies designation if molecular analyses were unsuccessful, resulting in 84 taxa (Appendix S3). The fungal taxa accumulation and rarefaction curves for the entire survey, each *Phragmites* lineage, and individual populations (Appendix S6) indicated that we captured less than half of the total *Phragmites* endophyte and pathogen diversity in the sampled area (estimated as 182 ± 44 fungal taxa using the Chao1 diversity estimator). Of the fungal taxa that were sampled, only 7% were isolated from all four *Phragmites* lineages, whereas 67% were isolated from just a single lineage (Appendix S7).

The raw abundance of foliar fungi isolates differed among *Phragmites* lineages ($F_{3,31}$ = 3.67, P = 0.023), and was 51% higher from the native than Delta lineage (P = 0.022) (Table 1). Model selection identified a single plausible model (ΔAICc ≤ 2) containing only *Phragmites* lineage to explain variation in isolate abundance per population (AICc weight = 1.00, Appendix S5), with post-hoc Tukey analyses finding 18% higher fungal isolate abundance from the native than European lineage (P = 0.024). In contrast, rarefied richness did not differ among *Phragmites* lineages ($F_{3,31}$ = 1.14, P = 0.347) (Table 1) and model selection deemed none of our explanatory variables to be influential (Appendix S5). Shannon diversity also did not significantly differ among *Phragmites* lineages ($F_{3,31}$ = 2.58, P = 0.071) (Table 1). However, two plausible models were identified to explain variation in Shannon diversity, with the best-fitting model containing only the intercept (AICc weight = 0.73, Appendix S5) and the second model containing the main effects of latitude and latitude^2 (AICc weight = 0.27). Post-hoc analyses revealed that the linear trend between latitude and Shannon diversity was not significant ($F_{1,17}$ = 0.40, P = 0.534) and the hump-shaped relationship was only marginally significant ($F_{1,17}$ = 3.37, P = 0.084). Finally, relative abundance of pathogens also differed among lineages ($F_{3,31}$ = 6.04, P = 0.002) and was 2.3 and 2.8 times higher for the native than the Gulf (P = 0.008) and Delta (P = 0.014) lineages, respectively (Table 1). Model selection identified two plausible models (ΔAICc ≤ 2) to explain variation in pathogen relative abundance, containing the intercept only (i.e., no explanatory variables were considered to be influential) (AICc weight = 0.63, Appendix S5) and *Phragmites* lineage (AICc weight = 0.37), respectively, with post-hoc analyses revealing no significant difference between the native and European lineages (P = 0.221).

Foliar fungi community composition varied strongly among *Phragmites* lineages ($F_{3,31}$ = 6.87, P < 0.001) (Fig. 3) and subsequent pairwise PERMANOVA of Bray-Curtis dissimilarities revealed that all lineages differed from one another (all comparisons P <

0.003). Homogeneity of dispersion analysis revealed that all lineages had similar beta diversity ($F_{3,31}$ = 2.03, P = 0.131). Latitudinal gradients in community composition of foliar fungi depended on *Phragmites* lineage (latitude × lineage interaction; $F_{1,16}$ = 2.20, P = 0.029), with similarity of populations increasing at higher latitudes for the native lineage ($F_{1,8}$ = 2.21, P = 0.046) but not the European lineage ($F_{1,8}$ = 1.13, P = 0.344). These biogeographic patterns were further reflected in our findings that community dissimilarity increased with distance between populations for the native ($F_{1,88}$ = 4.44, P = 0.038) and Gulf ($F_{1,88}$ = 63.27, P < 0.001) lineages, was marginally significant for the European lineage ($F_{1,88}$ = 3.13, P = 0.080), and had no relationship for the Delta lineage ($F_{1,18}$ = 1.73, P = 0.205).

Pathogen susceptibility varied with lineage latitude

In the common garden, we detected differences in pathogen damage (infection area) among *Phragmites* lineages for three of the four pathogens investigated: *Alternaria alternata* from Maine ($F_{3,68}$ = 4.86, P = 0.004), and *Cladosporium* sp. 1 ($F_{3,75}$ = 4.40, P = 0.007) and *Cladosporium* sp. 2 ($F_{3,79}$ = 3.52, P = 0.019), both from Louisiana. The European lineage suffered 36% (P = 0.007) and 52% (P = 0.005) less damage than the Gulf lineage against *A. alternata* and *Cladosporium* sp. 1, respectively, and 34% (P = 0.030) less damage than the native lineage against *Cladosporium* sp. 2 (Fig. 4). No other differences among lineages were detected.

Model selection identified four plausible models (ΔAICc ≤ 2) to explain variation in pathogen damage from *A. alternata* (Appendix S8). The full model was the best-fitting, containing the lineage × latitude and lineage × latitude^2 interactions, along with the associated main effects (AICc weight = 0.32). Pathogen damage to the European lineage demonstrated positive and u-shaped relationships with latitude ($t_{40.6}$ = -1.89, P = 0.067) and latitude^2 ($t_{40.8}$ = 1.92, P = 0.062), respectively, although these relationships were not quite significant at the α = 0.05 level. Conversely, pathogen damage of the native lineage significantly decreased with latitude ($t_{38.5}$ = 2.04, P = 0.049) and latitude^2 ($t_{38.4}$ = -2.17, P = 0.037) (Fig. 5a). For both putative pathogens *Cladosporium* sp. 1 and *Cladosporium* sp. 2, model selection identified the top plausible model as containing only the *Phragmites* lineage main effect (AICc weights = 0.37 and 1.00, respectively, Appendix S8). For *Cladosporium* sp. 1, there was a nonsignificant trend towards lower damage on the European than native *Phragmites* lineage ($F_{1,47}$ = 3.11, P = 0.084), whereas for *Cladosporium* sp. 2, there was significantly lower damage on the native compared to the European lineage ($F_{1,49}$ = 6.17, P = 0.016). For *Cladosporium* sp. 1, additional plausible models included main effects of latitude

and latitude^2 (Appendix S8), although post-hoc analyses found that their negative relationships with damaged area were only marginally significant at the $\alpha = 0.05$ level ($F_{1,46}$ = 3.43, $P = 0.071$ and $F_{1,46} = 3.46$, $P = 0.069$, respectively). Finally, model selection identified five plausible models to explain variation in pathogen damage from *Stagonospora* sp. (Appendix S8). The two top models comprised the lineage × latitude (AICc weight = 0.31) and lineage × latitude^2 (AICc weight = 0.26) interactions and main effects. The European lineage showed a positive relationship with latitude ($t_{74} = 2.81$, $P = 0.006$) and latitude^2 ($t_{25.1}$ = 2.75, $P = 0.011$), whereas pathogen damage of the native lineage declined with latitude (t_{74} = -2.46, $P = 0.016$) and latitude^2 ($t_{25.2} = -2.42$, $P = 0.029$) (Fig. 5b).

DISCUSSION

Recent studies have demonstrated the presence of biogeographic heterogeneity in enemy release resulting from nonparallel latitudinal gradients in the strength of plant-herbivore interactions (Cronin et al., 2015; Allen et al., 2017; Bhattarai et al., 2017; Bhattarai, Meyerson & Cronin, 2017; Lu et al., 2018; Lu et al., 2019). In the first study to examine this possibility for plant-pathogen interactions, we found a positive latitudinal gradient in pathogen damage to *Phragmites* in the field, but despite possessing different fungal communities, the native and European lineages of *Phragmites* exhibited parallel gradients. This finding indicates that there is little biogeographic heterogeneity in enemy release from pathogens for European *Phragmites* along the Atlantic Coast of the USA. Conversely, in a controlled common garden environment, we found a mixture of parallel and nonparallel genetically-based latitudinal gradients in pathogen susceptibility, depending upon the pathogen isolate being investigated. These mixed results demonstrate that biogeographic patterns in escape from enemies depend on the type of interaction (i.e., herbivores vs. pathogens) and focal species, suggesting that a more comprehensive assessment of species interactions may improve future studies that investigate latitudinal gradients in enemy release and biotic resistance. Furthermore, the differing results between the field and common garden indicate that the local environment can obscure genetically-based patterns in plant traits associated with enemy interactions such as susceptibility to pathogens (see also Allen et al., 2017; Bhattarai et al., 2017). This study is the first to empirically examine the contribution of genetics and local environmental conditions to latitudinal gradients in plant-pathogen interactions.

We found a high diversity of fungi associated with diseased *Phragmites* leaves; 717 fungal isolates comprising 84 fungal taxa. In contrast to our first prediction, fungal

abundance, richness, and diversity did not differ among *Phragmites* lineages. However, foliar fungi communites diverged among all four *Phragmites* lineages, overriding local environmental conditions associated with latitude. In other words, *Phragmites* populations of the same lineage (native or European) located at opposite ends of their distribution (i.e., North Carolina and Maine) generally had more similar fungi communities than plants of different lineages growing together in the same wetland, mirroring results for *Phragmites* rhizosphere bacteria (Bowen et al., 2017). Fungal communites also differed between the Gulf and Delta *Phragmites* lineages. However, their latitudinal ranges are very narrow (0.2° and 3.7°, respectively) and do not overlap with the distribution of the native and European lineages, potentially confounding biogeographic comparisons involving these two lineage groups. Our study adds to a growing body of evidence supporting plant lineage as a key determinant of foliar fungi community structure in multiple plant species (Whitham et al., 2012; Sapkota, Knorr, Jørgensen, O'Hanlon & Nicolaisen, 2015; Materatski et al., 2019; but see Busby, Newcombe, Dirzo & Whitham, 2014; Whitaker, Reynolds & Clay, 2018). We expand on these earlier findings as one of the first studies conducted outside of an agricultural context, by comparing closely-related native and invasive taxa over a broad geographic scale, and quantifying damage from the fungal pathogen community. For *Phragmites*, the divergent fungal communities for all lineage comparisons indicates a high degree of specialisation of plant-fungi interactions at the lineage level, consistent with previous findings comparing rhizosphere oomycete (Nelson & Karp, 2013; Crocker et al., 2015), bacteria (Bowen et al., 2017), and archaea (Yarwood et al., 2016) communities among *Phragmites* lineages, but contrasting with a recent study of endophytic root fungi, bacteria, and oomycete communities in the Great Lakes Region (Bickford et al., 2018). However, our study is the first to examine differences among *Phragmites* lineages in their foliar microbiome, where leaf tissue provides a substrate different from that of roots or the rhizosphere, and that varies among *Phragmites* lineages in leaf chemistry (Cronin et al., 2015; Pyšek et al., 2019).

Our results from the field survey and common garden experiment present contrasting evidence for our first prediction and the enemy release hypothesis. Despite divergent foliar fungi communities and pathogen abundance, pathogen damage did not differ among *Phragmites* lineages in the field, suggesting that release from fungal pathogens does not contribute to the invasion success of the European lineage (Bickford et al., 2018). However, the common garden experiment identified genetically-based differences in susceptibility to some putative pathogens that favoured the European lineage over the Gulf and native

lineages. This mismatch between field and common garden results may be because pathogen damage in the field is a cumulative measure of all pathogen damage, likely to be highly dependent upon local environmental conditions (e.g., edaphic and climatic factors, and the plant and pathogen community), whereas the common garden experiment assessed plant susceptibility to spore solutions from single isolates. Regardless, the combination of divergent fungi communities and genetically-based differences in pathogen susceptibility among lineages raises the potential for the development of beneficial or pathogenic fungi to promote or control the native and European lineages, respectively. For example, fungal pathogens have previously been used for biological control (Evans, 2013; Harding & Raizada, 2015), although never at the subspecific level. Our study indicates that plant-associated fungi may represent a more diverse and tractable system for biological control ventures than insect herbivores, which to date, have not proven sufficiently specialised for sub-specific biological control (Bhattarai, Allen, Cronin, Kiviat & Meyerson, 2016; Cronin, Kiviat, Meyerson, Bhattarai & Allen, 2016; Kiviat et al., 2019). However, future research should focus on individual pathogen taxa and the link between pathogen damage and plant fitness.

Based on large-scale studies that have found higher plant pathogen and endophyte diversity at lower latitudes (Wellman, 1968; Terhonen et al., 2011; Tedersoo et al., 2014; but see Millberg et al., 2015), our second prediction was that *Phragmites* foliar fungi communities would vary along a latitudinal gradient. Contrary to this prediction, we found little evidence for latitudinal gradients in the abundance, richness, and diversity of foliar fungi. These opposing results may be because many large-scale studies do not account for the confounding effect of concomitant gradients in host diversity, whereas we controlled for this effect by focussing on a single species. However, we observed latitudinal variation in fungi community structure for the native but not European *Phragmites* lineage, indicating that the native lineage may be locally co-adapted with its fungal communities along the latitudinal gradient, whereas the European lineage may predominantly be interacting with widespread, generalist fungi, such as *Arthrinium* sp., isolated from six of the ten European populations. Populations of the native lineage have a longer coevolutionary history with resident fungal communities in North America than the invasive lineages (millennia vs ~150 years; Orson, 1999), potentially leading to these more specialised interactions or altered plant resistance or tolerance to natural enemies. Interestingly, Lu et al. (2018) found a similar result for invasive *Alternanthera philoxeroides* (alligatorweed) and its native congener *A. sessilis* (sessile joyweed) in China, where the native *A. sessilis* exhibited latitudinal gradients in the

composition and impact of rhizosphere soil bacteria and fungi communities, whereas no latitudinal gradients were observed for the invasive species. Taken together, these findings indicate that coevolutionary history may play a substantial role in determining the composition and impact of the plant microbiome over large spatial scales.

Pathogen damage observed in the field survey was positively related to latitude, demonstrating biogeographic heterogeneity in plant-pathogen interactions. However, because the native and European *Phragmites* lineages exhibited parallel latitudinal gradients in pathogen damage, we find no evidence to support biogeographic heterogeneity in enemy release from pathogens for European *Phragmites* in North America. Our findings are consistent with the limited evidence for stronger pathogen pressure at higher latitudes (Nguyen et al., 2016), but contrast with the absence of latitudinal gradients in fungal pathogen diversity on *Phragmites*, indicating that the positive latitudinal gradients in pathogen damage may be driven by other factors such as increased pathogen virulence (Oates, Burdon & Brouwer, 1983) or decreased plant resistance to pathogens (Burdon et al., 1983; but see Björkman, 1963; Hamilton et al., 2013) at higher latitudes. More broadly, our results contribute to the growing body of evidence supporting that several biotic interactions are not consistently stronger in the tropics, including meta-analyses of terrestrial herbivory (Moles et al., 2011), marine herbivory (Poore et al., 2012), seed predation (Moles & Westoby, 2003), and parasitism (Hawkins, 1994). To date, studies of plant-pathogen interactions have been scarce (Schemske et al., 2009). In contrast, both field experiments (Freestone, Ruiz & Torchin, 2013) and meta-analysis (Kimbro, Cheng & Grosholz, 2013) have presented evidence of stronger biotic resistance to invasive species at lower latitudes. However, a recent study showed that other influential invasion mechanisms (i.e., propagule pressure) can overwhelm latitudinal variation in predator impacts on the population growth of bryozoans and tunicates (Cheng, Ruiz, Altieri & Torchin, 2019), supporting other meta-analyses that revealed that biotic resistance of bird and freshwater communities did not vary with latitude (Blackburn & Duncan, 2001; Alofs & Jackson, 2014).

By simultaneously considering closely-related native and invasive taxa, our study expands on these findings by demonstrating that native and invasive *Phragmites* experience identical latitudinal gradients in biotic resistance from foliar pathogens in the field. This lack of latitudinal variation in relative pathogen impact between native and invasive taxa does not favour the invader throughout its range and suggests that this type of comparative approach is important when investigating biotic resistance as a potential mechanism of invasion success. Regardless, it remains an interesting question whether mechanisms of invasion success

frequently change across the invaded range, such as has been proposed with *Spartina alterniflora* (smooth cordgrass) in China (Liu, Strong, Pennings, & Zhang, 2017). Interestingly, a recent analysis found ubiquitous support for biotic resistance using data on native and non-native species richness from 24, 456 plots from 153 sites across the United States (Beaury et al., 2020), but did not address whether its strength varied with latitude. Our findings of opposing latitudinal gradients in pathogen diversity (no gradient) and damage (positive gradient in the field, variable in the common garden) do not support natural enemy diversity as a driver of biotic resistance at the species level, or the widely-accepted notion that latitudinal gradients in diversity and interaction strength are related (Schemske et al., 2009).

We found mixed evidence for latitudinal gradients in plant-pathogen interactions in the common garden experiment, where genetically-based latitudinal gradients in pathogen susceptibility differed between the native and European lineages (supporting our third prediction) and from those observed in the field (contrary to our fourth prediction). Native and European *Phragmites* lineages exhibited nonparallel latitudinal gradients in susceptibility to damage from two of the four isolates investigated in the common garden experiment (*Alternaria alternata* and *Stagonospora* sp.), indicating that the European *Phragmites* lineage may benefit from pathogen enemy release at lower latitudes, such as has been observed with some *Phragmites* herbivores (Cronin et al., 2015) and in the *Alternanthera* study system (Lu et al., 2018, Lu et al., 2019). However, these patterns in susceptibility did not translate to pathogen damage in the field, indicating that the local environmental conditions and plant and pathogen communities may be stronger determinants of pathogen damage than plant lineage alone. This conclusion is supported by several studies that demonstrate strong variation in pathogen damage and resistance along environmental gradients including temperature, rainfall, humidity, and elevation (Kniskern & Rausher, 2006; Busby et al., 2014; Spear, Coley & Kursar, 2015), all of which may be expected to covary with latitude in the field. However, because the experiment was conducted in only one common garden, we cannot assess how changing environmental conditions in multiple common gardens could alter the latitudinal gradients in *Phragmites* pathogen susceptibility. Finally, the presence of a latitudinal gradient in susceptibility of the European lineage to *Stagonospora* sp. supports other research that has showed that invasive plants can evolve rapidly along latitudinal gradients (Bhattarai et al., 2017; Liu et al., 2020).

Conclusions and future directions

This is the first study to compare foliar fungi communities, pathogen damage, and genetically-based pathogen susceptibility of native and invasive plant taxa along a latitudinal gradient. We show that host plant lineage has a strong influence on foliar fungi community structure and pathogen susceptibility, but that this does not necessarily translate to differences in fungal diversity or pathogen damage in the field. Second, our results did not support the presence of latitudinal gradients in fungal abundance, richness or diversity. However, we show that pathogen damage of *Phragmites australis* was positively related with latitude in North America, and that native and invasive plant taxa can evolve nonparallel latitudinal gradients in foliar fungi community structure and pathogen susceptibility, the latter varying among pathogen isolates. Finally, these pathogen-specific, genetically-based latitudinal gradients in pathogen susceptibility may be dominated in the field by the influence of the local environmental conditions and pathogen community, resulting in no detectable biogeographic heterogeneity in enemy release for the European *Phragmites* lineage. Like with plant-herbivore interactions (Anstett et al., 2016), the jury remains out on the existence and direction of latitudinal gradients in plant-fungi interactions and the potential implications for fungal diversity. This uncertainty is largely due to the influence of many confounding factors and the methodological difficulties associated with assessing species interactions over such a large scale (Moles et al., 2011; Anstett et al., 2016). Therefore, because of the high variation in latitudinal gradients of species interactions, future research might aim to take a comprehensive, community-level, and standardised approach to assessing latitudinal gradients in enemy release and biotic resistance and the role of species interactions in biological invasions in general. For example, this could be achieved by large-scale collaborative field surveys and exclusion experiments that quantify the impact on plant fitness of entire suites of important interaction partners (e.g., foliar fungi, mycorrhiza, herbivores) and also evaluate abiotic drivers of global change, such as salinity, nutrient availability, and climate variables.

ACKNOWLEDGEMENTS

We thank the organisations and people that granted permission to visit field sites: Rachel Carson National Wildlife Refuge, Choptank Nature Conservancy, Alice Welford, Mackay Island National Wildlife Refuge, Pettipaug Yacht Club, Estell Manor State Park, and Palm Beach County Parks Department. Thanks also to G. Bhattarai, R. Harman, N. Harms, J. Croy, K. Manley, S. Campbell, F. Scaglia Drusini, M. Blackwell, M. Hoy, and J. Shearer for field, lab, and greenhouse assistance. Thank you to the two anonymous reviewers for

comments on the manuscript. This project was supported by NSF grants DEB 1050084 and DMS 1516833 to JTC and DEB 1501775 to JTC and WJA. Additional support was provided to WJA from the Louisiana Environmental Education Commission, Sigma Xi Scientific Research Society, and LSU Biograds. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The authors declare no conflicts of interest.

BIOSKETCH

Warwick Allen is a post-doctoral fellow at The Bio-Protection Research Centre and is based at University of Canterbury, New Zealand. This work was carried out during his Ph.D. at Louisiana State University. His research interests centre around understanding how species interactions influence communities and ecosystems, with a particular focus on the role of direct and indirect species interactions in the causes and consequences of biological invasions. This research group aims to study the role of biogeography, multitrophic species interactions, plant genetics, and plasticity in the success, impacts, and management of plant invasions at large spatial scales.

SUPPLEMENTARY MATERIALS

Appendix S1. *Phragmites australis* populations visited during the field survey.
Appendix S2. Additional methodological details of culturing and molecular identification of foliar fungi of *Phragmites australis*.
Appendix S3. Fungal taxa isolated from *Phragmites australis* leaves.
Appendix S4. *Phragmites australis* populations used for the common garden experiment.
Appendix S5. Model selection results for the field survey.
Appendix S6. Taxa accumulation and rarefaction curves for foliar fungi of *Phragmites australis*.
Appendix S7. Sharing of foliar fungi among *Phragmites australis* lineages.
Appendix S8. Model selection results for the common garden experiment.

REFERENCES

Allen, W. J., Meyerson, L. A., Flick, A. J., & Cronin, J. T. (2018). Intraspecific variation in indirect plant-soil feedbacks influences a wetland plant invasion. *Ecology*, 99, 1430-1440.

Allen, W. J., Meyerson, L. A., Cummings, D., Anderson, J., Bhattarai, G. P., & Cronin, J. T. (2017). Biogeography of a plant invasion: drivers of latitudinal variation in enemy release. *Global Ecology and Biogeography*, 26, 435-446.

Alofs, K. M., & Jackson, D. A. (2014). Meta-analysis suggests biotic resistance in freshwater environments is driven by consumption rather than competition. *Ecology*, 95, 3259–3270.

Anstett, D. N., Nunes, K. A., Baskett, C., & Kotanen, P. M. (2016). Sources of controversy surrounding latitudinal patterns in herbivory and defense. *Trends in Ecology and Evolution*, 31, 789-802.

Bartoń, K. (2019). MuMIn: Multi-model inference. R package version 1.43.6. http://CRAN.R-project.org/package=MuMIn.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., … Green, P. (2019). lme4: linear mixed-effects models using 'Eigen' and S4. R package version 1.1-21. http://CRAN.R-project.org/package=lme4.

Bezemer, T. M., Harvey, J. A., & Cronin, J. T, (2014). Response of native insect communities to invasive plants. *Annual Review of Entomology*, 59, 119–141.

Beaury, E. M., Finn, J. T., Corbin, J. D., Barr, V., & Bradley, B. A. (2020). Biotic resistance to invasion is ubiquitous across ecosystems of the United States. *Ecology Letters*, in press.

Bhattarai, G. P., & Cronin, J. T. (2014). Hurricane activity and the large-scale pattern of spread of an invasive plant species. *PLoS ONE*, 9, e98478.

Bhattarai, G. P., Meyerson, L. A., Anderson, J., Cummings, D., Allen, W. J., & Cronin, J. T. (2017). Biogeography of a plant invasion: genetic variation and plasticity in latitudinal clines for traits related to herbivory. *Ecological Monographs*, 87, 57-75.

Bhattarai, G. P., Meyerson, L. A., & Cronin, J. T. (2017). Geographic variation in apparent competition between native and invasive *Phragmites australis*. *Ecology*, 98, 349-358.

Bhattarai, G. P., Allen, W. J., Cronin, J. T., Kiviat, E., & Meyerson, L. A. (2016). Response to Blossey and Casagrande – ecological and evolutionary processes make host specificity at the subspecies level exceedingly unlikely. *Biological Invasions*, 18, 2757–2758.

Bickford, W. A., Goldberg, D. E., Kowalski, K. P., & Zak, D. R. (2018). Root endophytes and invasiveness: no difference between native and non-native *Phragmites* in the Great Lakes Region. *Ecosphere*, 9, e02526.

675 Björkman, E. (1963). Resistance to snow blight (*Phacidium infestans* Karst.) in different
676 provenances of *Pinus sylvestris* L. *Studia Forestalia Suecia*, 5, 1–16.
677 Blackburn, T. M., & Duncan, R. P. (2001). Determinants of establishment success in
678 introduced birds. *Nature*, 414, 195-197.
679 Bowen, J. L., Kearns, P. J., Byrnes, J. E. K., Wigginton, S., Allen, W. J., Greenwood, M., …
680 Meyerson, L. A. (2017). Lineage overwhelms environmental conditions in
681 determining rhizosphere bacterial community structure in a cosmopolitan invasive
682 plant. *Nature Communications*, 8, 433.
683 Bunn, R. A., Ramsey, P. W., & Lekberg, Y. (2015). Do native and invasive plants differ in
684 their interactions with arbuscular mycorrhizal fungi? A meta-analysis. *Journal of*
685 *Ecology*, 103, 1547-1556.
686 Burdon, J. J., Oates, J. D., & Marshall, D. R. (1983). Interactions between *Avena* and
687 *Puccinia* species. I. The wild hosts: *Avena barata* Pott Ex Link, *A. fatua* L., *A.*
688 *ludoviciana* Durieu. *Journal of Applied Ecology*, 20, 571–584.
689 Burnham, K. P., & Anderson, D. R. (2010). *Model selection and multimodel inference: a*
690 *practical information-theoretic approach* (2nd ed.). New York, NY: Springer.
691 Busby, P. E., Newcombe, G., Dirzo, R., & Whitham, T. G. (2014). Differentiating genetic
692 and environmental drivers of plant-pathogen community interactions. *Journal of*
693 *Ecology*, 102, 1300-1309.
694 Cheng, B. S., Ruiz, G. M., Altieri, A. H., & Torchin, M. E. (2019). The biogeography of
695 invasion in tropical and temperate seagrass beds: Testing interactive effects of
696 predation and propagule pressure. *Diversity and Distributions*, 25, 285-297.
697 Coley, P. D., & Kursar, T. A. (2014). On tropical forests and their pests. *Science*, 343, 35-36.
698 Colin, R., & Eguiarte, L. E. (2016). Phylogeographic analyses and genetic structure
699 illustrate the complex evolutionary history of *Phragmites australis* in Mexico.
700 *American Journal of Botany*, 103, 876-887.
701 Crocker, E. V., Karp, M. A., & Nelson, E. B. (2015). Virulence of oomycete pathogens from
702 *Phragmites australis*-invaded and noninvaded soils to seedlings of wetland plant
703 species. *Ecology and Evolution*, 5, 2127–2139.
704 Cronin, J. T., Bhattarai, G. P., Allen, W. J., & Meyerson, L. A. (2015). Biogeography of a
705 plant invasion: plant-herbivore interactions. *Ecology*, 96, 1115-1127.
706 Cronin, J. T., Kiviat, E., Meyerson, L. A., Bhattarai, G. P., & Allen, W. J. (2016). Biological
707 control of invasive *Phragmites australis* will be detrimental to native *P. australis*.
708 *Biological Invasions*, 18, 2749–2752.

709 Dickie, I. A., Bufford, J. L., Cobb, R. C., Desprez-Loustau, M.-L., Grelet, G., Hulme, P. E.,
710 … Williams, N. M. (2017). The emerging science of linked plant-fungal invasions.
711 *New Phytologist*, 215, 1314-1332.
712 Dobzhansky, T. (1950). Evolution in the tropics. *American Scientist*, 38, 209-221.
713 Elton, C. S. (1958). *The ecology of invasions by animals and plants*. London, UK: Methuen.
714 Evans, H. C. (2013). Biological control of weeds. In F. Kempken (Ed.) *Agricultural*
715 *applications* (pp. 145–172). Berlin, Germany: Springer Verlag Berlin Heidelberg.
716 Flory, S. L., Alba, C., Clay, K., Holt, R. D., & Goss, E. M. (2018). Emerging pathogens can
717 suppress invaders and promote native species recovery. *Biological Invasions*, 20, 5-8.
718 Freestone, A. L., Ruiz, G. M., & Torchin, M. E. (2013). Stronger biotic resistance in tropics
719 relative to temperate zone: Effects of predation on marine invasion dynamics.
720 *Ecology*, 94, 1370-1377.
721 Hamilton, M. G., Williams, D. R., Tilyard, P. A., Pinkard, E. A., Wardlaw, T. J., Glen, M., …
722 Potts, B. M. (2013). A latitudinal cline in disease resistance of a host tree. *Heredity*,
723 110, 372-379.
724 Harding, D. P., & Raizada, M. N. (2015). Controlling weeds with fungi, bacteria, and viruses:
725 a review. *Frontiers in Plant Science*, 6, 659.
726 Hawkins, B. (1994). *Pattern and process in host-parasitoid interactions.* Cambridge, UK:
727 Cambridge University Press.
728 Hauber, D. P., Saltonstall, K., White, D. A., & Hood, C. S. (2011). Genetic variation in the
729 common reed, *Phragmites australis*, in the Mississippi River Delta marshes: evidence
730 for multiple introduction. *Estuaries and Coasts*, 34, 851-862.
731 Hillebrand, H. (2004). On the generality of the latitudinal diversity gradient. *The American*
732 *Naturalist*, 163, 192-211.
733 Keane, R. M., & Crawley, M. J. (2002). Exotic plant invasions and the enemy release
734 hypothesis. *Trends in Ecology and Evolution*, 7, 164–170.
735 Kimbro, D. L., Cheng, B. S., & Grosholz, E. D. (2013). Biotic resistance in marine
736 environments. *Ecology Letters*, 16, 821–833.
737 Kinlock, N. L., Prowant, L., Herstoff, E. M., Foley, C. M., Akin-Fajiye, M., Bender, N., …
738 Gurevitch, J. (2018). Explaining global variation in the latitudinal diversity gradient:
739 Meta-analysis confirms known patterns and uncovers new ones. *Global Ecology and*
740 *Biogeography*, 27, 125-141.
741 Kiviat, E., Meyerson, L. A., Mozdzer, T. J., Allen, W. J., Baldwin, A. H., Bhattarai, G. P., …
742 Cronin, J. T. (2019). Evidence does not support the targeting of cryptic invaders at the

743 subspecies level using classical biological control: the example of *Phragmites*.
744 *Biological Invasions*, 21, 2529-2541.
745 Knight, I. A., Wilson, B. E., Gill, M., Aveles, L., Cronin, J. T., Nyman, J. A., … Diaz, R.
746 (2018). Invasion of *Nipponaclerda biwakoensis* (Hemiptera: Aclerdidae) and
747 associated *Phragmites australis* dieback in southern Louisiana. *Biological Invasions*,
748 20, 2739–2744.
749 Kniskern, J. M., & Rausher, M. D. (2006). Environmental variation mediates the deleterious
750 effects of *Coleosporium ipomoeae* on *Ipomoea purpurea*. *Ecology*, 87, 675-685.
751 Kowalski, K. P., Bacon, C., Bickford, W., Braun, H., Clay, K., Leduc-Lapierre, M., …
752 Wilcox, D. A. (2015). Advancing the science of microbial symbiosis to support
753 invasive species management: a case study on *Phragmites* in the Great Lakes.
754 *Frontiers in Microbiology*, 6, 95.
755 Kulmatiski, A., Beard, K. H., Meyerson, L. A., Gibson, J. R., & Mock, K. E. (2010).
756 Nonnative *Phragmites australis* invasion into Utah wetlands. *Western North*
757 *American Naturalist*, 70, 541-552.
758 Lacap, D. C., Hyde, K. D., & Liew, E. C. Y. (2003). An evaluation of the fungal
759 'morphotype' concept based on ribosomal DNA sequences. *Fungal Diversity*, 12, 53-
760 66.
761 Lambertini, C., Mendelssohn, I. A., Gustafsson, M. H. G., Olesen, B., Riis, T., Sorrell, B. K.,
762 & Brix, H. (2012). Tracing the origin of Gulf Coast *Phragmites* (Poaceae): a story of
763 long-distance dispersal and hybridization. *American Journal of Botany*, 99, 538-551.
764 Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2019). emmeans: Estimated
765 marginal means, aka least-squares means. R package version 1.3.5.1. http://CRAN.R-
766 project.org/package=emmeans.
767 Li, H., Zhang, X. M., Zheng, R. S., Li, X., Elmer, W. H., Wolfe, L. M., & Li, B. (2014).
768 Indirect effects of non-native *Spartina alterniflora* and its fungal pathogen (*Fusarium*
769 *palustre*) on native saltmarsh plants in China. *Journal of Ecology*, 102, 1112–1119.
770 Liu, W., Strong, D. R., Pennings, S. C., & Zhang, Y. (2017). Provenance-by-environment
771 interaction of reproductive traits in the invasion of *Spartina alterniflora* in China.
772 *Ecology*, 98, 1591-1599.
773 Liu, W., Zhang, Y., Chen, X., Maung-Douglass, K., Strong, D. R., & Pennings, S. C., &
774 (2020). Contrasting plant adaptation strategies to latitude in the native and invasive
775 range of *Spartina alterniflora*. *New Phytologist*, in press.

776 Lu, X., He, M., Ding, J., & Siemann, E. (2018). Latitudinal variation in soil biota: testing the
777 biotic interaction hypothesis with an invasive plant and a native congener. *The ISME*
778 *Journal*, 12, 2811-2822.
779 Lu, X., He, M., Tang, S., Wu, Y., Shao, X., Wei, H., … Ding, J. (2019). Herbivory may
780 promote a non-native plant invasion at low but not high latitudes. *Annals of Botany*,
781 mcz121.
782 Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., & Bazzaz, F. A. (2000).
783 Biotic invasions: causes, epidemiology, global consequences and control. *Ecological*
784 *Applications*, 10, 689-710.
785 Mangla, S., Inderjit, & Callaway, R. M. (2008). Exotic invasive plant accumulates native soil
786 pathogens which inhibit native plants. *Journal of Ecology*, 96, 58–67.
787 Materatski. P., Varanda, C., Carvalho, T., Dias, A. B., Campos, M. D., … Félix, Md. R.
788 (2019). Spatial and temporal variation of fungal endophytic richness and diversity
789 associated to the phyllosphere of olive cultivars. *Fungal Biology*, 123, 66-76.
790 Meyerson, L. A., Cronin, J. T., & Pyšek, P. (2016). *Phragmites* as a model organism for
791 studying plant invasions. *Biological Invasions*, 18, 2421-2431.
792 Meyerson, L. A., Lambertini, C., McCormick, M. K., & Whigham, D. F. (2012).
793 Hybridization of common reed in North America? The answer is blowing in the wind.
794 *AoB Plants*, 4, pls022.
795 Meyerson, L. A., Saltonstall, K., & Chambers, R. M. (2009). *Phragmites australis* in eastern
796 North America: a historical and ecological perspective. In B. R. Silliman, E.
797 Grosholz, & M. D. Bertness (Eds.) *Human impacts on salt marshes: a global*
798 *perspective.* Los Angeles, CA: University of California Press.
799 Millberg, H., Boberg, J., & Stenlid, J. (2015). Changes in fungal community of Scots pine
800 (*Pinus sylvestris*) needles along a latitudinal gradient in Sweden. *Fungal Ecology*, 17,
801 126-139.
802 Mitchell, C. E., & Power, A. G. (2003). Release of invasive plants from fungal and viral
803 pathogens. *Nature*, 421, 625-627.
804 Moles, A. T., Bonser, S. P., Poore, A. G. B., Wallis, I. R., & Foley, W. J. (2011). Assessing
805 the evidence for latitudinal gradients in plant defence and herbivory. *Functional*
806 *Ecology*, 25, 380–388.
807 Moles, A. T., & Ollerton, J. (2016). Is the notion that species interactions are stronger and
808 more specialized in the tropics a zombie idea? *Biotropica*, 48, 141-145.

Moles, A. T., & Westoby, M. (2003). Latitude, seed predation and seed mass. *Journal of Biogeography*, 30, 105-128.

Nelson, E.B., & Karp, M. A. (2013). Soil pathogen communities associated with native and non-native *Phragmites australis* populations in freshwater wetlands. *Ecology and Evolution*, 3, 5254-5267.

Nguyen, D., Castagneyrol, B., Bruelheide, H., Bussotti, F., Guyot, V., Jactel, H., … Boberg, J. (2016). Fungal disease incidence along tree diversity gradients depends on latitude in European forests. *Ecology and Evolution*, 6, 2426-2438.

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., & Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, 20, 241-248.

Oates, J. D., Burdon, J. J., & Brouwer, J. B. (1983). Interactions between *Avena* and *Puccinia* species. II. The pathogens: *Puccinia coronata* CDA and *P. graminis* Pers.F.Sp. *avenae* Eriks, & Henn. *Journal of Applied Ecology*, 20, 585–596.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2019). vegan: community ecology package. R package version 2.5-5. https://CRAN.R-project.org/package=vegan.

Ollerton, J. (2012). Biogeography: are tropical species less specialised? *Current Biology*, 22, R914-R915.

Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J., … Zobel, M. (2010). The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist*, 188, 223–241.

Orson, R. A. (1999). A paleoecological assessment of *Phragmites australis* in New England tidal marshes: changes in plant community structure during the last few millennia. *Biological Invasions*, 1, 149-158.

Poore, A. G. B., Campbell, A. H., Coleman, R. A., Edgar, G. J., Jormalainen, V., Reynolds, P. L., … Duffy, J. E. (2012). Global patterns in the impact of marine herbivores on benthic primary producers. *Ecology Letters*, 15, 912–922.

Power, A. G., & Mitchell, C. E. (2004). Pathogen spillover in disease epidemics. *American Naturalist*, 164, S79–S89.

Pyšek, P., Skálová, H., Čuda, J., Guo, W.-Y., Doležal, J., Kauzál, O., … Meyerson, L. A. (2019). Physiology of a plant invasion: biomass production, growth and tissue chemistry of invasive and native *Phragmites australis* populations. *Preslia*, 91, 51-75.

842 Rasband, W. S. (2018). ImageJ, U.S. National Institutes of Health, Bethesda, Maryland,
843 USA, https://imagej.nih.gov/ij/, 1997-2018.
844 R Development Core Team. (2019). R: a language and environment for statistical computing.
845 Version 3.6.1. R Foundation for Statistical Computing, Vienna, Austria.
846 http://www.R-project.org.
847 Reinhart, K. O., & Callaway, R. M. (2006). Soil biota and invasive plants. *New Phytologist*,
848 170, 445–457.
849 Saltonstall, K. (2002). Cryptic invasion by a non-native genotype of the common reed,
850 *Phragmites australis*, into North America. *Proceedings of the National Academy of*
851 *Sciences of the United States of America*, 99, 2445-2449.
852 Sapkota, R., Knorr, K., Jørgensen, L. N., O'Hanlon, K. A., & Nicolaisen, M. (2015). Host
853 genotype is an important determinant of the cereal phyllosphere mycobiome. *New*
854 *Phytologist*, 207, 1134-1144.
855 Schemske, D. W., Mittelbach, G. G., Cornell, H. V., Sobel, J. M., & Roy, K. (2009). Is there
856 a latitudinal gradient in the importance of biotic interactions? *Annual Review of*
857 *Ecology Evolution and Systematics*, 40, 245–269.
858 Shearer, J. F., & Harms, N. E. (2012). Survey for pathogens of *Phragmites* in New York. US
859 Army Corps of Engineers Report ERDC/EL TN-12-1, U.S. Army Engineer Research
860 and Development Center, Vicksburg, Mississippi. 10p.
861 Spear, E. R., Coley, P. D., & Kursar, T. A. (2015). Do pathogens limit the distributions of
862 tropical trees across a rainfall gradient? *Journal of Ecology*, 103, 165-174.
863 Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., …
864 Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science*, 346,
865 1256688–1256688.
866 Terhonen, E., Marco, T., Sun, H., Jalkanen, R., Kasanen, R., Vuorinen, M., & Asiegbu, F.
867 (2011). The effect of latitude, season and needle-age on the mycota of Scots pine
868 (*Pinus sylvestris*) in Finland. *Silva Fennica*, 45, 301–317.
869 U'Ren, J. M., Riddle, J. M., Monacell, J. T., Carbone, I., Miadlikowska, J., & Arnold, A. E.
870 (2014). Tissue storage and primer selection influence pyrosequencing-based
871 inferences of diversity and community composition of endolichenic and endophytic
872 fungi. *Molecular Ecology Resources*, 14, 1032-1048.
873 van Kleunen, M., & Fischer, M. (2009). Release from foliar and floral fungal pathogen
874 species does not explain the geographic spread of naturalized North American plants
875 in Europe. *Journal of Ecology*, 97, 385-392.

Wellman, F. L. (1968). More diseases on crops in the tropics than in the temperate zone. *Ceiba*, 14, 17–28.

Whitaker, B. K., Reynolds, H. L., & Clay, K. (2018). Foliar fungal endophyte communities are structured by environment but not host ecotype in *Panicum virgatum* (switchgrass). *Ecology*, 99, 2703-2711.

Whitham, T. G., Gehring, C. A., Lamit, L. J., Wojtowicz, T., Evans, L. M., Keith, A. R., & Smith, D. S. (2012). Community specificity: life and afterlife effects of genes. *Trends in Plant Science*, 17, 271-281.

Yarwood, S. A., Baldwin, A. H., Gonzalez Mateu, M., & Buyer, J. S. (2016). Archaeal rhizosphere communities differ between the native and invasive lineages of the wetland plant *Phragmites australis* (common reed) in a Chesapeake Bay subestuary. *Biological Invasions*, 18, 2717-2728.

Data accessibility statement: Data have been archived in Dryad (doi: 10.5061/dryad.n2z34tmsv) and all sequences were deposited into GenBank (NCBI) under accession numbers MT000583-MY000643.

Table 1. Average foliar fungi richness and diversity metrics per population (± S.E.) for diseased leaves from each *Phragmites australis* lineage examined in the field survey. Different lowercase letters indicate significant differences between lineages for each diversity metric in post-hoc Tukey tests ($P \leq 0.05$).

	Phragmites australis lineage			
	Native	European	Gulf	Delta
Fungi isolate abundance	22.9 ± 1.09^{a}	19.4 ± 0.91^{ab}	22.0 ± 1.48^{ab}	15.2 ± 3.72^{b}
Total number of taxa	35	35	46	19
Singletons	11	8	13	4
Rarefied richness	4.1 ± 0.13^{a}	4.2 ± 0.20^{a}	4.5 ± 0.15^{a}	3.9 ± 0.42^{a}
Shannon diversity	1.8 ± 0.06^{a}	1.9 ± 0.12^{a}	2.0 ± 0.08^{a}	1.5 ± 0.21^{a}
Pathogen relative abundance	0.69 ± 0.04^{a}	0.58 ± 0.07^{ab}	0.30 ± 0.11^{b}	0.25 ± 0.09^{b}

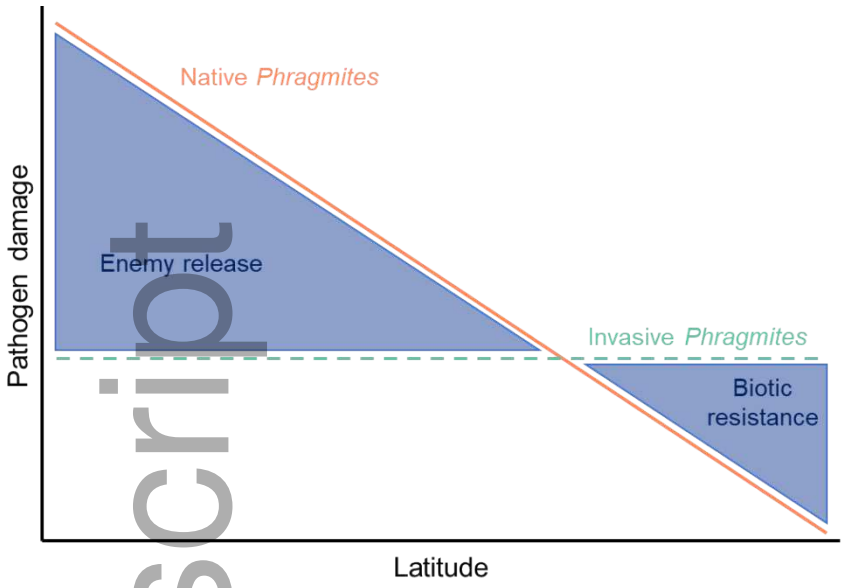

Figure 1. Predicted relationship between population latitude and pathogen damage for native (solid line) and invasive (dashed line) lineages of *Phragmites australis* in a North American field survey and Louisiana common garden experiment. Adapted from Bezemer, Harvey and Cronin (2014) and Cronin et al. (2015).

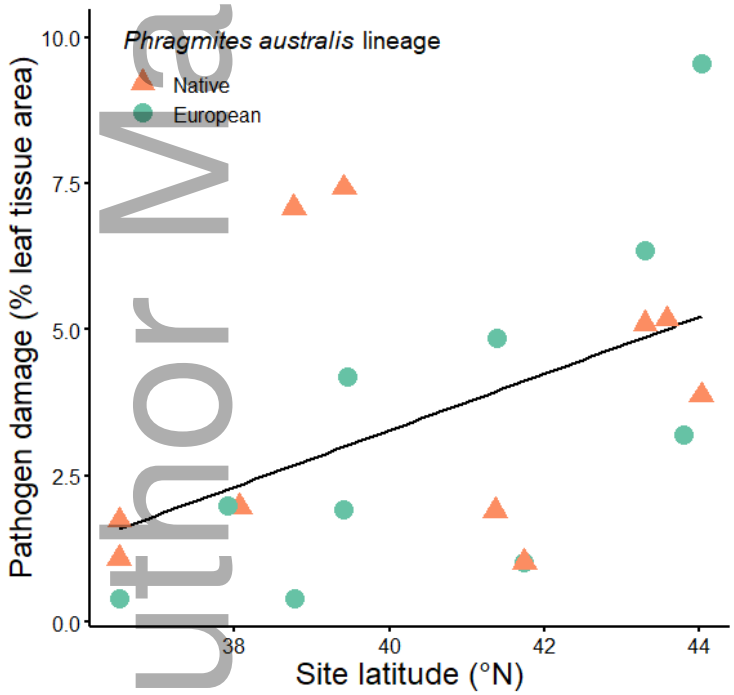

Figure 2. Relationship between population latitude and pathogen damage (% leaf tissue area with disease symptoms) for native (triangles) and European (circles) *Phragmites australis* lineages in the field survey. Line fit by least-squares regression for both lineages combined.

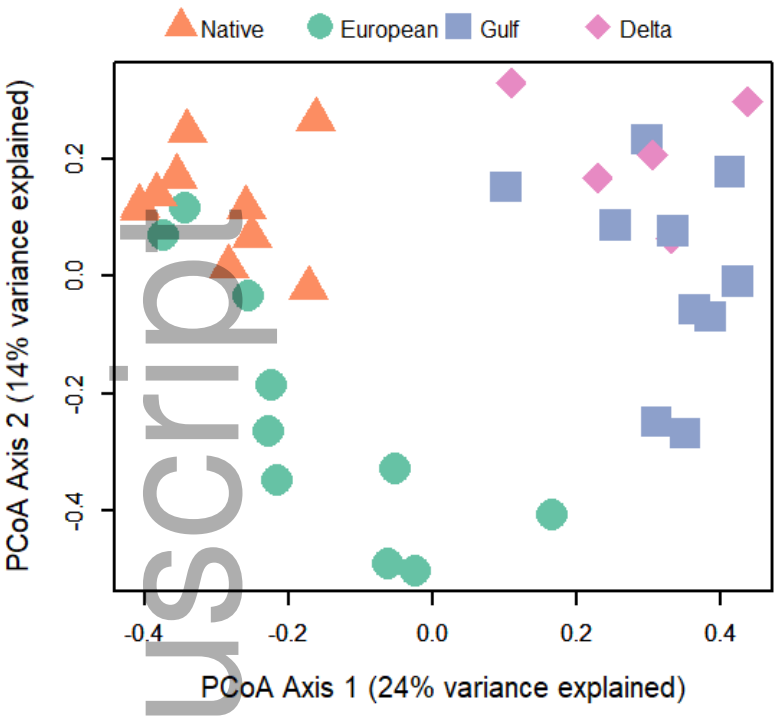

Figure 3. Ordination plot of principle coordinates analysis (PCoA) of Bray-Curtis dissimilarities among the fungi communities of diseased leaves from *Phragmites australis* populations belonging to four different lineages (native, European, Gulf, Delta). Each point represents a single *Phragmites* population, with points closer in ordination space having more similar fungal communities.

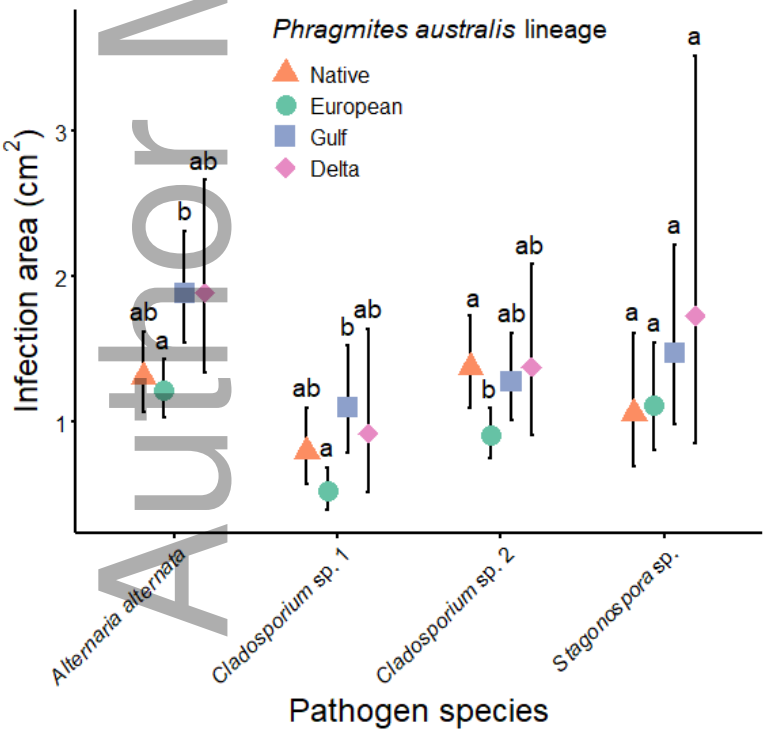

Figure 4. Area of infection (cm^2) (back-transformed least squares means ± 95% C.I.) of native, European, Gulf, and Delta lineages of *Phragmites australis*, inoculated using spore

solution from four foliar fungi isolates in a common garden experiment. Different letters indicate significant differences between lineages for each pathogen in post-hoc Tukey tests ($P \leq 0.05$).

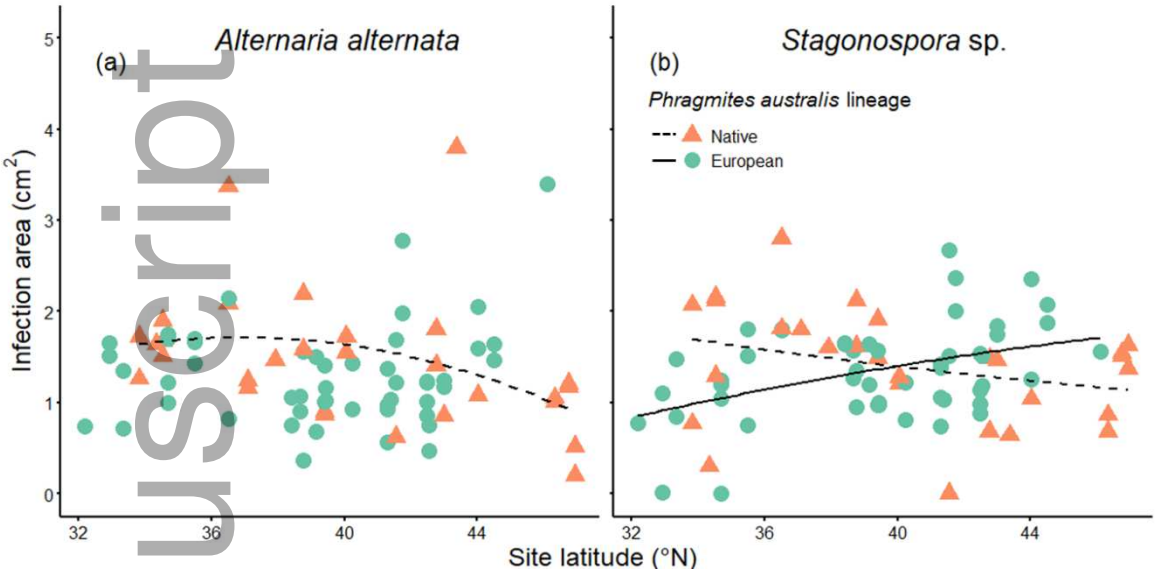

Figure 5. Relationships between population latitude and area of infection (cm^2) for populations of native and European *Phragmites australis* lineages inoculated with spore solutions from isolates of *Alternaria alternata* (a) and *Stagonospora* sp. (b) in a common garden experiment. Only lines (fit by least-squares regression) significant at the $\alpha = 0.05$ level are shown, meaning that there is no regression line presented for the European lineage in panel (a).