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Preface

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Geographic and genetic variation in susceptibility of *Butomus umbellatus* to foliar fungal pathogens

Abstract Large-scale patterns of plant invasions may reflect regional heterogeneity in biotic and abiotic factors and genetic variation within and between invading populations. Having information on how effects of biotic resistance vary spatially can be especially important when implementing biological control because introduced agents may have different impacts through interactions with host-plant genotype, local environment, or other novel enemies. We conducted a series of field surveys and laboratory studies to determine whether there was evidence of biotic resistance, as foliar fungal pathogens, in two introduced genotypes (triploid G1, diploid G4) of the Eurasian wetland weed, *Butomus umbellatus* L. in the USA. We tested whether genotypes differed in disease

attack and whether spatial patterns in disease incidence were related to geographic location or climate for either genotype. We surveyed 27 *B. umbellatus* populations (17 G1, 10 G4) to determine disease incidence and associated fungal pathogens. For a subset of plant populations, we isolated foliar fungi and tested pathogenicity of three isolates in laboratory assays. After accounting for location (latitude, climate), G1 plants had lower disease incidence than G4 plants in the field (38% vs. 70%) but similar pathogen richness. In contrast, bioassays revealed G1 plants consistently received a higher damage score and had larger leaf lesions regardless of pathogen. The seemingly contradictory results between the field and laboratory may be due to climatic differences between areas that limit the regional pool of pathogens or their effect on plant genotype. These results demonstrate that two widespread *B. umbellatus* genotypes exhibit different susceptibility to pathogens and effectiveness of pathogen biological controls may depend on local conditions.

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Introduction

Investigations into large-scale patterns of plant invasions are important for understanding variable impacts by invaders in the introduced range and for predicting potential management outcomes (Allen et al. 2017; Cronin et al. 2015; Gaskin et al. 2013; He and Rocchini 2013; Ordóñez and Olff 2013). Such spatial variation in invader impacts may be the result of differences in local-site characteristics (e.g., community composition or structure, soil type, resource availability), regional differences in climate or biotic limiting factors (e.g., novel predators or competitors) (Schaffner et al. 2011; Stricker et al. 2016; Wolfe et al. 2004), or genetically-based variation in key invader traits (e.g., dispersal, competitive ability, enemy resistance) (Maron and Vilà 2008; Rejmanek and Richardson 1996; Richardson and Pyšek 2006; Van Kleunen et al. 2010). In particular, geographic (e.g., latitudinal) differences in community resistance to invaders (i.e., biotic resistance) or susceptibility of the invader to novel enemies may be expected when the invaded range is very large (i.e., continental) (Cronin et al. 2015; Maron and Vilà 2008). Biotic resistance thus represents a spatially variable ecosystem service through prevention of establishment and consequent negative impacts by plant invaders (Levine et al. 2004). Although examinations of its role in invasion success often take place at the local or regional scale, ignoring possible geographic variation in the strength of biotic resistance, a number of authors have taken a larger-scale approach (Allen et al. 2017; Castillo et al. 2018; Cronin et al. 2015; DeRivera et al. 2005; Freestone et al. 2013; Parker et al. 2006).

Geographic variation in biotic resistance or invader success may result from genetic variation within the invading plant species that is spatially heterogeneous. Spatial variability in propagule pressure, or founder or bottleneck processes during plant introduction and establishment (Sax et al. 2005) may lead to multiple introduced genotypes that do not interbreed or have limited gene flow between them. Cryptic invasions involving multiple genotypes have been reported in a number of instances (Burrell et al. 2015; Morais and Reichard 2018; Mukherjee et al. 2012; Saltonstall 2002; Tano et al. 2015). An example is the aquatic invasive plant, *Hydrilla verticillata* (L.f.) Royle which was introduced at least twice into the U.S during the 20th century, resulting in two widespread ecologically

and genetically distinct haplotypes which vary in their response to introduced biological controls (Grodowitz et al. 2010; Madeira et al. 2004). Several studies have demonstrated that enemy release or biotic resistance can vary considerably among genotypes of the same plant species in the introduced range (Allen et al. 2017; Cronin et al. 2015; Maron and Vilà 2008; terHorst and Lau 2015). However, most studies examining these differences focused on generalist herbivores (e.g., Cronin et al. 2015; Liu et al. 2018; Siemann and Rogers 2003), specialist herbivores (e.g., Garcia-Rossi et al. 2003; Liu et al. 2018; Maron and Vilà 2008), or specialist pathogens (Burdon et al. 1981) in the introduced range, with limited examination of generalist pathogens (Maron and Vilà 2008). Additionally, there is evidence that intraspecific variation in chromosome number (i.e., ploidy) may generate patterns in which increased ploidy leads to broader environmental tolerances through an enhanced adaptive potential, creating a more invasive phenotype (Hahn et al. 2012; Hao et al. 2013; Levin 1983; Pandit et al. 2011; te Beest et al. 2012). For instance, increased chromosome numbers in the genus *Leucanthemum* resulted in greater resistance to herbivory by a specialist insect (Stutz et al. 2016). However, historical interactions with herbivores and local adaptation may be more important than ploidy in other cases (Meyerson et al. 2016). The importance of ploidy in plant invasions for structuring associated herbivore communities and influencing herbivore performance has received some attention (Pandit et al. 2014), but still relatively little is known about the role of plant genetic variation in invasive plant—disease dynamics. For an invading species with multiple ploidy levels in the invaded range, higher chromosome number is expected to produce disease-resistant phenotypes if increased ploidy is associated with higher allelic diversity at, or increased expression of, immune genes (King et al. 2012; Oswald and Nuismer 2007).

An ideal study system for investigating geographic and genetic variation in pathogen susceptibility in a plant invasion is *Butomus umbellatus* L. (Butomaceae; flowering rush), an invasive wetland plant of Eurasian origin. In North America, *B. umbellatus* populations are either diploid or triploid (Kliber et al. 2005). Populations in the Northwest, upper Midwest, and far Northeastern US comprise the widespread triploid cytotype (genotype 1; G1), whereas a diploid cytotype

(genotype 4; G4) occurs primarily in the Northeast and Great Lakes region (Lui et al. 2005). In total, seven AFLP genotypes (G1, G2, G3, G4, G5, G6, G9) have been documented thus far in North America (Gaskin, unpublished data). Other than G1 and G4, other introduced genotypes are exceedingly rare, many only identified from a single location. Although G1 and G4 plants have not been documented to co-occur at the same location, they are sympatric in the upper Midwest. As management tools are developed for *B. umbellatus* in the US, it is necessary to better understand the importance of genetic variability on factors that limit plant performance. Because biological control agents are under development currently in Europe, a better understanding of the variability of plant response to natural enemies would increase likelihood of using future agents effectively, potentially by allowing managers to choose agents based on host genotype.

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

Materials and methods

Study system

Butomus umbellatus is an introduced wetland monocot, first documented in North America in the Saint

Lawrence River in the late 1800s (Knowlton 1923). The earliest U.S. populations were reported from River Rouge, MI in 1918 (Anderson et al. 1974) and subsequently throughout the Great Lakes region (Witmer 1964). Although present for nearly 100 years, spread of *B. umbellatus* has been limited mostly northward from the Great Lakes into Canada, with the southernmost record in Colorado (Barger and Moorhead 2007). Infestations now persist across the northern tier of the U.S. and evidence for multiple introductions from separate source areas is strong (Anderson et al. 1974). Spread is primarily clonal, but reproductive ability varies between sexual (diploid plants) and vegetative (diploid and triploid plants) forms (Eckert et al. 2000, 2003). Although populations in the native European range are thought to be mostly triploid, in North America the diploid G4 cytotype is most common (Kliber et al. 2005).

Field survey: disease

To examine whether there were geographic and genotype differences in frequency of disease, we surveyed 27 *B. umbellatus* populations (17 G1 and 10 G4 populations; Fig. 1; Supplement 1) during mid-June to early September over 3 years (2014–2016). Sites were located by a variety of means, including internet database searches (e.g., www.eddmaps.org), consultation with state personnel (Minnesota Department of Natural Resources, Washington State Department of Ecology), and chance encounters during transit. Surveyed sites spanned approximately 9 degrees latitude (~ 1000 km). At each site, leaf tissues were collected from at least 10 plants separated by ~ 1 m for genotyping by amplified fragment length polymorphism polymerase chain reaction (AFLP-PCR). Ploidy determination was made by flow cytometry (e.g., Bohanec 2003; Delaat et al. 1987). Previous results confirmed that plants within sites were the same genotype (i.e., we have not detected multiple co-occurring genotypes in our sites) (J. Gaskin, unpublished data). Sites were sampled between mid-July and early-September.

Sampling and examination protocols were as described in Harms and Shearer (2015). At each site, 20 whole *B. umbellatus* ramets were excavated for examination and damage assessment. Ramets were collected by hand on shore, by wading in shallow water, or from a boat. Care was taken to sample

Fig. 1 Map of *B. umbellatus* census sites. Blue squares represent sites with the triploid genotype G1 and red triangles are sites with the diploid genotype G4. Counties where *B. umbellatus* has been recorded are yellow (EDDMaps.org)

separate plants, though in some instances plants may have been connected underground. Within 12 h of sampling, plants were examined with a hand lens and presence or absence of disease symptoms (leaf lesions, discoloration or distinct leaf spots) (Harms and Shearer 2015) was recorded. For each site, we determined the proportion of sampled plants that displayed disease symptoms.

Field survey: fungal richness

To determine whether fungal richness varied with *B. umbellatus* genotype or environment, during 2016, we collected diseased leaf material. At 18 sites (9 G1 and 9 G4) (Supplement 1), we excised approximately 5 cm leaf sections from five plants per site. Leaves were kept refrigerated and were processed in the laboratory within five days of collection. Sections of tissue were surface sterilized in 10% bleach for one minute then rinsed in sterile water. The sections were subsequently inserted into slits cut into Martin's Agar (Martin 1950) plates and incubated in the dark at room temperature (20–22 °C) for 1 week. Fungal isolates that emerged from the tissues were transferred to Potato Dextrose Agar (PDA) and Corn Meal Agar (Difco, Detroit MI) slants for preservation. They were also plated onto PDA and Potato Carrot Agar (Dhingra and Sinclair 1995) for identification purposes. Isolates were identified using morphological characteristics and taxonomic literature (Domsch et al. 1980; Ellis 1971; Weir

et al. 2012). Using literature reports, we categorized each fungal species as pathogenic or not. In some cases, the literature was ambiguous (i.e., a species may be a facultative pathogen). In cases where we suspected the species was not pathogenic, or only sometimes pathogenic, we categorized it as non-pathogenic. For some taxa, we were unable to obtain satisfactory taxonomic resolution (e.g., a number of Dematiaceous or Moniliaceous Ascomycetes) so made no determination on their pathogenicity. With categorization of isolates, pathogen richness was determined for each site and compared between genotypes.

Climatic environmental data

Climate data for surveyed locations were extracted from the first three principal components (PCs) of the 35 bioclimatic variables in the CliMond 1975H dataset (Kriticos et al. 2014). The three PCs differ in the influence of various climate variables, with PC1 (Bio36) being primarily a temperature variable, PC2 (Bio37) a wetness index, and PC3 (Bio38) a dryness index (Kriticos et al. 2014). We used these PCs to obtain climate information for each survey location and as potential explanatory variables in statistical model selection below.

Leaf infection experiment

From fungal species isolated during 2016, we experimentally tested whether G1 plants were more resistant to infection by fungal pathogens than G4 plants. Plants used in this experiment were field-collected in 2016 and propagated repeatedly at the Engineer Research and Development Center (ERDC), Vicksburg, MS until July 2017. Plants were initially grown outdoors in commercially-available topsoil supplemented with Osmocote® slow release fertilizer (15-9-2; Scotts Miracle-Gro, Marysville, OH). After a season of growth, main rhizomes or rhizome branches were split into ~ 3 cm pieces and planted into new topsoil. Diploid plants produce relatively little rhizome material so in addition to rhizome splitting, we planted the corm-like bulbils. This procedure was repeated 2 times over the course of 1 year to reduce maternal effects (Roach and Wulff 1987).

For each genotype, we used four replicate populations from our garden (Table 1), chosen because sufficient plant material was available for the experiment. Plants were grown in two shallow tanks in a greenhouse for 6 weeks before the experiment. Charcoal-filtered water was delivered from the local municipal water supply and maintained at 5 cm below the sediment surface prior to the experiment.

To test for differences between genotypes in resistance to foliar pathogens, we conducted an excised-leaf assay. This type of assay has been validated in other pathogen-plant systems (Bussey and Stevenson 1991; Pratt 1996) (including *Colletotrichum sublineolum* P. Henn. on sorghum and *Alternaria solani* (Ell. and Mart.) Jones and Grout. on potatoes) and comparisons with traditional greenhouse whole-plant assays are consistently similar (Prom et al. 2015). In addition to the excised leaf experiment,

we conducted a smaller whole-plant experiment which generated similar results (Supplement 2) but report only the excised-leaf experiment here. We inoculated leaves with one of three plant fungal pathogens, *Plectosphaerella cucumerina* Kleb., *Colletotrichum fioriniae* Marcelino & Gouli ex R.G. Shivas & Y.P. Tan, and the ubiquitous *Alternaria alternata* (Fr.) Keissl. These fungal species were chosen because they have previously been reported as plant pathogens (Agrios 2015; Uecker 1993). *Plectosphaerella cucumerina* was present in three G1 and seven G4 sites from the northeastern to northwestern USA during our surveys; *C. fioriniae* was identified from two G4 and a single G3 site in the northeastern and upper Midwestern US and *A. alternata* from all surveyed sites. Fungal species were isolated from G4 plants at Kildeer Pond, OH (*P. cucumerina* and *A. alternata*) and G3 plants in Springbrook Pond, IL (*C. fioriniae*), then cultured in bulk for this experiment using previously reported methods detailed in Supplement 2. A potential drawback of using pathogens isolated from G4 plants (*P. cucumerina* and *A. alternata*) is that they are adapted to that genotype. Colony forming units (CFUs) for all isolates were 1×10^5 CFUs. Leaf sections (10 cm) were cut from culture plants and randomly assigned to one of three pathogen treatments: *A. alternata*, *C. fioriniae*, or *P. cucumerina*. Leaf pieces were lightly abraded with 200 grit sandpaper then placed on water agar in petri dishes (six per treatment combination). Previously prepared inoculum (100 μ l) was applied to the abraded leaf area. Petri dishes were covered and left on the benchtop at room temperature (~ 23 °C) for 48 h. After 48 h, petri dish lids were removed and leaf photographs were taken with a Nikon D60 digital camera. Photographs were imported into ImageJ image processing software (Rasband 2016) then lesion area (mm²) and damage score were determined.

Table 1 Flowering rush populations used in this study

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

The damage score used here is similar to that previously applied by Shearer et al. (2011) and is a qualitative assessment of leaf condition on an ordinal scale (Table 2). We defined the damage scale so that levels of damage were approximately equally-spaced on the scale. The same observer (NEH) made all damage assessments. A higher damage rating represents lower resistance to infection. Examples of infected leaves assigned to various scores are provided in Supplement 3.

Statistical analyses

Field survey: disease

To test whether there were latitudinal gradients in the proportion of infected *B. umbellatus* plants and whether they differed with plant genotype or climate, we used a generalized linear model with beta error distribution and log-link function. Proportion of diseased plants was the dependent variable in the model, genotype (G), latitude (L), the genotype by latitude (GxL) interaction, and each of the three bioclimatic PC's (PC1, PC2, PC3) were included as predictors. The interaction between genotype and latitude was included in the model because nonparallel gradients in species interactions can result from genotype-specific differences in disease or herbivore resistance across latitudes (e.g., Cronin et al. 2015). Although in some cases longitude is an appropriate spatial predictor variable, in this case, longitude and PC3 were highly correlated ($r = 0.91$), and so longitude was removed from the analysis. Sampling occurred over multiple years with nine sites out of 28 sampled during 2 years. To account for this, Year (Y) was included in the model as a random effect (Kwong et al. 2017).

Additionally, we used Akaike information criterion adjusted for small sample size (AICc) to select the

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

Field survey: fungal richness

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

Leaf infection experiment

We did not have enough common garden populations to provide a rigorous test for genetic-based latitudinal clines in disease. Therefore, in laboratory trials we tested only for genetic differences in disease resistance. We predicted that our results would support

Table 2 Damage rating applied to infected *B. umbellatus* leaves

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

field observations that G4 plants are more susceptible to disease than G1 plants. As such, the former genotype was predicted to have greater lesion size and higher damage score than the latter genotype. To test for differences in lesion size (excised-leaf experiment) we used generalized linear models with normal distribution and log-link function. In models, genotype was a fixed effect and population was a random effect to account for the nesting of populations within a genotype (Bhattarai et al. 2017). To test whether disease rating was higher in G4 plants, we used generalized linear mixed models with multinomial error distribution and cumulative logit link function (Gbur et al. 2012). In the excised-leaf disease rating model, population within genotype was random and genotype was a fixed effect. Separate models were used for each pathogen species in both experiments. Additionally, because we used two pathogens (*A. alternata*, *P. cucumerina*) originally isolated from a diploid G4 population (Kildeer Pond) in our experiment, we conducted a comparison of damage rating and lesion size between diploid populations only. A difference in damage rating or lesion size between plants from Kildeer Pond and other diploid populations might suggest local adaptation in these fungal pathogens to *B. umbellatus* populations. As above, to test for differences in damage rating between diploid populations, we used a generalized linear model with multinomial error distribution and cumulative logit link function with population as a fixed effect. To test for differences in lesion size, we used generalized linear models with normal distribution and log-link function and population was a fixed effect. As above, separate models were used for each pathogen.

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

Results

Field surveys: disease incidence

Across the northern tier of the US, latitude, genotype, and climate (separate from latitude) influenced patterns of disease incidence in *B. umbellatus*. Specifically, variation in the proportion of plants with disease symptoms was equally explained by two top candidate models which included genotype, latitude, the

genotype x latitude interaction and either the temperature (PC1; adj. $R^2 = 0.48$, AICc = -9.61 , Akaike weight = 0.26; Table 3) or wetness (PC2; adj. $R^2 = 0.45$, AICc = -9.69 , Akaike weight = 0.27) principal component. Regarding our first hypothesis that latitude would influence disease occurrence, the relationship was nonparallel between genotypes (Fig. 2; GxL: $F = 6.74$, $P = 0.02$). Separate models for each genotype detected a significant relationship between latitude and disease for G1 but not G4 populations (G1: $F = 6.31$, $P = 0.02$; G4: $F = 1.4$, $P = 0.27$). Our second hypothesis, that triploid G1 plants would display less disease incidence than G4 plants, was confirmed. The proportion of plants with disease symptoms was nearly double for G4 (0.75 ± 0.1 , mean $\pm$ SE) than G1 (0.39 ± 0.05) plants after accounting for effects of latitude and climate in the top model (Fig. 2). Bioclimatic variables were influential in five out of the top six models. In the top model, PC2 (wetness index) was significantly influential (df = 1, $F = 5.44$, $P = 0.03$). In general, disease incidence was positively correlated with PC2 ($r = 0.25$).

Field surveys: pathogen richness

We recovered 39 species of fungi from *B. umbellatus* during our surveys, including 20 that were deemed likely pathogenic. The AICc top model explained little variation in fungal richness among *B. umbellatus* populations and included only a single variable (Genotype) (Table 3; AICc = 69.49, Akaike weight = 0.21, Adj. $R^2 = 0.009$). G4 plants had, on average, 37% more associated pathogen species than G1 plants (G4 pathogen richness: 3.33 ± 0.43 mean $\pm$ SE; G1 pathogen richness: 2.44 ± 0.43). In addition to the top model, seven other candidate models emerged as having substantial support (Δ AICc ≤ 2); five included climate variables, two included latitude, and six included plant genotype (range of AICc = 69.89–71.49, Akaike weight = 0.21–0.08). The inclusion of genotype in the majority of top models, and its large effect size, strongly support its importance in determining the number of pathogens infecting *B. umbellatus* in the USA.

Table 3 Top best-fit models for the proportion of diseased *B. umbellatus* plants collected during field surveys and *B. umbellatus*–associated pathogen richness, based on AICc selection procedure

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

G Genotype, L latitude, PC1, PC2 and PC3 are bioclimatic principal components (see “Methods”)

Fig. 2 From field surveys for disease, the relationship between latitude and the proportion of plants with disease symptoms for the two common introduced *B. umbellatus* genotypes (based on the AICc-best model; Table 1). The solid black line (and square points) represent genotype G1, and the gray line (and gray dots) is genotype G4. The AICc-best model includes the interaction term (G x L). Genotype means ($\pm$ SE) are displayed in the inset and statistically significant differences between means noted with an asterisk (df = 1, 27; $F = 6.89$, $P = 0.01$). Lines are fit by least-squares regression (G1: Proportion plants diseased = $2.58 - 0.050 \times \text{Latitude}$; G4: Proportion plants diseased = $-1.69 + 0.055 \times \text{Latitude}$)

Laboratory excised-leaf experiment

By multiple measures, G1 leaves were more susceptible to infection than G4 leaves in the excised-leaf experiment (Fig. 3). Damage ratings assigned to G1 leaves were approximately 100%, 150%, and 45% higher than G4 when infected by *P. cucumerina* (df = 1, $F = 10.95$, $P = 0.02$), *C. fioriniae* (df = 1, $F = 10.72$, $P = 0.02$), and *A. alternata* (df = 1, $F = 5.14$, $P = 0.06$), respectively. Mean lesion size, although not significantly different, was 80% larger in G1 leaves infected by *C. fioriniae* (df = 1, $F = 4.15$, $P = 0.09$), 24% for *P. cucumerina* (df = 1, $F = 2.57$, $P = 0.17$), and 7% for *A. alternata* (df = 1, $F = 0.16$, $P = 0.70$). Within diploid plants, there was no evidence of local adaptation for either *A. alternata* or *P. cucumerina* (Fig. 3). Damage rating and lesion sizes were not significantly different between diploid populations (*A. alternata* damage rating: df = 3, $F = 0.42$, $P = 0.74$; lesion size: df = 3, $F = 0.2$, $P = 0.89$; *P. cucumerina* damage rating: df = 3, $F = 1.27$, $P = 0.31$; lesion size: df = 3, $F = 0.5$, $P = 0.69$).

Fig. 3 Mean ($\pm$ SE) leaf lesion area for leaves of *B. umbellatus* infected by the generalist pathogens *Plectosphaerella cucumerina*, *Colletotrichum fioriniae*, and *Alternaria alternata*. Genotype means are indicated by dashed lines. Site abbreviations are as follows: FL flathead lake, PO pend oreille river, RP rose pond, YR yakima river, KP kildeer pond, OR oswegatchie river, PRM point rosa marsh, UI unity Island

Discussion

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

Geographic and genetic variation in the effects of biotic resistance during plant invasions is most likely common, and support that biotic resistance is important in determining large-scale patterns of invasion is gaining (Allen et al. 2017; Bhattarai et al. 2017; Cronin et al. 2015; Freestone et al. 2013; Parker et al. 2006). For example, recent investigations of the grass *Phragmites australis* (Cav.) Trin. Ex Steud. have found nonparallel latitudinal gradients in foliar and stem-herbivore impacts between native and invasive haplotypes of *P. australis* in marshes of North America (Cronin et al. 2015). In that case, native

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

Genetic variation in disease susceptibility and implications for biological control

In contrast to the two-fold higher pathogen incidence on G4 versus G1 plants in the field, we found pathogenicity in the laboratory was higher for G1 plants. Reasons for these seemingly contradictory findings are currently under investigation but may reflect differences in residence time between introduced taxa and associated pathogen accumulation, variable developmental stage-resistance relationships, novel associations with pathogens, environmental variation across the invaded range, or some combination of the above. Escape from pathogens is likely to explain invasion success in some taxa (Torchin and Mitchell 2004), but the importance of pathogen escape for *B. umbellatus* is unknown because native range surveys for damaging pathogens is lacking. Separate from latitudinal patterns, the difference in pathogen accumulation and impact between introduced genotypes in the US may be due to differences in residence time between them (Mitchell et al. 2010). Thus, the

older populations of G4 plants could be expected to have a larger pathogen pool associated with them, a pattern which was confirmed during our study. This may help explain why disease was more common on G4 plants during field surveys but not in laboratory experiments. Another possible explanation for contrasting field and laboratory results is that susceptibility to natural enemies varies during developmental stages and between genotypes. The importance of developmental susceptibility has been demonstrated in a number of plant systems and is actually widespread (Develey-Rivière and Galiana 2007). If disease susceptibility changes with age, but the rate of change differs between genotypes, then this could explain the pattern we observed because greenhouse experiment plants were all less than 1 year old (i.e., planted just before experiments) and plants at field sites were likely a range of ages.

Despite using pathogens isolated from multiple genotypes, consistent damage patterns were documented regarding pathogenicity to G1 and G4 plants. Local adaptation to host plant taxa by pathogens (Croll and McDonald 2017; Gandon and Van Zandt 1998), if occurring in populations of *B. umbellatus*, may have produced the opposite pattern than we observed in the laboratory and isolates should have performed better on their local hosts. We used strains of *P. cucumerina* and *A. alternata* isolated from G4 plants at Kildeer Pond, OH, one of the populations used in our experiments. Damage to Kildeer Pond experimental plants was not significantly higher than damage to other G4 populations for either pathogen. Likewise, our conclusions based on observed genotypic differences in infection would remain the same if only taking into account *C. fiorinae*. Local adaptation by pathogens has been previously observed in other plant-pathogen systems and is indicated by increased performance of the pathogen on the local host over foreign hosts (Bowen et al. 2017; Hokkanen and Pimentel 1989; Laine 2005, 2007).

Spatial variation in biotic interactions has clear importance to management of invasive plants using biological control agents. Currently, a number of insect herbivores and the rust fungus *Doassansia niesslii* De Toni (Exobasidiomycetes: Doassansiaceae) are under examination for their potential as biological control agents of *B. umbellatus* in North America. From research in Europe, there is an indication that *B. umbellatus* plants of different ploidy

levels vary in their susceptibility to infection, although the range of plant populations used so far has been limited. In our study, we used generalist pathogen taxa encountered during domestic surveys in the US. Unfortunately, we do not know whether results of the current study using generalist pathogens will be transferrable to predict impacts of specialist biological control pathogens if approved for introduction in the US. Additionally, many European populations are genotypes which have not been found yet in the US. To develop better predictive assays, a broader range of North American genotypes should be included in efficacy tests for prospective biological control agents.

It is now well known that both host- and agent-genotype effects on biological control success can be substantial and spatially variable (Boughton and Pemberton 2011; Mukwevho et al. 2017, 2018). For example, biological control agents of *Hydrilla verticillata* vary in performance between dioecious and monoecious genotypes in the US (i.e., host-genotype effects), genotypes which occur mostly in separate geographic areas (i.e., monoecious hydrilla has a northern US distribution and dioecious hydrilla has a southern distribution). This has generated interest in introducing agents that are better-adapted to specific host genotypes and led to additional overseas exploration for new agents (Grodowitz et al. 2010; Harms and Grodowitz 2011; Harms et al. 2017; Purcell et al. 2019). Likewise, cryptic species of *Diorhabda* beetles were introduced for control of saltcedars (*Tamarix* spp.) in the western US, leading to variable control in introduced areas due to both climatic limitations on beetles and variable host-agent interactions between the beetles and several saltcedar species (DeLoach et al. 2007; Tracy and Robbins 2009). Similarly, unsuccessful biological control of giant salvinia (*Salvinia molesta* Mitchell) and waterhyacinth (*Eichornia crassipes* (Mart.) Solms) in some southern US locations due to climate limitations has led to exploration for more cold-hardy (*Cyrtobagous salviniae* Calder and Sands) (Russell et al. 2017) or heat-tolerant (*Megamelus scutellaris* Berg) (Foley et al. 2016; Freedman and Harms 2017) agents. These examples highlight management programs in which spatially variable control has been attributed to genetic or climate limitations on agents. In the current study, we demonstrated that introduced *B. umbellatus* genotypes have different susceptibilities to foliar fungal pathogens and disease incidence varied with latitude for one

but not the other genotype. This suggests that it may be necessary to consider biological control agents of *B. umbellatus* that are genotype, climatic, or latitude-specific.

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14. ABSTRACT Large-scale patterns of plant invasions may reflect regional heterogeneity in biotic and abiotic factors and genetic variation within and between invading populations. Having information on how effects of biotic resistance vary spatially can be especially important when implementing biological control because introduced agents may have different Impacts through interactions with host-plant genotype, local environment, or other novel enemies. We conducted a series of field surveys and laboratory studies to determine whether there was evidence of biotic resistance, as foliar fungal pathogens, in two introduced genotypes (triploid G1, diploid G4) of the Eurasian wetland weed, <i>Butomus umbellatus</i> L. in the USA. We tested whether genotypes differed in disease attack and whether spatial patterns in disease incidence were related to geographic location or climate for either genotype. After accounting for location (latitude, climate), G1 plants had lower disease incidence than G4 plants in the field (38% vs. 70%) but similar pathogen richness. In contrast, bioassays revealed G1 plants consistently received a higher damage score and had larger leaf lesions regardless of pathogen. These results demonstrate that two widespread <i>B. umbellatus</i> genotypes exhibit different susceptibility to pathogens and effectiveness of pathogen biological controls may depend on local conditions.					
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