Multiple Potential Stressors and Dieback of *Phragmites australis* in the Mississippi River Delta, USA: Implications for Restoration

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Abstract

In 2016, widespread dieback of *Phragmites australis* was reported in the Mississippi River Delta (MRD), Louisiana. We conducted two common-garden experiments to investigate several potentially important factors associated with this dieback: scale insects, water depth, fertilization and *P. australis* genetics (three lineages: Gulf, Delta and invasive EU). Predictions tested were scale abundance is lower in high water, at low fertilization, and for EU; plant biomass is negatively impacted by scales, high water and high fertilization; and EU suffers the least damage from the three potential stressors. Scale abundance was 41% lower in high water and decreased 2.7 fold as fertilization increased. Also, EU had 1.5–2.6 times fewer scales than Gulf, but had similar scale abundance to Delta. Impacts of scales, water depth and fertilizer on plant biomass depended strongly on lineage. Scales reduced biomass of Delta, EU and Gulf by 38%, 32% and 10%, respectively. In comparison, biomass was 30% higher for EU, 46% lower for Gulf and unchanged for Delta in high versus low water. Finally, at high fertilization levels, Gulf produced 57% more biomass than EU. Owing to its greater tolerance to scales and high water, EU may be most suitable for use in restoration of the MRD.

Keywords Dieback · Herbivory · Fertilization · *Nipponaclerda biwakoensis* · Scale insects, water depth

Introduction

The fragility of coastal wetlands and marsh ecosystems is clearly evident from the relatively common occurrence of widespread dieback of dominant or foundation plant species. Dieback is typically characterized by stunted growth of stems, premature senescence of leaf tissue, the presence of dead meristematic tissue, and increased patchiness in the distribution of plants, usually over broad spatial scales (Mendelssohn and McKee 1988; Armstrong et al. 1996b; Clevering 1997; van der Putten 1997; Alber et al. 2008; Gigante et al. 2011). Dieback has occurred with salt-marsh cordgrass (*Spartina alterniflora* Loisel = *Sporobolus alterniflora*; Poaceae) in the USA (Mendelssohn and McKee 1988; McKee et al. 2004; Alber et al. 2008; Elmer et al. 2013), common reed (*Phragmites australis* (Cav) Trin. ex Steud.; Poaceae) throughout Europe (van der Putten 1997; Brix 1999) and in China (Li et al. 2013), seepweed (*Suaeda salsa* L.) in China (He et al. 2017), and mangrove forests in Australia, South Africa and the Caribbean (Breen and Hill 1969; Duke et al. 2017; Lovelock et al. 2017; Rossi et al. 2020). These ecosystems have tremendous value because they are as productive as tropical forests and coral reefs, provide habitat and shelter for an extraordinary diversity of animals, play an essential role in global CH_4 and CO_2 sequestration and exchange, stabilize and prevent erosion of river, lake and coastal margins, absorb excess nitrogen and phosphorus from sewage and fertilizer runoff, and are vital to the fisheries industry (Zedler and Kercher 2005; Bertness 2007; Mitsch and Gosselink 2015). Understanding the causes of large-scale diebacks is an essential step toward the development and implementation of a management plan that restores these ecosystem services.

Phragmites australis has a cosmopolitan distribution and is often the dominant plant species in freshwater marshes and mesohaline coastal wetlands (Clevering and Lissner 1999;

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Lambertini et al. 2006). In Europe, scientists observed dieback of common reed dating back to at least the 1950s and over the ensuing years dieback has been observed throughout much of the continent (Ostendorp 1989; van der Putten 1997; Brix 1999; Gigante et al. 2014). It should come as no surprise that virtually all of the proposed causes for *P. australis* dieback tie directly back to human activities – eutrophication, hydrological changes in the environment (e.g., sea-level rise, flooding), increasing temperature, loss of genetic diversity, toxic chemicals and introduced natural enemies (e.g., Armstrong et al. 1996a; van der Putten 1997; Brix 1999; Nechwatal et al. 2008; Li et al. 2014). According to van der Putten (1997) and Brix (1999), the prevailing opinion is that waterlogging, primarily via sediment anoxia and high sulfide concentrations, and/or eutrophication through the accumulation of organic matter are responsible for the dieback. Eutrophic conditions result from an excess of nutrients derived as runoff from nearby agricultural or urban areas (Khan and Ansari 2005). To date, natural enemies have been implicated as causes of dieback in several instances (Ostendorp 1989; Armstrong et al. 1996a; Nechwatal et al. 2008; Li et al. 2014). Herbivorous insects mostly have been dismissed as factors in *P. australis* dieback (van der Putten 1997; Brix 1999; but see Armstrong et al. 1996a; Tscharntke 1999) despite reports of them being responsible for localized dieback; i.e., of single stands (Cronin et al. 2015).

In North America, dieback of *P. australis* was first reported in Michigan in the early 1950s, concurrent with the dieback of other emergent wetland species, and was attributed to rising and prolonged high water levels (McDonald 1955). More recently, large scale dieback of *P. australis* was detected in the Fall of 2016, affecting stands across the Mississippi River Delta (MRD), Plaquemines Parish, Louisiana, USA (Knight et al. 2018). Through remote sensing and the analysis of NDVI (normalized difference vegetation index) from LandSat imagery, widespread dieback of *P. australis* in the MRD has been confirmed (Ramsey III and Rangoonwala 2017; Suir et al. 2018). Concomitant with the dieback syndrome was the discovery of a non-native scale insect, *Nipponaclerda biwakoensis* (Kuwana) (Hemiptera: Aclerdidae; hereafter, scale insect), that spanned the MRD and occurred at outbreak levels (Knight et al. 2018). During the summer, at peak scale activity, nearly 100% of stems are infested, with live scales per meter of stem averaging 150 (Knight et al. 2020). Support for the role of scale insects in the dieback of *P. australis* is that NDVI is negatively correlated with scale abundance (I. A. Knight, G. Suir and R. Diaz, unpublished data). Interestingly, the MRD consists of multiple genetically, morphologically and ecologically distinct *P. australis* lineages, including both native and invasive lineages (see below), that appear to differ in their susceptibility to scales and dieback (Knight et al. 2018; Knight et al. 2020).

Previously, Knight et al. (2020) investigated the interactive effects of scale insects and salinity on *P. australis* performance and found that both factors influenced scale abundance and *P. australis* growth parameters. However, salinity levels in the MRD average < 3 ppt (2007–2019; www.lacoast.gov/crms) and it is unlikely that elevated salinity levels are solely responsible for the current dieback. In this study, we experimentally investigated the independent and interactive effects of four key factors potentially associated with *P. australis* dieback in the MRD: infestations of *N. biwakoensis* scales, water depth, fertilizer level and *P. australis* lineage. As excess nutrients are a precursor to eutrophication, the use of fertilizer as a treatment is only a first step toward understanding the role of eutrophic conditions in *P. australis* dieback. However, numerous studies with plants have shown that excess nutrient enrichment of the soil can be detrimental to plant performance and increase the plant's vulnerability to other stressors (e.g., Limpens and Berendse 2003; Scheirs and De Bruyn 2004; Lovelock et al. 2009; Tao et al. 2014).

In a common garden in Louisiana, we conducted two experiments, one in which scales (added, no-scale control), water depth (low, high) and *P. australis* lineage (Delta, EU and Gulf) were manipulated and the other in which scales (added, no-scale control), fertilizer (low, medium, high) and lineage (EU, Gulf) were manipulated in fully crossed designs. After one growing season, we measured scale abundance and aspects of *P. australis* growth (aboveground biomass, number of stems per pot, mean stem height and mean basal stem diameter). For the first experiment ("Scales, water-depth and lineage experiment"), we tested the following set of predictions: (1) scale abundance is lower for plants when water depth is high and plants are of the putatively resistant invasive lineage, EU (Knight et al. 2020); (2) *P. australis* aboveground biomass and other growth measures are negatively impacted by scale infestations and high water depth regardless of lineage; and (3) as a highly successful invader of habitats varying in wide range of environmental conditions (Eller et al. 2017; Knight et al. 2018; Knight et al. 2020), the EU lineage of *P. australis* will be less susceptible to impacts from scales and high water depth. For the second experiment ("Scales, fertilization and lineage experiment"), we tested three analogous predictions: (4) scale abundance is lower for plants grown at the lowest fertilizer level and on the invasive lineage; (5) for all lineages, plant biomass is negatively impacted by scale infestations and high fertilization; and (6) the EU lineage of *P. australis* will be more tolerant of scales and high fertilizer levels. We conclude with recommendations for

the restoration of marsh habitats that have suffered recent dieback.

Materials and Methods

Study System

Phragmites australis is a large (3–5 m tall) perennial grass that is distributed across all continents except Antarctica (Clevering and Lissner 1999; Lambertini et al. 2006). It is a common inhabitant of freshwater and brackish wetlands where it grows clonally and typically forms dense monospecific stands. In the MRD, several phylogenetically distinct lineages exist (Saltonstall 2002; Lambertini et al. 2008). The Gulf lineage (haplotype I, *P. australis berlandieri*) is widely distributed along the Gulf Coast (Saltonstall 2002; Hauber et al. 2011). An invasive Eurasian lineage (haplotype M; *P. australis australis*) first appeared in herbarium records about 150 years ago and spread westward over the ensuing years (Chambers et al. 1999; Saltonstall 2002). Today, the Eurasian lineage is recognized as a major threat to wetland ecosystems and successful management has so far been challenging and expensive (Martin and Blossey 2013). Additional haplotypes have been introduced to North America from Europe, North Africa (Lambertini et al. 2012; Meyerson and Cronin 2013) and Asia (Lambert et al. 2016) but have relatively localized distributions. One such haplotype, Delta, is genetically similar to populations found in North Africa and the Mediterranean and has, so far, only been reported from Louisiana (Hauber et al. 2011; Lambertini et al. 2012; Knight et al. 2018).

The Mississippi River Delta (MRD) is the seventh largest river delta on earth and is a hotspot for *P. australis* diversity (Lambertini et al. 2012; Couvillion et al. 2017). The Delta lineage is the dominant vegetation in low elevation, fresh-to-slightly brackish marshes but the Eurasian lineage M (hereafter, EU) occurs in the same habitats in scattered and smaller stands (Hauber et al. 2011; Lambertini et al. 2012; Knight et al. 2018). Gulf is also present but typically at higher elevation and in relatively dry habitats. It has low salt tolerance and is rarely found in brackish or saline marshes (Achenbach et al. 2013). The MRD has been experiencing significant increases in nitrate and phosphorus concentrations since the 1950s (Rabalais et al. 1996), and a steady increase in relative sea-level since the early 1900s (Shea and Karen 1990). In just the past five years (2014 to 2019), water levels during the early growing season were 1–1.5 m higher than in the previous four years (NOAA tides and currents, https://tidesandcurrents.noaa.gov/map/index.html).

Nipponaclerda biwakoensis is native to Japan, China and Korea where it has been reported as a pest where *P. australis* is grown commercially (Kuwana 1907; Qin et al. 2003; Brix et al. 2014). The outbreak of this scale in the MRD is the first record of its establishment in North America (Knight et al. 2018). Although it feeds on a number of grass species, in the USA, the scale has been confined to *P. australis* (L. Aviles and R. Diaz, unpublished data). The scale attaches to the stem, which is protected by the leaf sheaths, and overwinters in the nymphal and adult stages. In its native range, *N. biwakoensis* has 3–6 generations per year (Kaneko 2004). First instars are the dispersive, "crawler" stage and subsequent nymphal and adult stages are sedentary.

A garden plot containing populations of *P. australis* collected from all over North America has been maintained at Louisiana State University since 2010 (Bhattarai et al. 2017; Croy et al. 2020). Each population was established from a small clump of rhizomes, planted in sand in 76 L pots, and subjected to the same fertilization and watering regime to minimize maternal effects. For this study, three source populations were selected from each of the three lineages commonly found in Louisiana, Delta, Gulf and EU (Supplementary Material, Table S1). Populations had been in culture for at least two years (since 2017). To increase the likelihood that our populations from the same lineage were not all derived from the same clone, the EU populations were obtained from Louisiana (LA), California (CA) and Arkansas (AK), Gulf was collected from Texas and two sites in LA that were separated by >200 km. Delta, which has only been reported from LA, were collected from two sites in the southwest (Cameron, Louisiana) and one site from the southeast in the MRD.

Scales, Water Depth and Lineage Experiment

In mid-April, 2019, rhizomes were harvested from the garden, rinsed to remove all soil and cut into 12–15 cm length sections (35–50 g wet mass). A single rhizome cutting was inserted upright into a 16.2 cm diameter pot (18.5 cm tall) and filled with a peat-based garden soil. Eighty-four rhizome cuttings per population were planted. Afterward, pots were placed in 1.2 m diameter plastic pools filled with water to a depth of ≈ 15 cm. After one week, we fertilized each pot with 28 g of Osmocote® (9-month, slow-release 15-9-12 NPK). To each pool, we also added a 36 ml solution comprised of 45 g of Miracle Gro (24-8-16 NPK, The Scotts Miracle-Gro Company®, Marysville, Ohio), 132 ml of Liquinox® (iron and zinc supplement; Liquinox Co., Orange, California) and 11.3 L of water.

Five weeks later, potted plants that were growing vigorously and had at least one stem >0.5 m tall were transferred to twenty-four 568 L cattle tanks (126 cm long × 94 cm wide × 61 cm tall; Supplemental material, Fig. S1). At this time, we inspected a randomly chosen stem from each pot, peeled back the leaf sheaths and found no evidence of scales. Tanks were spaced at least 5 m apart to minimize movement of scales among tanks (Fig. S1-A). Each tank could fit 27 pots and

our goal was to add three replicate pots from each of the nine source populations. Owing to some losses, specifically with regard to one population per lineage (CJM, BCI and ARM1; Table S1), eight of the tanks were not represented by all nine populations. For those tanks, we substituted other populations from the same lineage when possible and for all tanks there was a minimum of two populations per lineage and 21 pots. To minimize bias in our results from positional effects, pots were added to each tank in a random fashion.

Tanks were randomly assigned to water-depth (low, high) and scale-insect treatments (scales added, no-scale control). Water depth was controlled by the presence of a 5 cm drain hole at the bottom of the tank. An elbow joint with attached 0.7 m length of pvc tubing was screwed onto the threaded drain opening (Fig. S1-B). With the pvc tubing lowered horizontal to the ground, the depth in the tanks never exceeded 10 cm. Tanks in the low-water treatment were set at 10 cm water level. Tanks in the high-water treatment were set at 40 cm, which was accomplished by rotating the pvc tubing upward so that the drain height was 40 cm. Tanks were filled to their appropriate level with tap water (0 ppt salinity). Water levels were checked three times per week and tap water was added as necessary. For the low-water depth tanks in the summer, tanks often went dry but the soil was always damp. High-water tanks never dropped below 32 cm. Every month until the end of the study, tanks were fertilized with the Miracle Gro – Ironrite mixture at the concentration described above.

The scale-addition treatment was initiated on 27 June, 2019 (7 weeks after planting) at a time when stems were thick and tall enough to support scale insect growth, development and concealment beneath the leaf sheaths (mean height $\pm$ SE: 1.07 $\pm$ 0.05 m, $n = 322$). Tanks were randomly assigned to either a scale addition or control treatment. At this time, a random sampling of several stems per tank confirmed that potted plants were still scale-free. We collected scales from a nearby natural source (Lake Pontchartrain), a roadside stand of the Gulf lineage, 70 km east of the garden. Infested portions of stems were cut into 0.3–0.5 m sections (including 1–2 nodes). A total of 32 stem cuttings were distributed evenly within each tank, either by leaning them upright against existing stems (low-water tanks) or by attaching them to live stems with tie-wire. This method of scale inoculation allowed for crawlers to disperse from cut stems to live stems (Knight et al. 2020). Based on a dissection of 20 stem cuttings, drawn haphazardly from our collection, there were 66.0 $\pm$ 12.2 (mean $\pm$ SE) live adult female scales per stem section. On 31 July, the process was repeated. This time, 10 stem sections from the same source location were added to each tank. During this peak period of scale activity, we estimated that there were 209.8 $\pm$ 34.7 adult female scales per stem section. Overall, we estimated that we added 4208 adult females per tank for those tanks assigned to the scale-addition treatment. Our goal was to achieve scale abundances that were comparable to the summertime highs observed in the MRD (150 per meter of stem on Detla; Knight et al. 2020). Because of the low rate of successful transfer of crawlers to experimental plants (Knight et al. 2020), the multiple high-density introductions of adult scales was warranted.

Scales, Fertilization and Lineage Experiment

Concurrent with the previous experiment, we also conducted a garden experiment in which we manipulated scale insects and fertilizer levels and measured the response by three populations from two *P. australis* lineages. Ideally, this and the previous study would have been combined into one fully crossed design but it proved to be logistically impractical. As before, we sourced our plant material from common garden at LSU. In this case, only populations of the Gulf and EU lineage were available (Table S1). The Delta lineage suffered unexpectedly high mortality during the sprouting stage and had to be omitted from the experiment. Populations of the EU lineage were sourced from South Carolina, Arkansas and Louisiana. The Gulf lineage was sourced from California and two locations in Louisiana separated by 103 km.

Using the same methods as described previously, rhizome cuttings from each of the six *P. australis* populations were potted on 25–26 February, 2019. In order to better standardize nutrient content, we used sand as a potting medium. After one week, all pools were fertilized with the Miracle Gro – Liquinox solution. This low-level fertilization application was repeated on 29 March and 22 April. On 4 June (14 weeks after planting), we began implementing the experimental treatments. Four replicates of each of the six source populations were added to each of twelve 1.2 m diameter pools (Fig. S1-C). Owing to space limitations, pools were separated by only a minimum of 3 m and assigned at random to one of three fertilization treatments applied weekly: Low (4.2 g Miracle Gro mixed with 3.8 L of water and poured evenly throughout the pool), Medium (15 g Miracle Gro in 3.8 L of water), and High (45 g Miracle Gro in 3.8 L of water). At the first signs of iron deficiency, chlorosis of the leaves, Liquinox was added to all pools (2.75 ml per pool on 4 June, 31 July, 18 September). The medium-fertilizer treatment is the fertilization level we normally use for maintaining healthy and vigorous potted *P. australis* (Bhattarai et al. 2017). The high-fertilizer treatment is triple the medium-fertilizer treatment and was meant to represent an excess of nutrients; one that could lead to eutrophic conditions if the experiment ran for a long enough period of time. Scales were added in two pulses, on 27 June and 31 July, at the same time and the same numbers as for the previous experiment.

Data Collection

At the end of the growing season, on 21 October, the experiments were terminated and the following variables were measured. (1) *Number of live and dead stems* - every pot was

removed from each tank or pool and all live (green) and dead stems were counted. (2) *Stem heights, basal diameter and scale counts* - we haphazardly selected three green stems from two pots per population and tank/pool, clipped them at the soil surface and returned them to the laboratory. From each stem, we measured the height (base to the tip of the uppermost green leaf, in cm) and basal diameter of each stem (in mm) and the leaf sheaths were carefully peeled back and the number of live adult female and juvenile scales were enumerated. For this study, we focused only on total scales per stem (adult females + juveniles). We also standardized our measure of scale abundance by dividing the mean number of scales per three stems by the mean stem length (i.e., mean number of scales per m of stem). Stems were then placed in paper bags and transferred to the greenhouse to dry. (3) *Aboveground biomass* - for every pot in each tank or pool, we clipped all aboveground biomass and transferred the stem bundle to a nearby greenhouse to dry out (temperatures ranged from 19 to 39 C). After all stems were completely dry (two months later; confirmed by re-weighing the same samples at 3-d intervals for two weeks and observing no change in biomass), we weighed the stem bundles (and associated trio of stems for scale counts) using a hanging scale (Pesola©, Schindellegi, Switzerland; precision of ±0.3%).

Data Analysis

Our scale treatment was not absolute. Even though plants at the start of the experiment were confirmed to be free of scales, scales proved to be capable of moving between scale-infested and control tanks and pools separated by 3–5 m of mowed grass, either by crawling or carried by winds or avian vectors. The distribution of scale abundance (mean number per m of stem) was approximately normal with the exception of an excess of zeros. Using only the pots in which scale counts were performed (two replicate pots per lineage and population for each experiment), we classified pots as having no scales (= 0) or scales (= 1). We then conducted a simple χ^2 test for independence to assess whether pots assigned to the scale-addition treatment were statistically more likely to have scales than pots assigned as controls. Then, using pots with >0 scales, we conducted separate generalized linear mixed model (GLMM) analyses to test whether scale abundance (*ln*-transformed) was a function of our experimental treatments: lineage, scale addition, and either water depth or fertilizer. Cattle tank (or pool) number and *P. australis* population were treated as random effects to account for position effects within the garden plot and within-lineage variation, respectively. Our predictions were that pots in tanks/pools assigned to the scale-control treatment would have more zeros and lower scale counts than pots in tanks/pools assigned to the scale-addition treatment. We also tested the prediction that the EU lineage would have fewer scales than the other lineages

(Cronin et al. 2015; Knight et al. 2020) and assessed whether water depth or fertilizer treatment influenced scale abundance.

Next, using the same GLMM framework, we tested whether end-of-season aboveground biomass per pot was affected by lineage, scales and either water depth or fertilizer (a separate test for each of the experiments). Even though scale abundance varied continuously among pots, for both analyses, we opted to treat it as a categorical variable because the treatment was assigned to the whole tank/pool and we only measured final scale abundance on a subset of the pots per tank and pool, respectively. Considerable replication would have been lost if actual scale counts per pot were used. For both experiments, aboveground biomass (hereafter simply biomass) was *ln*-transformed to normalize the data distribution and homogenize variances. Finally, we conducted additional analyses to determine what aspects of biomass – stem number per pot (*ln*-transformed), stem height, and/or basal diameter - were affected by our treatments. Separate GLMMs were performed for each response variable.

GLMM analyses were performed using R v3.6.2 (R Core Team, 2019) and package lme4. Diagnostics for the GLMM were assessed using residual plots (variance in the Pearson's residuals is constant with respect to predicted values of the response variable) and quantile-quantile plots (for normality of residuals). ANOVA tables for fixed effects were obtained using the anova function in package lmerTest. Type III sums-of-squares were used and the denominator degrees of freedom for the *F*-test were computed using the Satterthwaite method. Model goodness-of-fit was computed using package MuMIn and the method of Nakagawa and Schielzeth (2013). Here, the proportion of variance explained is divided into two components: 1) the marginal R^2 which measures the variance explained by all fixed effects combined and 2) the conditional R^2 which measures the variance explained by the model; i.e., all the fixed and random effects combined. Marginal means ± SE for each treatment or treatment combination were obtained using the ggemmeans function in package ggeffects. Finally, contrasts between pairs of means were assessed using package emmeans and *P* values were adjusted for multiple comparisons using Tukey's method.

Results

Scales, Water Depth and Lineage Experiment

Scales were surprisingly capable of traversing 5 m of mowed grass and colonizing tanks assigned to our no-scale control treatment. Scales were found in all tanks and for pots in which scales were counted, only 6% (23 of 373 pots) had no scales recorded on them. Pots in tanks assigned to the no-scale control treatment had more than twice as many pots with zero scales per m of stem (9.6% and 3.8%, respectively) but the

difference was not statistically significant ($\chi^2 = 3.30$, $df = 1$, $P = 0.069$). However, excluding pots with zero scales (to achieve normality), there was a very strong effect of scale treatment on the number of scales per m of stem, independent of lineage (Table 1A, $P < 0.001$; Fig. 1a). Based on back-transformed estimates of scale abundance, stems in the scale-addition treatment had 5.0 times the number of scales
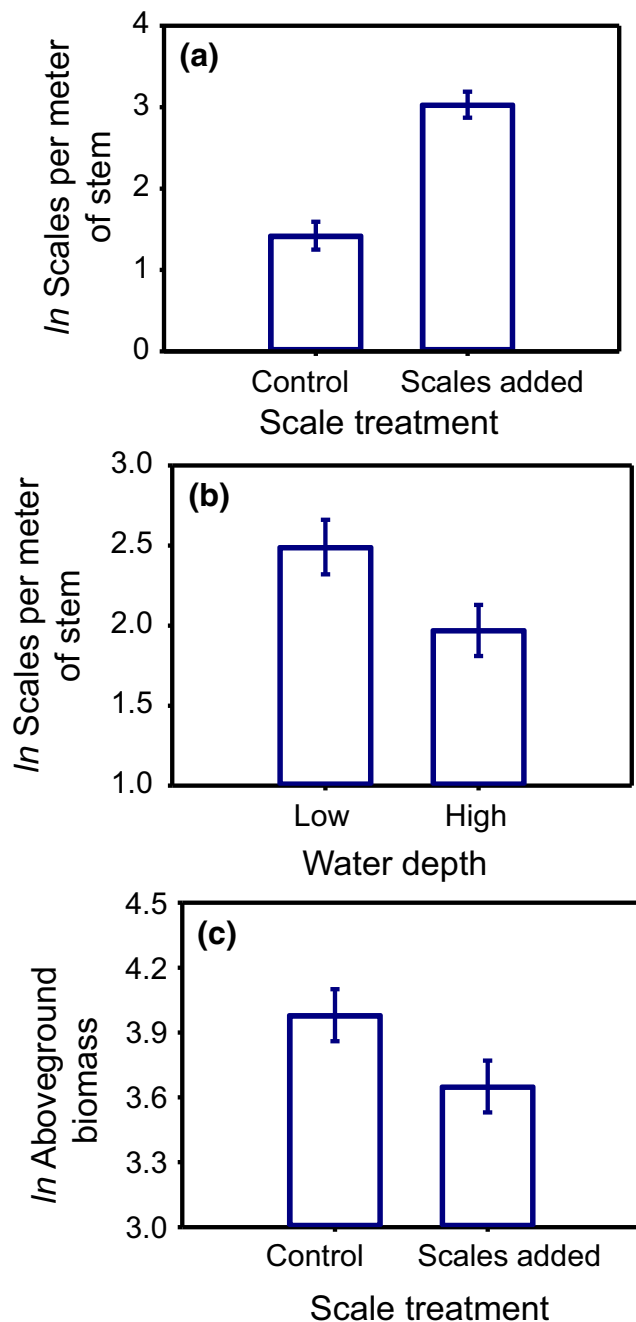

Fig. 1 (a) *ln* number of *N. biwakoensis* (scales) per m of stem for the scale treatment treatment (Control = no scales added, Scale addition = adult female scales added), (b) *ln* number of scales per m of stem in response to water-depth treatment (Low, High), and (c) *ln* biomass per stem for the scale treatment. Marginal means ± SE (adjusting for all other factors in the model) are reported

per m than stems in the control treatment (20.7 vs. 4.1 scales per m of stem).

In support of prediction 1, we found that plants in high water, irrespective of lineage, had an average of 41% fewer scales than plants in low water (Table 1A, Fig. 1b). Although not significantly different (Table 1A), EU had the lowest mean number of scales per m (7.9 ± 1.2), followed by Delta (8.2 ± 1.2) and then Gulf (12.2 ± 2.0). Overall, the fixed effects explained 42.7% of the variation in the model (based on the marginal R^2) and the fixed and random effects combined explained 57.8% of the model variance (based on the condition R^2).

By the end of the growing season, pots of *P. australis* had accumulated 16.7 ± 0.52 (range: 0–109) live stems at a mean height of 120 ± 1.5 cm ($n = 371$ pots). *Phragmites australis* end-of-season *ln* biomass was significantly influenced by all three treatments (scale addition, lineage and water depth; see Table 1B). We predicted that *P. australis* aboveground biomass would be negatively impacted by scale infestations and high water depth, regardless of lineage (prediction 2). In support of this prediction, we found that the scale-addition treatment resulted in a 28.1% reduction in biomass per pot relative to the control treatment (based on back-transformed estimates of the marginal means of *ln* biomass; Fig. 1c, $F_{1,16.02} = 8.29$, $P = 0.011$). However, the lineages varied in how strongly they were impacted by scales (i.e., a Scales × Lineage interaction; Table 1B). Biomass of the Delta, EU and Gulf lineage was 38%, 32% and 10% lower in the scale-addition versus control tanks, respectively but the difference was statistically significant only for the Delta lineage (Delta: $t_{79.8} = 2.98$, $P = 0.043$; EU: $t_{76.5} = 2.38$, $P = 0.176$, Gulf: $t_{102.5} = 0.66$, $P = 0.986$; Fig. 2a). Contrary to prediction 3, that the EU lineage would be least impacted by scale insects, it was the Gulf lineage that suffered the least biomass loss in the scale-addition relative to control treatments. Finally, the loss of biomass in the scale-addition treatment was the result of changes in stem production, height and basal diameter that was lineage dependent (Supplementary Information, Table S2, Fig. S2). The loss of biomass in the scale-addition treatment for the Delta lineage was associated with 13% and 19% reductions in stem height and diameter, respectively; for the EU lineage, it was primarily associated with an 28% reduction in stem production; and for the Gulf lineage, it was primarily due to a 9% reduction in stem diameter (Fig. S2).

Lineage and water depth also interacted to affect *ln* biomass (Table 1B, Fig. 2b); partially supporting prediction 2. Whereas the Delta lineage was unaffected by water depth ($t_{79.8} = 1.00$, $P = 0.915$), the EU and Gulf lineages exhibited the opposite responses to water depth. The EU lineage had 30% more biomass in the high- relative to the low-water tanks ($t_{76.5} = 2.27$, $P = 0.220$) and the Gulf lineage had 46% more biomass in the low- than high-water tanks ($t_{102.6} = 3.61$, $P = 0.005$). Finally, there was a scale × water × lineage interaction.

Table 1 Results from separate generalized linear mixed models for the effects of scales (addition, control), water depth (Low = 10 cm, High = 40 cm), *P. australis* lineage (Gulf, Delta, EU) and all possible interactions on (A) *ln N. biwakoensis* (scale) abundance per m of stem and (B) *ln* biomass

Source of variation	MS	Num df	Den df	F	P
(A) ln Scales per m of stem					
Scales	**40.858**	**1**	**20.36**	**54.902**	**<0.001**
Water depth	**4.190**	**1**	**20.35**	**5.631**	**0.028**
Lineage	2.360	2	6.62	3.171	0.108
Scales × Water depth	0.019	1	20.35	0.026	0.873
Scales × Lineage	0.602	2	313.23	0.809	0.446
Water × Lineage	0.108	2	313.46	0.145	0.865
Scales × Water × Lineage	0.303	2	313.81	0.407	0.666
Error	0.744				
Marginal R^2	0.427				
Conditional R^2	0.577				
(B) ln Biomass					
Scales	**8.671**	**1**	**16.02**	**8.288**	**0.011**
Water depth	1.652	1	16.03	1.579	0.227
Lineage	0.913	2	7.39	0.873	0.457
Scales × Water depth	0.036	1	16.02	0.035	0.855
Scales × Lineage	**4.155**	**2**	**565.35**	**4.088**	**0.017**
Water × Lineage	**11.605**	**2**	**565.34**	**11.093**	**<0.001**
Scales × Water × Lineage	**3.199**	**2**	**565.52**	**3.058**	**0.048**
Error	1.046				
Marginal R^2	0.083				
Conditional R^2	0.160				

Notes: Goodness-of-fit statistics, marginal and conditional R^2, were computed using the method of Nakagawa and Schielzeth (2013) where the marginal R^2 estimates the variance explained by the fixed effects, and the conditional R^2 estimates the variance explained by the fixed effects plus random effects

Sources of variation highlighted in bold are statistically significant ($P \leq 0.05$). Mean squares (MS), numerator and denominator degrees of freedom (Num df, Den df), *F*-statistic and *P*-values are reported

Most notably, Gulf biomass was higher in the low- than high-water treatment but the effect size differed between scale treatments (Fig. S3). In the scale-control tanks, biomass was 60% higher in the low-water tanks ($t_{97.7} = 3.72$, $P = 0.017$) and in the scale-addition tanks, it was only 29% higher and not statistically significant ($t_{107.6} = 1.39$, $P = 0.963$; Fig. S3). The reduced biomass of the Gulf lineage in the high-water treatment was due to significant reductions in both stem height and diameter (17% and 11%, respectively; Supplemental Information, Table S2, Fig. S4). In contrast, in high water, the EU lineage had a 13% increase in stem diameter (Fig. S4). Overall, the full model explained only 16% of the variation in *ln* biomass, with one half of that variation explained by the fixed factors (Table 1B).

Scales, Fertilization and Lineage Experiment

Despite one half of the pools being assigned to the scale-addition treatment, all pools had live scales at the end of the study and only 10 of 305 pots had zero scales (2 in the scale-addition and 8 in the scale-control treatments; an insufficient number for statistical tests). However, the scale manipulation had the desired effect (Table 2A): *ln* scales per m of stem were 2.7 times higher in the scale-addition than control pools (based on back-transformed means; Fig. 3a).

In support for the first part of prediction 4, we found that the EU lineage supported 2.6 times fewer scales than the Gulf lineage (Table 2A, Fig. 3b). However, contrary to second part of prediction 4, scales were 2.7-fold more abundant in the low- (12.6 per m of stem) compared to the high-fertilizer treatment (4.6 per m of stem) ($t_{5.4} = 3.39$, $P = 0.039$, back-transformed counts; Fig. 3c). All other pairwise comparisons involving the fertilizer treatment were not significant ($P > 0.20$). The fertilizer treatment effect was also strongly influenced by the scale treatment (i.e., a fertilizer × scale addition interaction; Table 2A). For pools in which scales were added, fertilizer level had no effect on scale abundance (all paired contrasts, $t < 1.09$, $P > 0.5$; Fig. 3d). However, in the pools with the scale-control treatment, fertilization strongly influenced scale abundance: the high-fertilizer treatment reduced scale abundance by an average of 4.6 fold relative to the low- or medium-fertilizer treatments ($t > 3.28$, $P < 0.04$;
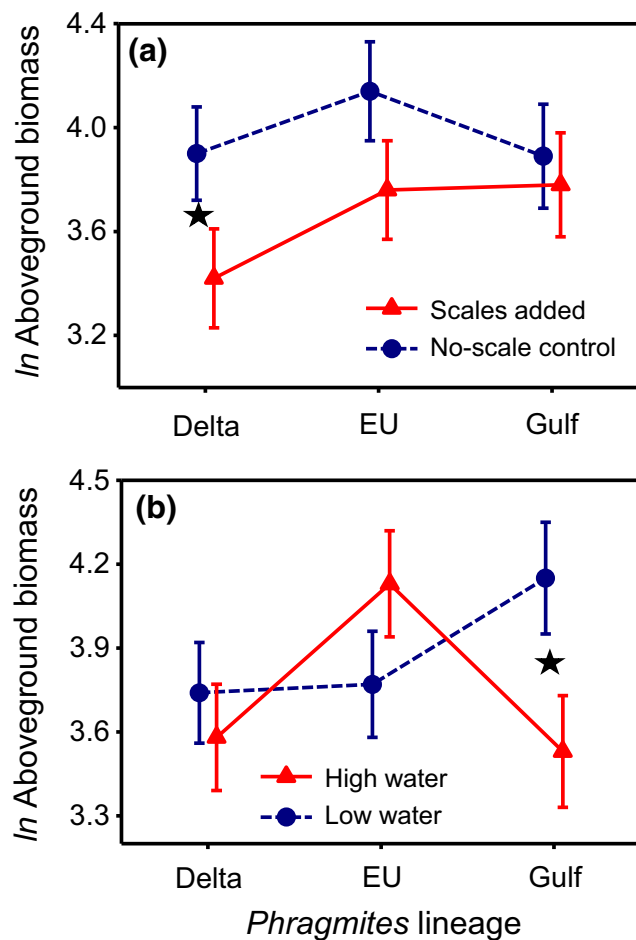
Fig. 2 *ln* biomass per pot as a function of *P. australis* lineage and (**a**) *N. biwakoensis* scale-addition treatment and (**b**) water-depth treatment. Marginal means ± SE (adjusting for all other factors in the model) are reported and a star indicates a significant difference between means within a lineage ($P \leq 0.05$)

Fig. 3d). Focusing only on the high-fertilizer pools, there were 7 times as many scales in the scale-addition than control treatment (1.8 versus 12.2 live scales per m of stem, respectively; $t_{6.12} = 4.80$, $P = 0.024$). Overall, the GLMM model explained 30% of the variation in scale abundance (random effects accounted for <3% of the variation).

The GLMM for *P. australis ln* biomass failed to converge on a solution when all 2- and 3-way interactions were included. However, the model did converge on a solution when the fertilizer × scale × lineage interaction was removed. Based on Akaike's Information Criteria, the reduced model was more plausible than the full model (AICc-reduced: 532.2, AICc-full: 533.2). Below, we report the results for the reduced model.

We had predicted that *P. australis* biomass would be negatively affected by scale insects and high fertilizer levels (prediction 5) and that EU would be more tolerant to these potential stressors (prediction 6). However, we found mixed support for the former prediction and rejected the latter prediction.

Biomass in this experiment was only influenced by lineage and lineage × fertilizer treatment (Table 2B). We found no evidence that the biomass of EU and Gulf lineages were negatively or differentially affected by our scale treatment. Across all fertilizer treatments, the Gulf lineage averaged 46% more biomass than the EU plants ($F_{1,4.8} = 22.91$, $P < 0.001$; Table 2B, Fig. 4). Gulf were also 33% taller and had 2.1 times thicker stems but produced 59% fewer stems than the EU lineage (Supplementary Information, Table S3, Fig. S5). Fertilization also affected plant biomass through its interaction with lineage (Table 2B). As the fertilizer level increased, the proportional difference in biomass between the Gulf and EU lineages increased - from 35% in the low-fertilizer treatment to 57% in the high-fertilizer treatment. Clearly, the EU lineage is more sensitive to increasing fertilization than the Gulf lineage. However, neither the Gulf nor EU lineage exhibited a significant change in biomass across the fertilization treatments (all pairwise contrasts: $t < 2.6$, $P > 0.05$). According to the GLMM, 27% of the variation in *ln* biomass was explained by the fixed factors in this model and 42% by the full model.

Discussion

Impact of Scale Insects on *P. australis*

Several reports have implicated herbivores or other natural enemies as potential drivers or contributors to *P. australis* dieback. Armstrong et al. (1996a) argued that through their feeding damage, herbivores have the potential to cause dieback syndrome in *P. australis*. In other cases, natural enemy impact on *P. australis* was strongest through its interaction with other environmental stressors. For example, Nechwatal et al. (2008) suggested that flooding and rising water temperature exacerbated the effects of the reed pathogen *Pythium phragmitis* (Nechwatal et al. 2005) on *P. australis* dieback. Similarly, Li et al. (2014) found that invasive *S. alterniflora* in China indirectly affected *P. australis* dieback through a shared fungal pathogen (*Fusarium palustre* Elmer and Marra). In a related system, dieback of smooth cordgrass along the Atlantic and Gulf Coasts of the USA has been linked to both herbivores and pathogens, although both may have been too localized to explain the observed large-scale dieback (Silliman and Bertness 2002; Elmer et al. 2007; Alber et al. 2008).

In the Mississippi River Delta (MRD), the nonnative scale *N. biwakoensis* has been linked to the loss of marsh primary productivity (as measured by changes in NDVI; I. A. Knight and R. Diaz, unpublished data). As predicted (prediction 1), in our Scales, water-depth and lineage experiment, a mean of 20 live scales per m of stem, resulted in a 28% reduction in *P. australis* biomass relative to the control treatment. This is comparable to the experimental findings by Knight et al. (2020) wherein they reported significant scale-induced

Table 2 Results from separate generalized linear mixed models for the effects of scales (addition, control), fertilizer level (Low, Medium, High), *P. australis* lineage (Gulf, EU) and all possible 2-way interactions on (A) *ln N. biwakoensis* (scale) abundance per m of stem and (B) *ln* biomass

| Source of variation	MS	Num df	Den df	F	P
(A) ln Scales per m of stem					
Scales	**29.069**	**1**	**5.11**	**16.300**	**0.010**
Fertilizer	**10.703**	**2**	**5.13**	**6.002**	**0.045**
Lineage	**37.817**	**1**	**167.40**	**21.205**	**<0.001**
Scales × Fertilizer	**11.292**	**2**	**5.13**	**6.332**	**0.043**
Scales × Lineage	0.616	1	167.40	0.345	0.558
Fertilzer × Lineage	0.200	2	165.85	0.112	0.894
Scales × Fertilizer × Lineage	1.476	2	165.85	0.828	0.439
Error	1.783				
Marginal R^2	0.427				
Conditional R^2	0.577				
(B) ln Biomass					
Scales	0.119	1	6.28	0.369	0.565
Fertilizer	0.690	2	6.26	2.138	0.196
Lineage	**22.906**	**1**	**4.80**	**71.017**	**<0.001**
Scales × Fertilizer	0.104	2	6.27	0.322	0.736
Scales × Lineage	0.036	1	266.84	0.112	0.738
Fertilzer × Lineage	**1.164**	**2**	**266.95**	**3.609**	**0.028**
Error	0.322				
Marginal R^2	0.083				
Conditional R^2	0.160				

Notes: Goodness-of-fit statistics, marginal and conditional R^2, were computed using the method of Nakagawa and Schielzeth (2013) where the marginal R^2 estimates the variance explained by the fixed effects, and the conditional R^2 estimates the variance explained by the fixed effects plus random effects. For ln biomass, the Scales × Fertilizer × Lineage term was omitted from the analysis because the model could not converge on a solution

Sources of variation highlighted in bold are statistically significant ($P \leq 0.05$). Mean squares (MS), numerator and denominator degrees of freedom (Num df, Den df), F-statistic and P-values are reported

reductions in plant height and the proportion of stem's length that had green leaf tissue.

A previous study of ours (Knight et al. 2020) demonstrated that scale abundances in the MRD were 2–7 times higher in

Fig. 3 Effects of (**a**) *N. biwakoensis* scale treatment, (**b**) *P. australis* lineage, (**c**) fertilizer treatment and (**d**) scale-by-fertilizer treatment interaction on the marginal mean ± SE (adjusting for all other factors in the model) *ln* scales per m of stem. Star indicates a significant difference between means within the high-fertilizer treatment ($P = 0.003$). Different letters associated with bars in (**c**) denote significant differences between means ($P < 0.05$)
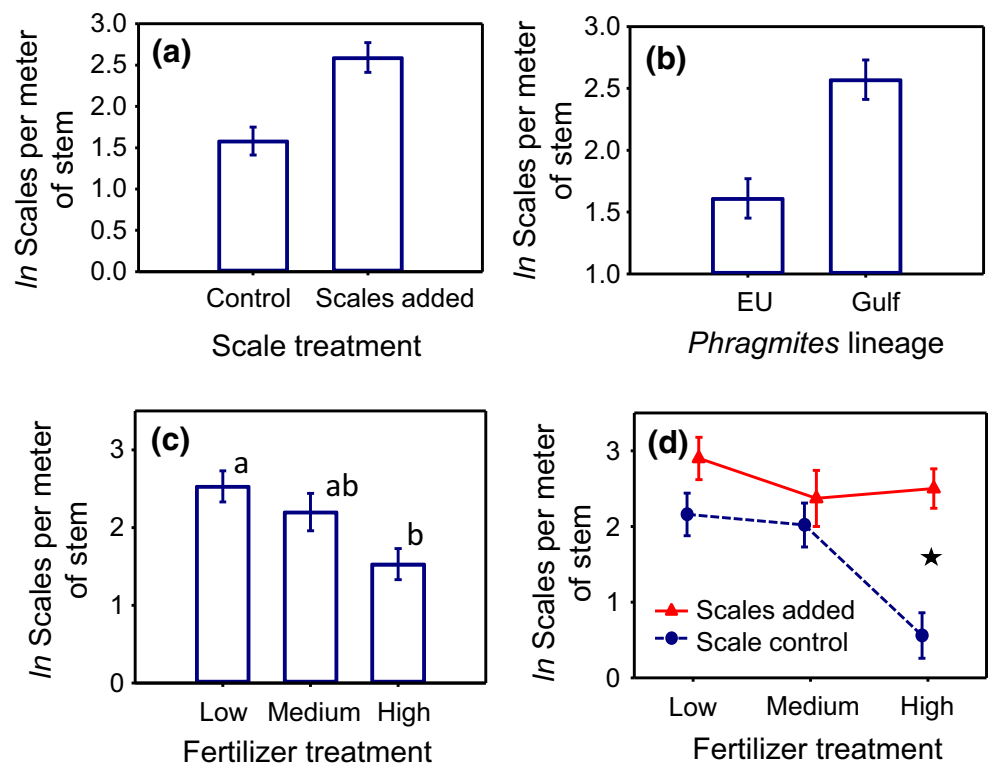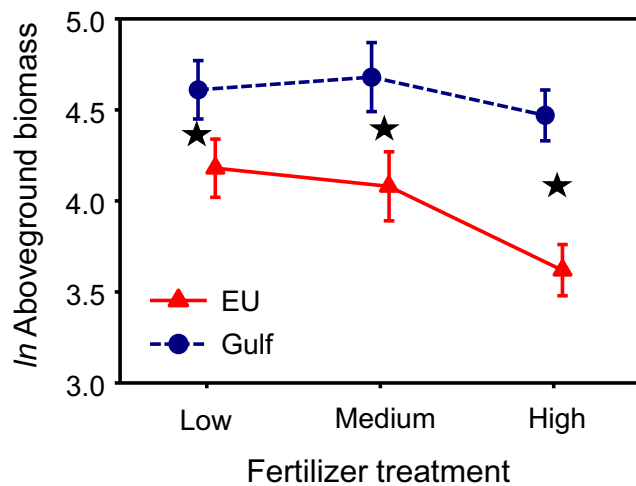
Fig. 4 The effect of fertilizer treatment and *P. australis* lineage on *ln* biomass per pot. Marginal means ± SE (adjusting for all other factors in the model) are reported

Delta than EU stands and these results were corroborated in a mesocosm experiment. Across a broad spectrum of herbivore species and feeding guilds, the EU lineage appears to be highly resistant (Lambert and Casagrande 2007; Lambert et al. 2007; Cronin et al. 2015; Allen et al. 2017). As such, we predicted that the EU lineage would be more resistant to scales than the other lineages (prediction 1, 4). Although we did find that scale abundance was lowest on the EU lineage, it was only 4% lower than on the Delta lineage; a non-significant difference. In comparison, the Gulf lineage had 35–260% more scales than the EU lineage (depending on experiment), suggesting that the EU lineage is more resistant to scales than the Gulf lineage (consistent with Knight et al. (2020)). Perhaps if the experiment ran longer, or we focused on other fitness metrics (e.g., seed production, belowground biomass, long-term survival), greater differences between Delta and EU would have been revealed.

Contrary to prediction 4, as fertilizer levels increased, scale abundance decreased. This occurred independent of scale-addition treatment but was most strongly evident in the scale-control pools. One possible explanation for this result is that in resource-rich environments, plants may produce more nitrogen-based chemical defenses (Coley et al. 1985; Herms and Mattson 1992; Miller and Woodrow 2008). As our findings in the scale-control pools suggest (where scale abundances were reduced almost 5-fold in the high- relative to low-fertilization treatment), those defenses may be most effective when population sizes are initially small. Although grasses like *P. australis* are thought to have less diverse chemical defenses (McNaughton and Tarrants 1983; Quigley and Anderson 2014), *P. australis* does exhibit tremendous variation in resistance to herbivory and pathogen damage (Allen et al. 2017; Bhattarai et al. 2017; Allen et al. 2020; Croy et al. 2020). The chemical basis of that resistance is not well

understood. Regardless of the underlying mechanism, nutrient runoff from nearby cattle ranches or up-river farms may favor production of nitrogenous-based defenses and inhibit the buildup of scale insects in the MRD.

In support of our prediction 2, we found that *P. australis* lineage played a critical role in whether aboveground biomass was impacted by the scale insect. In our Scale, water-depth and lineage experiment, the biomass of all three lineages were negatively impacted by the addition of scales. The Delta lineage was most strongly impacted, having 38% less biomass in the scale-addition than control treatment. Although we did not observe much change in stem production between scale treatments, when scales were abundant, Delta plants were 13% shorter and had 19% thinner stems. In the MRD, the Delta lineage comprises >95% of the area covered by *P. australis* (Hauber et al. 2011; Lambertini et al. 2012; Knight et al. 2020). According to Knight et al. (2020), field-based estimates of average scale abundance on the Delta lineage can reach as high as 150 per m of stem – 7.5 times higher than the densities used in this study! It remains an open question whether experimental scale densities, comparable to those found in the field, would be sufficient to induce mortality in *P. australis*. Currently, we also do not know the long-term impact of scale outbreaks on the energy storage (in roots and rhizomes), and the precise physiological mechanisms underlying scale damage to *P. australis*.

Consistent with our prediction 2, all three lineages in the Scales, water-depth and lineage experiment were negatively impacted by the addition of scales. Also, in support of the study by Knight et al. (2020), the aboveground biomass of the EU and Gulf lineages were less impacted by scale insects (32% and 10% reductions relative to controls, respectively) than the Delta lineage (38% reduction). As such, the significant scale × lineage interaction found in this experiment was largely driven by the greater damage to the Delta lineage. Furthermore, the absence of a scale × lineage interaction in the Scale, fertilizer and lineage experiment is easily explainable – this experiment did not include the Delta lineage; only EU and Gulf.

In our experiments, the Delta and EU lineages had similarly low scale loads but the Delta lineage suffered proportionately more damage, suggesting that Delta has lower tolerance of herbivory than EU. Here, we define tolerance as the degree to which a plant can grow or reproduce in response to herbivory (Strauss and Agrawal 1999). The Gulf lineage, which had the highest herbivore loads and lowest proportional loss of biomass (in the scale-addition treatment), would appear to be most tolerant of herbivory. The EU lineage fell somewhere in between and consequently prediction 3, that the invasive EU lineage would be most tolerant of scales, was only partially supported. In the only study to examine herbivore tolerance in *P. australis*, Croy et al. (2020) found that the North American native lineage (*P. australis americanus*)

was more tolerant than the EU lineage. In that study, there was a strong tradeoff between tolerance and resistance between the EU and native lineages. Qualitatively, such a tradeoff may exist between the Delta and Gulf lineages (i.e., the Gulf is more tolerant but less resistant than the Delta lineage). In support of classic plant defense models, tolerance and resistance in *P. australis* may be mutually exclusive defense strategies (Strauss and Agrawal 1999; Tiffin 2000; Croy et al. 2020).

Water Depth Effects

Climate change models predict greater variability in precipitation, more extreme precipitation events, and an increase in the frequency and magnitude of inland and coastal floods (Craft et al. 2009; Kirwan and Megonigal 2013; Schile et al. 2017). *Phragmites australis* is generally considered to be tolerant of high water levels because it can aerate flooded tissues by transporting oxygen through the aerenchyma, creating an extensive network of internal airspaces (Armstrong and Armstrong 1991; Jackson and Armstrong 1999; Eller et al. 2017). To date, there is limited information regarding whether *P. australis* genotypes or lineages vary in their gas transport efficiencies or response to high water or flooding (but see Engloner and Major 2011; Tulbure et al. 2012).

In our study, the high-water treatment maintained water depth at 40 cm, well within the level found in the MRD where water depths can range from 0 cm to at least 150 cm (J. T. Cronin, personal observation). Although we predicted that high water depth would negatively impact aboveground biomass of all three lineages (prediction 2) and have the smallest effect on the EU lineage (prediction 3), only the latter prediction was supported by our data. Surprisingly, the EU lineage actually increased in aboveground biomass by 30% in the high as compared to the low water-depth treatment (but not significantly). Aboveground biomass of the Delta lineage was unaffected by our water-depth treatment and the Gulf lineage responded negatively, decreasing in biomass by 46%. How the different lineages would respond belowground to changes in water depth remains an open question. However, it is known that root:shoot ratios for *P. australis* can change in response to changing abiotic conditions (e.g., Lissner et al. 1999; Holdredge et al. 2010; Eller and Brix 2012) and, therefore, it is possible that the response to high water for aboveground plant parts would differ from that for the roots and rhizomes.

The highly efficient gas transport system of the EU lineage (Tulbure et al. 2012) likely predisposes it to being able to thrive in flooded/high-water environments. Currently, nothing is known about gas and nutrient transport in the Delta and Gulf lineages but they appear to be adapted to different environments. Our results may explain why the Gulf lineage is scarce in the MRD except at high elevation, usually on roadside and river embankments on the margins of the MRD (J. T. Cronin personal observation). Interestingly, in a study of Louisiana clones of *P. australis* (of undetermined lineage), Howard and Rafferty (2006) found considerable clonal variation in tolerance to high-water depth but averaged among clones, higher water levels resulted in significantly taller plants with more aboveground biomass. Genetic variation within a plant species for flood tolerance is commonly reported (Davy et al. 1990; Bailey-Serres and Voesenek 2008) and includes coastal marsh plants such as *Spartina alterniflora* (Gallagher et al. 1988; Deng et al. 2007), various mangrove species (Guo et al. 2018) and *Salicornia* species (Davy et al. 1990).

High water levels in our experiment reduced scale insect abundance by 41% (supporting prediction 1). A decrease in abundance of stem-feeding herbivores would be expected under a flooding regime simply because of the reduced availability of stem tissue to feed upon. Scales were not found below the water line in our experiments nor in the field (J. T. Cronin, personal observation). However, we cannot exclude the possibility that the high-water effect on scale abundance was mediated by changes in plant chemistry or morphology (Lower et al. 2003; Bailey-Serres and Voesenek 2008).

Fertilization Effects

Coastal and wetland marshes have been subjected to nutrient inputs from agricultural, sewage and urban-development runoff and elevated atmospheric deposition owing to climate change (Galloway et al. 2004; Day et al. 2008). *Phragmites australis* is highly efficient at up-taking and assimilating nutrients and this is thought to be one reason for why it has been a successful invader (Chambers et al. 1999; Romero et al. 1999; Tho et al. 2016; Eller et al. 2017). In Europe, the cascading effects of excess nutrients, leading to eutrophic conditions - anoxic sediments, phytotoxin production from algal blooms and increased litter production (Armstrong et al. 1996c; Brix 1999) - were commonly implicated in causing the dieback syndrome for *P. australis*. Given the short-term nature of the Scales, fertilization and lineage experiment (4.5 months), the use of shallow pools that were well oxygenated, and minimal algal activity, eutrophic conditions were unlikely to be significant. However, high fertilizer levels can be detrimental to plants in other ways; e.g., by either increasing their palatability to herbivores or pathogens or making the plants more susceptible to other abiotic stressors (e.g., Limpens and Berendse 2003; Scheirs and De Bruyn 2004; Lovelock et al. 2009; Tao et al. 2014).

Under high-nitrogen conditions, the EU lineage in North America has been shown to be a better competitor, superior in nutrient assimilation and more plastic in its response to nutrient levels than the North American native lineage (Saltonstall and Stevenson 2007; Holdredge et al. 2010; Mozdzer and

Megonigal 2012; Mozdzer et al. 2013). Neither the EU nor Gulf lineage was significantly affected by increasing fertilization levels and, therefore, we reject prediction 5 as it pertains to plant biomass. We were also surprised to find that the highly invasive EU lineage of *P. australis* was outperformed by the Gulf lineage under all fertilization levels (no support for prediction 6). Across fertilizer treatments, Gulf plants produced an average of 46% more biomass than the EU lineage. They were also 33% taller and had 2.1 times thicker stems. Interestingly, as the fertilizer level increased, the Gulf lineage performed proportionately better than the EU lineage, in terms of productivity.

The Gulf lineage is widely distributed in Central America (Saltonstall 2002; Lambertini et al. 2012; Colin and Eguiarte 2016) but it is currently under debate as to whether it is native or introduced into North America. However, it clearly has shown "invasive" like behavior in North America. Since the early 1980s, the Gulf lineage has exhibited rapid clonal growth within higher-elevation and inland marshes along the Gulf Coast in response to disturbances by hurricanes (Bhattarai and Cronin 2014). Over that same time period, it has also exhibited rapid range expansion from the Gulf Coast to the West Coast (Meyerson et al. 2010). In retrospect, it may not be so surprising that the Gulf lineage can perform at least as well, if not better, than the EU invasive lineage under various fertilization regimes.

Implications for Restoration

Phragmites australis in the MRD is a bulwark against soil erosion, land loss, and damage from storm surges and waves. Additionally, it provides numerous ecosystem services regardless of lineage (Kiviat 2013). It is present at the mouth of the Mississippi River and dominates virtually all distributary passes from the river and appears to grow in water too deep for other plant species to survive (Hauber et al. 1991; Hauber et al. 2011; Knight et al. 2018). Dieback is extensive throughout the MRD (Knight et al. 2018; Suir et al. 2018), with previously vegetated sites being converted to open water or becoming dominated by less desirable shallow-rooting or floating invasive plants; e.g., water hyacinth [*Pontederia crassipes* (Mart.) Solms] or elephant ear [*Colocasia esculenta* (L.) Schott]. Restoration of the marsh vegetation in the MRD is imperative and replanting *P. australis* seems to be the best economical option. Restoration trials have been made previously with *P. australis* in Louisiana (Howard et al. 2008; Howard and Turluck 2013).

If restoration is going to be part of the management plan for the MRD, we offer the following general recommendations based on the findings from this study. First, most dieback in the MRD has occurred in areas that are flooded, with water depths ranging from 0.5 m to 1.5 m. Replanting using the Gulf lineage should not even be a consideration given its lower

tolerance for high water. Between the Delta and EU lineage, land managers outside of Louisiana would probably be aghast if we were to recommend replanting with the EU lineage (haplotype *M*) that has proven to be such a vexing and costly invader in many other parts of North America (Martin and Blossey 2013). However, the EU lineage is already patchily distributed in the MRD and exhibits fewer symptoms of dieback than the more widespread and common Delta lineage (Knight et al. 2020). In addition to the EU lineage being more resistant to *N. biwakoensis* scales and a suite of other common herbivores (Lambert and Casagrande 2007; Cronin et al. 2015; Bhattarai et al. 2017; Knight et al. 2020), it is also more tolerant of herbivory and high water than the Delta lineage. Unfortunately, it remains unknown whether the EU lineage performs better than Delta under high-fertilizer conditions, as Delta was not included in the fertilization study. Finally, fertilization of plants used in restoration, at a time when scale densities are low, may help to limit scale outbreaks on those plants.

In the restoration work performed by Howard et al. (2008), after 14 months, their "Eurasian haplotype" outperformed the Gulf lineage almost five to one. In this case, the Eurasian haplotype was sourced from the MRD where Delta is most common and the molecular tools used at the time were not sensitive enough to distinguish EU from Delta. Regardless, their study confirms that the Gulf lineage is not the best choice for restoration in the MRD.

Conclusions

Dieback in the MRD is a complex phenomenon, perhaps more so than in Europe because of the unusual genetic diversity of *P. australis*, relatively deep water, and high-energy storms that impact the affected area. A modest infestation by the introduced scale insect, *N. biwakoensis*, resulted in a 28% reduction in *P. australis* biomass. As scale infestations are persistent and densities are substantially higher in the MRD (Knight et al. 2020), we conclude that *N. biwakoensis* is likely a key factor in the dieback of this system. Other potential stressors can impact scale abundance – scale populations achieve lower densities in high water and nutrient-rich environments. Perhaps our most important finding is that the genetic diversity of *P. australis* in the MRD is central to understanding how scales, high water and fertilizer impact *P. australis* biomass. Between our study and that of Knight et al. (2020), we found strong lineage-specific differences in *P. australis* resistance to and tolerance of herbivory. Most notably, the Delta lineage, which dominates the MRD, was moderately resistant but had the lowest tolerance to scales. High water benefited the EU lineage but was detrimental to the Gulf lineage, whereas increased fertilization became increasingly more detrimental to the EU as compared to the Gulf

lineage. Other stressors, such as salt intrusion, have also been shown to interact with *P. australis* lineage and scale insects to affect plant performance (Knight et al. 2020). Without intervention and a speculative absence of major abiotic stressors (tropical storms, elevated river levels), the MRD may recover from this dieback event but it is likely that the genetic composition of *P. australis* will be drastically different, dominated by the EU lineage. Although controversial, the EU lineage does provide similar beneficial ecosystem services to other *P. australis* lineages (Kiviat 2013) and its use in restoration could facilitate recovery.

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