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Investigating Plant Phenotype, Salinity, and Infestation by the Roseau Cane Scale as Factors in the Die-Back of *Phragmites australis* in the Mississippi River Delta, USA

Ian A. Knight[1] · James T. Cronin[2] · Madeline Gill[1] · John A. Nyman[3] · Blake E. Wilson[1] · Rodrigo Diaz[1]

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Abstract
Die-back of *Phragmites australis* in the Mississippi River Delta (MRD), Louisiana, USA, is associated with large populations of nonnative Roseau Cane Scale (RCS), *Nipponaclerda biwakoensis*. Initial observations suggested different *P. australis* phenotypes displayed different susceptibilities to scale infestation and die-back, but the role of scale infestation on die-back was unknown. To understand the effect of RCS on *P. australis*, paired stands of Delta and European phenotypes in the MRD were monitored for stem heights, densities, and scale infestation over two years. A mesocosm experiment was conducted to assess whether RCS abundance and *P. australis* growth were dependent on water salinity and phenotype. Three *Phragmites australis* phenotypes were grown in small pools under fresh or mesohaline conditions, and RCS infestated or non-infested treatments. Scale densities were up to 7 times greater on the Delta compared to the European phenotype. Scale infestation resulted in 22%–39% reductions in the proportion of stems with green leaf tissue for all phenotypes, and 12% reduction in stem heights for Delta-type. Salinity was detrimental to all phenotypes, reducing stem heights by 20% compared to freshwater. Our results provide evidence that the RCS can result in die-back symptoms similar to what is observed in the MRD.

Keywords Die-back · *Phragmites australis* · Mississippi River Delta · Scale insect · Salinity · Phenotype

Introduction

Die-back of *Phragmites australis* (Cav.) Tren. Ex Steud. was detected in the Fall of 2016 affecting stands across the Mississippi River Delta (MRD), Plaquemines Parish, Louisiana, USA (Knight et al. 2018). Symptoms of die-back include retreat from deep water, reductions in stem density, a more clumped distribution of stems within a stand, and premature senescence of leaf tissue (Armstrong et al. 1996a; van der Putten 1997). Widespread declines in plant health have also been observed through remote sensing where an analysis of NDVI (normalized difference vegetation index) from Landsat imagery has suggested that declines may have begun as early as 2015 (Ramsey III and Rangoonwala 2017). While *P. australis* is often considered invasive across much of North America (Chambers et al. 1999), it can also provide several ecosystems services (reviewed in Kiviat 2013). *Pragmites australis* is the dominant emergent vegetation in the Mississippi River Delta (Hauber et al. 2011), and declining stands of *P. australis* raise concerns over the stability of the regional wetlands.

Although die-back of *P. australis* is new to the MRD, the phenomenon has been reported from across its cosmopolitan distribution. Research by the European Research Project on Reed Die-back and Progression concluded that a significant factor in the decades of die-back in Europe was eutrophication, exacerbated by stagnant water tables (reviewed in van der Putten 1997). This was by no means the only factor identified in the die-back across this continent. Stress from soil phytotoxins, plant pathogens, and insect feeding damage were all implicated in producing symptoms associated with die-back (Armstrong et al. 1996a, b). In China, die-back of stands in the Dongtan wetlands of the Yangtze River estuary were

✉ Ian A. Knight
ian.knight88@gmail.com

[1] Department of Entomology, Louisiana State University, 402 Biological Sciences Bldg, Baton Rouge, LA 70803, USA

[2] Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

[3] School of Renewable Natural Resources, Louisiana State University, Baton Rouge, LA 70803, USA

attributed to the invasion of *Spartina alterniflora* (Loisel.). The only North American account of *P. australis* die-back occurred at Pointe Mouillee, Michigan, concurrent with catastrophic die-back of other emergent wetland species, and was attributed to rising and prolonged high water levels (McDonald 1955).

Increasing salinity can also cause die-back of *P. australis* stands. Lissner and Schierup (1997) report die-back of *P. australis* stands in Europe at sites where soil water salinity was greater than 15% [sic]. Concentrations of 20 ppt and greater have been shown to reduce shoot production (Eller et al. 2014; Achenbach and Brix 2014), though no differences were observed below 20 ppt. Soil water salinities of 28 ppt promoted replacement of invasive *P. australis* by native salt marsh species (Sun et al. 2007). Symptoms such as stunted plant growth are exacerbated by greater salinities and prolonged exposure (Hellings and Gallagher 1992; Howard and Rafferty 2006). Saltwater intrusion resulting from eustatic sea level rise, subsidence, and changes in weather patterns as a result of global climate change are expected to exacerbate salinity stress (reviewed in Day Jr et al. 2005).

The discovery of extensive die-back in the MRD precipitated the discovery of heavy infestations of the non-native Roseau Cane Scale (hereafter referred to as RCS), *Nipponaclerda biwakoensis* (Kuwana) (Hemiptera:Aclerdidae) (Knight et al. 2018). Subsequent sampling by our group revealed that the RCS was present in 11 parishes in southeastern Louisiana (Knight et al. 2018). The scale appears to have arrived with three Encyrtid parasitoid wasps known from their native range, *Astymachus japonicus* Howard, *Neastymachus japonicus* Tachikawa, and *Boucekiella depressa* Hoffer (Tachikawa 1970; Kaneko 2004; Knight et al. 2018). Native to Japan, China, and Korea, RCS is of little to no concern to local *P. australis* populations except as a pest where *P. australis* is grown commercially (Kuwana 1907; Qin et al. 2003; Brix et al. 2014).

The effect of the RCS on *P. australis* health has not been studied; however, feeding by sap sucking insects can result in physiological changes, which may reduce airflow to submerged roots (Armstrong et al. 1996b). Other damage by sap-sucking insects includes injury by direct removal of plant nutrients and sugars, as well as reduced photosynthesis from the accumulation of sooty mold growing on excreted honeydew (Bowling et al. 2016). Severe infestations of invasive sap-sucking, scale-like, insects in bamboo can result in death of the stand (Hodges and Hodges 2004). Currently, there are no data on the role of RCS infestations in *P. australis* die-back, but initial observations of relatively low scale densities and better apparent health of certain *P. australis* phenotypes in the MRD implicate RCS as a potential factor in the die-backs.

Within Louisiana there are several genetic lineages with distinctive phenotypes. Delta-type (haplotype M1) is genetically similar to populations found in North Africa and the Mediterranean, and is the dominant lineage found in the MRD (Hauber et al. 2011; Lambertini et al. 2012). Die-backs in the MRD appear to primarily, or at least more severely impact the Delta-type (Knight et al. 2018). Also found within the MRD is a phenotype of European lineage (EU-type, haplotype M) (Saltonstall 2002; Lambertini et al. 2012). The EU-type *P. australis* is a lineage that is often considered invasive throughout much of North America and is frequently reported to be less susceptible to arthropod herbivores than native North American haplotypes (Park and Blossey 2008; Cronin et al. 2015). Beyond the MRD, Land-type (haplotype I) is found in more upland habitat, is widely distributed along the Gulf Coast (Saltonstall 2002; Hauber et al. 2011), and has recently spread westward to California (Meyerson et al. 2010). Considered to be a distinct subspecies (*P. australis berlandieri*), Land-type occurs in less saline conditions, potentially explaining its rarity in the MRD (Achenbach and Brix 2014).

The goal of this study is to investigate the role of RCS in the die-back of *P. australis* by determining the susceptibility of different *P. australis* phenotypes to the scale, and testing how scale herbivory and salt stress interact to affect plant health. The first objective was to evaluate stand conditions and determine whether the two common *P. australis* phenotypes found in the MRD (EU-type, Delta-type) differ in RCS abundance (number of scales per stem) and frequency of parasitism. Five contiguous pairs of EU- and Delta-type stands were censused over two years. For the second objective, a controlled mesocosm experiment was conducted to test whether salinity level and *P. australis* phenotype influenced susceptibility to the RCS and whether the RCS alone or in combination with salinity could cause the die-back symptoms observed in the MRD.

Materials and Methods

Paired Stands of EU- and Delta-Type Phenotypes

Pass-a-Loutre Wildlife Management Area (Pass-a-Loutre) is a 46,540 ha reserve located in the Mississippi River Delta and managed by the Louisiana Department of Wildlife and Fisheries. Pass-a-Loutre is a coastal freshwater marsh with average salinity levels less than 1 ppt that is fringed by beach and barrier island habitats with *P. australis* being the dominant vegetation of the region (CPRA 2019). Mean water depth across sites at the time of sampling ranged from 7 to 44 cm. Mean annual air temperatures over the duration of the study ranged from 17.2 to 25 °C and sites consisted of Balize and Larose soils (Natural Resources Conservation Service 2019).

Across its invasive range, exotic Haplotype M exhibits growth habits that distinguish it from native haplotypes. Relative stem heights are comparable among haplotypes for

young shoots (Vasquez et al. 2005), but non-native *P. australis* are significantly taller later in the season, as well as having greater stem densities and overall aboveground biomass (League et al. 2006; Saltonstall and Stevenson 2007; Park and Blossey 2008; Mozdzer and Zieman 2010; Price et al. 2014). In the MRD the European haplotype is between 1.5 and 2.5 m shorter than either the Land haplotype (I) or the dominant Delta haplotype (M1) (Hauber et al. 2011).

Paired populations of Delta and European *P. australis* phenotypes (Delta-type and EU-type respectively) were selected from within Pass-a-Loutre (Table 1). Sites were chosen by the presence of large stands of EU-type, distinguished from Delta-type by shorter more gracile stems, purple inflorescences present during the early summer, and sparse or absent ligule hairs, which are dense on Delta-type stems (Hauber et al. 2011). Each pair of study plots was established along the channel edge and was accessible by boat. Sites were identified, established, and first sampled on 31 May 2017. Repeating approximately every two months, subsequent sampling occurred on 11 July 2017, 13 October 2017, 25 January 2018, 28 March 2018, 15 May 2018, 07 July 2018, and 18 September 2018, repeating approximately every two months. Sampling did not occur between 11 November 2017 and 21 January 2018, because Pass-a-Loutre was off limits to researchers during the migratory bird and waterfowl hunting season.

Measurements of stand health and growth were taken on each sample date. Stem heights were measured to the nearest centimeter from the soil surface for 10 live stems randomly chosen from within each plot. Live and total *P. australis* stem densities were recorded from five 0.25m^2 quadrats randomly located throughout the plot. Quadrats consisted of a 1.77 m length of plastic tubing connected at each end with a barbed coupler to form a 0.25m^2 loop. Three criteria were used to determine whether an area would be used for a stem density quadrat: the sample areas must occur within the bounds of the plot, the sample area must contain at least a single *P. australis* stem, and sample areas were located ≥2 m apart. On the final sample date, a percent green measurement was taken as an index of degree of plant senescence. Percent green was estimated visually as the percentage of a *P. australis* stem located above the surface of the water that was covered by green leaf sheath tissue (measured from the node of the lowest green leaf), and was obtained from the same stems used to measure height. The proportion of stems infested with RCS was determined from 30 stems haphazardly collected from within each plot. Leaf sheaths were peeled back from the stem to observe the scales underneath. Ten infested stems from this collection were brought to the lab in sealed plastic bags. If fewer than 10 stems were infested (i.e., when scale incidence was low), all infested stems were returned to the lab. Total live scale counts, including adult and immature scales, as well as scales with visible parasitoid wasp larvae or pupae were enumerated on each stem within a week of collection. Scales that were desiccated, predated upon, or with obvious parasitoid exit holes were assumed to be dead and not counted. Live scale counts are reported as densities per meter stem.

Table 1 GPS coordinates for paired-plot sites in the Pass-a-Loutre Wildlife Management Area of the Mississippi River Delta, Plaquemines Parish, Louisiana, USA

Plot	Delta-type		EU-type	
	Latitude	Longitude	Latitude	Longitude
1	29.13586	−89.1935	29.13698	−89.193
2	29.14009	−89.1916	29.13983	−89.1918
3	29.15063	−89.1987	29.14991	−89.1996
4	29.14363	−89.1503	29.14374	−89.1506
5	29.15029	−89.1889	29.15059	−89.189

Mesocosm Experiment

To investigate whether scale infestations and their interaction with salinity produced significant declines in plant health, a mesocosm study was established at Louisiana State University Innovation Park in Baton Rouge, Louisiana (30.3580, −91.1425). Rhizome material from three, four, and three populations from each *P. australis* phenotype (Delta-, EU-, and Land-type, respectively) were propagated in January 2018 (Table 2). *Phragmites* populations had been collected from field sites a minimum of one year prior to propagation, and rhizome materials used in the mesocosm experiments were harvested from cultures of these populations. Clones of two of the three Land-type *P. australis* did not successfully propagate and this phenotype was only represented by a single source population for this study. Propagation consisted of clipping rhizomes into 10–20 g sections containing between 1 and 4 nodes each. The rhizome cuttings were planted in 5.7-

Table 2 Phenotype, collection date, and GPS coordinates of collection sites of rhizome material used to propagate *Phragmites* clones used for the mesocosm experiment

Clone	State	Phenotype	Latitude	Longitude	Date collected
D1	Louisiana	Delta	29.07972	−89.2981	03 Apr 2017
D2	Louisiana	Delta	29.17649	−89.2864	18 Apr 2017
D3	Louisiana	Delta	29.05301	−89.3327	18 Apr 2017
E1	Louisiana	EU	29.17608	−89.2866	18 Apr 2017
E2	Louisiana	EU	29.65813	−92.5199	22 Aug 2017
E3	Louisiana	EU	29.21948	−89.3006	08 Feb 2017
E4	Louisiana	EU	29.14991	−89.1996	11 Jul 2017
G1	Texas	Land	29.55194	−94.3895	21 Apr 2017

L nursery pots filled with sand and placed into 75-L plastic pools. In total there were 24 pots per clone. Pools were filled with tap water and fertilized with 15 mL of Miracle-Grow™ All Purpose Plant Food (Stern's Miracle-Gro Products, Port Washington, NY, USA) and 11 mL of Liquinox Iron and Zinc (Liquinox Co, Orange, CA, USA) and potted rhizomes were allowed to grow in a greenhouse for approximately 3 months (see Bhattarai et al. 2017). Pots that had successfully sprouted were moved to outdoor mesocosms (pools) on 16 April, 2018 with one pot of each population per pool.

Experimental treatments were arranged in a split plot design. Whole plot treatments were applied at the pool level that received a combination of either 'fresh' or 'salt' salinity treatments and 'Infested' or 'Non-Infested' RCS infestation treatments. Whole plots were replicated six times, for a total of 24 pools. Split plots were by clone, with each pool containing a single clone from each population, excepting the two Land-populations that failed to grow. There were a total of eight unique clones per pool, a total of 24 pots per clone, and 192 pots in total. Pools were spaced at least one meter apart to minimize movement of scales between pools, and arranged in a 4 by 6 grid. Salinity treatments began 1 week after pots were placed in the pools. Salinity was manipulated through the addition of Instant Ocean® (Spectrum Brands, Blacksburg, VA, USA) aquarium salt to the pools, and maintained between 10 and 15 ppt for the duration of the study for half of the 24 pools. Water levels were maintained roughly equivalent with the surface of the pots. Pool salinity was monitored weekly, and salt or water was added to reestablish the target salinity level when necessary. Scale inoculum for the scale-infestation treatment was collected the last week of June. Prior experimentation found that direct inoculation with mobile first instar nymphs or gravid females did not reliably result in establishment of scale populations. Instead it was determined that inoculation using stem segments bearing gravid females, though difficult to control the exact volume of inoculum, was sufficient to result in establishment of scales in all treated pots. Inoculum was collected from the MRD and consisted of 5–15 cm long segments of infested *P. australis* stems containing a minimum of 5 gravid female scales per segment. Three infested stem segments were placed on the soil in direct contact with stems in each pot within a pool. Half of each of the fresh and salt treated pools were designated for the infestation treatment, and every pot within a pool receiving the treatment was innoculated. Stem segments used for inoculation were not removed. Successful infestation was determined a week after inoculation, by gently peeling back leaf sheaths of stems in infested pots to confirm the presence of settled nymphs. After infestation, pots were left to grow undisturbed until October 16, 2018 when plants were harvested.

Harvesting consisted of removal of all *P. australis* aboveground biomass. Harvested material was returned to the lab where the total number of live stems per pot was enumerated. A subsample of up to ten live stems per pot was randomly selected and each stem was measured for height (cm) and the percentage of the stem with green leaf tissue. Percent green was measured as the proportional length of green tissue to the total length of the stem. The stems were then inspected for the presence or absence of scales. If there were fewer than ten live stems in a pot then percent infestation, stem height, and percent green were determined from the total number of live stems per pot. Up to five infested stems per pot were randomly selected and total live scales per meter of stem were enumerated. Stems were then transferred to paper bags and allowed to dry for two weeks before being weighed for dry biomass.

Data Analysis

All observations were pooled by plot and sample date (field survey) or by pot (mesocosm experiment) and means analyzed with generalized linear mixed models (PROC GLIMMIX; SAS Institute, Cary, NC), and effects were considered significant when p-values were less than 0.05. For paired plots, the effects of phenotype, sample date, and their interaction on mean stem heights, live stem densities, and scale densities were analyzed with repeated measures using a compound-symmetry covariance structure and the Kenward-Rogers (Kenward and Roger 1997) correction for degrees of freedom. A repeated measures model would not converge for analysis of proportion of infested stems, so differences between phenotype were analyzed by each date individually.

For the mesocosm study, the effect of salinity, infestation treatment, and phenotype and their interactions on stem height, live stem densities, proportion of stems infested with scale, percentage of the stem with green tissue, and scale counts per meter stem using generalized linear mixed models (PROC GLIMMIX). Stem heights and live stem densities for both paired stands and mesocosms were normally distributed and were modeled with a Gaussian distribution. The proportion of stems infested, proportion of parasitized scales, and percent green estimates were modeled with a binomial distribution. Counts of live scales per meter of stem were modeled with a Poisson distribution. Effects of individual clones within phenotype were not examined. All means are presented with standard error, and means separation ($\alpha = 0.05$) was done with the LSMEANS statement in PROC GLIMMIX adjusted with the Games and Howell (1976) method for pairwise multiple comparisons.

Results

Paired Plots at the Mississippi River Delta

The two *P. australis* phenotypes observed in the paired stands exhibited different growth habits over the course of the

season. Mean stem heights of the Delta-type (247.0 ± 4.5) were 22.4% taller than adjacent EU-type (202.0 ± 3.3) (Table 3), and mean heights increased over the growing season (Fig. 1a). No interaction was observed between phenotype and date with regard to stem height. Mean stem densities did not differ between phenotypes. Stem densities generally increased over the season, with the EU-type increasing in density more rapidly than the Delta-type (Fig. 1b). For the final sample date in September 2018 (the only date for which percent green data were recorded), stems of EU-type plots were 1.9 times greener (Delta-type = 0.241 ± 0.022; EU-type = 0.489 ± 0.032). The two varieties flowered at different times with EU-type flowers appearing as early as May, while Delta-type flowered in late September – October.

Phenotype was a major factor in the differences in infestation by *N. biwakoensis* observed in the MRD. The proportion of infested stems was generally lower for the EU-type, though close to 100% of inspected stems were infested by the final sample date of each year (Fig. 1c). Delta-type had 42.4 and 48.0% greater proportion of stems infested for three of the seven sample dates (07/2017, 03/2018, and 05/2018), and EU-type stands had 46.4% greater proportion of stems infested in January 2018. Scales per meter of stem were between 2 and 7 times greater in Delta- vs. EU-type stands on five of the seven sample dates (Fig. 1d). Densities generally increased over the course of the growing season, declining over the winter, and differences by date were significant. A significant phenotype by date interaction was also observed for scale densities, with changes in densities between dates being much more in Delta- than those for EU-type, which did not change significantly across all samples. Mean proportion of parasitized scales (0.11 ± 0.02) was generally higher later in the season; however, neither phenotype nor sample date was significantly affected.

Mesocosm

Results from the garden mesocosm experiment were similar to those from the field survey with respect to differences between *P. australis* phenotypes. A significant main effect of phenotype was observed for stem heights, and Delta-type stems (112.8 ± 2.9 cm) were 20.4% taller than the Land-type (89.9 ± 5.2 cm) and 31.0% taller than the EU-type stems (78.9 ± 1.4 cm), though each responded differently to salinity or infestation treatments (Table 4) (Fig. 2a). Stem densities, as measured by the number of live stems per pot, were also 2.4 and 1.8 times greater in EU-type pots compared to Delta- or Land-type, respectively, which themselves were not significantly different (Fig. 2b). Lastly, percent green significantly differed by phenotype (Fig. 2c). EU-type (0.36 ± 0.01) had a 1.9 and 1.4 fold greater proportion of green stem than Delta-type (0.19 ± 0.01) and Land-type (0.28 ± 0.02), respectively.

Table 3 Type III test of fixed effects for all models reported in the paired plots study

Variable	Effect	df	F	p
Stem Height	Phenotype	1, 3.7	112.92	< 0.001
	Date	6, 46.53	15.04	< 0.001
	P * D	6, 46.99	0.62	0.715
Live Stem Density	Phenotype	1, 4.146	6.99	0.055
	Date	6, 46.51	8.35	< 0.001
	P * D	6, 46.63	4.91	< 0.001
Proportion Stems Infested(by sample date)	P - 07/2017	1, 8	45.61	< 0.001
	P - 10/2017	1, 8	0.92	0.366
	P - 01/2018	1, 8	33.66	< 0.001
	P - 03/2018	1, 8	29.62	< 0.001
	P - 05/2018	1, 8	8.17	0.021
	P - 07/2018	1, 8	< 0.01	0.974
	P - 09/2018	1, 6	< 0.01	0.980
Live RCS per Meter Stem	Phenotype	1, 54	46.82	< 0.001
	Date	6, 54	81.45	< 0.001
	P * D	6, 54	8.81	< 0.001
Proportion Parasitism	Phenotype	1, 26.02	0.17	0.680
	Date	6, 48.85	0.82	0.561
	P * D	6, 48.85	0.51	0.797
Percent Green	Phenotype	1, 75	35.11	< 0.001

The effect of phenotype on the proportion of stems infested was modeled separately for each date. For interaction effects, Phenotype and Date are abbreviated as "P" and "D", respectively. For the proportion of stems infested section of the table the effect of Phenotype (P) is individually modeled for the date shown

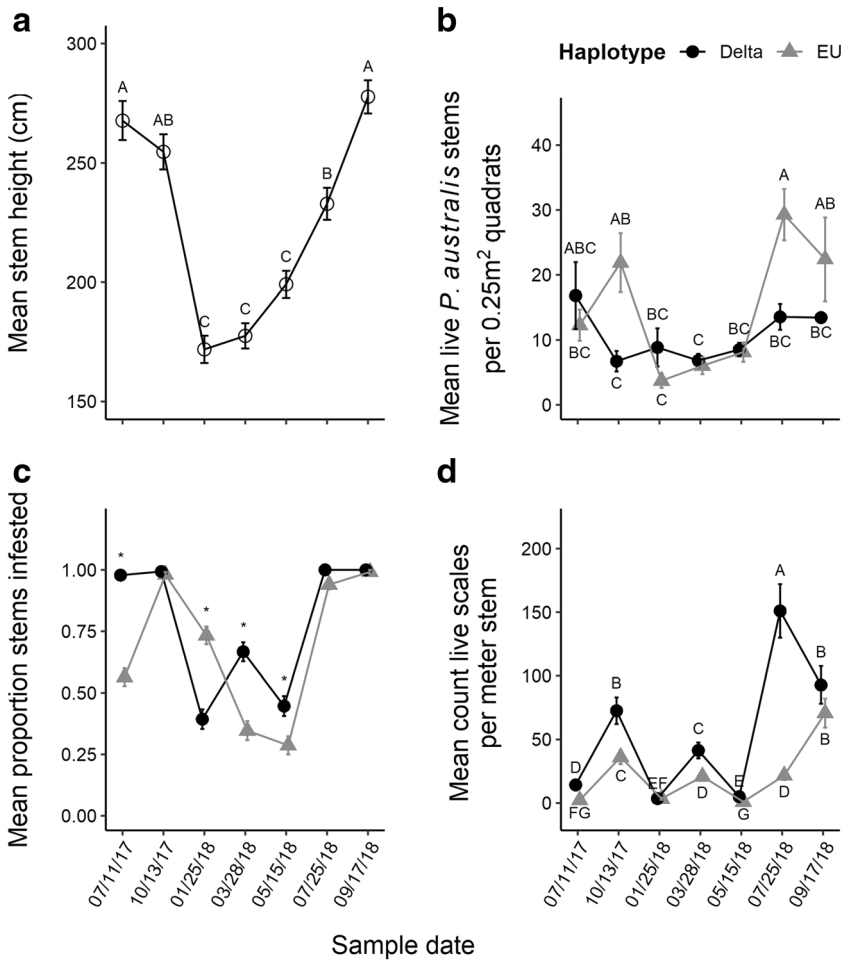

Fig. 1 Measurements of *P. australis* stand condition and *N. biwakoensis* infestation obtained from paired stands of EU-type and Delta-type *Phragmites* by sample date. A) Mean stem height (cm) ± SE, B) mean live stem density of Delta- (black circles) and EU-type (grey triagles) stands per 0.25m^2 quadrat ± SE, C) mean proportion ± SE of stems infested with *N. biwakoensis* and D) mean live scale counts ± SE per meter of stem for EU-type and Delta-type *Phragmites*. Letters in Fig. 1a correspond to the main effect of sample date, and dates that share letters are not significantly different ($P \leq 0.05$). Letters for Fig. 1b, d correspond to the interaction of phenotype and sample date, and phenotype by sample date combinations that share letters are not significantly different ($P \leq 0.05$). Figure 1c only show individual comparisons between phenotypes by sample date. Differences between phenotypes were significant on sample dates marked with an asterisk ($P \leq 0.05$)

Scale infestation and salt stress also had varying effects on plant growth. The 10–15 ppt salt reduced mean stem heights of all haplotypes, though the magnitude of this reduction differed by haplotype (Table 4). Percent reductions in height due to salinity were 20.5, 16.2, and 32.4 for the Delta-, EU- and Land-type respectively. No significant main effect of infestation treatment was observed for stem heights. A significant phenotype by infestation treatment interaction was observed, with infested Delta phenotype having 12.7% shorter stems than non-infested Delta-, while EU- and Land-type were unaffected (Fig. 2a). Salt treatment also reduced the number of live stems per pot by 13.2% (fresh = 27.6 ± 3.2; salt = 22.3 ± 3.3). Infestation treatment had no effect on stem density. Conversely, infestation treatments resulted in a 34.9% reduction in percent green regardless of phenotype (Fig. 2c). No significant main effect of salinity was observed for percent green, but a significant salinity by phenotype interaction was observed with salt treatments, reducing percent green by 13.7% for EU-type compared to fresh treatments, but no differences by salt treatment for Land- or Delta-type.

Although the non-infested pools were free of scales at the start of the experiment and were isolated from other pools by a minimum of 1 m, at the end of the study, scales were observed in all but a single pot. The percentage of stems per pot infested with scales was 17.6% higher in the infested compared to the non-infested treatments (Fig. 2d). Percentage of stems infested also differed by phenotype, with Delta-type having 14.1 and 9.52 greater percent stem infestation rate compared to EU- or Land-type, respectively. The EU- and Land- phenotypes did not differ. Salinity did not affect the percentage of stems infested. Scale densities were also 2.5 times lower in the non-infested plots and varied by phenotype (Fig. 2e). A significant phenotype by infestation treatment was also observed, with the change in densities between infested and non-infested treatments being much more pronounced for Delta- and Land- compared to EU-type. Scale densities were 94.3% greater in the saline pools compared to the freshwater pools.

Discussion

Differences among *P. australis* Phenotypes

An important distinction when determining the role of RCS in the die-back symptoms observed in the MRD, was determining whether observed differences in the health between

Table 4 Type III test of fixed effects for all models reported in the mesocosm study

Variable	Effect	df	F	p
Stem Height	Infestation	1, 160	1.25	0.265
	Phenotype	2, 160	96.69	< 0.001
	Salinity	1, 160	40.37	< 0.001
	I * P	2, 160	5.54	0.005
	I * S	1, 160	0.72	0.397
	P * S	2, 160	4.7	0.010
	I * P * S	2, 160	0.9	0.409
Live Stem Density	Infestation	1, 148	0.01	0.921
	Phenotype	2, 148	9.98	< 0.001
	Salinity	1, 148	5.42	0.021
	I * P	2, 148	0.53	0.592
	I * S	1, 148	0.04	0.852
	P * S	2, 148	0.63	0.533
	I * P * S	2, 148	0.15	0.857
Percent Green	Infestation	1, 160	16.71	< 0.001
	Phenotype	2, 160	61.94	< 0.001
	Salinity	1, 160	0.45	0.504
	I * P	2, 160	0.3	0.740
	I * S	1, 160	2.33	0.129
	P * S	2, 160	3.88	0.023
	I * P * S	2, 160	2.56	0.081
Proportion Stems Infested	Infestation	1, 155	19.41	< 0.001
	Phenotype	2, 155	11.1	< 0.001
	Salinity	1, 155	0.05	0.822
	I * P	2, 155	0.94	0.393
	I * S	1, 155	2.7	0.102
	P * S	2, 155	0.85	0.429
	I * P * S	2, 155	3.03	0.051
Live RCS per Meter Stem	Infestation	1, 160	17.14	< 0.001
	Phenotype	2, 160	21.97	< 0.001
	Salinity	1, 160	8.32	0.005
	I * P	2, 160	5.72	0.004
	I * S	1, 160	0.44	0.510
	P * S	2, 160	2.73	0.068
	I * P * S	2, 160	2.28	0.105
Dry Weight	Infestation	1, 160	17.14	< 0.001
	Phenotype	2, 160	21.97	< 0.001
	Salinity	1, 160	8.32	0.005
	I * P	2, 160	5.72	0.004
	I * S	1, 160	0.44	0.510
	P * S	2, 160	2.73	0.068
	I * P * S	2, 160	2.28	0.105

For interaction effects, Infestation, Phenotype and Salinity are abbreviated "I", "P", and "S", respectively

phenotypes is due to inherent differences in plant growth. Differences in stem heights observed in both the field and mesocosm studies match those typical of their phenotype as described by Hauber et al. (2011). While all stem heights were considerably shorter in the mesocosm compared to the field, similar differences between phenotypes were observed in the mesocosm experiment. With respect to stem densities, less has been reported about differences between Louisiana phenotypes. Achenbach and Brix (2014) observed no differences in stem densities between young plants. In our study, this was not the case with either the field or mesocosm results, with EU-type having significantly greater stem densities. This discrepancy may be due to the fact that both field and mesocosm populations had a greater length of time to develop these differences relative to the 28 day old plants used by Achenbach and Brix (2014). In its invasive range, EU-type *P. australis* is widely reported as having greater stem densities than its native counterparts, so greater stem densities of EU-type in Louisiana is unsurprising. Achenbach and Brix (2014) do report substantially lower shoot height and biomass for the Land-type relative to other Louisiana haplotypes, which was not the case in this study. It should be noted that Land-type was only represented by a single source population, so inferences that can be made about this phenotype in the context of this study are limited.

Percent green, as it was measured for this study, is not a metric that has been reported in previous literature. It does provide a quick and useful measure of senescence, which is one of the more striking symptoms of die-back in the MRD. A similar measure made by Park and Blossey (2008) observed greater number of leaves per stem in exotic compared to native *P. australis*. Similarly, Eller et al. (2014) observed that leaf production rate and overall leaf dry matter to be greater in EU- compared to Delta-type. Relatively greater stem elongation rates in Delta-type and greater leaf production rates in EU-type may provide an explanation for the greater percent green observed in the EU-type, and highlights the need for caution when comparing these measures between phenotypes. Interestingly, in a review of differences between introduced and native lineages of *P. australis*, above ground biomass production is reported as higher in the introduced lineages (Mozdzer et al. 2013). No such differences were observed here, and it appears that, at least in the mesocosm pots, above-ground production was equivalent between the Louisiana phenotypes despite the differences in growth habit.

Salt Stress

Though salt is a known stressor, *P. australis* is generally tolerant to a wide range of salinities. Lissner and Schierup (1997) report die-back of European stands with greater than 15% soil water salinity, and stunted plant growth with increasing salinity (Hellings and Gallagher 1992) or prolonged exposure to elevated salinity (Howard and Rafferty 2006) has been documented. Similarly, Sun et al. (2007) report that soil water salinity of 28 ppt was necessary to promote replacement of

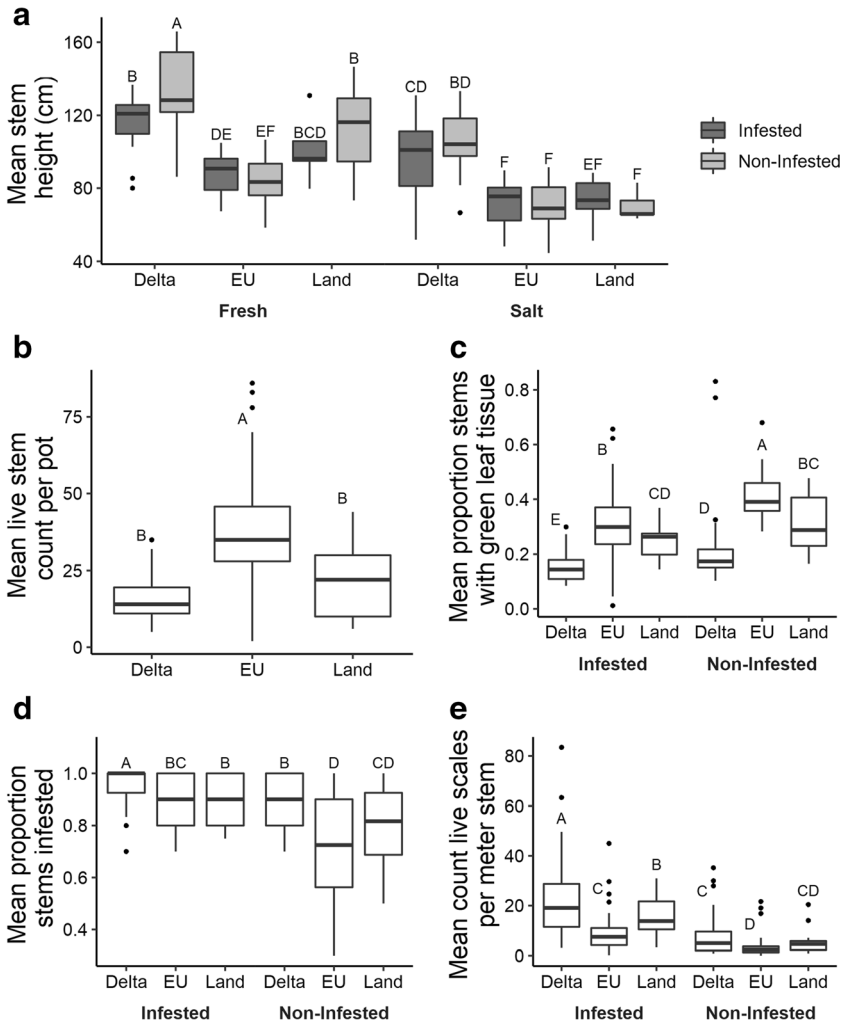

Fig. 2 Box plots depicting the condition of *P. australis* plants and *N. biwakoensis* (RCS) abundance in experimental mesocosms. **a** *Phragmites* stem height (cm), (**b**) live stem density per pot, (**c**) percent green per pot, (**d**) proportion of infested stems per pot and (**e**) live scale counts are reported. Means that share letters are not significantly different ($P \leq 0.05$)

invasive *P. australis* by salt marsh species. Concentrations of 20 ppt and greater have been shown to reduce shoot production (Eller et al. 2014; Achenbach and Brix 2014), and no differences were observed below 20 ppt. Soil salinity was not measured in the paired stands for this study, and water salinity was measured infrequently. However, no readings taken over the course of sampling the paired stands were greater than 1 ppt (Knight unpub. obs.). This is supported by hourly salinity data collected by the Coastwide Reference Monitoring System, where salinities are reported at ≤1 ppt for stations nearest to the paired stands, though sites closer to the Gulf of Mexico can occasionally reach salinities of greater than 20 ppt (CPRA 2019). Because the mesocosms were maintained between 10 and 15 ppt, the results reported here are consistent with the literature with respect to stem density.

Differences in tolerance to increasing salinities with respect to haplotype/ lineage are also consistently reported in the literature, with the introduced Eurasian lineages generally having greater tolerance or growth under saline conditions (Vasquez et al. 2005; Price et al. 2014). These observations do not seem to be the case with Louisiana haplotypes as Achenbach and Brix (2014) observed almost no differences between Delta-type and EU-type shoots, though both were more tolerant to salinity than Land-type. Interestingly, though the reduction in stem height by salinity was the smallest for the EU-type, salt treatments only reduced percent green of EU-type. It is unclear why this was observed, though Eller et al. (2014) suggesting the Delta-type may be more salt tolerant. It is difficult to make too many inferences about the Land-type from this study, as it was only represented by a single clone in our mesocosm, the relatively higher susceptibility to salt stress of the Land-type reported by Achenbach and Brix (2014) was not observed in this study, though this may be due to differences in what measurements were taken and the duration of the study.

The differences in susceptibility of *P. australis* phenotypes to herbivory reported here has interesting implications for understanding the role of RCS in the die-back syndrome. While both the effects of salinity or herbivory on invasive and native *P. australis* lineages has been well studied (reviewed in Hazelton et al. 2014), there does not appear to be any

information regarding interactions between salinity or other stressors on susceptibility of *P. australis* to arthropod herbivores. Salt stress is known to modulate phytohormones involved in plant responses to herbivore damage (Wang et al. 2001), and may result in induced resistance. In the case of the non-halophyte *Brassica juncea* (L.) Czern, salt stress was not found to affect induced resistance or tolerance, but resulted in greater constitutive resistance due to a decrease in host plant quality (Renault et al. 2016; see also Thaler and Bostock 2004). Alternatively, Eichele-Nelson et al. (2017) report improved fecundity of two spotted spider mites (*Tetranychus urticae* Koch) on corn (*Zea mays* L.) and soybean (*Glycine max* L.) under increasing salinity stress. Despite the apparent reduction in host quality, the increase in scale densities reported here more closely resemble those of Eichele-Nelson et al. (2017).

Scale Herbivory

Reports of the invasive EU-type lineage in North America have generally found that it is more resistant to arthropod herbivores than native haplotypes. Cronin et al. (2015) observed lower feeding damage on the European haplotypes by leaf chewing herbivores, reduced infestation by gall forming midges (*Lipara* spp., Diptera, Chloropidae), and reduced densities of mealy plum aphid (*Halyopterus pruni,* Hemiptera, Aphididae) relative to native haplotypes at lower latitudes. When comparing stem heights of native (Haplotype E) and introduced (Haplotype M) *P. australis*, Park and Blossey (2008) observed no differences between haplotypes grown in a common garden. At their field site, though both varieties were taller than in the common garden, the introduced haplotype was significantly taller than the native; this difference in height was attributed to higher incidence of gall forming *Lipara* spp. on the native stems (see also Allen et al. 2017). Similarly, Lambert and Casagrande (2007), observed lower susceptibility to *H. pruni* in European (Haplotype M) compared to native haplotypes (S and E), where feeding damage and sooty mold growth on honeydew has been attributed to plant mortality. Results from this study align with most (but see Blossey et al. 2018) reports of reduced susceptibility of EU-type to herbivory, and differences in susceptibility to RCS between the dominant Delta phenotype and the sporadic populations of the EU-type in the MRD of two magnitudes or greater were frequently observed over two growing seasons across all paired sites and in the mesocosms. This is particularly interesting considering both are non-native lineages, though no study to date has assessed the relative susceptibility of Delta-type to native North American lineages. Honeydew production and its effect on plant fitness was not measured as part of this study; however, at sites in the MRD heavy infestations can often be detected by the smell of fermenting honeydew before stems are even inspected for scale (I. Knight personal observations).

Armstrong et al. (1996a) suggested that insect feeding damage on *P. australis* could cause die-back, because it reduces or prevents airflow through induction of premature senescence and callus formation in the aerenchyma. In addition to loss of nutrients to insect feeding and loss of photosynthetic potential with prematurely senesced leaves, reduced airflow to rhizomes could increase stress from phytotoxins in the soil, reduce development of horizontal rhizomes during the growing season, and cause permanent anoxia at buds and root tips (Armstrong et al. 1996a). Culm airflow and callus formation were not measured as a part of this study; however, the reduction in percentage of stem with green leaf tissue in infestation treatments, and across phenotypes indicates that scale infestation can be a significant factor driving premature senescence. The fact that the reduction in green tissue was observed independent of phenotype and salt stress provides support for a role of RCS in the die-backs symptoms observed in the MRD.

Conclusions

While initial investigations into the die-backs in the MRD were centered on the scale due to the high populations observed at affected sites in 2016 (Knight et al. 2018), it was important to observe how scale infestations were interacting with abiotic stressors. Studies of *P. australis* die-back in Europe hinted at various potential causes including phytotoxins in the sediment (Armstrong et al. 1996b), eutrophication (van der Putten 1997), and prolonged winter flooding (McDonald 1955). As addressed above, salinity is also a known stressor, which may exacerbate scale infestations in areas where tides or storms push saline water into the marsh. Whether salinity is a significant factor in die-back in the upper reaches of the MRD, where paired plot sites were located is not clear; however, increasing storm intensity and significant relative sea level rise may increase susceptibility of all *P. australis* phenotypes.

The role of phenotype is also a significant factor in determining susceptibility to scale infestations and die-back. No previous work has addressed the relative susceptibility of Delta-type to herbivory relative to other *P. australis* phenotypes. There is, however, an abundance of literature describing reduced susceptibility of EU-type, and the results reported here are consistent with those accounts. EU-type has generally been reported as being more tolerant of adverse conditions; however, a recent comparison of Louisiana phenotypes suggested that Delta-type was better adapted to conditions in the MRD (Eller et al. 2014).

The results of this study support multiple conclusions about the relationship between RCS and *P. australis* in the MRD. First, Delta-type *P. australis* has been confirmed to be more

susceptible to scale infestations. Second, scale infestations have been shown to reduce the proportion of stem with green leaf tissue for all phenotypes, and reduces mean stem heights for the susceptible phenotypes, despite generally lower populations in controlled mesocosms compared to the field. The reduced stem density symptoms associated with die-back were not observed in scale-infested plants in the mesocosms, suggesting that other factors may be involved in the dieback in the MRD or that scale induced changes in stem density may not be observable over a single growing season. Lastly, results support the conclusion that abiotic stress like salinity can increase susceptibility of all phenotypes to herbivores like RCS; however, additive effects of stressors on plant health were not observed.

Going forward, the greater susceptibility of the dominant Delta- phenotype and potential for increasing susceptibility of all phenotypes with increasing salinity raises questions about the role different *P. australis* phenotypes in Louisiana. If the effects of scale infestations and other potential stressors cannot be effectively mitigated, how will EU-type, native marsh grasses, and other aquatic invasives such as taro (*Colocasia esculenta*), alligatorweed (*Alternanthera philoxeroides*), and water hyacinth (*Eichornia crassipes*) respond to reduced competition by Delta-type affected by scale and abiotic stressors? Research is currently underway at Louisiana State University to further elucidate the potential impact of other known stressors of *P. australis*, modeling the long term effects of *P. australis* die-backs on the MRD, evaluation of restoration efforts and succession of plant communities, and investigating the biology and potential role of natural enemies for mitigating scale infestations in the MRD.

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