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Determination of common reed (*Phragmites australis* (Cav.) Trin. ex Steudel) varieties in Florida

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INTRODUCTION

The common reed (*Phragmites australis* [Cav.] Trin. ex Steudel, hereafter referred to as *Phragmites*) has a cosmopolitan distribution, occurring on all continents except Antarctica, and may be the most widely distributed flowering plant in the world (Tucker 1990). Over the past 150 years, *Phragmites* has become much more widespread and abundant in North America and is considered invasive at many locations (Meyerson et al. 2009a). The spread of *Phragmites* has been attributed to changing land use patterns, increased nutrient availability, and to the cryptic invasion of a lineage of European or Asian origin that was recently identified using genetic typing (Marks et al. 1994, Saltonstall 2002, Meyerson et al. 2009a). The varieties of *Phragmites* currently found in North America can be identified based on chloroplast DNA sequences (Saltonstall 2002): a native variety found along the east coast and inland in North America, a Gulf Coast variety, and a nonnative Eurasian variety.

The Eurasian variety was introduced to the east coast of the United States in the late 1700s or early 1800s and now dominates much of the Atlantic coast (Saltonstall 2003a). It has also invaded regions around the Great Lakes and is found in the Mississippi River Delta and a few western sites around major metropolitan areas (Saltonstall 2002, 2003a, 2003b). The Eurasian variety outcompetes native varieties and decreases the diversity of other native plant species by forming dense monocultures (Meyerson et al. 2009a). Hybridization between native and nonnative varieties was originally thought to be nonexistent, but recent studies suggest that hybridization does take place and could represent an additional threat to the genetic integrity of North American varieties (Meyerson et al. 2009b, Paul et al. 2010).

Historically, the Gulf Coast variety was the only one known to occur along the Gulf Coast and eastern Atlantic Coast of Florida; however, it is not clear if this variety is native to North America. This variety has been present along the Gulf Coast since at least the 1800s, but it is also common in South America and is closely related to a variety from Asia (Saltonstall 2002, 2003a). One author has assigned Gulf Coast *Phragmites* to another species, *Phragmites karka* (Retz.) Trin. ex Steudel, which also occurs in Australia, Polynesia, and tropical Asia (Ward 2010). The Eurasian variety has only been found in the Mississippi Delta along the Gulf Coast and may have been there since at least the 1970s (Pellegrin and Hauber 1999, Saltonstall 2003a, Howard et al. 2008). Genetic examination of five early herbarium samples and seven modern samples from Florida found only the Gulf coast variety (Ward and Jacono 2009). *Phragmites* has not been extensively collected and genotyped from across the state of Florida or other areas east of the Mississippi; therefore, it is possible that the Eurasian variety may be present but undetected in these areas (Ward and Jacono 2009). There have been reports of *Phragmites* increasing its local range in some areas of Florida, although it is unknown whether this is due to the presence of the invasive Eurasian variety (Matt Phillips and Don Schmitz, pers. comm.). If these local expansions are due to the presence of the Eurasian variety, it may be possible to remove these patches before they spread further in the state. The objectives of our study were to (1) genetically determine if the exotic Eurasian variety has invaded Florida or how close it currently is to Florida, and (2) determine if there are morphological characteristics that can be used to easily distinguish the Gulf Coast variety from the exotic Eurasian variety in the field.

MATERIALS AND METHODS

Leaf tissue samples were collected from *Phragmites* from October 2009 to November 2010 from 69 locations in Florida, 4 in Alabama, 4 in Mississippi, 16 in Louisiana, 2 in Georgia, and 5 in South Carolina (Figure 1; Table 1). We selected the Florida sampling locations based on data contained within the 2008 and 2009 annual surveys of aquatic plants conducted by the Florida Fish and Wildlife Conservation Commission (FWC unpublished report). We visited all water bodies (72) that were reported by FWC to contain *Phragmites* in Florida and found the plant at 69 locations. Additional samples outside of Florida were collected opportunistically from stands of *Phragmites* observed near major roadways. At each location, young leaf samples (~2 cm^2) were usually collected from each of five stems (n = 518 samples total; Table 1). The stems were separated by at least 5 m. Individual leaf samples were preserved in small ziplock bags containing silica gel. At sample locations, we recorded the GPS coordinates, stem density (counted in one randomly selected 0.25 × 0.25m quadrat per population), stem height, stem texture (smooth or ribbed), color of exposed stem at base (i.e., not hidden behind a leaf sheath), panicle architecture (compact or open), and growth stage. For samples collected in

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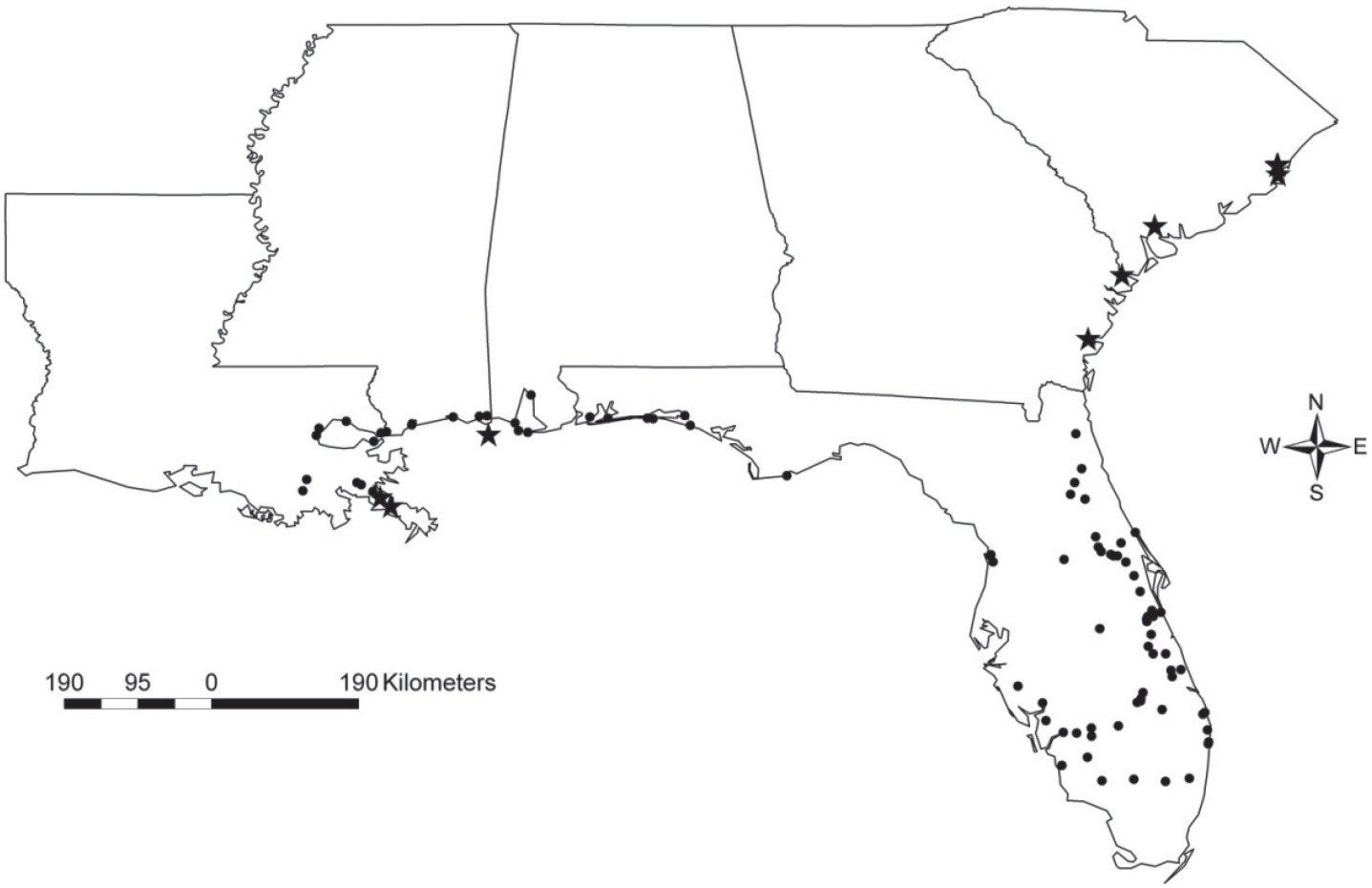

Figure 1. Sampling locations of *Phragmites*. Circles indicate the presence of the Gulf Coast variety and stars indicate the presence of the invasive Eurasian variety.

fall 2010, upper and lower glumes were measured on 4 to 5 spikelets from 1 to 10 panicles at each sampling site. Ligule length was measured on the second leaf from the top of the plant.

DNA was extracted using the Genomic DNA Mini plant kit (IBI Scientific, Peosta, IA). We first determined the variety of a subset of samples (n = 20) by sequencing two previously described chloroplast regions (*trnT(UGU)–trnL(UAA)* and *rbcL–psaI*; Saltonstall 2002) using BigDye v3.1 chemistry (Applied Biosystems, Carlsbad, CA) and an ABI3130 Genetic Analyzer (Applied Biosystems, Carlsbad, CA). We then screened all 518 individuals by amplifying a portion of the *rbcL* chloroplast region using the polymerase chain reaction (PCR), and then used the restriction enzyme *HhaI* to cut the PCR product using the methods of Saltonstall (2003c). Only the PCR product from the invasive variety is cut by the *HhaI* enzyme and is therefore visualized as a smaller product compared to the uncut Gulf coast variety PCR product when run on an agarose gel. PCR products were stained with GelRed (Biotium, Hayward, CA), separated on 1% agarose gels, and visualized using a UV transilluminator. We retested 96 of the 518 samples, and all yielded the same results. All varieties identified by complete sequencing were also correctly identified by the restriction enzyme method.

RESULTS

Stem texture, color of exposed stems, and panicle architecture did not vary between locations in Florida or locations in other states that were identified as the Gulf Coast variety. All stems examined were very smooth and shiny; the color of exposed stems at the base was red and the panicle architecture was open and often dropping (Table 1; Figure 2). Conversely, all plants identified as the invasive Eurasian variety by genetic data had noticeably ribbed stems and slightly dull color, the color of exposed stems at the base was green, and the panicles were compact and often erect (Table 1; Figure 2). No seeds were observed on panicles of either variety.

Stem density of the Eurasian variety was significantly higher (167 ± 41 m^{-2}, mean ± SE) than density of the Gulf coast plants (93 ± 8 m^{-2}; $F_{1,90} = 7.4$, $P = 0.007$). Gulf coast plants were taller than Eurasian plants (3.57 ± 0.11 m compared to 2.74 ± 0.27 m; $F_{1,62} = 7.5$, $P = 0.008$). Upper glume length was not different between the varieties (Eurasian = 6.04 ± 0.11 mm, Gulf Coast = 5.95 ± 0.06 mm; $F_{1,120} = 0.38$, $P = 0.54$), but the lower glumes of the Eurasian variety were longer than those of the Gulf coast variety (Eurasian = 3.92 ± 0.12 mm, Gulf Coast = 4.38 ± 0.05 mm; $F_{1,120} = 13.67$, $P < 0.001$). Ligule length did

Table 1. Sampling localities of *Phragmites*. Variety: E – Eurasian, G – Gulf Coast, determined genetically for all samples (N) at a given locality. Other abbreviations include: S – smooth stem, R – ribbed stem, O – open panicle, C – closed panicle, ND – not determined, R - stem color red at base when leaf sheath removed, G - stem color green at base when leaf sheath removed.

Locality Id	Latitude	Longitude	State	Variety	N	Stem	Panicle	Color
VOL01	28.93786	-81.0942	FL	G	5	S	O	R
BRO002	26.14656	-80.5773	FL	G	5	S	O	R
COL002	26.15524	-81.3156	FL	G	5	S	O	R
COL001	26.17154	-80.9448	FL	G	5	S	O	R
BRO001	26.18475	-80.3014	FL	G	5	S	O	R
LEE01	26.33393	-81.779	FL	G	5	S	O	R
COL003	26.42942	-81.4821	FL	G	5	S	O	R
PBC005	26.58793	-80.0815	FL	G	5	S	O	R
PBC004	26.6109	-80.076	FL	G	5	S	O	R
HEN001	26.67717	-81.436	FL	G	5	S	O	R
LEE03	26.71404	-81.6098	FL	G	5	S	O	R
LEE02	26.7219	-81.7615	FL	G	5	S	O	R
PBC003	26.75096	-80.089	FL	G	5	S	O	R
HEN002	26.77103	-81.4376	FL	G	5	S	O	R
GL001	26.79858	-81.1245	FL	G	5	S	O	R
CH01	26.85783	-81.9632	FL	G	5	S	O	R
PBC001	26.93364	-80.1418	FL	G	5	S	O	R
PBC002	26.95502	-80.1217	FL	G	5	S	O	R
MRT001	26.9869	-80.6166	FL	G	5	S	O	R
BB01	27.06256	-80.92643	FL	G	5	S	O	R
DES01	27.06708	-82.0038	FL	G	5	S	O	R
BB02	27.07323	-80.90561	FL	G	5	S	O	R
BB03	27.08827	-80.86596	FL	G	5	S	O	R
BB04	27.11399	-80.86198	FL	G	5	S	O	R
OKE001	27.188	-80.8384	FL	G	5	S	O	R
SR01	27.26359	-82.2884	FL	G	5	S	O	R
SLC002	27.3741	-80.4976	FL	G	5	S	O	R
SLC001	27.4478	-80.5136	FL	G	24	S	O	R
WP059e	27.4546	-80.39771	FL	G	5	S	O	R
IRC003	27.6395	-80.5758	FL	G	5	S	O	R
IRC001	27.6414	-80.7209	FL	G	5	S	O	R
IRC002	27.7268	-80.7758	FL	G	5	S	O	R
BC02	27.86647	-80.74146	FL	G	5	S	O	R
PLK01	27.93405	-81.3386	FL	G	5	S	O	R
BRV003	28.01848	-80.7925	FL	G	5	S	O	R
BRV004	28.04836	-80.7947	FL	G	5	S	O	R
BRV005	28.06651	-80.7864	FL	G	5	S	O	R
BRV006	28.07812	-80.7813	FL	G	5	S	O	R
BRV001	28.07888	-80.7205	FL	G	5	S	O	R
BRV002	28.08492	-80.7527	FL	G	5	S	O	R
WP060	28.12563	-80.63112	FL	G	5	S	O	R

Table 1.(Continued) Sampling localities of *Phragmites*. Variety: E – Eurasian, G – Gulf Coast, determined genetically for all samples (N) at a given locality. Other abbreviations include: S – smooth stem, R – ribbed stem, O – open panicle, C – closed panicle, ND – not determined, R - stem color red at base when leaf sheath removed, G - stem color green at base when leaf sheath removed.

Locality Id	Latitude	Longitude	State	Variety	N	Stem	Panicle	Color
BRV007	28.1478	-80.7338	FL	G	5	S	O	R
BRV009	28.36786	-80.8714	FL	G	5	S	O	R
BRV008	28.37021	-80.871	FL	G	5	S	O	R
ORG001	28.55281	-80.9427	FL	G	5	S	O	R
WP058	28.71467	-81.03656	FL	G	5	S	O	R
CIT003	28.7166	-82.5784	FL	G	5	S	O	R
LK01	28.74521	-81.7542	FL	G	5	S	O	R
SEM01	28.78628	-8101806	FL	G	5	S	O	R
SC01	28.78687	-81.13403	FL	G	5	S	O	R
CIT002	28.7908	-82.6126	FL	G	5	S	O	R
CIT 001	28.8012	-82.603	FL	G	5	S	O	R
WP056	28.80222	-81.21075	FL	G	5	S	O	R
WP055	28.83653	-81.32384	FL	G	5	S	O	R
VC02	28.88924	-81.35522	FL	G	5	S	O	R
VC01	29.01172	-81.38757	FL	G	5	S	O	R
PC03	29.44989	-81.51167	FL	G	5	S	O	R
PC02	29.50765	-81.67751	FL	G	5	S	O	R
PC01	29.64366	-81.63138	FL	G	5	S	O	R
FRK001	29.7261	-84.9699	FL	G	5	S	O	R
SJ01	29.8051	-81.55048	FL	G	5	S	O	R
FDC001	30.21553	-81.61638	FL	G	5	S	O	R
WL002	30.3129	-86.0924	FL	G	5	S	O	R
WP062	30.39101	-86.5241	FL	G	5	S	O	R
SR01	30.39754	-87.0482	FL	G	5	S	O	R
OIOK	30.39817	-86.5914	FL	G	5	S	O	R
WP063	30.41112	-87.25803	FL	G	5	S	O	R
WP061	30.42775	-86.15547	FL	G	5	S	O	R
WL001	30.42823	-86.1553	FL	G	5	S	O	R
WP064	30.23191	-87.97785	AL	G	5	S	O	R
MODI	30.25293	-88.0855	AL	G	5	S	O	R
WP065	30.34199	-88.12601	AL	G	5	S	O	R
BSF	30.67047	-87.9419	AL	G	5	S	O	R
WP075	29.36909	-89.56648	LA	E	5	R	C	G
WP075f	29.36909	-89.56648	LA	G	5	S	O	R
WP074	29.47279	-89.68533	LA	E	5	R	C	G
WP074f	29.47279	-89.68533	LA	G	5	S	O	R
WP073	29.54039	-89.77699	LA	G	5	S	O	R
WP078	29.54758	-90.58734	LA	G	5	S	O	R
WP076	29.61863	-89.91122	LA	G	5	S	O	R
WP072	29.64307	-89.96192	LA	G	5	S	O	R
WP077	29.6815	-90.54378	LA	G	5	S	O	R

Table 1.(Continued) Sampling localities of *Phragmites*. Variety: E – Eurasian, G – Gulf Coast, determined genetically for all samples (N) at a given locality. Other abbreviations include: S – smooth stem, R – ribbed stem, O – open panicle, C – closed panicle, ND – not determined, R - stem color red at base when leaf sheath removed, G - stem color green at base when leaf sheath removed.

Locality Id	Latitude	Longitude	State	Variety	N	Stem	Panicle	Color
WP071	30.12643	-89.76379	LA	G	5	S	O	R
WP079	30.19677	-90.43211	LA	G	5	S	O	R
WP070	30.22575	-89.68087	LA	G	5	S	O	R
WP069	30.23889	-89.6171	LA	G	5	S	O	R
WP080	30.27881	-90.40023	LA	G	5	S	O	R
WP081	30.36166	-90.08533	LA	G	5	S	O	R
WP082	30.41953	-88.54069	LA	G	5	S	O	R
PBJ	30.20685	-88.4298	MS	E	5	ND	ND	G
WP068	30.32022	-89.32394	MS	G	5	S	O	R
WP067	30.41135	-88.84159	MS	G	5	S	O	R
WP066	30.42613	-88.45146	MS	G	5	S	O	R
GLY001	31.32788	-81.47091	GA	E	4	R	C	G
GCC001	32.07415	-81.07753	GA	E	5	R	C	G
BFT001	32.64779	-80.69691	SC	E	5	R	C	G
GRT004	33.25115	-79.26961	SC	E	5	R	C	G
GRT003	33.35609	-79.27979	SC	E	5	R	C	G
GRT002	33.36072	-79.27925	SC	E	5	R	C	G
GRT001	33.36855	-79.26856	SC	E	5	R	C	G

not vary between Gulf coast and Eurasian varieties ($F_{1,122}$ = 0.8, P = 0.38) and averaged 0.9 ± 0.02 mm.

For the 20 samples that were completely sequenced at the *trnT(UGU)–trnL(UAA)* and *rbcL–psaI* chloroplast regions, only the samples collected on Petit Bois Island, Mississippi, were the invasive Eurasian variety (chloroplast haplotype M in Saltonstall 2002), and only these samples were cut by *Hha I*. All other samples were the Gulf Coast variety (haplotype I in Saltonstall 2002) and were not cut by *Hha I*. We used several Petit Bois Island samples as positive controls for the invasive Eurasian variety on all subsequent gels. No samples from Florida or Alabama were cut by *HhaI* (Table 1). Samples from 2 of the 16 sites in the lower Mississippi River Delta of Louisiana were cut by *HhaI*, indicating they were the invasive Eurasian variety (Figure 1; Table 1). Both the sites in Georgia and all sites in South Carolina contained only the invasive variety (Figure 1; Table 1). The closest site in Georgia to Florida was 68 km north of the state border.

DISCUSSION

The Gulf Cost variety can be easily identified in the field by three morphological characters; (1) smooth stem, (2) stems red at base when exposed to the sun, and (3) open panicle architecture. In contrast, the invasive Eurasian variety has a ribbed stem that is green at the base when exposed to the sun and a compact panicle architecture. The Gulf Coast variety is taller and less dense than the invasive Eurasian variety, although these growth characteristics could not be used as reliable identification characteristics in the field. Our results for upper and lower glume length and ligule length are the same as those reported in Saltonsall et al. (2004). Although lower glume length was significantly different between the haplotypes, it cannot be used as a diagnostic character to separate varieties due to overlapping ranges. The lack of seed production has been previously reported for the Gulf Coast variety (White et al. 2004, Ward 2010). The Eurasian variety does produce seed (e.g., Meyerson et al. 2009b), but some authors have noted a lack of seed production in some populations (Ward 2010).

Our genetic testing suggests that the invasive Eurasian variety has not yet reached Florida waterways or areas east of the Mississippi Delta. It is perhaps surprising that the Eurasian variety is not more widespread along the Gulf Coast or in Florida given its close proximity to these areas and because at least one study suggests it may be a more effective colonizer than the Gulf Coast variety (Howard et al. 2008). The Eurasian variety may not be well adapted to the environment of these areas, or it may not be able to effectively compete in established stands of Gulf Coast plants.

Reports of *Phragmites* expanding its range in some areas of Florida such as in Apalachicola Bay (Matt Phillips, pers. comm.) are not due to the presence of the invasive variety. From a management perspective this means (1) it will not be necessary to attempt removal of an invasive variety, and (2) other factors such as habitat alteration or increased nutrient loading may need to be considered as potential facilitators for the spread of the Gulf Coast variety, especially if it begins to invade and displace native vegetation. Most studies

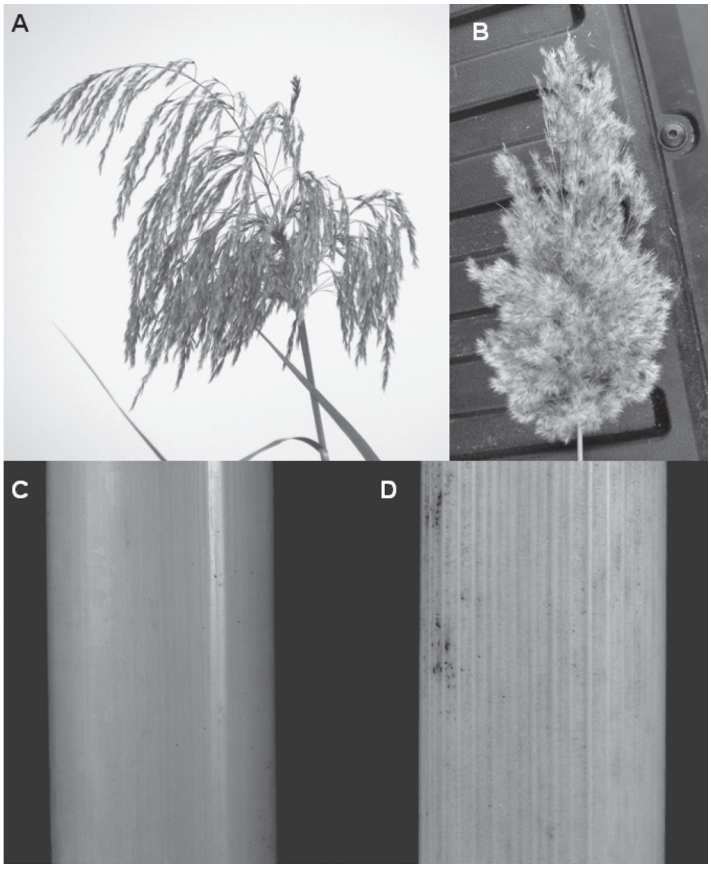

Figure 2. Characters to distinguish Gulf Coast and Eurasian *Phragmites australis*. A. Gulf Coast panicles open and often dropping; B. Eurasian panicles compact and often erect; C. Gulf Coast stem smooth and shiny; D. Eurasian stem ribbed and slightly dull.

have focused on the native North American and invasive varieties (Meyerson et al. 2009a), and very little work has been conducted specifically with the Gulf Coast variety to understand the factors that may be responsible for its spread and colonization dynamics (Howard et al. 2008). Future work on the effects of nutrient loading and habitat alteration on the growth of the Gulf Coast variety needs to be conducted, and common garden experiments in Florida could also be used to determine if the Eurasian variety has the ability to invade stands of the Gulf Coast variety in Florida or elsewhere along the Gulf Coast where it currently does not occur.

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