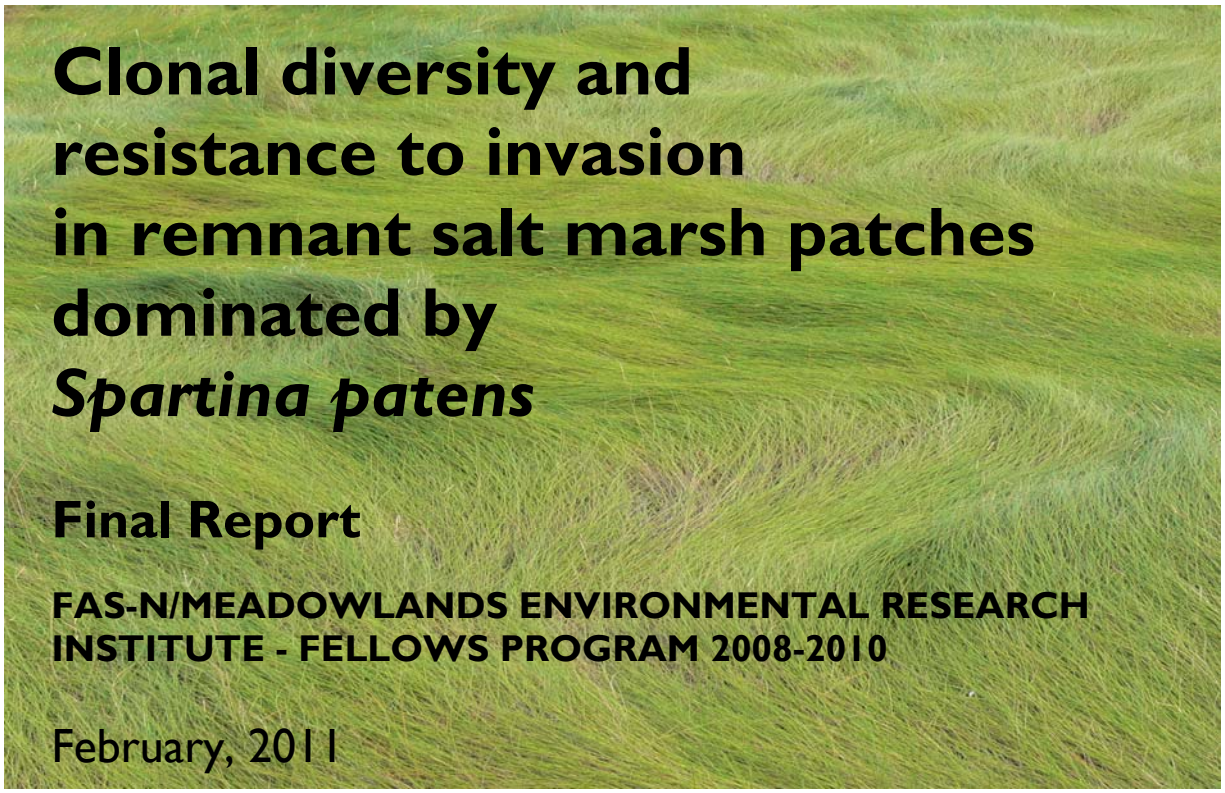

Clonal diversity and resistance to invasion in remnant salt marsh patches dominated by *Spartina patens*

Final Report

FAS-N/MEADOWLANDS ENVIRONMENTAL RESEARCH INSTITUTE - FELLOWS PROGRAM 2008-2010

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Funding periods

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Clonal Diversity and Resistance to Invasion in Remnant Salt Marsh Patches Dominated by *Spartina patens* (Holzapfel)

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Community Dynamics, Molecular Ecology and Root Ecology of Restored and Remnant *Spartina patens* High Marshes in the NJ Meadowlands (Holzapfel-Kirby)

Projects in 2010
Mechanisms of root antagonism in the native salt marsh grass *Spartina patens* (Holzapfel-Kirby)

ABSTRACT

Restoration efforts are attempts at creating and assembling local communities that have vanished. Recent studies have shown the importance of using local ecotypes of species as building blocks in these assemblies and the need of including information on genotypic differentiation has been stressed. Large portions of brackish east coast marshlands have been invaded by non-native, European genotypes of the common reed, *Phragmites australis*. As a result, only a small fraction of the NJ Hackensack Meadowlands is now dominated by native marsh species and only isolated patches of *Spartina patens* remain.

As these patches vary in size and seem to resist encroachment by *Phragmites* differentially, we are investigating (a) whether larger patches are able to resist invasion more than smaller patches and (b) whether large-patch clones are better suited for restoration efforts. We aim at understanding whether the extant mosaic of small and large patches is the result of a random disintegration of one former large *Spartina* stand or whether it is the result of a network of patches that resisted invasion differently .

In a combined approach that includes remote-sensing, surveys of permanent transects, common garden transplant experiments, competition experiments and genetic analysis, we are monitoring border dynamics and assessing genetic identity and performance of clones of different patch sizes. Results indicate that (a) border zones between the invader and *Spartina* tend to be more defined in large *Spartina* remnant patches than in small patches, and (b) that these overlap zones expand in small *Spartina* patches but decrease in large patches, thereby indicating that large-patch *Spartina* stalls *Phragmites* invasion.

There are large genetic differences between adjacent large- and small-patch ramets (clonal fragments) that are often more pronounced than differences between ramets of different regions. Matched small and large Spartina patch ramets are not related to each other and clonal diversity in large patches is larger than in small-patches.

In contrast, small-patch ramets grow faster than large-patch clones in a common garden setting but repress growth of clonal fragments of *Phragmites australis* to a lesser extent than large-patch ramests. When grown in direct competition in containers, small-patch *Spartina* is reduced by *Phragmites*.

Based on community dynamics and genetic data we conclude that small patches are not the remnants of the extant large patches that formerly were even larger. Rather, the current mosaic of small and large patches is the result of a large number of clones that resisted invasion differentially. These results indicate that clonal identity within *Spartina patens* populations plays a role in the interaction with a non-native competitor. Ramets from large patches tend to be more successful in competitive interactions and this suggests that restoration efforts should prefer these clones.

Little is known about the underlying mechanisms, and clone-specific root exudates were explored but no clear differences between clones from successful and less successful patches were found. Even though a large number of exudates was found in hydroculture and some unidentified compounds are patch-size specific, no overall patch-size dependent differences in exudates groups is apparent.

STRUCTURE OF RESEARCH

General Background

Large portions of brackish marsh have been invaded by non-native genotypes of the common reed (*Phragmites australis).* Within the Hackensack Meadowlands only fractions are still (or again) dominated by low marsh *Spartina alterniflora* and high marsh *Spartina patens* (both are native species). From the latter species only isolated patches remain that nevertheless appear to be stable (Artigas, personal communication). These patches vary in size and seem to resist encroachment by other species through their peculiar growth form: the formation of dense "cowlick" mats consisting of prostrate grass shoots. This high marsh has been used in historic time for hay making (Smith 1942) and burned to facilitate goose grazing and or muskrat trapping. Current dense mats of *S. patens* apparently developed after these management practices ceased. Today, the presence of the *Spartina* high marsh is contributing to the landscape diversity and species diversity in the Meadowlands and is attracting a wide range of wildlife (e.g., threatened bird species, for instance Short-eared Owls and Northern Harriers, that use these open patches to forage). The effect of clonal integration and clonal identity on community interactions that include competitive interactions is one of the emergent themes in current research on clonality in plants (Gough et al. 2001) and is connected to the importance of genetic identity to restoration efforts (Hufford and Mazer 2003). For instance, Silander showed in his classic analysis of the clone structure of *Spartina patens* in North Carolina that strong genetic differentiation appeared to correlate with differential competitive ability (Silander 1979). We explore the factors that allow these patches to persist in the presence of dominant and highly competitive *Phragmites*. In particular we investigate whether larger patches of *S. patens* consist of more competitive clones than small patches.

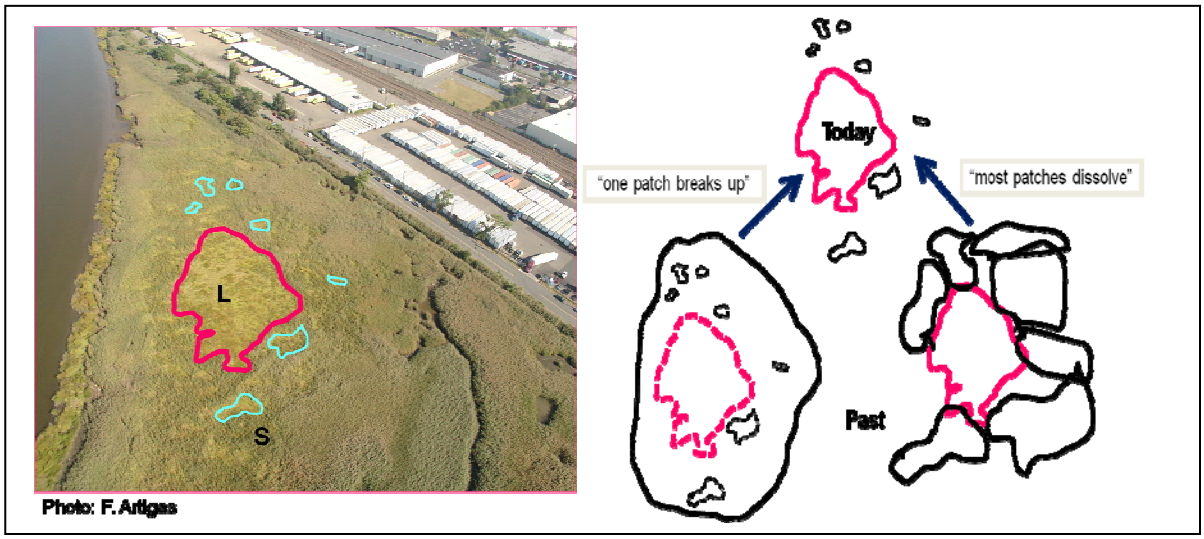


Fig. 1: Current large and small patch systems (left at Hawk Property) and development scenarios (right)

General Hypotheses:

The overarching goal is to understand the current mosaic of large and small *Spartina patens* patches in a matrix of the invasive *Phragmites* haplotype. In particular we hypothesize that pattern is due to two possible dynamics (as illustrated in Fig.1):
(H_1) a breakup of single large patches ("**one patch breaks up" hypothesis**)
(H_2) a differential dissolving of most of many connected patches ('**most patches disolve" hypothesis**)

Goals

We are interested in exploring the factors that allow *Spartina patens* patches to persist in the presence of dominant and highly competitive (invasive) *Phragmites*. In particular we would like to investigate (a) whether the peculiar prostate growth form of *S. patens* allows it to compete with other species and (b) whether larger patches of *S. patens* consist of more competitive genotypes than small patches. Four goals drove the initial stages of this research:

1) Establishment of permanent transects along the border zone between *Phragmites* and *S. patens*, allowing documentation of medium and long-term community development along these borders.
2) Establishment of molecular methods for the analysis of the genetic structure of genotypes of large and small patches with the goal of identifying molecular markers that will aid in selection of genotypes of *S. patens* that will be successful in restoration of the high marsh communities and in determining whether these patches consist of distinct genotypes differing in competitive potential.
3) Field and lab testing of performance of clonal fragments (ramets) from various remnant patches in the Meadowlands in a common garden at the Secaucus High School Wetland Enhancement Site (SHSWES).
4) Further trial-stage investigations to establish methodology to measure relative fitness and function of *Phragmites* along the ecotone zone in the edges of present *S. patens* patches

Collaborators & Personnel

The research is conducted in collaboration with **Dr. Huimin Man**, **Dr. Ildiko Pechmann**, and graduate students **TingMin Wu, Kimberly Plank, John Francois, Jessica Schnell, Gregg Burdulis and Sahil Wadhwa.**

1. SMALL-SCALE PATCH DYNAMICS: BORDER TRANSECTS

In the summer of 2007 we established 13 transects in 5 different locations along the lower Hackensack River (see Fig. 2). In three of these matched large (> 3,000m^2 = 0.74 acre) and small (< 200m^2 = 0.05 acre) *Spartina patens* patches were located. Transects were marked permanently to ensure consecutive sampling in the following years. The transects were placed perpendicular to the *Phragmites – S. patens* border with the center of the transect placed approximately at the center of the contact zone. Plant parameters for all occurring species (density, aerial cover, height) were measured and recorded for 10 50x50cm contiguous quadrats.

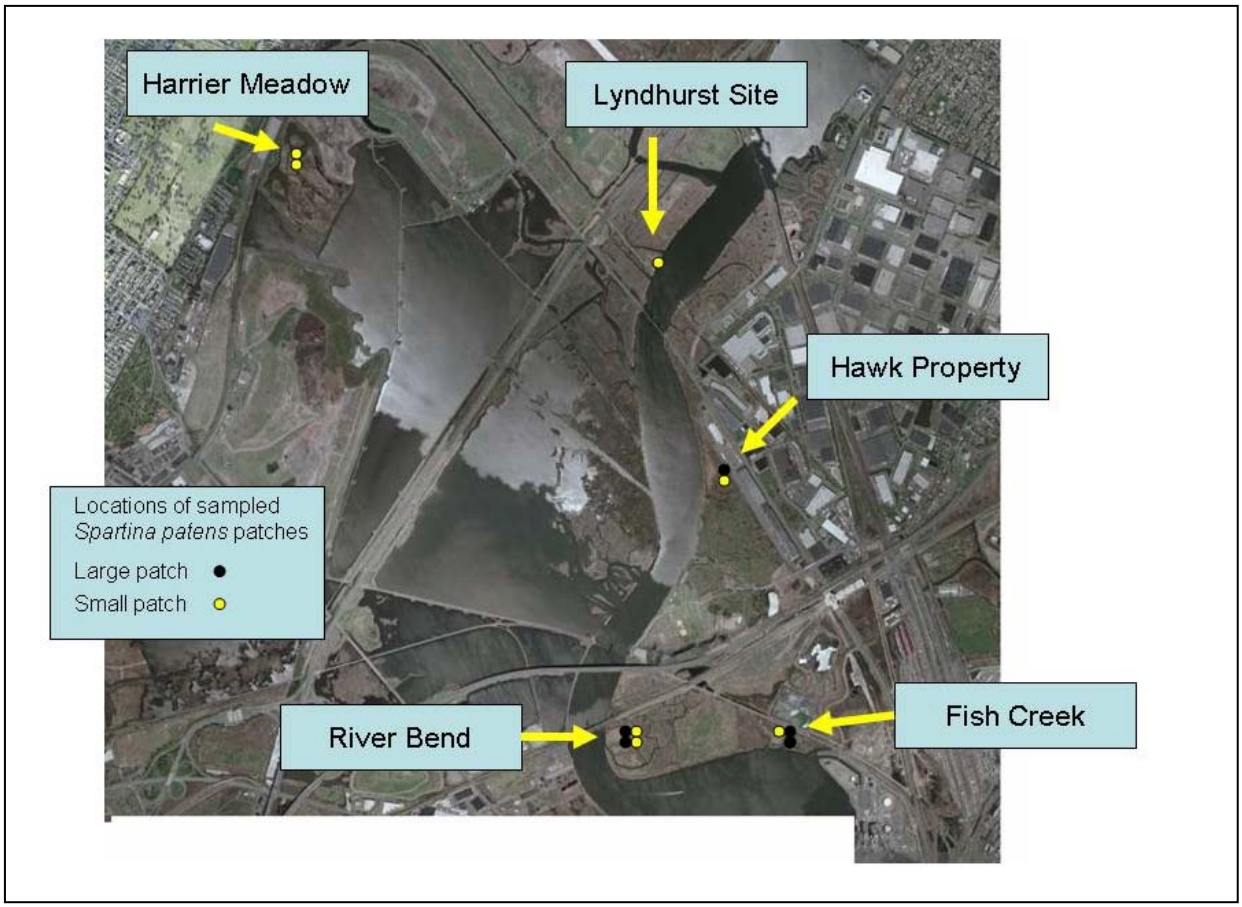


Fig. 2: Locations of transects in the Hackensack Meadowlands, NJ

The data from each transect are used to calculate the area that is dominated by the interacting species (A and B in Fig. 3); special consideration is given for the overlap zone (O). We use such transects in research on species interaction (especially native and non-native species) in a range of other areas in NJ as well. It has been shown that interpretations of these transects becomes more meaningful after data for a number of years are available. However the

analysis of the extent of overlap even for one year allows important conclusions in regard to the type of interaction present in the assembly under investigation.

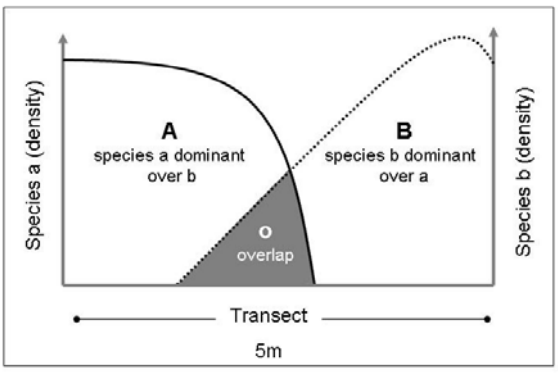


Fig. 3: Schematic view of transect data analysis. The integral area for the depicted areas – species A dominant over B and vice versa, overlap - are calculated and compared between transects and among years.

Exemplary results are shown in Fig. 4. For these transects it becomes evident that the overlap zone is more extensive for small patches than for large patches. An analysis of the relative extent of overlap revealed that this difference is statistically significant ($p>0.002$ (ANOVA), Fig 4).

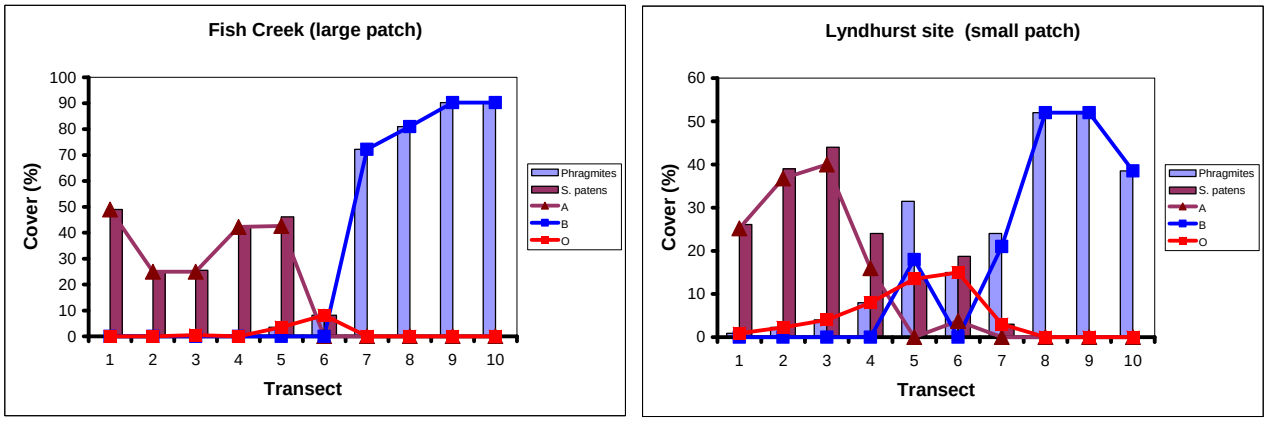


Fig. 4: Example of cover data for two *Spartina patens* (left site of transect) and *Phragmites* (right side) transects in 2007. A border is shown for a large *S. patens* patch (left) and small one (right). Note the differences between the sizes of the overlap zone (O): the integral area below the 0-curve amounts to 12 for the large patch vs. 46 for the small patch.

After 2007 the permanently marked transects were revisited and measured during late summer and fall in 2008, 2009 and 2010. The measured overlap zone between *Spartina* and *Phragmites* was significantly smaller in border zones of large *Spartina* patches (see Fig. 5) compared to borders at small patches. Clearly borders at large patches were therefore sharper. Even though year-to-year variation from 2007-2010 is notable in the data (especially in the

small patch bordes, no temporal effect was found in a repeated measurement ANOVA: time (years): F=0.984, df=2, p=0.347; time x sixe: F=1.833, df=2, p=0.209, size: F=4.342, df=1, p=0.043.

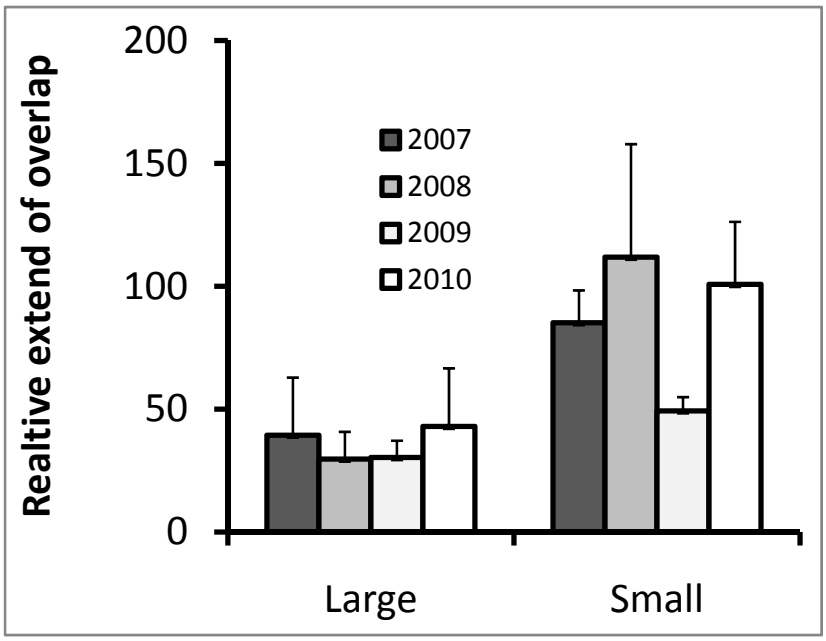


Fig. 5: Degree of border overlap in large and small *Spartina patens* patches during the four years of investigation Shown are means and 1 SE, n (large patch) = 6, n (small patch) =7.

The temporal dynamics of the transects, expressed as absolute change from 2007 to 2010 is shown in Fig. 6 (and Table 1). The largest changes occurred in the *Spartina* section of the transect at borders of small-patch stands where Spartina appears to give way to Phargmites. This was clearly not the case at borders of large patches.

Table 1: Results from 2-way ANOVA of percent changes in dependnecy of different zones (*Spartina* zone, *Phragmites* zone and overlap) and *Spartina* patch sizes (large and small). Note significant zone effect.

Dependent Variable: absolute change from 2007 to 2010

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	85235.022	5	17047.004	2.476	.057
Intercept	186.290	1	186.290	.027	.871
Size	8689.087	1	8689.087	1.262	.271
Zone	15477.845	2	7738.923	1.124	.340
Size * Zone	63205.006	2	31602.503	4.590	.019
Error	185889.908	27	6884.811		
Total	271151.930	33			
Corrected Total	271124.929	32			

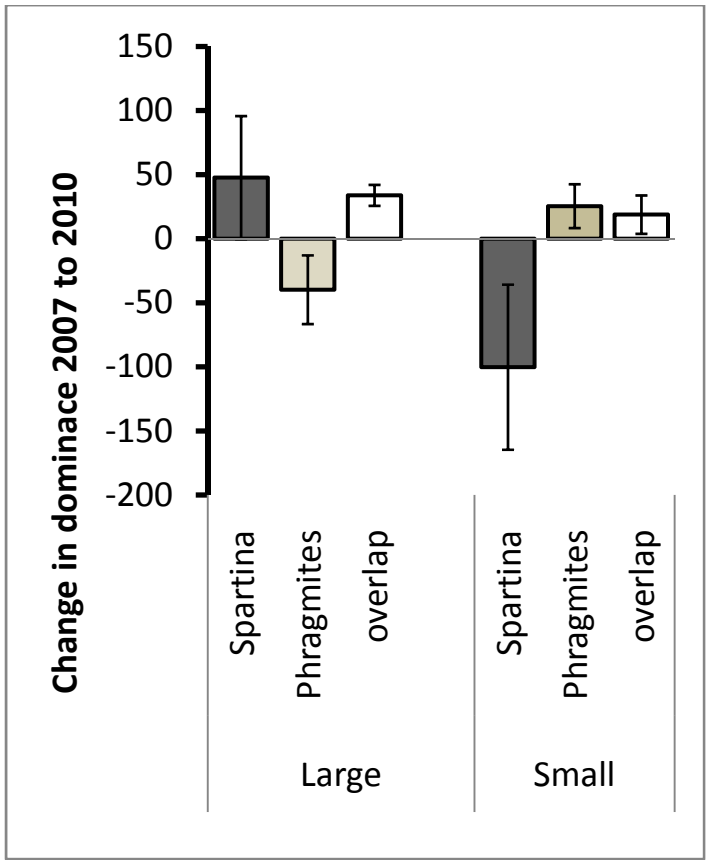


Fig. 6: Change of transect zones from 2007 to 2010 in large and small *Spartina patens* patches. Average values of all transects with 1 SE are shown. There is a significant statistical effect of zones (see Table 1) which is expressed in large changes in the *Spartina* zone (zone A) compared to the other zones.

2. GENETIC DESCRIPTION OF CLONAL STRUCTURE OF PATCHES

When local communities are degraded, damaged or destroyed through human activities, restoration plays an important role to preserve and maintain existing habitat and biodiversity. Recent studies have demonstrated the importance of using local ecotypes to build restoration blocks. Hufford and Mazer (2003) showed that genotypic differentiation in plant materials used in restoration can be crucial for the success. However, during restoration efforts, genetic identity of plant materials is often ignored due to the difficulty in identifying genotypes (Widen et al. 1994). Now, appropriate molecular tools that establish genetic markers, such as RAPDs (random amplified polymorphic DNA) and ISSRs (inter simple sequence repeats), are used for determining clonal diversity and population genetic structure. ISSRs have become an effective technique for measuring genetic diversity in plants (Wolfe and Liston 1998, Esselman et al.1999). The genetic diversity of *Spartina patens* in New Jersey is unkown. In order to assess the genetic information of *S. patens* and understand its genetic diversity, the following studies were conducted: (1) establish ISSR techniques for identifying molecular markers for *S. patens* in

New Jersey, (2) investigate the genetic diversity among *S. patens* populations from different locations.

Methods

Plant material has been sampled at random locations within selected small and large patches of *S. patens*. At least 10 samples from three matched small and large patches shown in Fig. 1 (Fish Creek =FC, River Bend=RB and Hawk Property=HP) were sampled. Genomic DNA from collected samples was extracted from fresh leaves and stems using the Qaigen DNeasy plant mini kit (Qiagen Company) and stored at -20^{o}C.

ISSR techniques utilize the DNA polymerase chain reaction (PCR) using microsatellite primers designed for di- or tri-nucleotide repeats with anchored one to three random nucleotides at the 3' end (Gupta et al. 1994).

In this study, 40 ISSR primers were screened on one randomly selected individual. Fourteen primers produced clear and reproducible fragments for Popgene analysis (Table 2).

Amplifications for ISSR analysis were conducted as follows: each PCR volume of 12.5 µl contained 6.25µl master mix (GoTaq Green Master Mix, Promega), 1µl 20 µM primer, 1µl (40 ng) DNA template. Amplifications were performed with a thermal cycler (PTC-100, MJ Research, Inc.) using the following program: denaturing at 94^{o}C for 1.5 minutes, followed by 40 cycles of denaturing (15 seconds at 94^{o}C), annealing 30 seconds at the appropriate primer melting temperature, elongation (2 minutes at 72^{o}C) and a final elongation step of 4 minutes at 72^{o}C. Final products were stored at 4^{o}C. Negative controls without DNA template were run with each experiment. PCR products were separated by electrophoresis on horizontal 1.2% (w/v) agarose gels in TAE buffer (90 Volt, 100mA) and visualized under ultraviolet light. Images were taken with Kodak image system1D 3.5.4. The molecular weight of each band was estimated using a 100 bp DNA ladder (Promega). The size of generated banding products ranges from 200 to 1600 base pairs. *Spartina patens* PCR products were analyzed by scoring the presence/absence of polymorphic bands. Ambiguous bands were not included in the analysis.

The percentage of polymorphic band (PPB), Nei's genetic diversity (*H*) and Shannon indices of diversity (*I)*, were used to calculate genetic diversity for each population by Popgene (version 1.32) (Yeh et al., 1997) (Table 3). *H* value in populations showed a range from 0.0539 (RBS) to 1.624 (HPL). PPB values among populations were from 15.15% (RBS) to 52.53% (HPL). *I* was from 0.0805 (RBS) to 0.2512 (HPL).

A dendrogram of the genetic relationship of each patch was prepared using Treecon for Windows (version 1.3b) (Van de Peer and De Wachter, 1994). The dengrogram was built based on the pairwise genetic distance among populations (Fig. 6).

Genetic differentiation within populations and among populations was estimated by using Analysis of Molecular Variance (AMOVA) (Excoffier et al.

1992). AMOVA is a method to calculate population differentiation directly from molecular data and to test hypotheses about genetic variation within the populations and among the populations. Variance estimates were based on 100 permutations. AMOVA was conducted using GenAlEx V6.3 (Peakall and Smouse, 2006)

Results

The analyses show that the value of Shannon's index of population of large patches is relatively higher than that of population of small patches (Tables 3 and 5), indicating that the genetic variation within populations found in large patches is larger than that of populations in small patches. This suggests (a) that individual genets (genetically identical parts of clones) tend to be smaller in large patches) and/or (b) more germination events occur in large patches, and/or (c) somatic genetic change occurred more often in large patches.

Furthermore, materials from matched small and large patches within individual locations are typically not closely related to each other (Table 4, Figs. 7 and 8). This demonstrates that genets of small and large patches, even though they are in close proximity to each other are genetically distinct from e ach other. Only few exceptions were found: one sample from Fish Creek large patch, the other from the Hawk Property large patch. They appear to form their own groups (Fig. 8).

Table 2: Primers, sequences and their annealing temperatures

ISSR primers	Sequences	annealing temperature (^{o}C)
849	GTG TGT GTG TGT GTG TYA	50.5
842	GAGAGAGAGAGAGAGAYG	47.2
854	TCT CTC TCT CTC TCT CRG	48
850	GTG TGT GTG TGT GTG TYC	53
840	GAGAGAGAGAGAGAGAYT	45.8
856	ACA CAC ACA CAC ACA CYA	49.8
857	ACACACACACACACACYG	63.3
855	ACACACACACACACACYT	49.8
869	GTTGTTGTTGTTGTTGTT	55.9
812	GAGAGAGAGAGAGAGAA	44.3
835	AGAGAGAGAGAGAGAGYC	45.6
844	CTCTCTCTCTCTCTCTRC	46.5
878	GGATGGATGGATGGAT	66.5
873	GAC AGA CAG ACA GAC A	45

Table 3. Genetic diversities in *Spartina* populations

Population ID	Number of polymorphic loci	PPB(%)	*H*	*I*
FCL	37	37.37	0.1075	0.1694
FCS	27	27.27	0.0804	0.126
RBL	37	37.37	0.1202	0.1849
RBS	15	15.15	0.0539	0.0.805
HPL	52	52.53	0.1624	0.2512
HPS	31	31.31	0.0982	0.1516
Total	83	83.84	0.2408	0.3697

PPB: percentage of polymorphic bands; *H*: Nei's genetic diversity; *I*: Shannon's information index; FCL: Fish Creek large patch; FCS: Fish Creek small patch; RBL: River Bend large patch; RBS: River Bend small patch; HPL: Hawk Property large patch; HPS: Hawk Property small patch.

Table 4: Nei's Original Measures of Genetic Identity and Genetic distance (bold)

Population	FCL	FCS	RBL	RBS	HPL	HPS
FCL	****	0.7752	0.8067	0.839	0.8434	0.7752
FCS	**0.2546**	****	0.8424	0.8387	0.8155	0.7742
RBL	**0.2148**	**0.1715**	****	0.8283	0.7965	0.753
RBS	**0.1756**	**0.1759**	**0.1883**	****	0.8516	0.7899
HPL	**0.1703**	**0.204**	**0.2275**	**0.1606**	****	0.798
HPS	**0.2547**	**0.2559**	**0.2838**	**0.2359**	**0.2257**	****

Nei's Original Measures of Genetic distance (bold)/ geographic distance(km)

Population	FCL	FCS	RBL	RBS	HPL	HPS
FCL	****	0.12	1.3	1.06	1.99	1.93
FCS	**0.2546**	****	1.1	0.97	1.89	1.83
RBL	**0.2148**	**0.1715**	****	0.07	2.2	2.4
RBS	**0.1756**	**0.1759**	**0.1883**	****	1.99	1.9
HPL	**0.1703**	**0.204**	**0.2275**	**0.1606**	****	0.07
HPS	**0.2547**	**0.2559**	**0.2838**	**0.2359**	**0.2257**	****

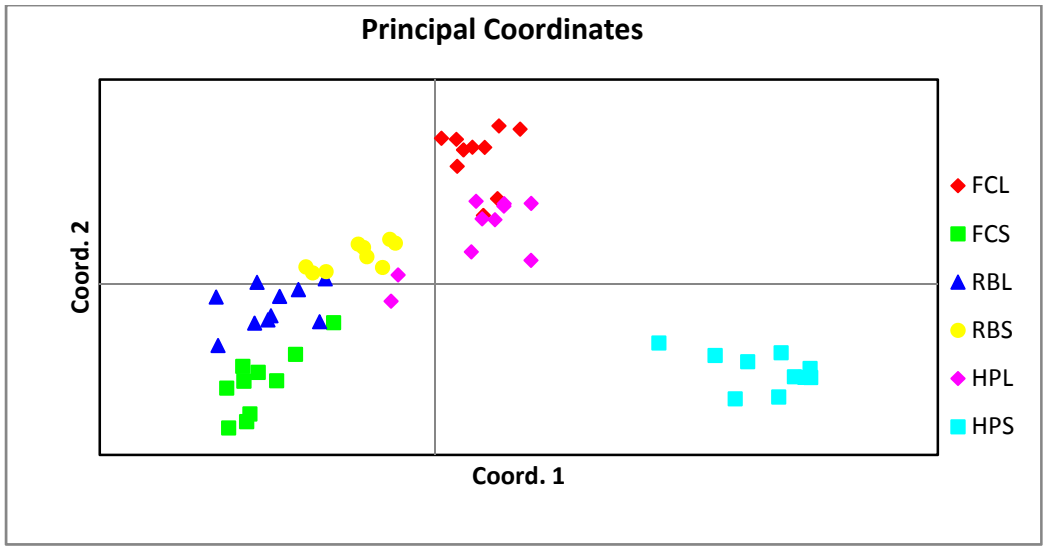


Fig. 7: Principal Coordinates Analysis (PCA) of six populations of *Spartina patens* sampled from Meadowlands in New Jersey based on ISSR fragments.

Table 5: Analysis of Molecular Variance (AMOVA) for six populations of *Spartina patens* in the Meadowlands, New Jersey.

Source	df	SS	MS	Est. Var.	%
Among Regions	2	158.133	79.067	0.000	0%
Among Pops	3	248.850	82.950	7.734	58%
Within Pops	54	302.900	5.609	5.609	42%

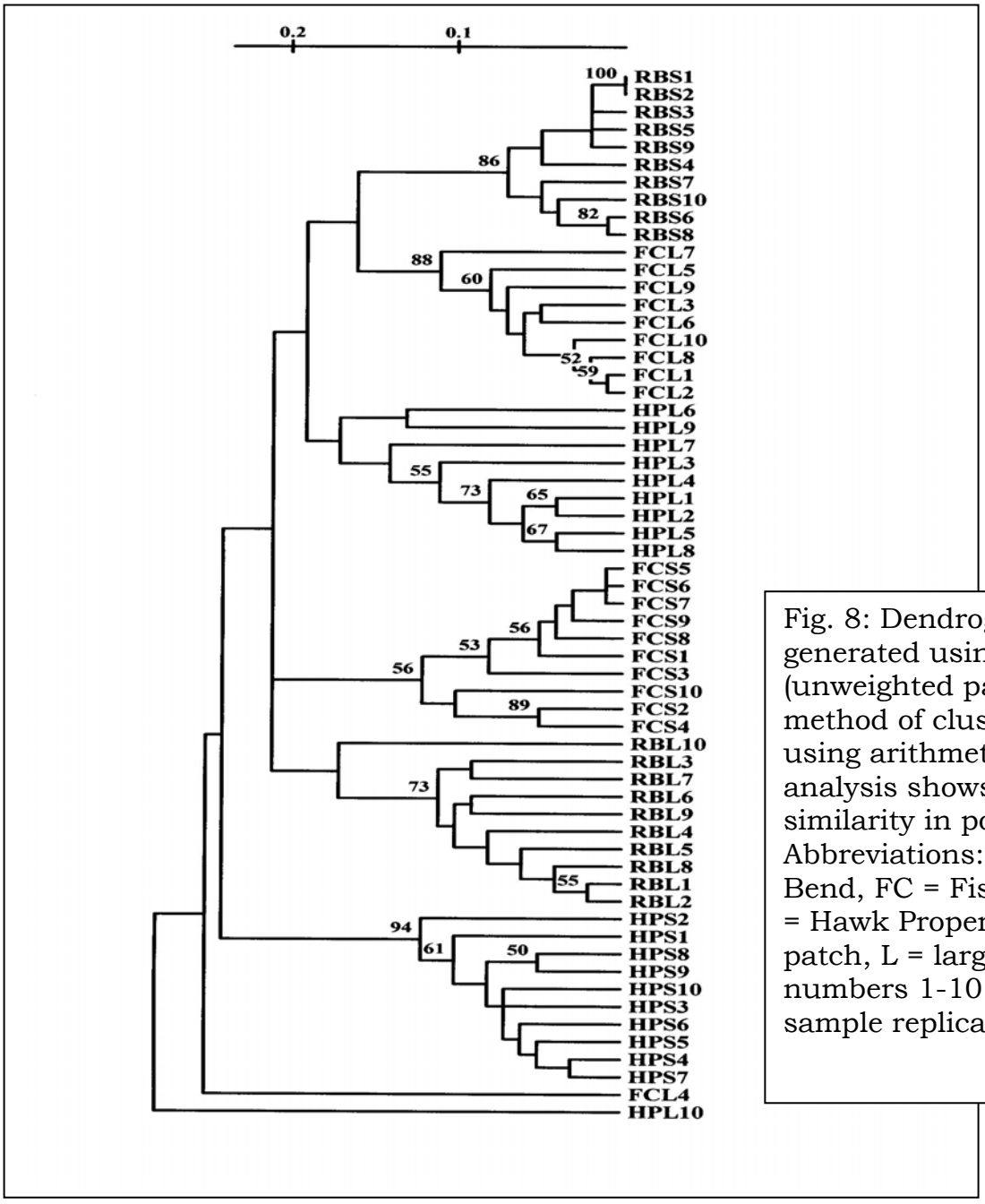


Fig. 8: Dendrogram generated using UPGMA (unweighted paired group method of cluster analysis using arithmetic average) analysis shows genetic similarity in populations. Abbreviations: RB = River Bend, FC = Fish Creek, HP = Hawk Property, S = small patch, L = large patch; the numbers 1-10 stand for sample replicates.

3. COMMON GARDEN AND *PHRAGMITES* COMPETITION EXPERIMENT

3.1 COMMON GARDEN

We planted clonal fragments (ramets) from three Meadowlands provenances with paired large and small *Spartina patens* remnant patches (and one outgroup taken from a large Spartina patch at the Raritan Bay (Conaskonk Point: N 40° 27' 21.15"; W 74° 10' 35.39"); see Fig. 9a) in a common garden design located at the edge of the Secaucus High School Marsh restoration site (Fig. 9b).

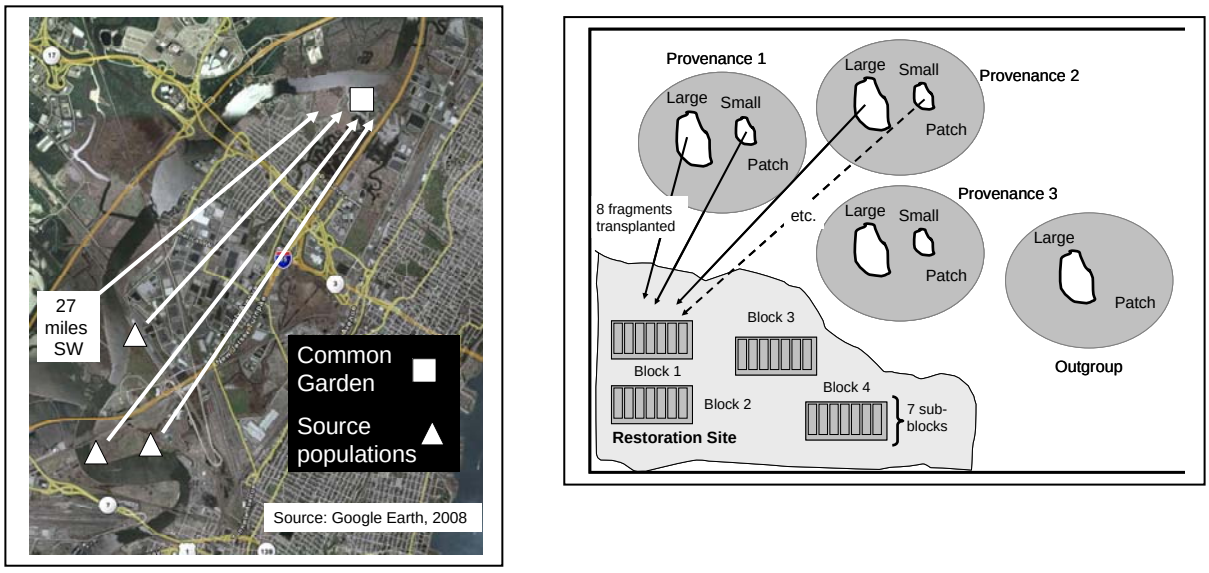


Fig. 9: Location of common garden and origin of clonal fragments (a, left panel). Common garden design (b, right panel).

Fig. 9b demonstrates the design in which small *Spartina patens*-plugs (taken with a 10 cm diameter root-soil auger) from different localities (provenances) within the Meadowlands (and one outgroup) were planted into 4 separate blocks at the edge of the ongoing restoration site.

Design:

- 4 blocks at the edge of the restoration site. Localities within the Meadowlands that have paired large and small *Spartina patens* remnant patches (Hawk Property, River Bend, Fish Creek, one outgroup: Raritan Bay)
- 9 replicates for each

- Block: (3 provenances x 2 patch sizes + 1 outgroup = 7 populations) x 8 replicates = 56 plant-plugs). Each population is placed in a sub block within each block
- The 8 plugs of a single 'provenance-patch-size-combination' have been planted together in a sub-block design. Each plug is separated from each other by 40 cm with an edge of 30 cm towards the outside.

Planting: Planting has been done in beginning of June 2008.

Monitoring: Performance of each *Spartina* plug after the first vegetative growing season has been assessed by measuring shoot height and basal diameter repeatedly.

Fig. 10: Views of the Common Garden at Secaucus Highschool Marsh

Results

Base-growth till the end of the season is shown in Fig. 10 varied between the four locations (ANOVA p=0.007, see Table 6) and more interestingly between the size of patch from which the ramets were taken. Small-patch ramets from the Meadowlands consistently show more rigorous growth than large-patch clones (p=0.008). The outgroup, ramets from a large patch at the Raritan Bay, grew as much as the small-patch ramets from the Meadowlands.

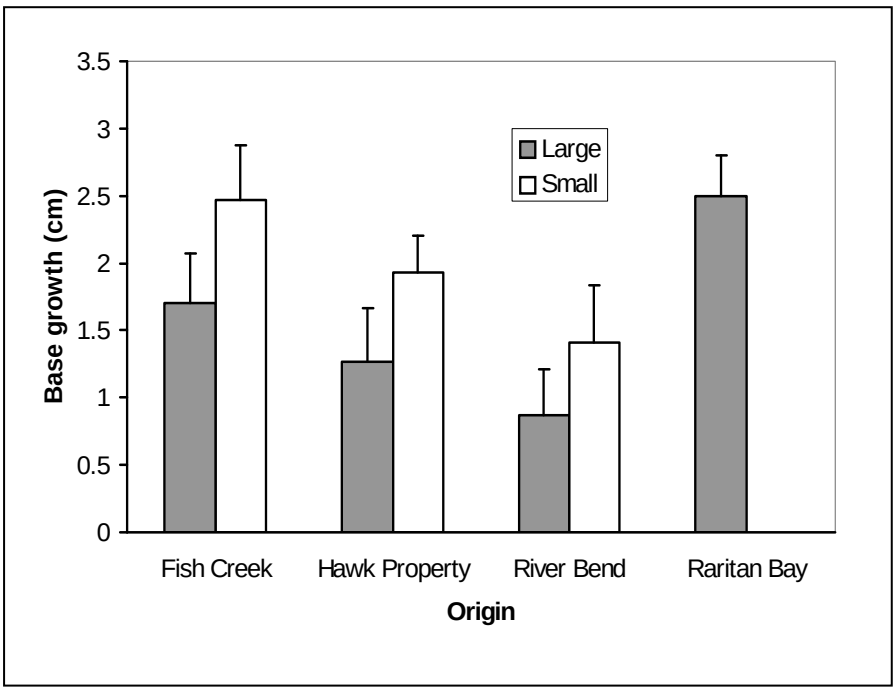


Fig. 11: Base growth of *Spartina* fragments in the first growing season. Shown are means with 1 SE.

Table 6: ANOVA results of first season base growth (2-way-block design).

Dependent Variable: Base growth

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	686.4990313	1	686.499	**7.347**	0.07272
	Error	281.793	3.10	93.442		
Origin	Hypothesis	28.607	2	14.304	**5.032**	0.00732
	Error	608.328	214	2.843		
Size	Hypothesis	20.475	1	20.475	**7.203**	0.00785
	Error	608.328	214	2.843		
Block	Hypothesis	305.807	3	101.936	**35.859**	0.00000

3.2 *PHRAGMITES* – *SPARTINA* COMPETITION EXPERIMENT

Methods

In May 13, 2009 single ramets of *Phragmites australis* rhizome fragments were harvested from a small area of the restoration site and planted within the common garden area. Rhizome fragments with one to three shoots were dug up, measured and weighed (washed wet weight) and planted between *Spartina patens* plantings. In each section of each block we planted 3 rhizome fragments in the first (eastern most) row of each provenance. Height measurements were taken in July 30 (78 days after planting) and Oct. 9 (149 days). Above-ground material was harvested at the later date and dry mass was determined.

Results

Above-ground biomass of *Phragmites* was significantly lower (see Fig. 12 and Table 7) when neighboring *Spartina patens* clones were taken from large patches. This pattern was found for all *Spartina* provenances.

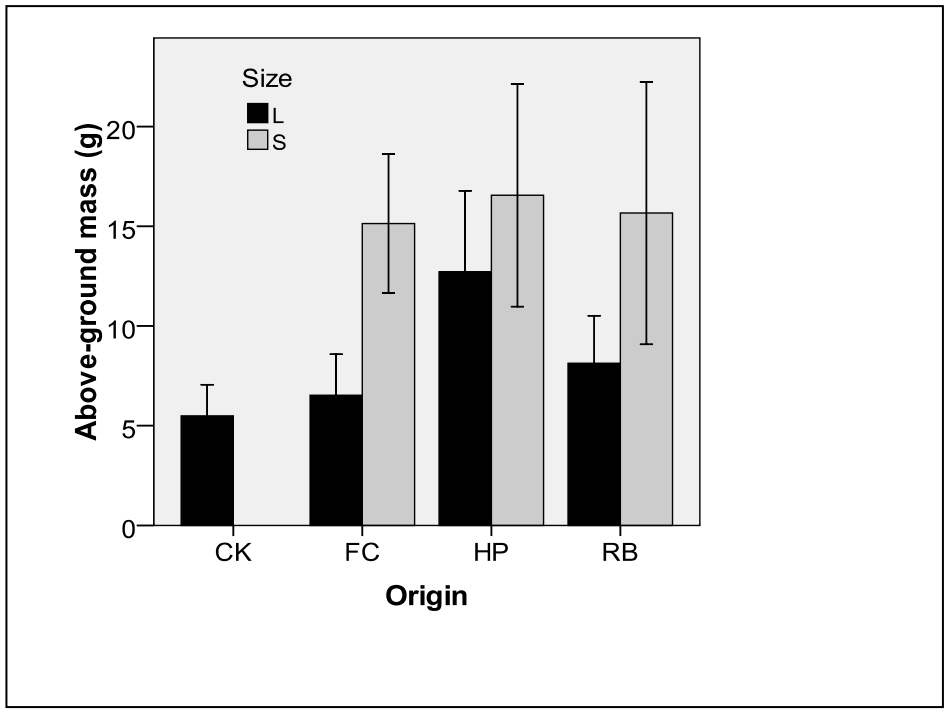


Fig. 12: Above-ground biomass of *Phragmites australis* in dependence of neighbor identity. Abbreviations: see above; shown are means and 1 SE.

Table 7: Results of 2-way ANOVA with patch size and origin as main effects. Block effects and start weight (as covariate) were removed from the model after non-significance was shown in prior block-based covariate analysis.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	3.179	24.570	.014
Size	1	68.170	4.499	.038
Origin	3	68.100	.646	.588
Origin * Size	2	68.135	.233	.793

a. Dependent Variable: har_ma_1.

4. POT COMPETITION EXPERIMENT (with Sahil Wadwha)

Methods

The experimental design (1b) for the study had (5) five different blocks, with each block carrying (5) five different treatments. The choice of five different treatments was made to test the competitive ability of both *Spartina* genotypes against exotic *Phragmites*. The design of each block was as follows: 1. **Ss**: Small patch *Spartina,* 2. **Ls**: Large patch *Spartina*, 3. **Ph**: *Phragmites, 4.* **Ph/Ls**: *Phragmites* against large patch *Spartina* and, 5. **Ph/Ss**: *Phragmites* against small patch *Spartina*. Treatment 1, 2 and 3 were used as controls in the experiment.

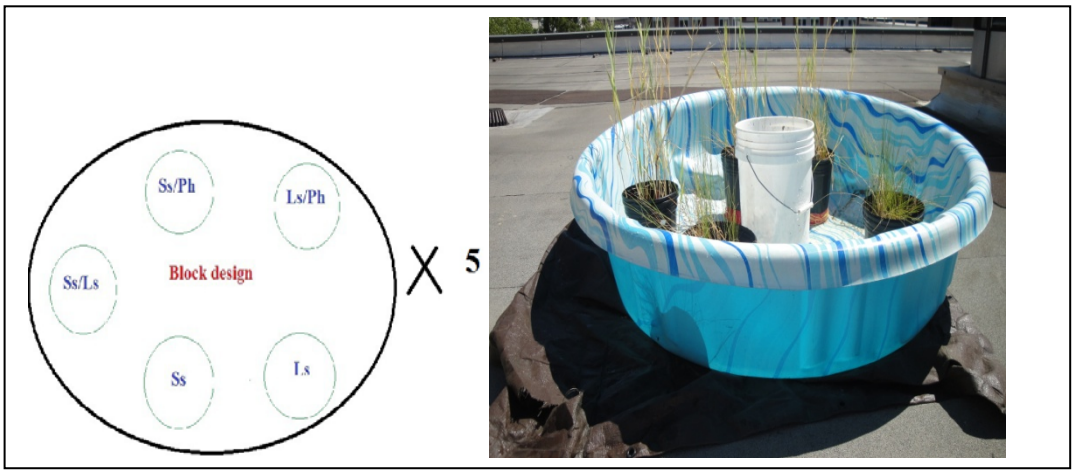


Fig. 13: Experimental setup: all five treatments were set up in individual blocks (wading pools) placed on the roof of Boyden Hall, Rutgers Newark.

Initial total weight of ramets for Small *Spartina*, Large *Spartina*, and *Phragmites* was standardized by using similar size ramets. The ramets were washed to remove soil before planting in pots (21 x 21cm), Soil used for the study was a 5:1 mix prepared by using washed play sand (source Home Depot) and field soil collected from the restoration site. Plants were grown for 3 months and harvested in the first week of September, 2010. The plants were removed from the pots and washed to remove the soil completely and then were carefully separated for below-ground and above–ground tissues. All the harvested samples were dried at 60^{o} C in an oven for seven days to remove the moisture and weighed.

Results

When grown alone ramets from Small-patch S*partina* developed higher Below-ground biomass followed by *Phragmites* and Large-patch *Spartina,* (Fig. 14)

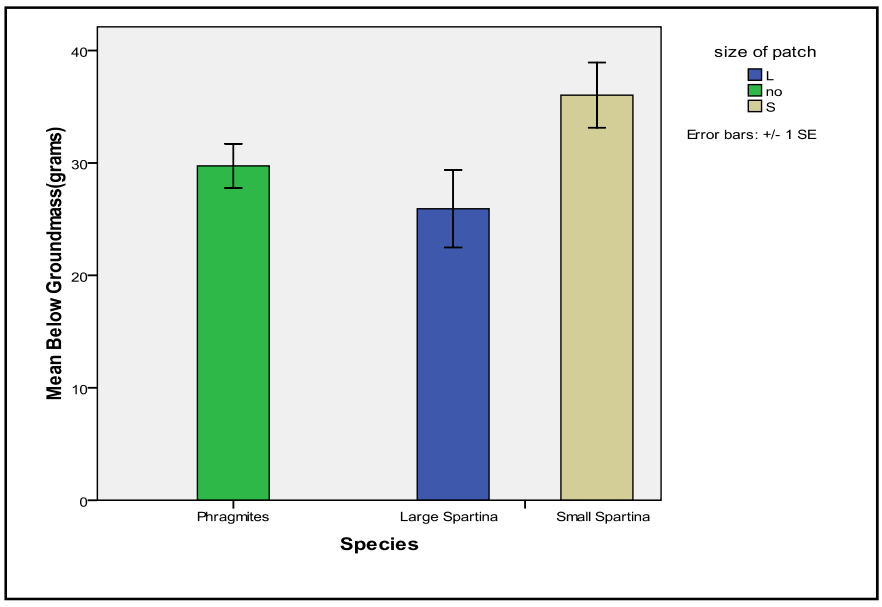


Fig. 14: Below-ground mass of Spartina types (large-patch and small-patch) and Phragmites when grown without a competitor.

In terms of mean root to shoot ratio (Fig. 15) indicates that small-patch *Spartina* invested most in root growth, large-patch *Spartina* less so, and *Phragmites* least.

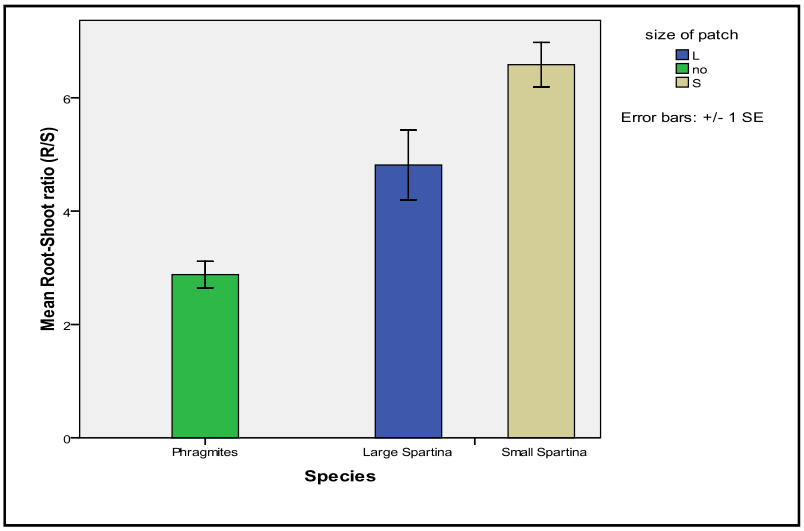


Fig. 15: Root-shoot ratio of *Spartina* types (large-patch and small-patch) and *Phragmites* when grown without a competitor.

When grown in competition, large-patch *Spartina* reduced the growth of *Phragmites* significantly more than large-patch *Spartina (*Fig. 16). Even though not significantly so, larger-patch *Spartna* gained larger mass than small-scale *Spartian* when grown with a *Phragmites* competitor.

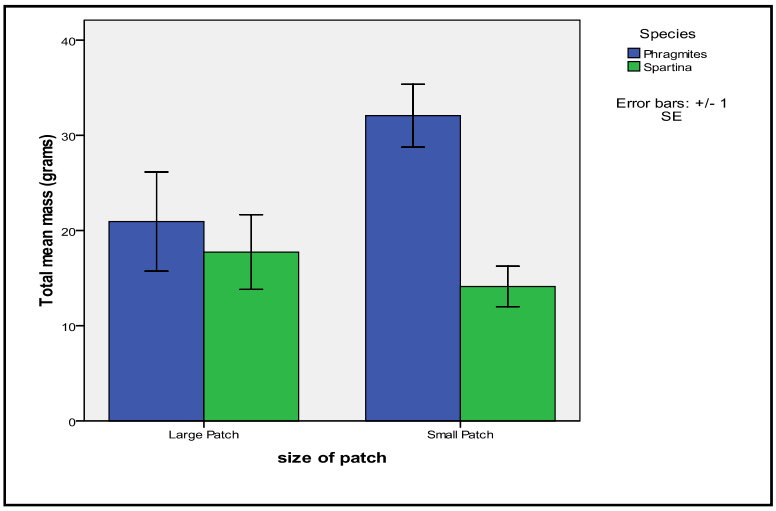


Fig. 16: Total mass of *Spartina* types (large-patch and small-patch) and *Phragmites* when grown in competition

It is quite evident that *Spartina* ramets from large-patch and small-patch respond differently during interaction with the non-native haplotype of

Phragmites. On one hand, where the small patch *Spartina* ramets show enhanced growth in absence of a competitor, the large patch *Spartina* ramets seem to have higher competition potential against *Phragmites*. It is interesting to note that the large-patch *Spartina* ramets show almost equal growth as *Phragmites*, which could be the reason for their ability to resist the invasion from exotic species, and can exist in large patches. In the pots where small-patch *Spartina* ramets were grown in the absence of competitor, the underground growth was higher than both large patch *Spartina* ramtes and *Phragmites* (Fig. 17). But in the presence of *Phragmites*, underground growth of small patch *Spartina* ramets was reduced.

a b c

Fig. 17 (a) Small Patch *Spartina* with no competitor, (b) Small Patch *Spartina* with *Phragmites* and (c) *Phragmites* with no competitor

5. *PHRAGMITES PERFORMANCE*

Methods

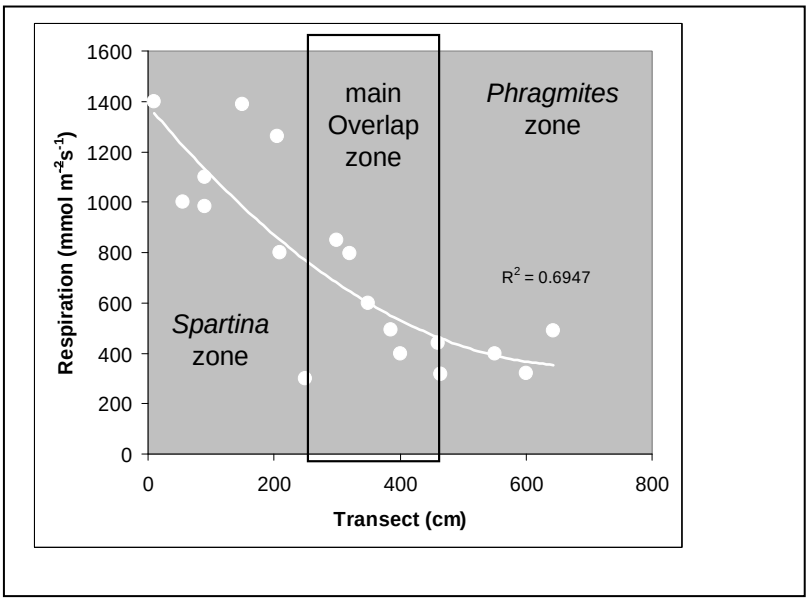
A number of above-ground structural and ecophysiological measurements were conducted to screen for additional negative (=competitive) effects of *S. patens* on emerging fronts of *Phragmites* in border zones. Leaf conductance was measured for leaves of *Phragmites* shoots (a) along border transects ranging from the Spartina to the *Phragmites* zone and (b) of shoots that differed in *Spartina*-caused bending. Measurements were taken in August 2007 (a) and in 2008 (b) with a hand-held Decagon Leaf Porometer.

Results

Respiration along transects: Leaf conductance measurements seem to indicate that isolated pioneer shoots of *Phragmites* show higher respiration rates and are more active than shoots in dense reed stands (the *Phragmites* zone, Fig. 18). These findings are supported by studies that our lab conducted in DeKorte Park, which indicated that edge shoots act as "carbon fixing-specialists" and are more active than shoots in the main stand (Zagajeski & Holzapfel in review). We conclude that possible negative interactions – if

existent– are more likely to take place below-ground and therefore require additional experimental investigations.

Fig. 18: Respiration rates of *Phragmites australis* along a border zone as measured with a Decagon Leaf porometer. Shown are data points from three transects combined. Rates were clearly higher for single shoots within the *Spartina* zone (left part of graph) than in the dense *Phragmites* zone (right).



Respiration of bent *Phragmites* culms: Leaf respiration of *Phragmites* increased with the increasing stem angle (Fig. 19, p=0.03). Vertical culms (90°) therefore had larger stomatal conductance than bent culms, indicating that bent culms tend to be more stressed.

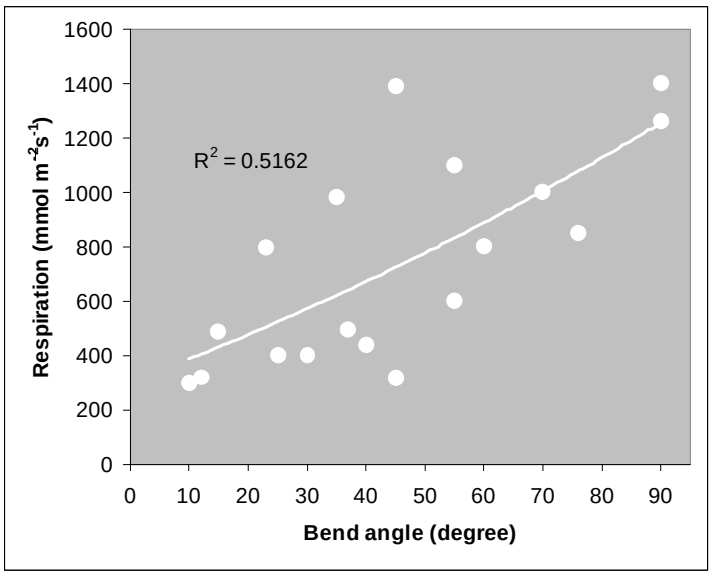


Fig. 19: Respiration rates of *Phragmites australis* in dependence of *Spartina*-caused bending as measured with a Decagon Leaf porometer. Shown are data points from measurements taken in the large *Spartina* patch of the Fish Creek site.

6. LARGE-SCALE, LONG-TERM PATCH DYNAMICS: REMOTE SENSING
(with Ildiko Pechmann)

Temporal development of *Spartina* patches: We used the historical aerial photo archive available at MERI to explore the history of the *Spartina* patches that we are currently investigating. Using GIS technology, we tried to determine whether the patches we observe today have changed in size and if so when. Starting with current imagery we spent considerable field work ground-truthing and attempted step-wise reconstruction of the history of a number of *S. patens* patches. It proved to be not possible to separate small patches from large patches on the available aerial imagery, as it appears that exact delineation of patches as visible in the field was unclear on historical aerial photos. The development of the *Spartina* high marsh at River Bend from 1930 to 2008 is shown in Fig. 20 as an example. Even though we were able to outline the *Spartina* dominated high marsh it was not clear whether small-size patches and areas with co-dominant *Phragmites* were included. Overall, a clear reduction in size was discernible (Fig. 21). The open question remains is whether the large patches we document today are indeed the remnant of continuous salt marsh areas. We need to combining this information with the molecular data on the genetic structure of those patches in order to shed light on the success or failure of certain patches over time.

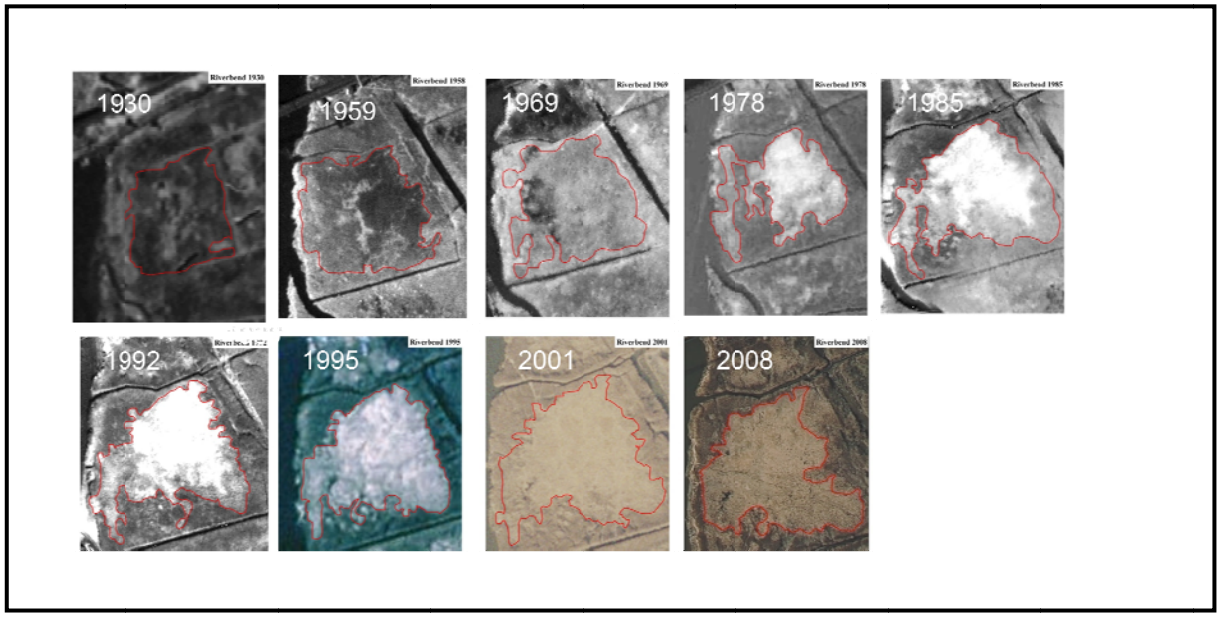


Fig. 20: Development of *Spartina patens* marsh at River Bend from 1930 to 2008 based on aerial imagery. The apparent borderline is marked in red.

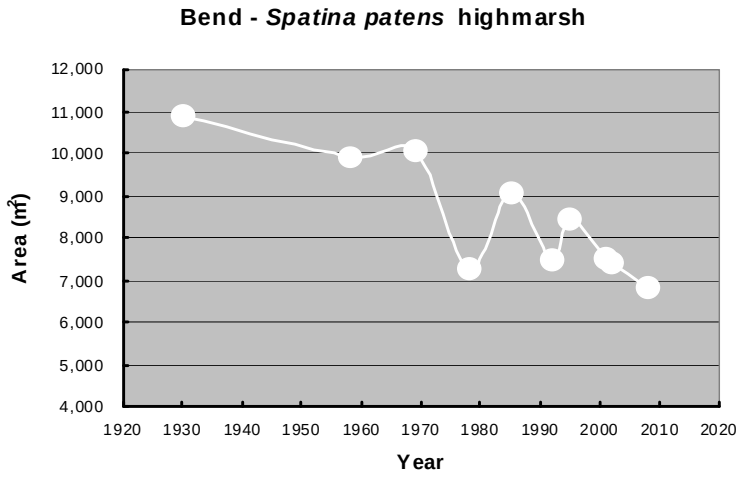


Fig . 21: Size development of *Spartina* high marsh at River Bend.

7. ROOT DYNAMICS OF *SPARTINA PATENS* IN A REMNANT AND A RESTORED HIGH MARSH (with Sahil Wadhwa)

We expect to gain important insight into the dynamics of competition between *S. patens* and *Phragmites*. Even more importantly, the ongoing research of other groups (in particular the group of Peter Jaffe, Princeton University) suggests that the redox profile and heavy metal immobilization is related to root activity in these marshes (Choi et al. 2006). Furthermore, metal retention is related to increases of organic carbon due to litter deposition and through decay of root and rhizome material. This is related to the amount of acid-volatile sulfide (AVS), a key partitioning phase controlling the immobilization of cationic heavy metals in sediment (Carapuca et al. 2004). In this context it is clear that detailed information about the below-ground dynamics of high marshes is crucial. Therefore, we propose adding studies of root- and rhizome-zone to our ongoing research investigation in the Meadowlands.

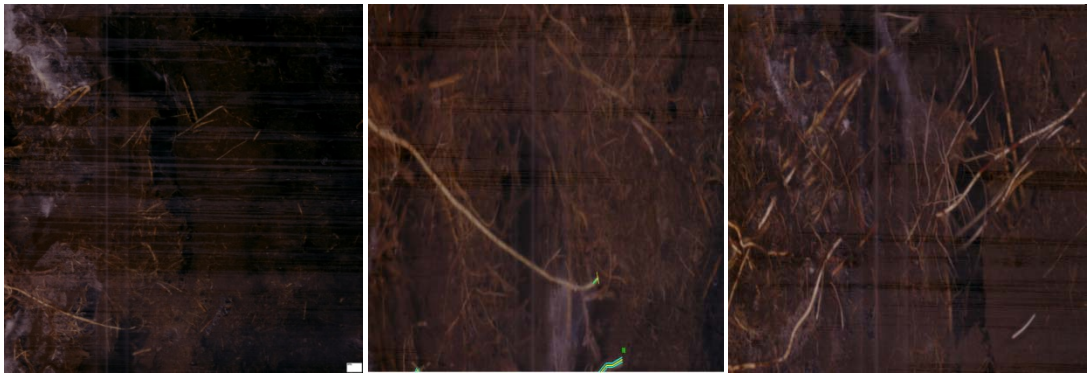
Direct root observation with root scan (micro-rhizotron) technology: We currently monitor root dynamics in high marsh communities. Using micro-rhizotron techniques (Smit et al. 2000) we will be able to describe the seasonal dynamics in established vegetation and the temporal dynamics on developing vegetation in restoration projects. Root observation tubes will be permanently installed in three different high marsh sites: 1) Established high marsh *Spartina patens* monotypic stands, (2) *Spartina patens*/*Phragmites* interfaces site (border zones) and (3) Restored and developing sites (possibly close to Peter Jaffe's sites that differ in organic content). We can directly observe root growth in the contact zones of interacting species. A CI-600 Root Scanner (CID, Inc.), designed to scan living roots in the soil, will be used for this observation. The

scanner system consists of a rotating linear scan head, a notebook computer and clear Plexiglas tubes. These tubes have been installed at the desired locations in the soil in root contact areas. To obtain an image, the scan head is inserted into the tube, and will automatically rotate a full circle creating a high resolution image of the soil and roots. The scan head can be moved to different depths to scan different sections of the soil profile. Images have been acquired in summer 2009 repeatedly and are currently analyzed digitally with the use the program WinRhizoTron (Regent Instruments Inc.), which calculates measurement of root growth, length and density over time. Data that we will obtain include: temporal dynamics of root development, depth profile of root development, and detailed root demographics for coarse and fine roots (root birth, death).

We installed a total of nine (9) tubes (6.5 cm diameter cellulose acetate butyrate tubes) at two different sites. Tubes were placed at an angle of 45^{o} from the soil surface to a meter depth with a soil auger. The tubes were placed in the large patch and small patch of *Spartina* and at the borders with *Phragmites* at one Hawk Property Site, Meadowlands, New Jersey and in a *Phragmites* patch in a restored site at Secaucus restoration site in Meadowlands, New Jersey. High resolution images (600 dpi) were taken by placing a rotating scanner inside the tubes twice. Images were collected twice every month from April 2010 to September 2010. Images were calibrated and analyzed by using WinRhizotron program in the laboratory to measure root length per given volume (716.170 cm^{3}) of the tube area. Two (2) images were taken within each tube of size (21.5 x 19.5 cm)

Results

During the period from April 2010 to September 2010, data from large patch at Hawk property showed a clear increase in the number and diameter size of below ground roots and rhizomes in the *Spartina* patch and at the border (Fig. 22).



April **May** **June**

Fig. 22: Top Images for Hawk Property *Spartina* patch from April-September, 2010.

Data obtained from these images suggest that total root length (TRL) increased from the beginning from the growing season and it hit a peak around the month of July, 2010, and later declined (Fig. 21), no clear difference was found between the two location (p=0.75)

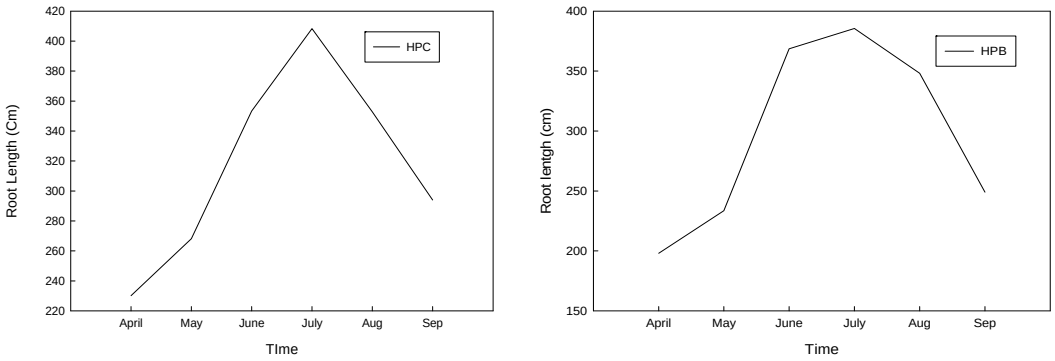
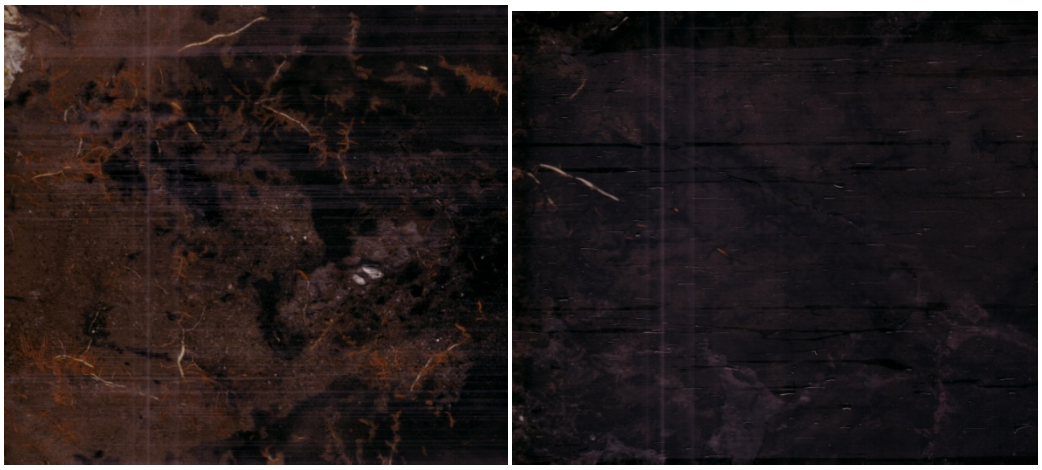


Fig. 23: Average Root lengths from April –September, 2010 from Hawk Property *Spartina* patch (left) and at the border to *Phragmites* (right).

There was no significant difference (p = 0.75) between the average TRL measured from tubes in the Large *Spartina* patch and at the borders (Fig. 23)

Data from tubes installed at *Phragmites* patches at Restored marsh site Secaucus Restoration Property are not reported here due to the fact that the natural conditions of growth were disturbed at this site due to herbicides application during the experimental period. The data collected from this site do not show clear trends of root or rhizome growth. For example, in some of the images collected for the month of June and July, most of the roots and underground rhizoids in upper 15-20 cm were missing (Fig. 24).



June **July**

Fig. 24: Soil-root scans from Secaucus Restoration Site (Top 20 cm from soil surface)

The below ground growth of roots and rhizomes showed a cyclic trend of increasing towards a peak and then declining. There was not a significant difference in below-ground root and rhizomes for the months of April and May, it could be due to an increase in the photosynthesis and a relative increase in the above ground tissue. A study in *Phragmites australis* stands showed a relatively lower increase in belowground tissue in the initial months of growing season (Asaeda et al. 2006)**.** Seasonal variations have previously been shown to occur in *Spartina alterniflora* where a peak in below ground tissue was recorded in mid-summer (Ellison et al. 1986).

8. POSSIBLE ROLE OF ROOT EXUDATES IN *SPARTINA-PHRAGMITES* CLONAL INTERACTION (with Kimberly Plank)

Mechanisms of root antagonism in the native salt marsh grass *Spartina patens*

The demonstrated differences in genetic structure and ecological performance raise important questions concerning the underlying mechanisms by which large-patch clones are able to resist the invaders. A clear understanding of these mechanisms, beside the intrinsic scientific merit, will likely advance further restoration efforts. One possible mechanism by which *Spartina* potentially antagonizes its competitor is through secretion of organic compounds by roots effecting rhizosphere redox conditions and raising rooting zone hydrogen sulfite levels (as studied by the Princeton group led by Peter Jaffe). The initial hypothesis we propose to explore relates to the possible role of *Spartina* root exudates. Do genetically distinct clones of *Spartina* differ in type and concentration of root exudates? There is some evidence that other

aquatic plants (seagrasses such as *Posidonia oceanica*, *Zostera marina*, and *Thalassia* spp.) can induce negative plant-substrate interactions by the accumulation of hydrogen sulfide in their root zones (Holmer & Nielsen 1997, Erskine & Koch 2000).

In order to test the hypothesis that clones of *Spartina patens* clones differ in phototoxic root exudates, we used a screening approach that utilizes our knowledge of genetic differentiation and performance of these clones in the Meadowlands.

Methods

General approach: Individuals of *Spartina* of the three previously investigated provenances (paired large and small patches within each provenance) have been grown hydroponically and solutions exposed to the rhizosphere have been collected and analysed for organic chemicals.

The source materials of *Spartina patens* were collected from their native environment in high marsh zones at Hackensack Meadowlands, New Jersey in May 2010. Sites include Fish Creek (FC), Hawk Property (HP), and River Bend (RB), as seen in Fig. 1. Each site contains both small and large patches of *Spartina patens*; small patches compose areas <200m^2 and large patches constitute areas > 3,000m^2. Transported in humid plastic bags to prevent desiccation of roots, the unearthed parts were brought to the laboratory where they were cut into plantlets. Plantlets consisted of ramet with attached roots. Plantlets were grown for four weeks in the greenhouse prior to experimentation.

Hydroponic setup: The air supply system consisted of four Petco-brand 100-120Volt, 60Hertz, 5Watt (9.0L/min exhaust) air pumps with check valves, four Petco 4-way gang valves, and sixty-four discard-a-stone, fine bubble airstone diffusers (Lee's Aquarium and Pet Products, catalog number 12521). Each component was connected by Topfin silicone airline tubing. The length of silicone tubing from each air pump to each check valve was 10.25 inches. The length of tubing from each check valve to each 4-way gang valve was 5.5 inches, and from each gang valve to each airstone diffuser was 13 inches. Silicone tubing-sized holes were dremeled into the side of each of sixty-four 64oz PPCO (copolymer of polypropylene and HDPE) specimen containers with LLDPE lids (Dynalon Products, www.usplastic.com); containers are inert and cannot be attacked by weak acids or bases. Airstones were threaded through the holes and sealed with 100% silicone sealant (GE Premium Waterproof Silicone II). Using a drill with 1.5" bit, holes were made in the container lids. Pieces of packing foam were cut into 1.5" x 1.5" pieces to support each plantlet through a hole cut in the lid of each specimen container.

Containers were filled with 1.875l of destilled water and brought to room temperature in the climate-controlled greenhouse. Air bubblers were activated. Soil and all debris were removed from plant material by washing prior to placement in containers. Plants were acclimated to the greenhouse for 4 weeks.

Fig. 25. Hydroponic setup. Ten replicates of each plant are from both small and large patches from Fish Creek (FC), Hawk Property (HP), and River Bend (RB).

Experimental design

We maintain that differences in species provenance determine components of root exudates which vary with remnant patch size. This hypothesis was tested by establishing hydroponic monoculture replicates of *Spartina patens* as in Fig. 25. Hydroponic growth methods have been validated by Bais *et al* (2001, 2002) as root secretion of secondary metabolites is comparable *in vitro* to that of soil. Three previously investigated source patches of provenances of *Spartina patens* with both small and large patches of each, and ten replicates for each site, were tested. Water levels were checked daily and adjusted as needed through water additions. Complete water changes were made weekly. Ambient temperatures were controlled in the greenhouse setting. Results and analysis of *Spartina patens* responses are described below.

Chemical analysis

Analysis of possible root exudates was executed in the Chemistry Department at Rutgers University (Newark, New Jersey). After 14 days of growth in milliQ in the hydroponic system, final analysis was done using Apex-ultra 70 hybrid fourier transform mass spectrometer (Bruker Daltonics). Conditions were as follows: 1 µL of water from each of sixty plant monocultures and two blanks were auto-injected into the machine. The method entailed a broadband mass scan from 118.2 to 1200.0 m/z, drying gas temperature was 190.0 °C, drying

gas flow rate was 4.0 L/min, ion accumulation time was 0.7 sec and nebulizer gas flow rate was 1.0 L/min (HyStar, Bruker Daltonics).

Statistical Analysis

Mass spectra were collected as a function of retention time and organized by both site and patch size using Microsoft Excel. SPSS was used to generate histograms (SPSS version 17.0). Data were subjected to Canoco detrended ordination correspondence analysis (Canoco CanoDraw). Surfer (version 8, Golden Software, Inc.) was used to generate contour and 3D surface maps. Both Mathematica and Gene Cluster were used for cluster analysis (Wolfram Mathematica version 7.0; Gene Cluster version 3.0). Results from Cluster were viewed in TreeView (Java).

Results

Similarities and differences in root exudate metabolite profiles were analyzed using several programs using different approaches. We anticipated differences in exudates among the different provenances of plants. Between groups of plants (small and large patches), we did not see differences (Figs. 26, 27). In comparison of the three sites, we did not see differences (Fig. 28). In both site and patch size, we did not see differences (Fig. 29). We did not see differences after a detrended ordination correspondence analysis in Canoco (Fig. 30). In Surfer, a program used to generate contour and 3D surface maps, we did not see patterns (Fig. 31).

After a cluster analysis using Gene Cluster 3.0, we did not see a distinct pattern of either site or remnant patch size (Fig. 32). Cluster analysis of individual results of all samples mass spectra data converted to binary showed no significant differences and no trends (Fig. 33). Again, the second two cluster trees have the blanks omitted, which includes the omission of all peaks in the blanks, from the samples so the samples are not skewed toward one of the two blanks. As seen from Fig. 21 in comparison to Fig. 33, there are differences in the generation of the clusters, however, no clear patterns in regards to patch size and site data.

In Microsoft Excel, high intensity peaks were selected and were plotted by patch size; results showed that patterns were not present (Fig. 34-35). Fisg. 34-35 show plots of the high intensities by mass and patch size. The 10 peaks for each patch size in Fig. 35 are somewhat different for the two patches, but is not sufficient to say the patches are different because the peaks “absent” in one patch size may actually still be present in the other patch size, just of lesser intensity-rank.

Conclusions: Although the results showed differences among individual plants, the overall three sites showed no differences. Similarly, the two patch sizes showed no differences. When the six groups were compared, there were no differences. No patterns of small and large patch size difference were observed in any of the analyses, and no correlations were detected using several strategies in cluster analysis. Results discussed here failed to confirm our hypothesis that genetically distinct patches of *Spartina patens* differ in root exudates.

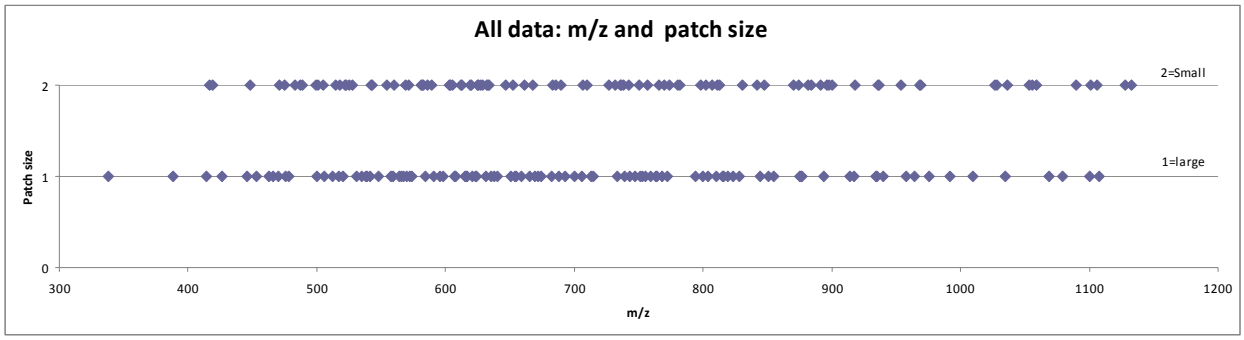


Fig. 26: Root exudates results of *Spartina patens* by patch size.

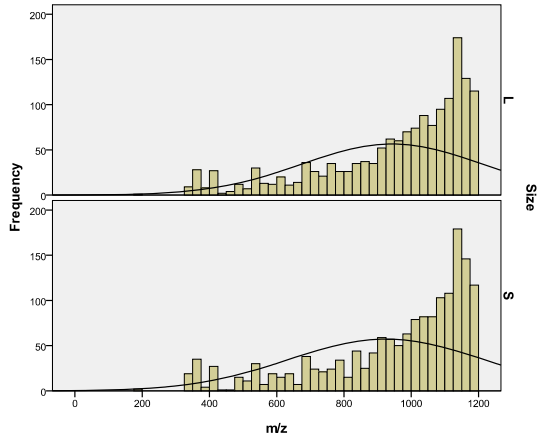


Fig. 27: SPSS-generated histogram of exudates of *Spartina patens* of small and large patches. Frequencies plotted on the y-axis represent number of occurrences of bins of mass values, plotted on the x-axis.

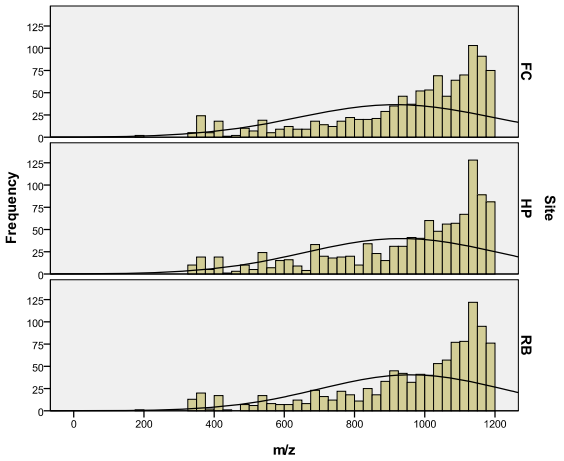


Fig. 28: SPSS-generated histogram of exudates of *Spartina patens* from the three source sites. Frequencies plotted on the y-axis represent number of occurrences of bins of mass values, plotted on the x-axis.

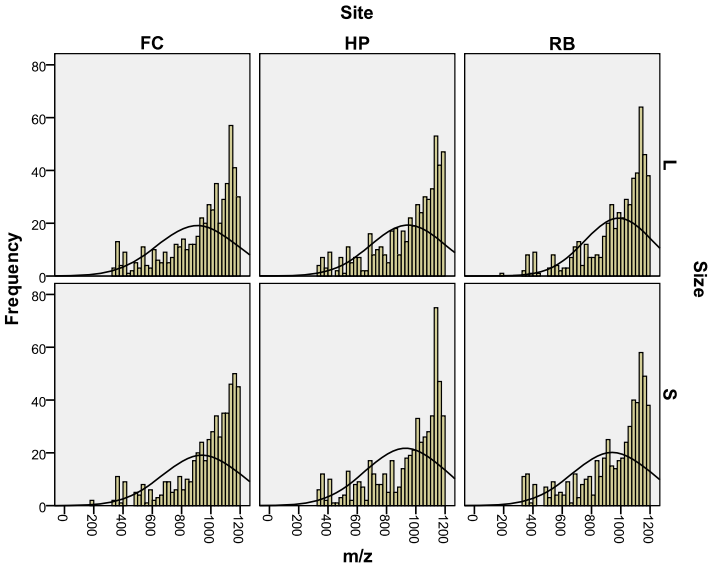


Fig. 29: SPSS-generated histogram of *Spartina patens* root exudates by both site and remnant patch size. Frequencies plotted on the y-axis represen number of occurrences of bins of mass values, plotted on the x-axis t.

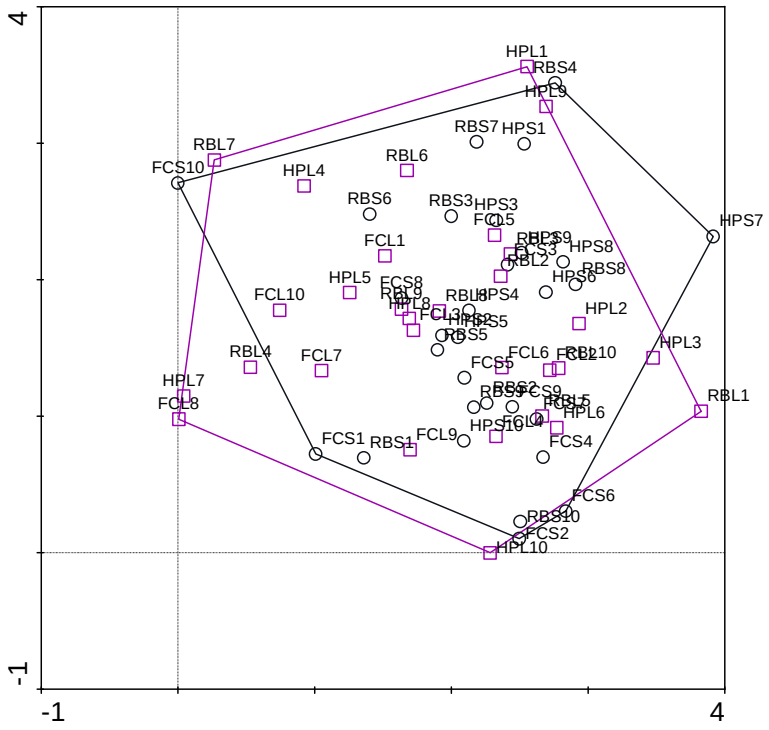


Fig. 30: Canoco-generated map of detrended ordination correspondence analysis of mass and intensity of *Spartina patens* root exudates by site, including all ten replicates of each site (FC=Fish Creek, HP=Hawk Property, RB=River Bend).

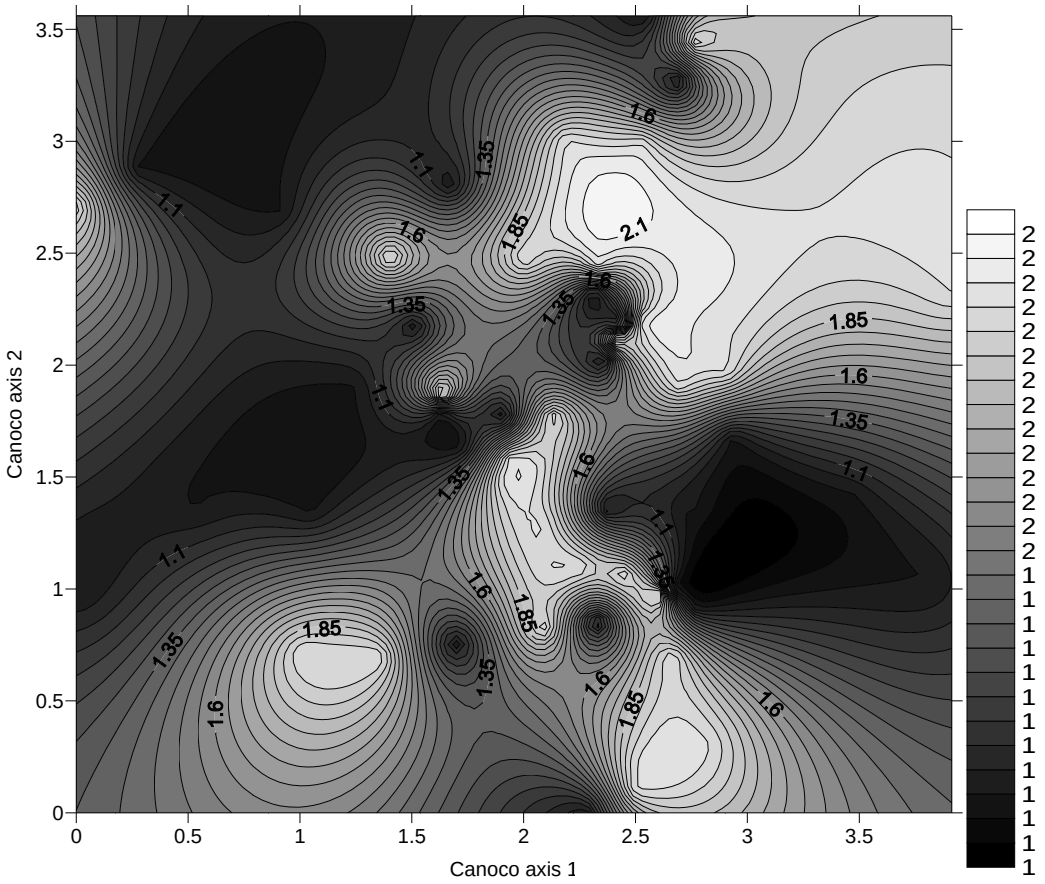


Fig. 31: Surfer-generated map of *Spartina patens* root exudates by patch. 1=large patch size, 2=small patch size. Canoco axis 1 is m/z and Canoco axis 2 is intensity of the peak in the mass spectra.

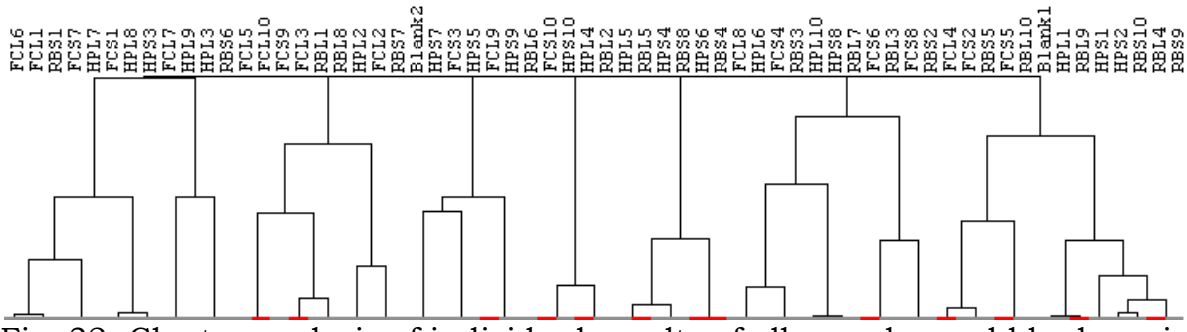


Fig. 32: Cluster analysis of individual results of all samples and blanks using all mass spectra data.

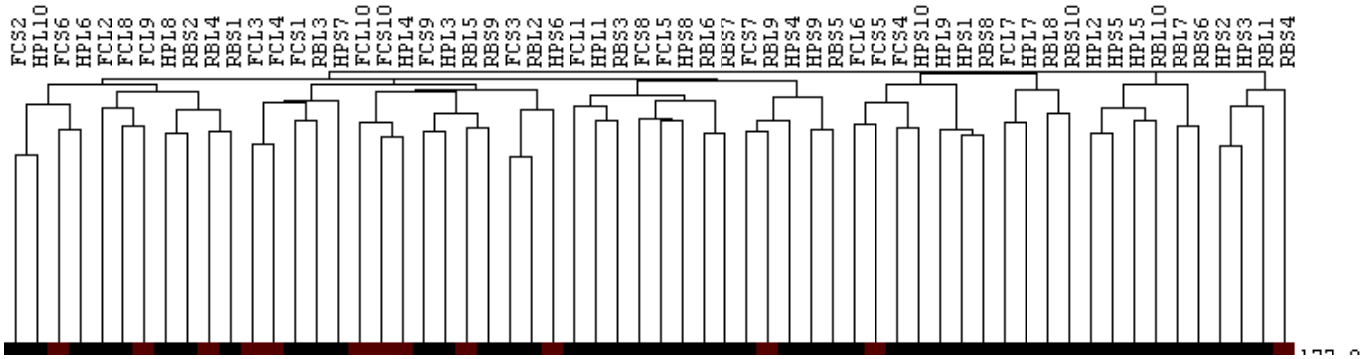


Fig. 33: Cluster analysis of individual results of all samples mass spectra data converted to binary.

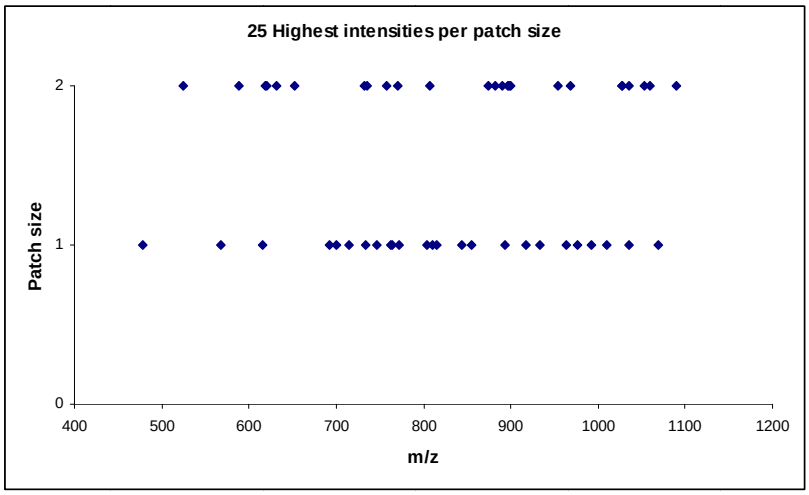


Fig. 34: High intensities of m/z per patch size (large=1, small=2).

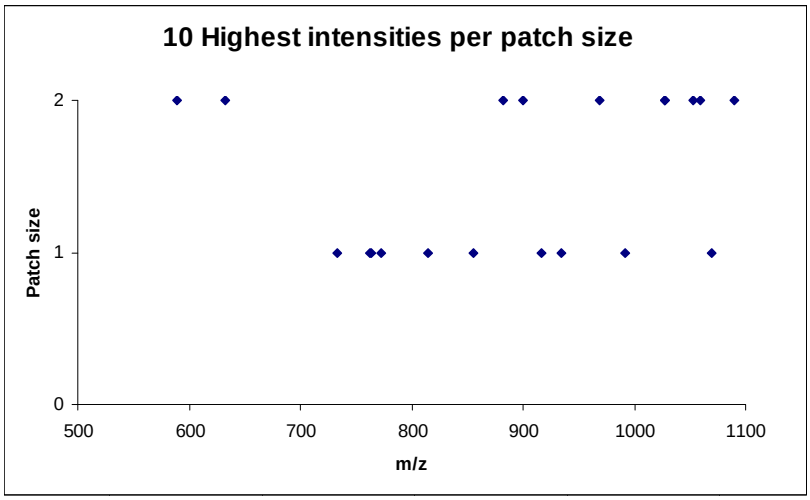


Fig. 35: High intensities of m/z per patch size (large=1, small=2).

OVERALL CONCLUSIONS

Our research efforts explored the factors that allow remnant patches of *Spartina patens* to persist in the presence of invasive and highly competitive and invasive *Phragmites*. Restoration efforts are attempts at creating and assembling local communities that have vanished. Recent studies have shown the importance of using local ecotypes of species as building blocks in these assemblies and the need to include information on genotypic differentiation has been stressed. Large portions of brackish east coast marshlands have been invaded by non-native, European genotypes of the common reed, *Phragmites australis*. As a result, only a small fraction of the NJ Hackensack Meadowlands is now dominated by native marsh species and only isolated patches of *Spartina patens* remain. As these patches vary in size and seem to resist encroachment by *Phragmites* differentially, we investigated (a) whether larger patches are able to resist invasion more than smaller patches and (b) whether large-patch clones differ genetically from small-patch clones. Current results of the project indicate (1) clear genetic differentiation between large and small-patch clones even when they are in close proximity to each other and (2) differential competitive capacities of these distinct clones. The latter has been demonstrated with descriptive and experimental approaches which indicate that (a) border zones between the invader and *Spartina* tend to be more defined in large *Spartina* remnant patches than in small patches, (b) *Spartina* increases in dominance at large-patch borders but decrease in small-patch borders, and (c) large-patch *Spartina* is able to reduce the growth of invading *Phragmites* fragments. These results strongly suggest that some genetically defined *Spartina* clones are more suitable for restoration efforts than others and we therefore anticipate that our result will have strong implication for future restoration projects.
The small patches appear not to be remnants of the large patches but clonally distinct patches. Revisiting the original alternative hypotheses:

(H_1) a breakup of single large patches
("**one patch breaks up" hypothesis**)
(H_2) a differential dissolving of most of many connected patches
('**most patches dissolve" hypothesis**),

we affirm that H_2 is more likely, at least H_1 can be fully rejected. It can be assumed that small patches are not remnants of homogenous large patches but distinct clones that fared less good under competition with an invasive marsh grass. As for an understanding of the mechanisms that cause the differential competitive behavior of *Spartina* clones, we have made but little advance.

Future studies need to address the underlying mechanisms of the interaction of *Spartina* with *Phragmites*. Based on our knowledge about genetic

structure and differential competitive ability of individual *Spartina patens* clones we intend to focus on three fields of investigation in order to approach the question: What mechanisms are responsible for the selective success of large-patch *Spartina patens* clones in the face of non-native *Phragmites* clone invasion?

(1) Aerenchyma hypothesis: Prior research indicated that one of potentially leading mechanism of species interaction is related to a differential redox profiles in the marsh soil and the availability of dissolved oxygen. With eco-physical experiment and microcosm approaches we intend to explore the idea that competitive *Spartina* clones reduce oxygen availability through a reduction in aerenchyma in root and rhizomes.

(2) *Chemical Defense Hypothesis*: Recently is was discoverd that the predominant invasive competitor of *Spartina patens*, *Phragmites australis*, exudes gallic acid (3,4,5-trihydroxybenzoic acid), a phenolic, which elevates levels of reactive oxygen species (ROS) (Rudrappa *et al* 2007). It has been suggested that phenolic compounds require oxidation for functioning in ecological interactions, such as those in the rhizosphere (Appel and Hirt 2004). We intend to investigate whether *Spartina* clones differ in their ability to oxidize and thereby reduce the potentially alleopathic effects of *Phragmites* exudations. We will build in this context on our knowledge gained from another MERI funded project that targeted such phenolic oxidation in grasses (Holzapfel et al. 2010).

(3) *Root Exudate Bioassay*: Building on our experimental setup used in our indecisive attempt to assay for root exudates, future experiments to further investigate root exudates presence include bioassay (Jiang-Hong et al. 2007). Removing aliquots from *Spartina patens* containers could be applied to *Arabidopsis thaliana* seed and compared to controls deficient in compounds, and germination rates would be checked for dose-response (Inderjit and Callaway 2003). Post-germination traits to be studied in separate experiments include morphological traits, such as stem density, height, basal growth, and photosynthetic ability, and reproductive abilities, such as seed production, new recruits, and fitness, a measure of the success of the genotype.

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