Performance of “Dominant” *Vallisneria americana* Genotypes in Greenhouse Mesocosm Competition Experiments

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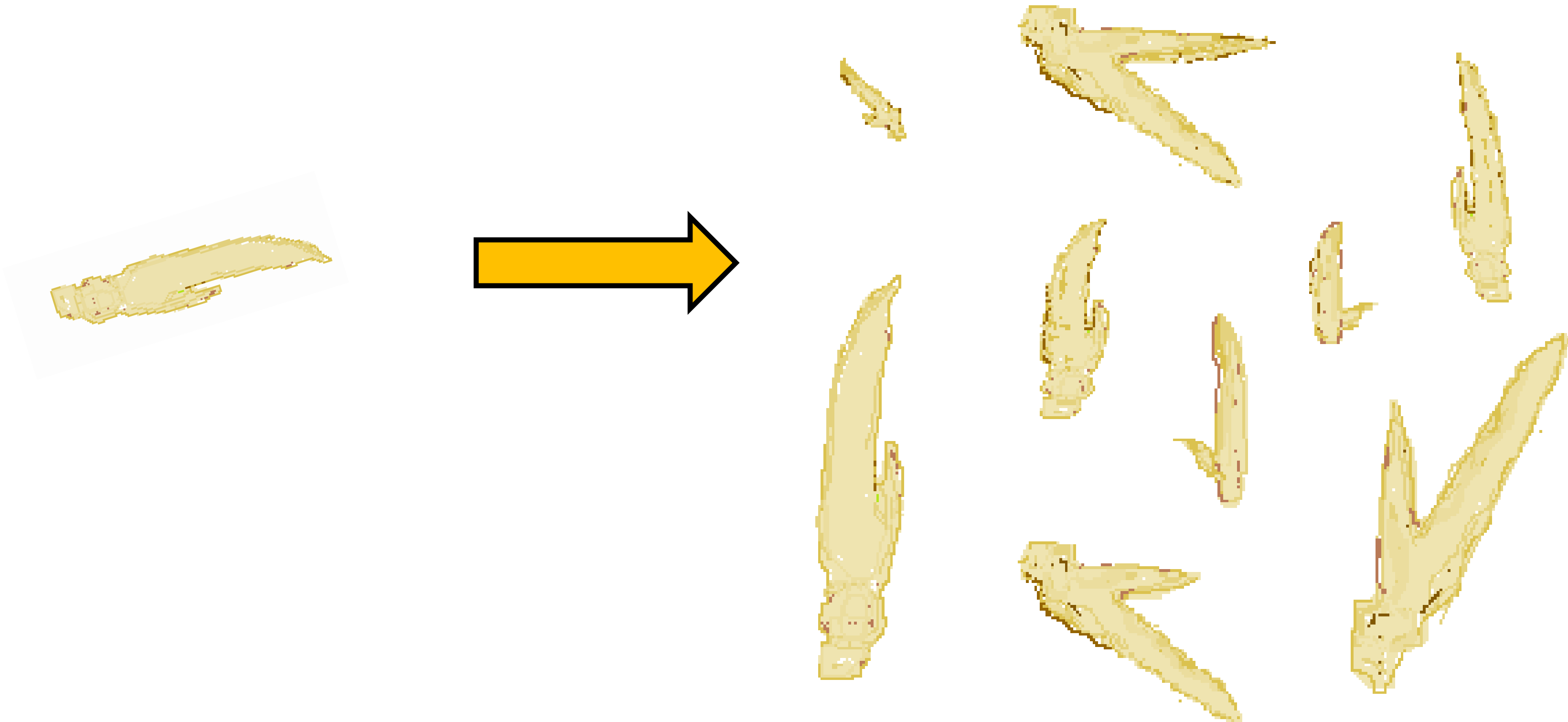
December 15, 2016

Vallisneria americana Michx

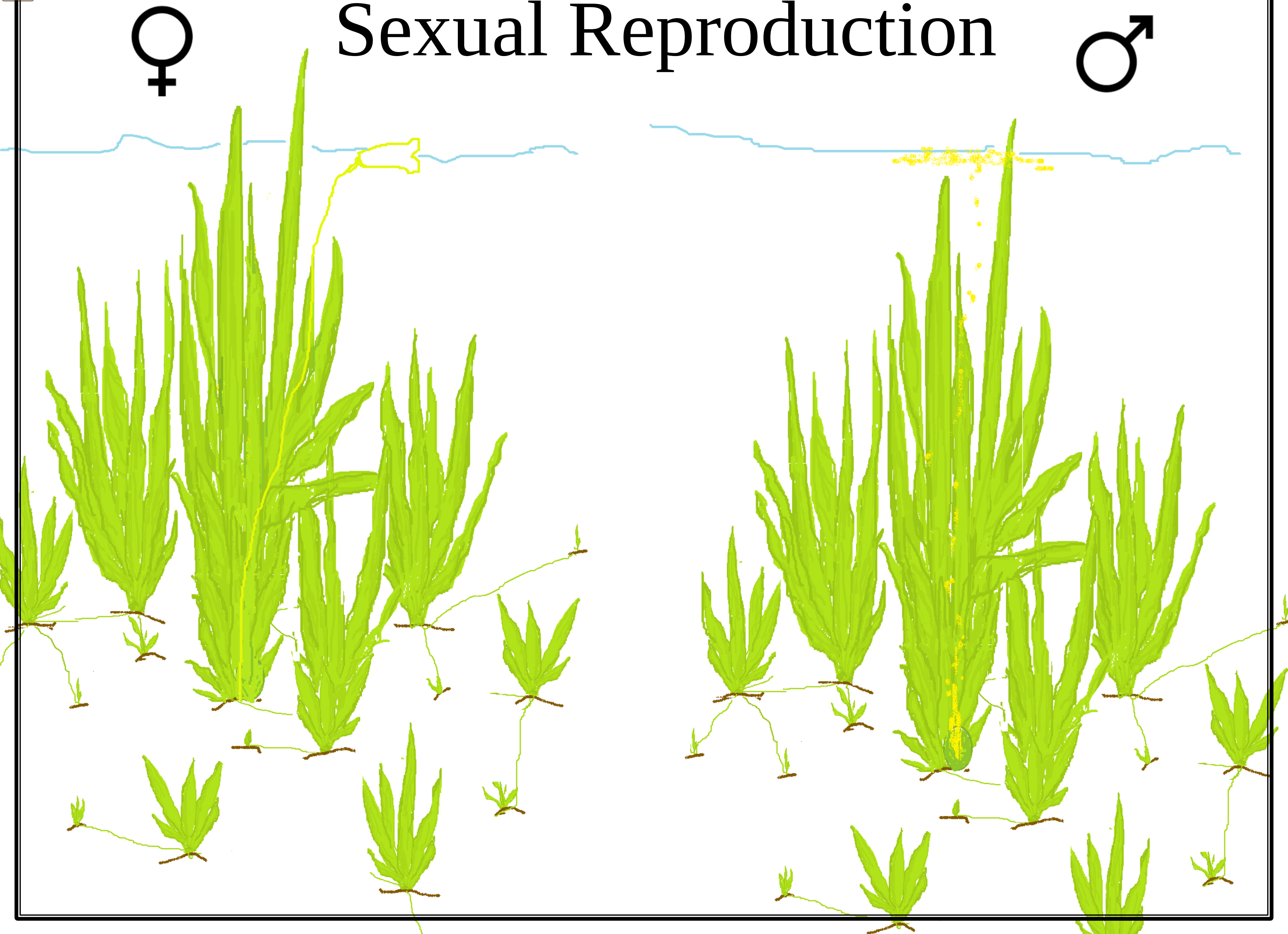
(Common names: wild celery, water-celery, tape grass, eelgrass)

Val Life Cycle

Asexual Reproduction



Sexual Reproduction

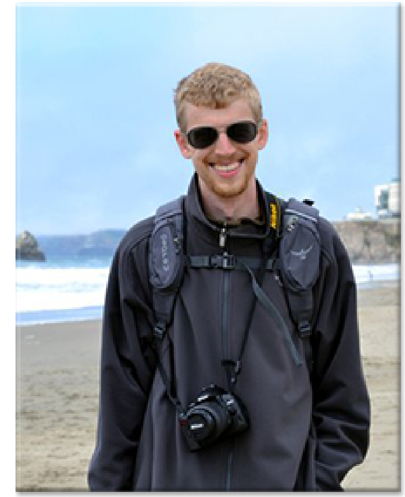


Prior Research

University of Maryland 1856

University of Maryland
Center for Environmental Science

Dr. Katia Engelhardt



Dr. Mike Lloyd

Dr. Maile Neel

Dr. Brittany Marsden

2007, 2009 & 2011 *V. americana* Samplings of the Upper Potomac River

-29 sites
-787 total individuals collected.
-401 different genotypes identified

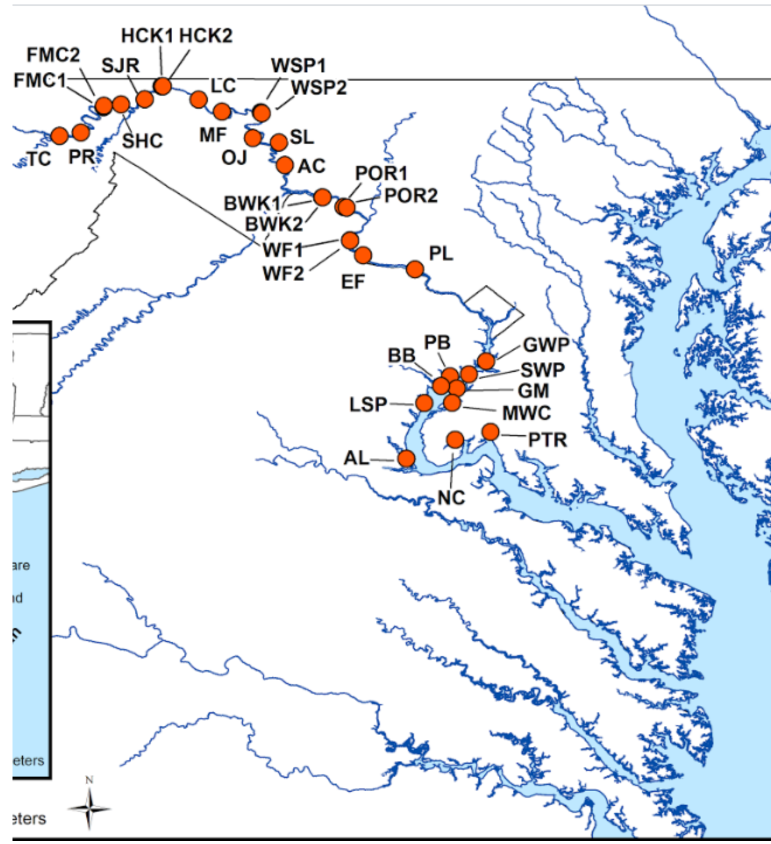
Number of Genotypes	Number of Occurrences
344	1
32	2
20	3-7
3	10-16
1	**89**
1	**104**

“Rare”

“D2”

“D1”

WOW!



Research Question

- Why are these two "dominant" genotypes (D1 and D2) so abundant in natural *V. americana* populations within the Potomac River?

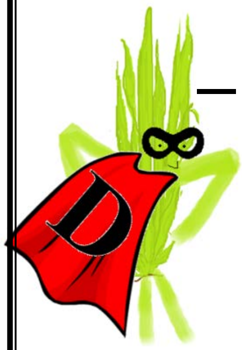
  - Are they phenotypically superior to other genotypes in terms of sprouting, growth, survival, asexual reproduction, or dispersal?

OR

  - Did chance events lead to low genetic diversity?

Restoration Implications

- If D1 and D2 are found to be "super-performers", including them in restoration plantings:
  - may increase the chance of success of plantings.
  - alternatively, might reduce diversity in restoration patches, making them less resilient over time.



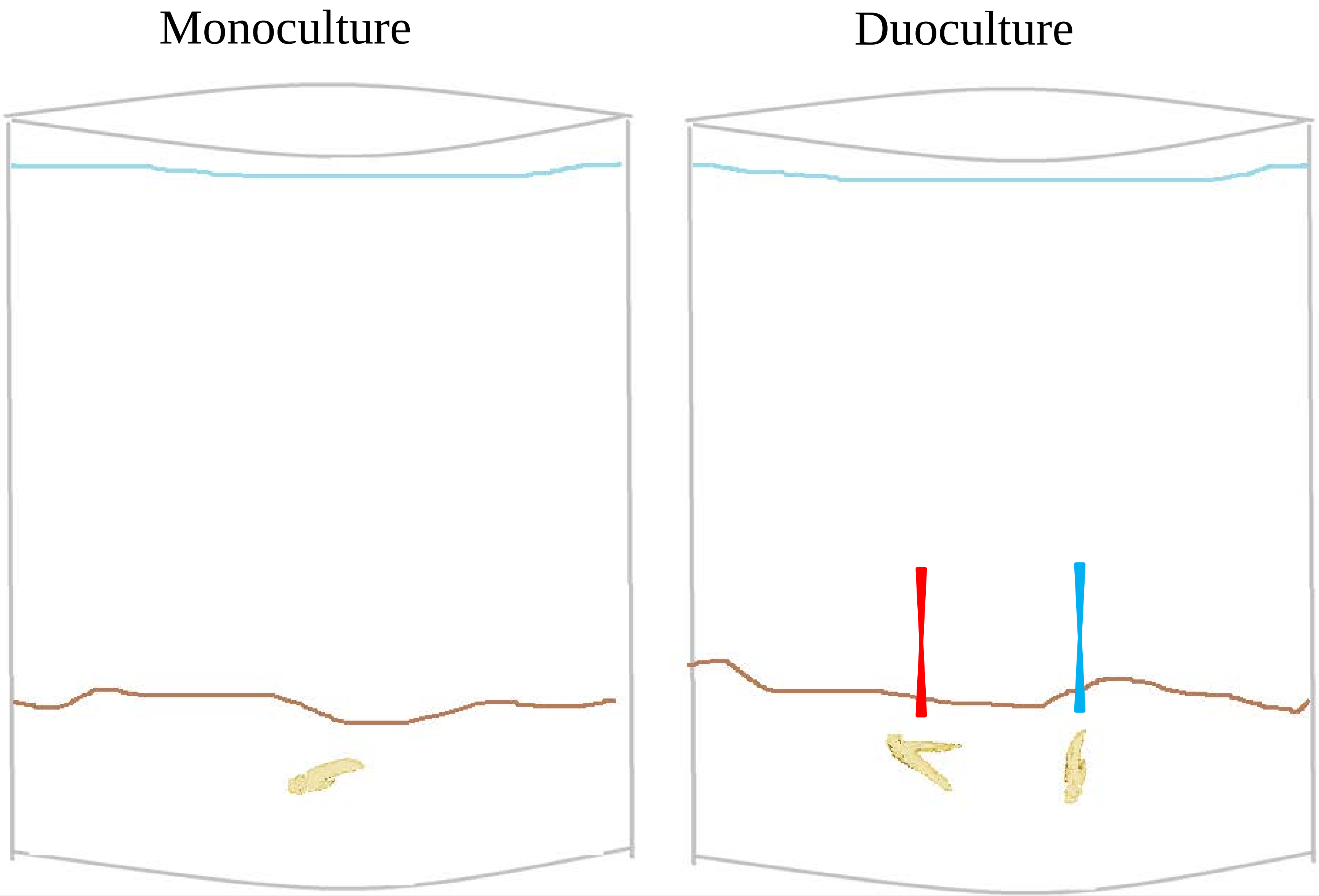
- If no difference in performance is observed, the lack of diversity may be viewed more negatively and could provide insight into why *V. americana* populations declined.

Hypothesis

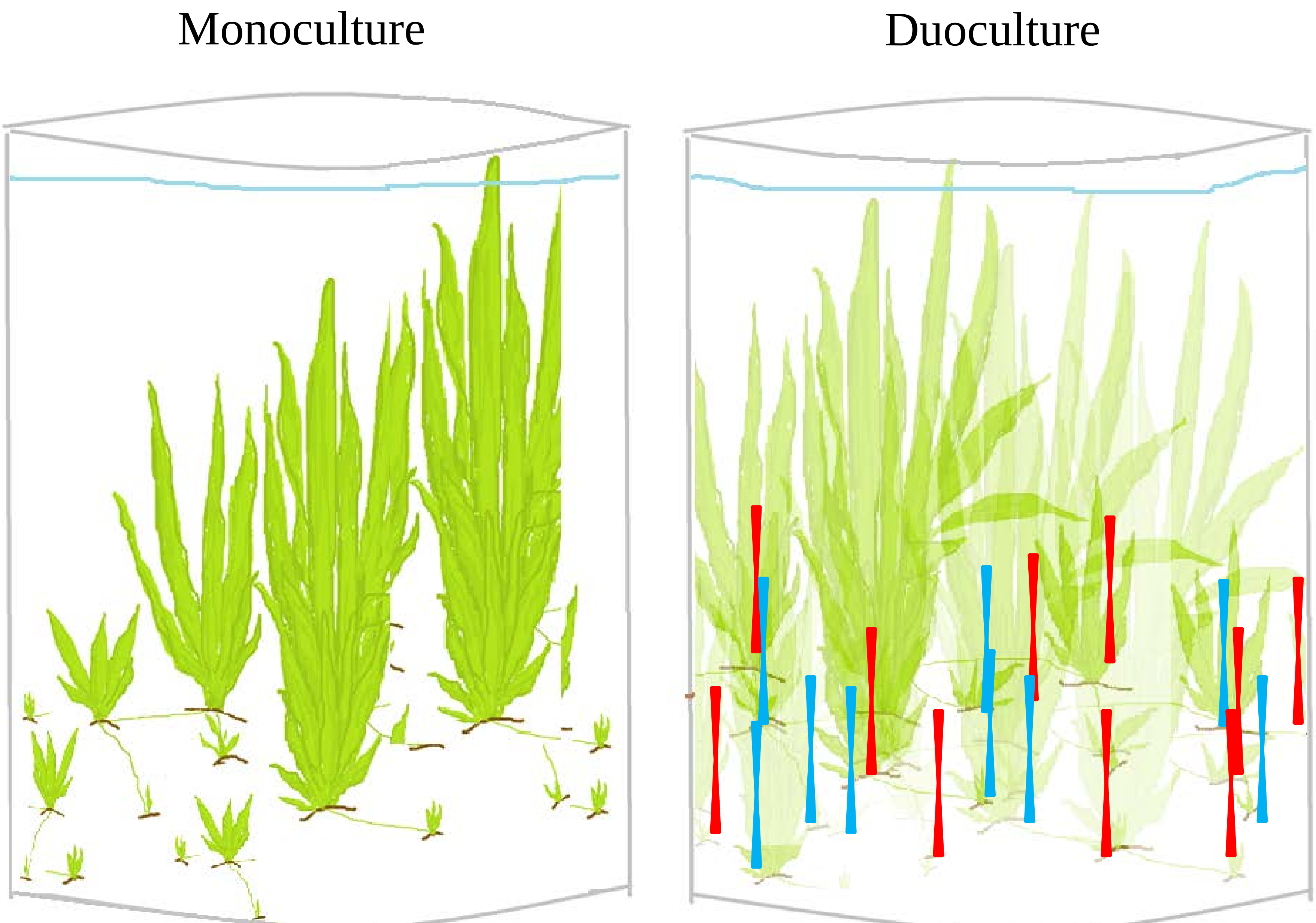
- Under the same conditions, dominant genotypes will outperform rare genotypes in <u>at least</u> one of the following traits:

  - percent success of sprouting (germ. rate)
  - ramet production
  - total biomass
  - turion production

Greenhouse Mesocosm Experiment



Greenhouse Mesocosm Experiment



Experimental Design

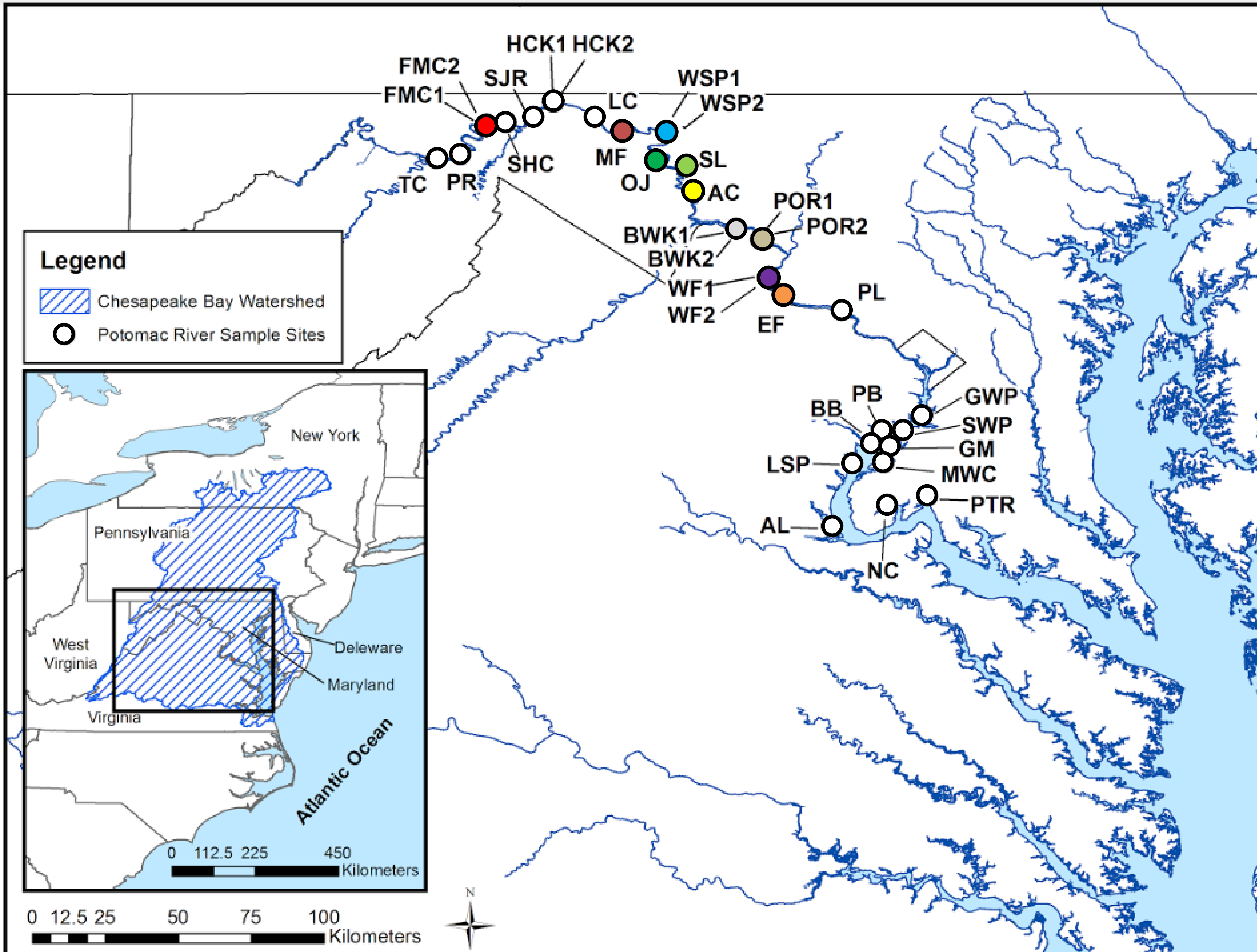
- 2 Dominant genotypes (“D1”&“D2”)
- 10 Rare genotypes (“R1”-“R10”)
- Monoculture of each genotype (n=4)
- Duoculture of each genotype vs. itself (n=4)
- D1 vs. D2 (n=4)
- Each Dominant vs. each Rare (n=4 per combination)
- Monoculture of each Dominant genotype sampled from 5 additional sites (n=6 per site)

Sample Selection

ID	# Turions	Site	Sample
D1	103	MF (D2)	2D05, 2D06, 2D10
D2	165	BWK2 (F2)	2F01, 2F02, 2F16, 2F19
R1	80	WF2 (H2)	2H04
R2	25	WF2 (H2)	2H24
R3	55	WSP2 (E2)	2E20
R4	50	WSP2 (E2)	2E25
R5	50	EF (H1)	1H03
R6	34	EF (H1)	1H08
R7	32	POR2 (G1)	1G01
R8	22	POR2 (G1)	1G22
R9	30	SL (E1)	1E10
R10	22	SL (E1)	1E03

ID Extra Sites D1	Site	Sample
D1s1	WSP2 (E2)	2E09
D1s2	POR2 (G1)	1G24
D1s3	AC (F1)	1F06
D1s4	OJ (D1)	1D06
D1s5	FMC1 (B2)	2B20

ID Extra Sites D2	Site	Sample
D2s1	MF (D2)	2D22
D2s2	EF (H1)	1H15
D2s3	POR2 (G1)	1G28
D2s4	SL (E1)	1E06
D2s5	OJ (D1)	1D03


HCK1 HCK2
FMC2
SJR
FMC1
LC
WSP1
WSP2
SHC
MF
TC
PR
OJ
SL
AC
POR1
POR2
BWK1
BWK2
WF1
WF2
EF
PL
Legend
Chesapeake Bay Watershed
Potomac River Sample Sites
PB
BB
GWP
SWP
GM
LSP
MWC
PTR
AL
NC
New York
Pennsylvania
West Virginia
Deleware
Maryland
Virginia
Atlantic Ocean
0 112.5 225 450
Kilometers
0 12.5 25 50 75 100
Kilometers
N

Experimental Practices

- Buckets with sterilized HWC Chesapeake Bay sediment, tap water
- 1 or 2 turions planted per bucket
- Random arrangement of buckets within greenhouse
- Irrigation system
- Weekly cleaning and removal of algae and flower monitoring
- Bi-weekly measurements and data collection
- Bi-weekly re-randomization
- Buckets with failed growth were replicated in week 9.



PRIVA

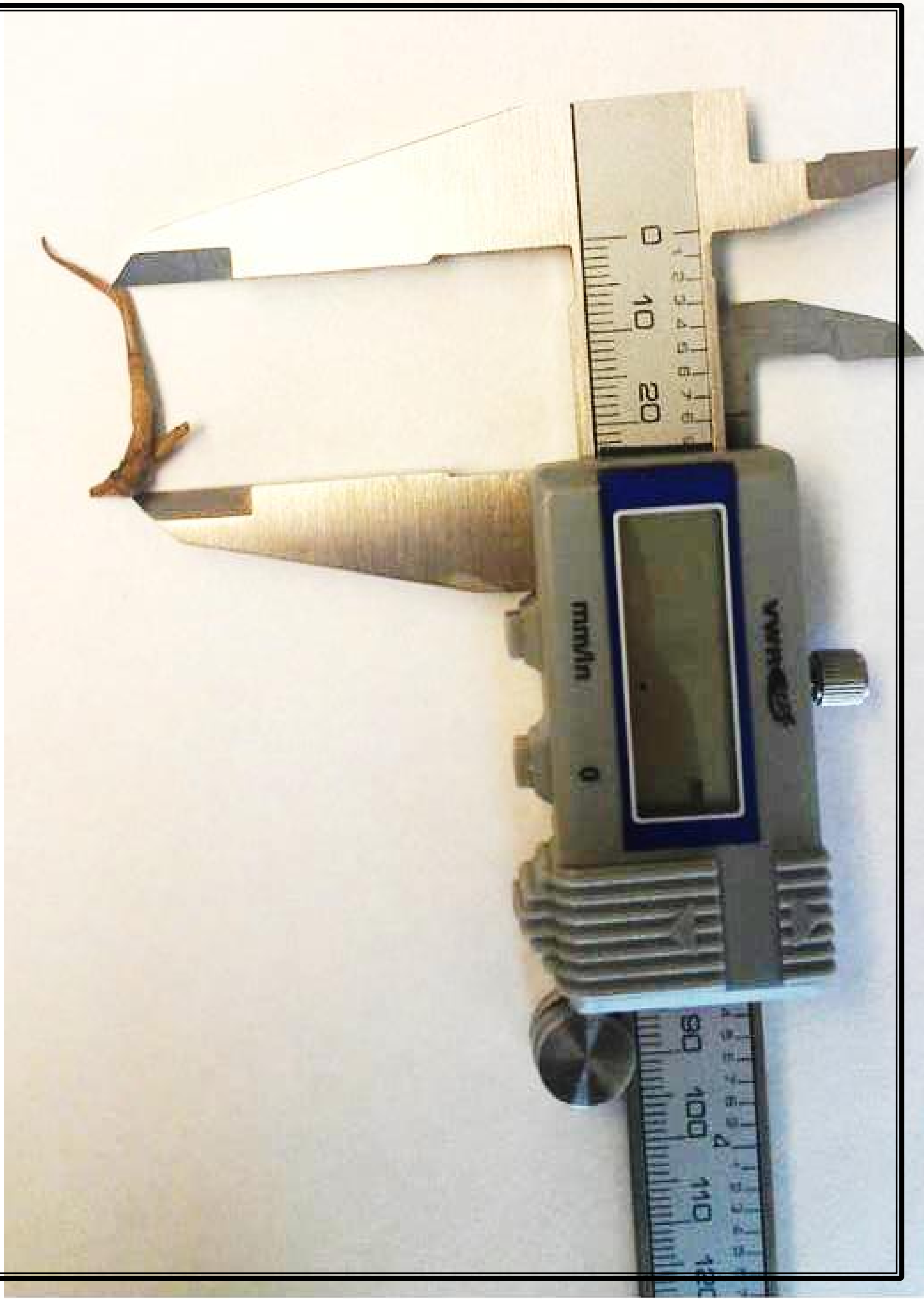
Data Collection

- Length/width of original turions
- Initial sprouting date
- Tracking of each genotype with colored toothpicks (identity unknown)
- Number of ramets
- Number of leaves per ramet
- Length and width of longest leaf per genotype
- Number of flowers, tracked with colored toothpicks
- 25 weeks from planting to senescence (June 14, 2013-December 2, 2013)


PRIVA

Harvest Data

- Harvest of all turions from each bucket
- All turions counted, length/width measured
- In competition buckets where both genotypes grew, all turions were genotyped to determine their source
- In competition buckets where only one genotype grew, a random sample of 10 turions were genotyped to determine which genotype grew



Genotyping Techniques

Single-Stranded DNA extraction:

- 10% Chelex slurry, manual tamping.
  Dilute DNA template 1:2
- LGC Sbeadx maxi plant DNA Extraction (Synergy?)

Microsatellite PCR:

- TDOWN2 touchdown program

Fragment Analysis:

- 3730XL 96-capillary high-throughput DNA sequencer

Electropherogram Software:

- GeneMapper

Distinguishing Microsatellite Loci

	atg002	aagx051	m13	aagx071	m49
D1	151157	184190	263271	224233	168180
D2	154157	178181	266271	230230	168168
R1	154154	178178	??????	230230	168168
R2	151157	178184	263271	224224	168180
R3	151157	184190	263271	224233	159180
R4	154157	178181	271271	230230	162168
R5	151154	190190	271271	230230	168168
R6	151171	184190	263271	224233	168180
R7	151157	184190	269271	224233	168180
R8	154154	178178	271271	230230	168168
R9	154157	178184	271271	230233	168168
R10	151154	178184	271271	224230	168180

Corrected Microsatellite Loci

	atg002	aagx051	m13	aagx071	m49
D1	151157	184190	263271	224233	168180
D2	154157	178181	266271	230230	168168
R1=D2	**154157**	**178181**	266271	230230	168168
R2=D1	151157	**184190**	263271	**224233**	168180
R3=D1	151157	184190	263271	224233	**168180**
R4=D2	154157	178181	**266271**	230230	**168168**
R5	151154	**178190**	271271	230230	168168
R6=D1	**151157**	184190	263271	224233	168180
R7=D1	151157	184190	**263271**	224233	168180
R8=D2?	**154157**	**178181**	**266271**	230230	168168
R9	154157	178184	271271	230233	168168
R10	151154	178184	**263271**	224230	168180

Sample Selection

ID	Site	Sample
D1	MF (D2)	2D05, 2D06, 2D10
D2	BWK2 (F2)	2F01, 2F02, 2F16, 2F19
R1 = D2	WF2 (H2)	2H04
R2 = D1	WF2 (H2)	2H24
R3 = D1	WSP2 (E2)	2E20
R4 = D2	WSP2 (E2)	2E25
R5	EF (H1)	1H03
R6 = D1	EF (H1)	1H08
R7 = D1	POR2 (G1)	1G01
R8 = D2	POR2 (G1)	1G22
R9	SL (E1)	1E10
R10	SL (E1)	1E03

ID Extra Sites D1	Site	Sample
D1s1	WSP2 (E2)	2E09
D1s2	POR2 (G1)	1G24
D1s3	AC (F1)	1F06
D1s4	OJ (D1)	1D06
D1s5	FMC1 (B2)	2B20

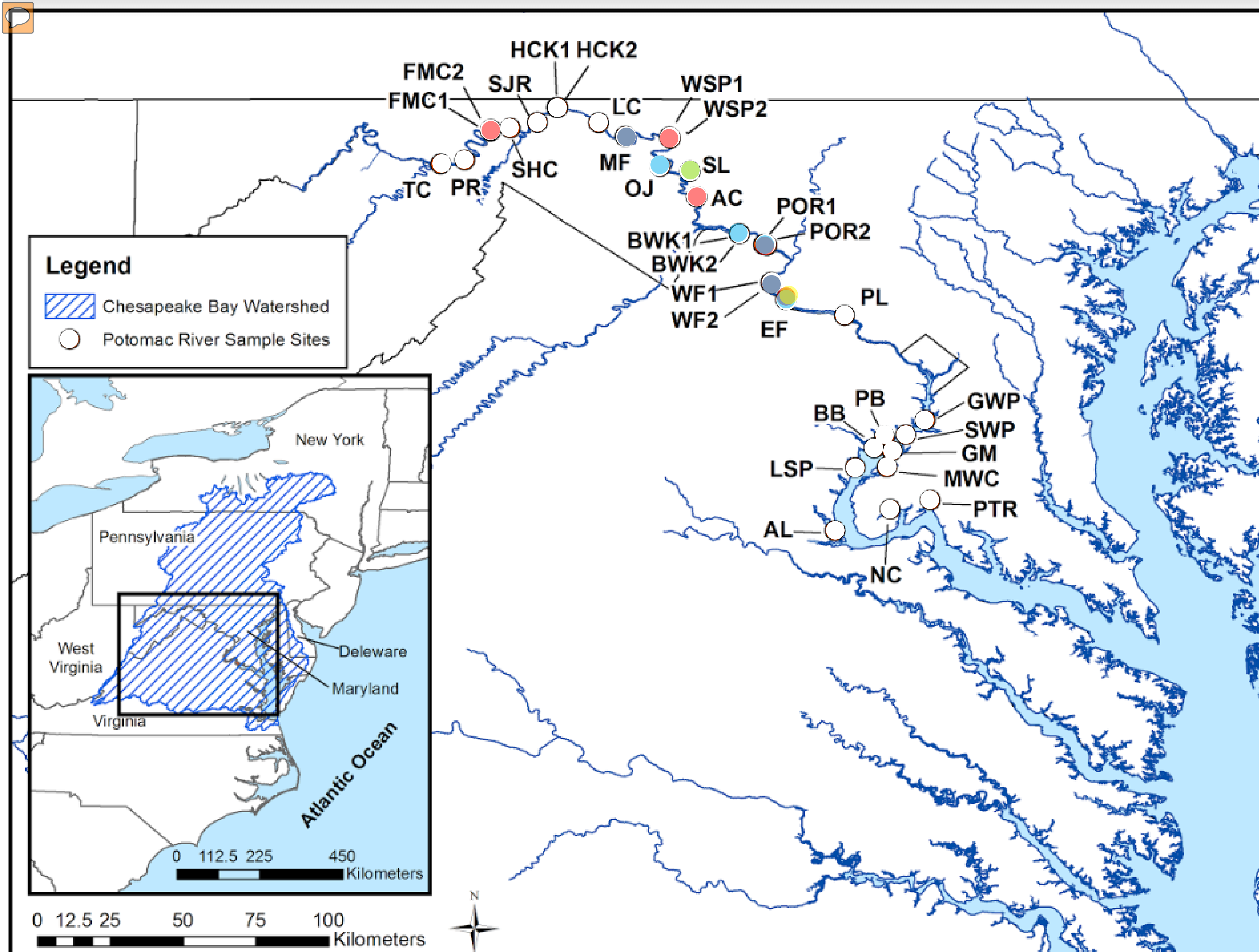
ID Extra Sites D2	Site	Sample
D2s1	MF (D2)	2D22
D2s2	EF (H1)	1H15
D2s3	POR2 (G1)	1G28
D2s4	SL (E1)	1E06
D2s5	OJ (D1)	1D03

Corrected Samples

Rares	Site	Sample
R5	EF (17)	1H03
R9	SL (11)	1E10
R10	SL (11)	1E03

D1	Site	Sample
D1	MF (8)	2D05, 2D06, 2D10
D1s1	WSP2 (9)	2E09
D1s2	POR2 (15)	1G24
D1s3	AC (12)	1F06
D1s4	OJ (10)	1D06
D1s5	FMC1 (3)	2B20
D1s6	WF2 (16)	2H24
D1s7	WSP2 (9)	2E20
D1s8	EF (17)	1H08
D1s9	POR2 (15)	1G01

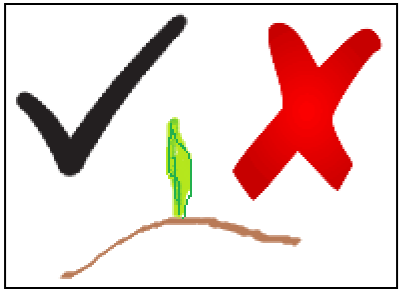
D2	Site	Sample
D2	BWK2 (13)	2F01, 2F02, 2F16, 2F19
D2s1	MF (8)	2D22
D2s2	EF (17)	1H15
D2s3	POR2 (15)	1G28
D2s4	SL (11)	1E06
D2s5	OJ (10)	1D03
D2s6	WF2 (16)	2H04
D2s7	WSP2 (9)	2E25
D2s8	POR2 (15)	1G22



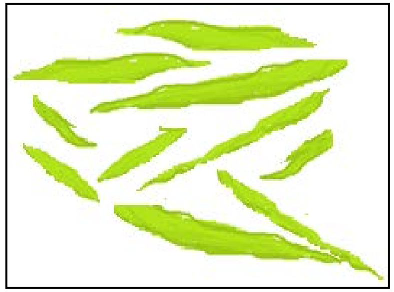
HCK1
HCK2
FMC2
SJR
FMC1
LC
WSP1
WSP2
SHC
MF
SL
TC
PR
OJ
AC
POR1
POR2
BWK1
BWK2
WF1
WF2
EF
PL
PB
BB
GWP
SWP
GM
LSP
MWC
PTR
AL
NC
Legend
Chesapeake Bay Watershed
Potomac River Sample Sites
New York
Pennsylvania
West Virginia
Deleware
Maryland
Virginia
Atlantic Ocean
0 112.5 225 450
Kilometers
0 12.5 25 50 75 100
Kilometers
N

Outcome Variables

Germ. Success Rate

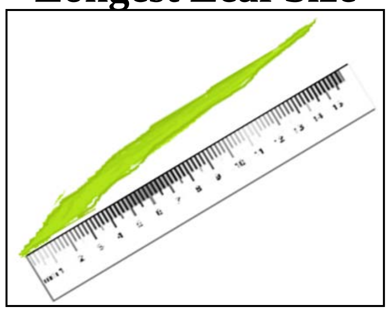


Leaf Production

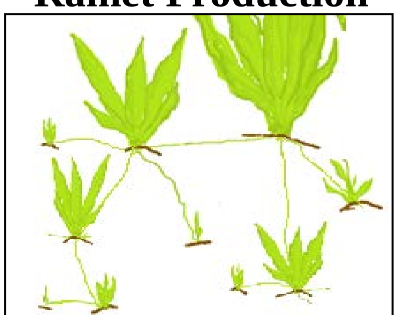


Germination Speed

Longest Leaf Size



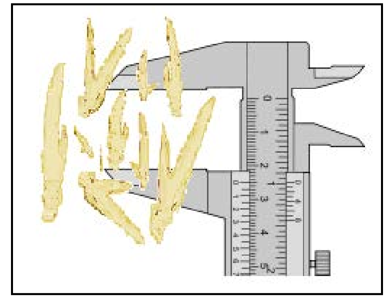
Ramet Production



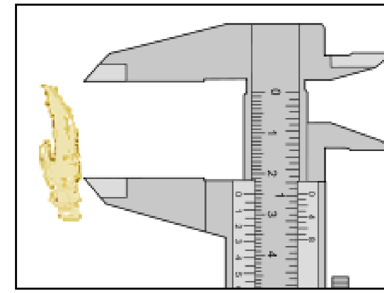
Turion Production

Turion Size

Total

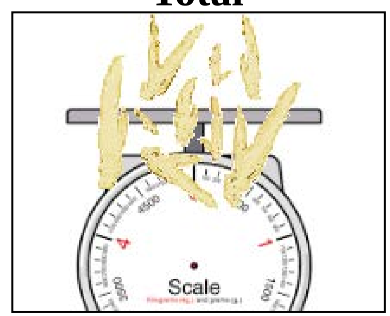


Average

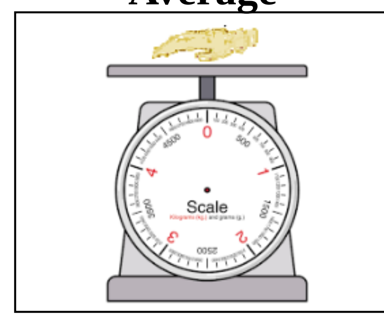


Turion Mass

Total



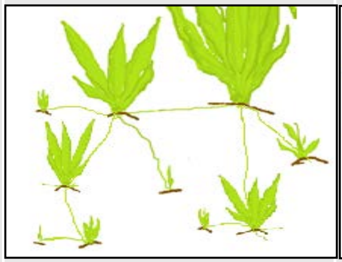
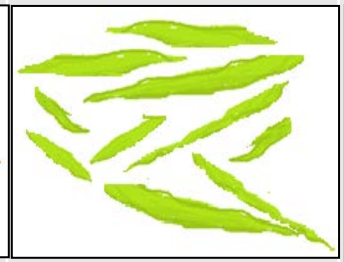
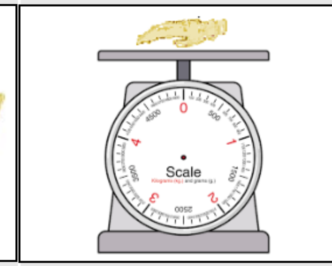
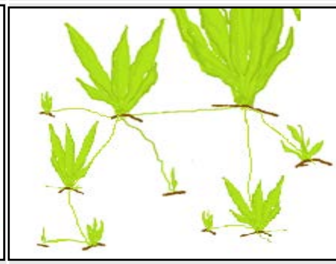
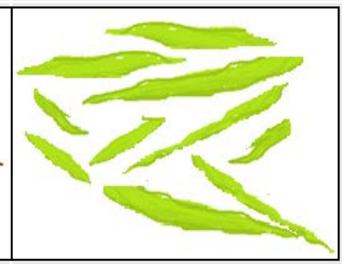
Average



Results: Questions of Interest

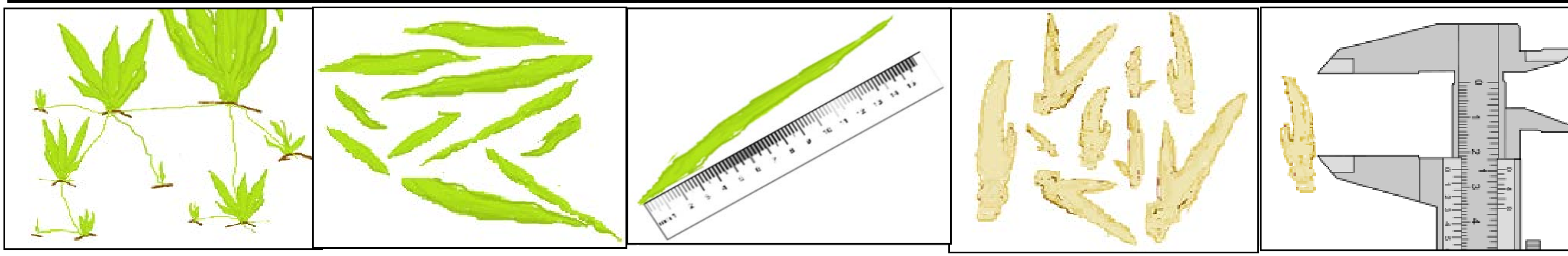
1. Dom Sites: Does site of origin affect dominant genotype performance?
2. Mono vs Duo: Does competition within the bucket limit performance of each genotype?
3. D1 v D2: How does performance of the two dominant genotypes compare?
4. Dominant v Rare: Do dominant genotypes perform better than rare genotypes?
5. How do DR competition buckets compare in overall performance to Dominant duocultures and Rare duocultures?

1. Dom Sites: Does site of origin affect dominant genotype performance?

Outcome Variables with Significant Site Differences					
D1					
D2					

2. Mono vs Duo: Does competition within the bucket limit performance of each genotype?

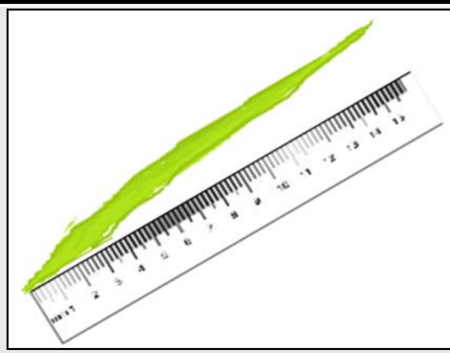
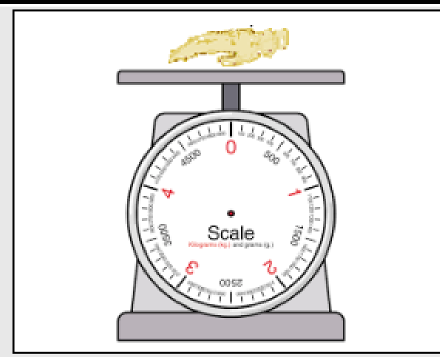
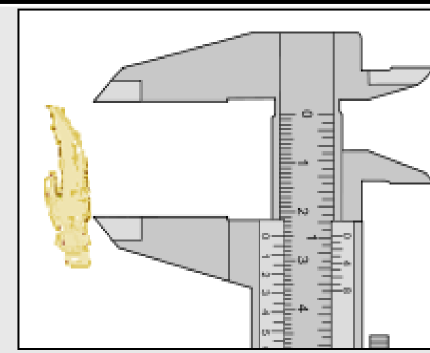
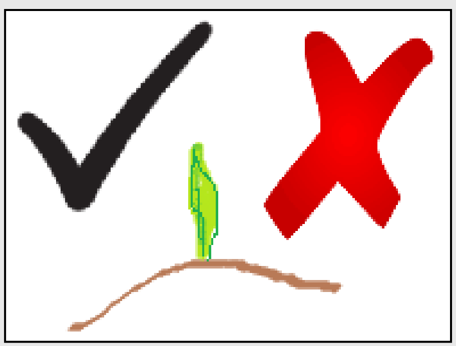
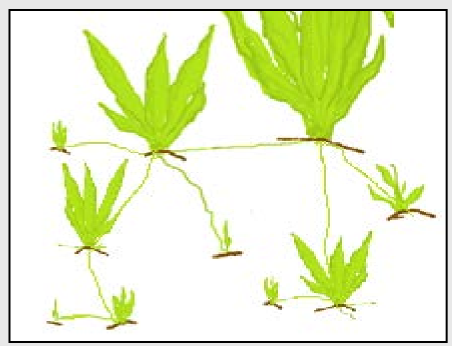
Outcome Variables Significantly Limited by Competition



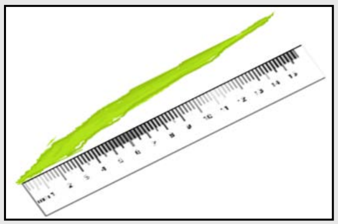
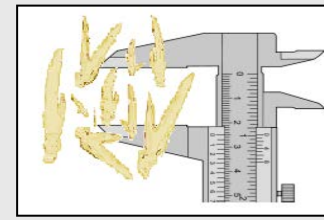
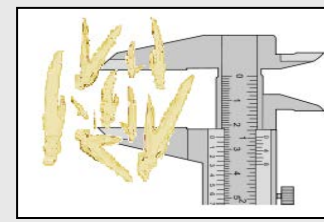
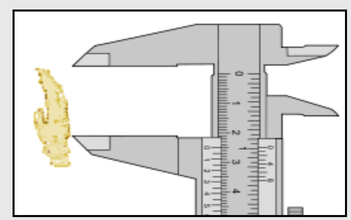
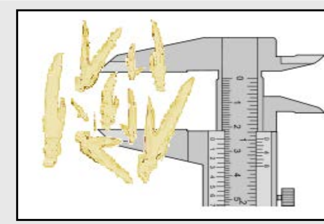
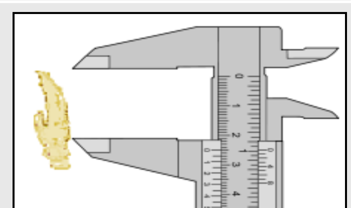
Outcome Variables NOT Limited by Competition

3. D1 v D2: How does performance of the two dominant genotypes compare?

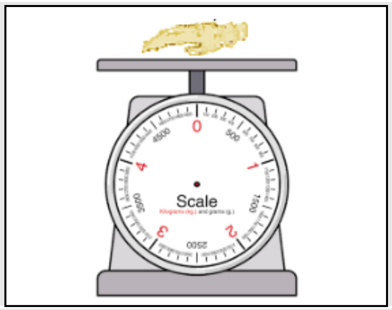
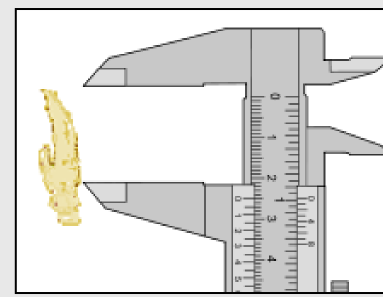
There are significant differences between D1 and D2, and each has its own strengths:

Outcome Variables with Significant Dominant Genotype Differences			
D1			
D2			

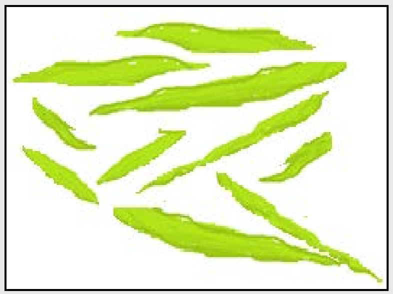
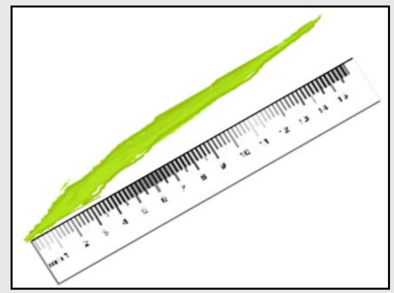
4. Dominant v Rare: Do dominant genotypes perform better than rare genotypes?

Outcome Variables with Significant Dominant vs Rare Differences				
D>R				
D1>R9				
D2>R5				
R10>D1				

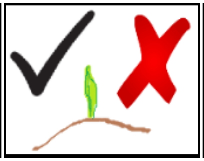
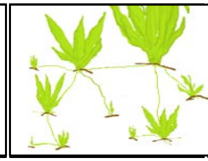
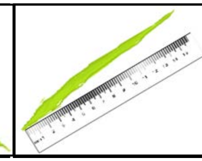
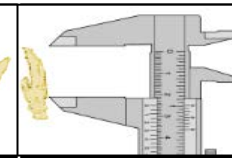
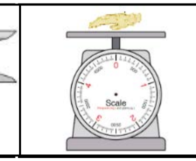
5. How do Competition buckets compare in overall performance to Dominant duocultures and Rare duocultures?

Outcome Variables with Significant SelfComp vs DRComp Differences			
D1D1 >			
D1D2 >			
D2D2			

5. How do Competition buckets compare in overall performance to Dominant duocultures and Rare duocultures?

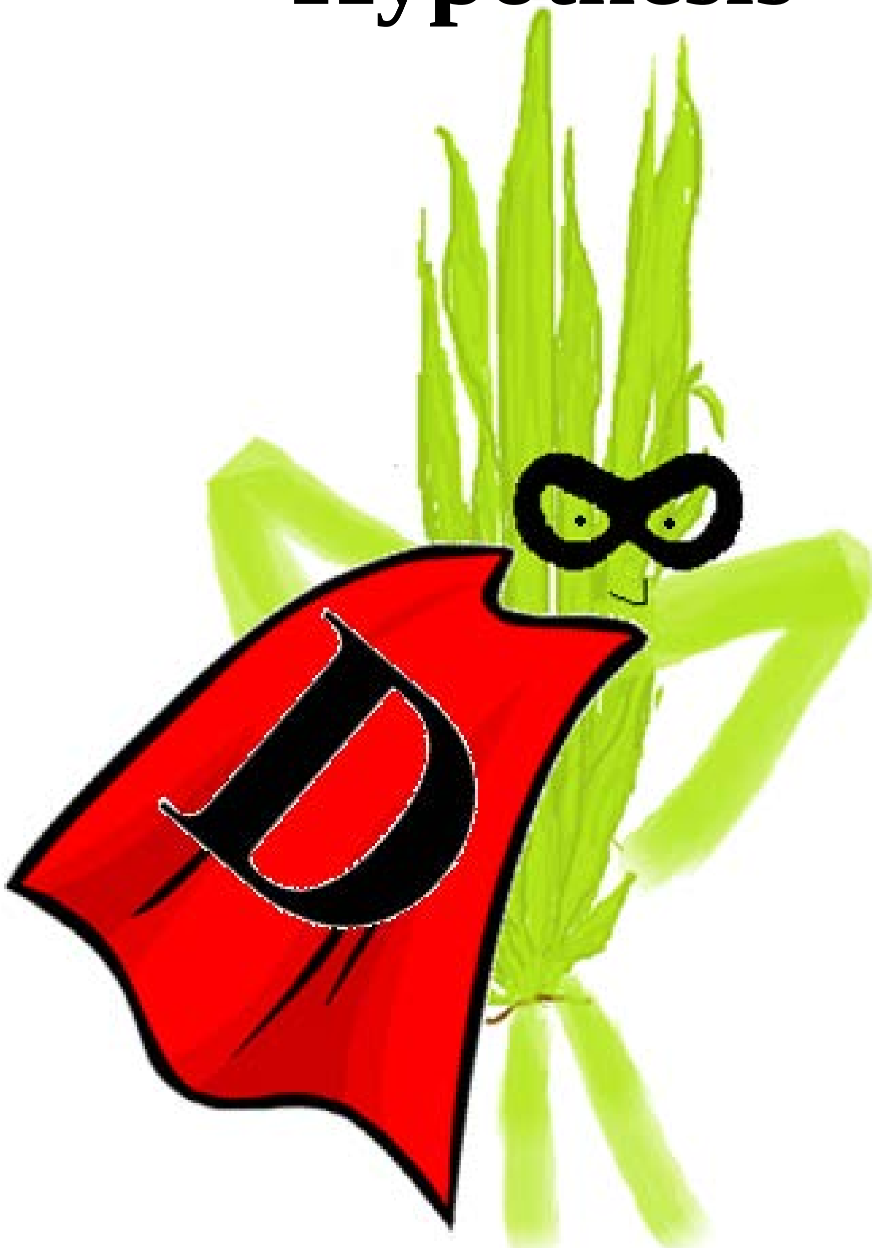
Outcome Variables with Significant Dominant vs Rare Differences			
DomDuo			
DRComp			
RareDuo			

Summary of Findings

								
Dom SiteDifs		D2	D1 & D2	D1 & D2		D1 & D2		D1
Comp Effect			Yes	Yes	Yes	Yes	Yes	
D1 vs D2	D2		D2		D1	D2	D1	D1
All D vs R				D				
Pairwise D vs R						D1>R9 D2>R5	D2>R5 R10>D1	
Dduos vs DRComp						D1D2> D2D2	D1D2> D2D2	D1D1> D1D2
Dduo vs DRComp				D2D2> D2R5	D1D1> D1R9			

Conclusions

Hypothesis



Result

Conclusions

- Important differences exist between and within dominant genotypes.
- D1 and D2 employ different resource allocation.
- D1 was found in more Potomac River samples than D2. However, from the results of this experiment we would expect D2 to dominate over D1. D1 allocates resources to leaf length and size/weight of turions. However, this investment in turions did not translate to higher germination success. D2 dominated in all of the most important categories: germination success, ramet production, and turion production.
- The impact of site of origin on performance is surprising, especially after 2 years of greenhouse propagation. This makes restoration recommendations even more difficult.
- Based on this limited experiment, D1 and D2 do no appear to be "superperformers". Few significant differences were detected between dominant and rare genotypes. However, sample size of rare trials may have impacted the results.
- This experiment was conducted in ideal water conditions and results may not reflect what is happening in the Chesapeake Bay.

Ongoing Questions

- How does genetic diversity relate to resilience in *V. americana* Chesapeake Bay populations?
- What were the important bottleneck events that affected the Chesapeake Bay, and when did they occur?
- Are D1 and D2 older than other genotypes?
- How related is each Chesapeake Bay genotype to D1 and D2? Possible development of additional primers?
- How does the condition of Chesapeake Bay waters impact the performance of these genotypes? (in situ vs. laboratory experiments needed)
- What restoration recommendations can be made regarding which genotypes should be used in plantings?

Interns & Acknowledgements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Sources

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Results: D1Site Differences

	s5 (n=4)	s0 (n=6)	s1 (n=4)	**s7 (n=7)**	s4 (n=5)	s3 (n=6)	**s9 (n=4)**	s2 (n=5)	s6 (n=4)	s8 (n=7)	avg	**Kruskal-W**
POSITION ON MAP	3	8	9	**9**	10	12	**15**	15	16	17		
Ramets (#)	15.75	20.83333	19.75	**25.42857**	19.4	21.83333	16.25	17	15.5	16.42857	18.81738	χ^2 = 25.4 **p = 0.002**
Turions (#)	29.25	37	32	**56.28571**	38.2	41	30.25	36.4	34.75	34.71429	36.985	χ^2 = 20.4 **p = 0.015**
Ave Turion Area (mm^2)	37.245	31.11167	32.0125	31.83714	31.598	31.98167	**37.9225**	31.358	28.6575	30.40571	32.41297	χ^2 = 11.4 p = 0.248
Tot Turion Area (mm^2)	1101.848	1149.62	1010.148	**1787.169**	1244.142	1311.272	1126.87	1142.774	1020.247	1088.776	1198.287	χ^2 = 16.4 p = 0.057
Ave Turion Mass (g)	0.0684	0.06056667	0.06855	0.0553	0.0545	0.05701667	**0.085775**	0.05976	0.045225	0.05552857	0.061062	χ^2 = 19.1 **p =0.023**
Tot Turion Mass (g)	2.02	2.245	2.19	**3.092857**	2.15	2.356667	2.54	2.206	1.6225	1.954286	2.237731	χ^2 = 10.7 p = 0.291
Num Leaves (#)	40.75	54	34.75	60.42857	43.8	**67**	45.5	45.6	35.5	41.28571	46.86143	χ^2 = 25.3 **p = 0.002**
Leaf Area (cm^2)	138.375	149.0833	128.75	136.1429	134.4	149.25	**183**	140.4	125.25	141.6429	142.6294	χ^2 = 6.89 p = 0.648
Germ Speed (weeks)	**2**	**2**	3	**2**	**2**	**2**	2.5	**2**	2.5	2.285714	2.228571	χ^2 = 13.1 p = 0.157
Germination (see other slide)												Yates Co χ^2=2.311 p=0.9855
Biomass	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a		n/a

Results: D2Site Differences

	s1 (n=5)	s7 (n=4)	s5 (n=6)	s4 (n=6)	s0 (n=6)	s8 (n=4)	s3 (n=4)	s6 (n=4)	s2 (n=4)	Avg	**Kruskal-Wallis χ^2**
POSITION ON MAP	8	9	10	11	**13**	15	**15**	**16**	17		
Ramets (#)	19	21.75	29.5	29.5	**32.83333**	21.5	22.25	20.5	20.5	24.14815	χ^2 = 15.4246, df = 8 **p = 0.0514**
Turions (#)	33.5	47.25	58.83333	**63.5**	59.66667	27.75	41	38	54	47.05556	χ^2 = 15.6366, df = 8 **p = 0.04789**
Ave Turion Area (mm²)	29.61	24.56	23.625	24.14	22.81	25.92	26.3225	**29.685**	26.1975	25.87444	χ^2 = 7.2963, df = 8, p = 0.505
Tot Turion Area (mm²)	1121.84	1139.4275	1412.72	**1539.8483**	1354.265	759.3225	1073.555	1115.535	1430	1216.279	χ^2 = 8.3902, df = 8, p = 0.3963
Ave Turion Mass (g)	0.0457	0.040225	0.04178333	0.04188333	0.03798333	0.044125	0.04195	**0.051275**	0.0475	0.043603	χ^2 = 7.1704, df = 8, p = 0.5184
Tot Turion Mass (g)	1.515	1.9225	2.568333	2.696667	2.248333	1.27	1.68	1.92	**2.575**	2.043981	χ^2 = 11.3659, df = 8 p = 0.1818
Num Leaves (#)	30.5	40.5	68.83333	72.83333	**74.66667**	50.25	39.75	53	40.25	52.28704	χ^2 = 17.7752, df = 8 **p = 0.02298**
Leaf Area (cm²)	127.75	157.625	152.25	121.1667	121.8333	126.75	130.25	**195.125**	177.625	145.5972	χ^2 = 14.0527, df = 8 p = 0.0804
Germ Speed (weeks)	4	**2**	**2**	**2**	**2**	3	**2**	**2**	**2**	2.333333	χ^2 = 17.9211, df = 8 **p = 0.02183**
Germination (see other slide)											Yates Corrected χ^2=8.068, df=8, p=0.42685604
Biomass	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a		n/a

Sample Selection

Rares	Site	Sample
R5	EF (H1)	1H03
R9	SL (E1)	1E10
R10	SL (E1)	1E03

D1	Site	Sample
D1	MF (D2)	2D05, 2D06, 2D10
D1s1	WSP2 (E2)	2E09
D1s2	POR2 (G1)	1G24
D1s3	AC (F1)	1F06
D1s4	OJ (D1)	1D06
D1s5	FMC1 (B2)	2B20
D1s6	WF2 (H2)	2H24
D1s7	WSP2 (E2)	2E20
D1s8	EF (H1)	1H08
D1s9	POR2 (G1)	1G01

D2	Site	Sample
D2	BWK2 (F2)	2F01, 2F02, 2F16, 2F19
D2s1	MF (D2)	2D22
D2s2	EF (H1)	1H15
D2s3	POR2 (G1)	1G28
D2s4	SL (E1)	1E06
D2s5	OJ (D1)	1D03
D2s6	WF2 (H2)	2H04
D2s7	WSP2 (E2)	2E25
D2s8	POR2 (G1)	1G22