

Innovative Control of *Phragmites* and Other Invasive Species: Species Specific Gene Silencing

Edward M. Golenberg Wayne State University Detroit, MI





Targeting *Phragmites* Success As Invasive Species



What is Gene Silencing?



Double stranded RNA (dsRNA) can block the expression of a gene by triggering methylation of the gene, by degrading the mRNA of the gene, or by blocking the translation of the mRNA of the gene

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Goal: Establish Gene Silencing for Control of Invasive Phragmites

- Develop robust mode for generation of dsRNA in *Phragmites*
- Develop efficient means of application to *Phragmites*
- Target genes involved in:
 - photosynthesis (biomass production)
 - flower development (sexual reproduction)
 - root development (asexual reproduction)
- Demonstrate reliable and effective gene silencing in *Phragmites*
- Test for reduction in competitive dominance of *Phragmites*

Develop robust mode for generation of dsRNA in *Phragmites*

- Viral Induced Gene Silencing-VIGS
- Hairpin generating vectors
- Artificial microRNA- amiR

VIGS- Viral Induced Gene Silencing

pWSRi is a Beet curly top virus (BCTV) based vector system (Golenberg et al 2009)





Examples of Gene Silencing Sterilizing a Flower



Wildtype Spinach Female Flower



Sterile Silenced Female Flower

Sather, Jovanovic, Golenberg 2010

Examples of Gene Silencing Changing Flower Gender



Wildtype Spinach Male Flower

Feminized Spinach Male Flower

Examples of Gene Silencing Changing Flower Gender



Wildtype Spinach Female Flower

Masculinized Female Flower

Examples of Gene Silencing Changing Flower Gender



Wildtype Spinach Male Flower

Hermaphroditized Spinach Male Flower

pWSRiMSV



pWSRiMSV:ZmPDS Agrobacterium infected



Hairpin Based Silencing Vectors



Himmelbach A et al. Plant Physiol. 2007;145:1192-1200



pIPKb007:ZmPDS Agrobacterium Infected



Silenced Photosynthetic abilities in model grass species

amIR



What's the advantage of switching to Artificial micro RNA (amiR)?

- Natural Micro RNA
 - Express genes in normal genomes
 - Used to regulate gene expression in development
 - Utilizes silencing machinery in the cell
- Artificial Micro RNA
 - Uses very small sequences for silencing
 - Gives the ability to design targets that are very specific
 - Can be species specific
 - Maybe even at the genotype level
 - (i.e. native vs. invasive *Phragmites*)

pEARLEYGATE100:ZmPDSmiR-1



How do we transfer from model species to *Phragmites*?



Building Phragmites Data Base

- Identify genetic code for particular traits in *Phragmites*
 - Phragmites transcriptome sequenced
 - Generated 4 RNAseq libraries: Inflorescence, Leaf, Root, Ramet meristem
 - Pair-end sequences for each library
 - Sequences each direction per library: Inflorescence and leaf 35 million each, Root and meristem 25 million each
 - Target traits Identified

Phragmites Transcriptome

Tissue	Transcriptome Length	Proportion of total
Inflorescence	32826103	0.262
Leaf	146724	0.001
Root	151693	0.001
Shoot tip	22670484	0.181
Shared	69294171	0.554

Why *Phragmites* Transcriptome is Important

- Once gene sequences are identified, specific traits can be targeted for silencing
- Differential expression patterns among strains of *Phragmites* (native vs. invasive) may identify specific targets
- Generates broad database for scientific community

Why is Gene Silencing Important?

- The Holy Grail- This will show first real proof of gene silencing as a viable management/control option
- If silencing vector is effective in mixed plant compositions, widespread application in the field could be an option
- If invasive *Phragmites* is affected and native is not, that makes widespread application more ecologically viable

Caveats and Concerns

- Penetrance and expressivity of miRs in *Phragmites*
- Persistence of silencing temporally
- Modes of applications
 - Mechanistically how to scale up
 - Environmentally are there unintended effects
- Development of management plans that includes guidelines for applications and reintroduction of native species

This could be BIG

If gene silencing is effective in a field setting and proves to be species specific, it will represent a <u>huge breakthrough</u> in invasive species management











