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

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Targeting *Phragmites* Success As Invasive Species

- *Phragmites* displays multiple life history parameters:
  - High seed output and small seed size
  - Rapid growth and upright structure: competition for sunlight and resources
  - Extensive vegetative reproduction: clones and rhizomes
Managers: Where (Who) are We?
Developing a New Arsenal
Gene Silencing

• What is gene silencing?
  – PTGS- Post Transcriptional Gene Silencing
  – RNAi- RNA interference

• Triggered by **double stranded RNA**
  – siRNA
  – miRNA

• Natural and common mechanisms in organisms
  – Viral defense
  – Gene regulation (development)
siRNA and Viral Defense

- Double Stranded Viral RNA
- Dicer-like Digestion
- Incorporation into RISC
- siRNA
- Activated RISC Binds and Degrades mRNA

Virus

Viral RNA

Viral RNA
1 A protein called exportin-5 transports a hairpin primary microRNA (pri-miRNA) out of the nucleus.

2.5 Meanwhile, one of the strands joins a group of proteins, forming an microRNA-protein complex. The other strand, known as a passenger strand is usually discarded. How this all happens is still not very well understood.

2 An enzyme called dicer (not shown) trims the pri-miRNA and removes the hairpin loop, leaving a double stranded microRNA duplex molecule.

3 In plant cells, the microRNA is usually perfectly complementary to its target mRNA molecule. The microRNA will bond with it, and cause the mRNA to break down.

4 In animal cells, the microRNA nucleotides typically don't pair up with the mRNA nucleotides as well. Their base pairing often follows a pattern though.

5 The microRNA-protein complex's presence blocks translation as well as speeding up deadenylation (breakdown of the Poly-A tail), which causes the mRNA to be degraded sooner and translated less.
What is Gene Silencing?

Plant processes, such as photosynthesis, are controlled by genetic material within the plant cells.

Healthy Phragmites plants are tall and robust, with abundant seeds, an aggressive root system, and exhibit high density growth.

Gene (DNA) → Transcription → mRNA → Translation → Protein → Phenotype
What is Gene Silencing?

**Gene Silencing** inhibits these intracellular processes resulting in muted trait expression.

Plants infected with the ‘gene silencing’ vector could have stunted growth, a yellow color, or no flowers (depending on which gene is silenced).

Gene (DNA) → Transcription → mRNA → Translation → Protein

Phenotype
Goal: Establish Gene Silencing for Control of Invasive Species

• Develop robust mode for the *transient* 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: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:ZmPDSAMP-1
amiR Trials in Sunflower

pEARLEYGATE100:HaKO1A1miR2
GS Trials in Phragmites
<table>
<thead>
<tr>
<th>Species</th>
<th>Gene</th>
<th>System/Function</th>
<th>Name</th>
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<tbody>
<tr>
<td><em>Helianthus annua</em> (Sunflower)</td>
<td><em>rbcS</em></td>
<td>Photosynthesis</td>
<td>HarbcSmR1</td>
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<td><em>Helianthus annua</em> (Sunflower)</td>
<td>ent-kaurenoic acid oxidase (KA01 gene), dwarf2 mutant</td>
<td>Gibberellin Production</td>
<td>HaKOAmiR1</td>
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<td>magnesium chelatase subunit 1 precursor (ChlII), oil yellow</td>
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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**

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<tr>
<th>Tissue</th>
<th>Transcriptome Length</th>
<th>Proportion of total</th>
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<td>Leaf</td>
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<td>Shoot tip</td>
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<td>Shared</td>
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Phragmites Transcriptome
Transcript Distribution by Tissue

- **Flower**
  - mt % of total
  - cp % of total
  - other % of total

- **Root**
  - mt % of total
  - cp % of total
  - other % of total

- **Meristem**
  - mt % of total
  - cp % of total
  - other % of total

- **Leaf**
  - mt % of total
  - cp % of total
  - other % of total
Transcriptome Analysis

*Phragmites* Differential Expression
## Transcriptome Analysis

**Phragmites** Differential Expression

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</table>

- Pattern 1 - All the same
- Pattern 4 - (FL)(MR)
- Pattern 5 - L(FMR)
- Pattern 8 - F(LMR)
- Pattern 14 - (F)(L)(MR)
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 huge breakthrough in invasive species management.
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