



WAYNE STATE  
UNIVERSITY



# Innovative *Phragmites*- Control Strategies

**Dr. Kurt P. Kowalski (USGS – GLSC)**

**Dr. Russell J. Rodriguez (USGS –WFRC)**

**Dr. Edward M. Golenberg (WSU)**

# Investigators



**USGS Project Lead:** Kurt P. Kowalski, 734/214-9308, [kkowalski@usgs.gov](mailto:kkowalski@usgs.gov)

**Lead Scientist:** D. Carl Freeman, 313/577-2793,  
[cfreeman@sun.science.wayne.edu](mailto:cfreeman@sun.science.wayne.edu)

**Investigators:** D. Carl Freeman<sup>1</sup>, Regina S. Redman<sup>2,4</sup>, Russell J. Rodriguez<sup>2,3,4</sup>, Edmond Van Hees<sup>5</sup>, Edward M. Golenberg<sup>1</sup>, Douglas A. Wilcox<sup>6</sup>, and Kurt P. Kowalski<sup>7</sup>

<sup>1</sup>Dept. of Biological Sciences, Wayne State University, Detroit, MI 48202

<sup>2</sup>Dept. of Biology, University of Washington, Seattle, WA 98195

<sup>3</sup>USGS-Western Fisheries Research Center, Seattle, WA 98115

<sup>4</sup>College of Forest Resources, University of Washington, Seattle, WA 98195

<sup>5</sup>Dept. of Geology, Wayne State University, Detroit, MI 48202

<sup>6</sup>Dept. of Environmental Science and Biology, SUNY-Brockport, Brockport, NY 14420

<sup>7</sup>USGS-Great Lakes Science Center, Ann Arbor, MI 48105

# *Phragmites australis* (Cav.) Trin. ex Steud (Common Reed)

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- Fast growing clonal grass
  - Wide leaves (1-5 cm) and large inflorescence
- Grows up to 6m high, often in high densities
- In North America for over 3000 years
- Invasive and exotic haplotype M is causing most of the problems





# Current Management Techniques

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# Innovative *Phragmites*-control strategies

## Two-pronged approach:

- Determine role of microbial community in competitive advantage during invasions
- Apply gene silencing technology to modify plant characteristics

### Partner driven:

U.S. Geological Survey  
Wayne State University  
University of Washington  
SUNY – Brockport

### Support:

GLRI  
USFWS  
Ducks Unlimited  
TNC – Michigan  
SEMCOG  
New York Dept. Conservation  
Healing Our Waters Coalition





# Endophytes

Microscopic Fungi ↘

Microscopic Fungi →



Microscopic Fungi ↗

## BENEFITS

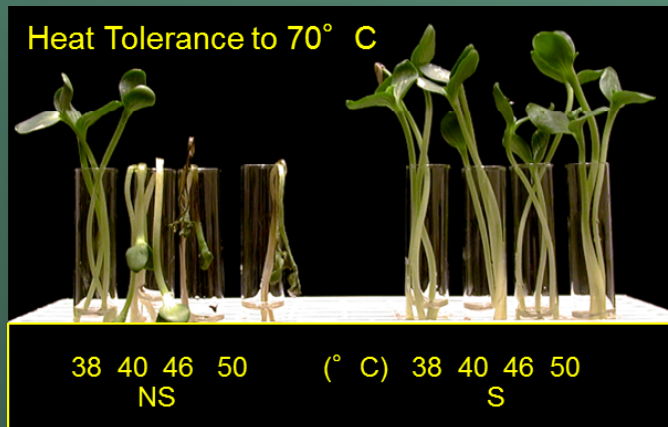
Tolerance

- Drought
- Temperature
- Salt

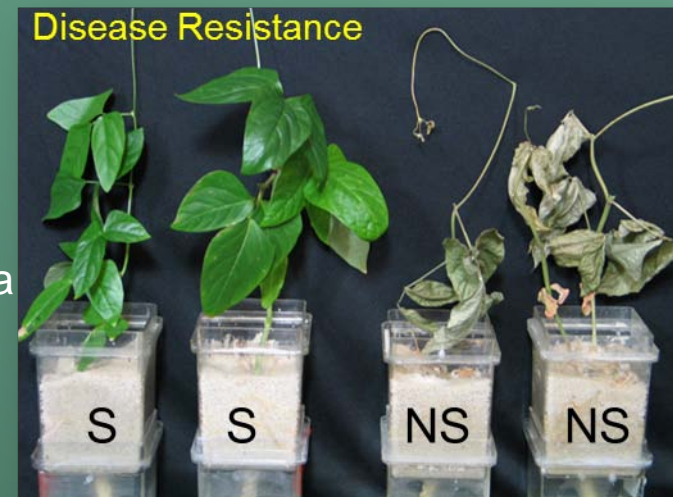
Accelerated Development of Seedlings

Increased Growth and Yield

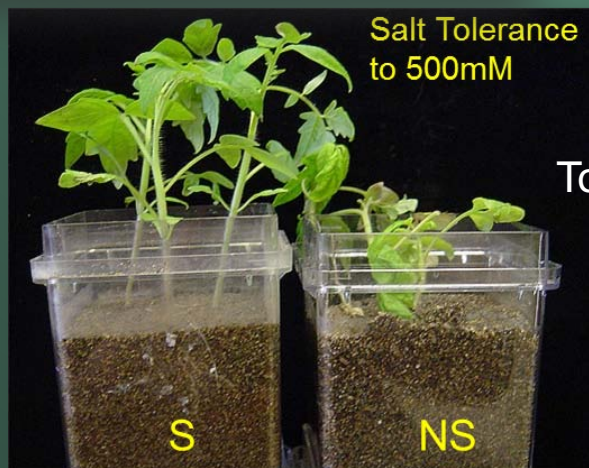
# Stress Tolerance Conferred by Fungal Endophytes



Watermelon



Cow Pea



Tomato



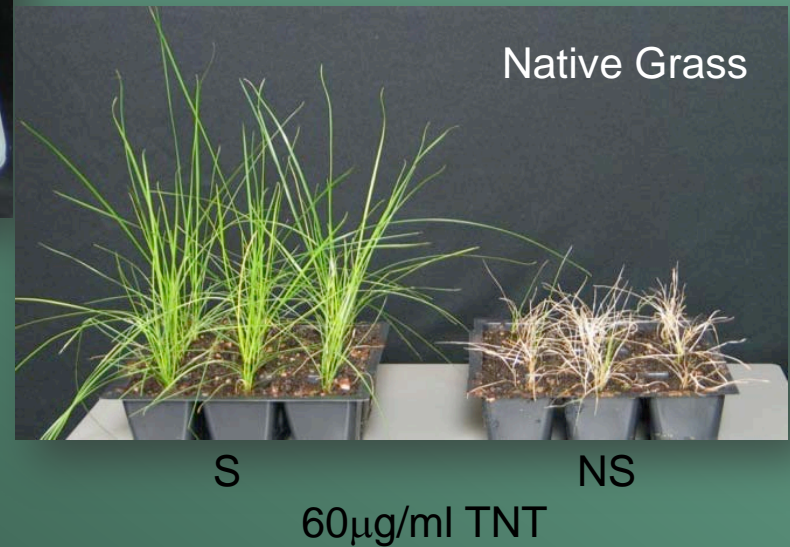


# Chemical Tolerance Conferred by Fungal Endophytes

## Heavy Metal Tolerance

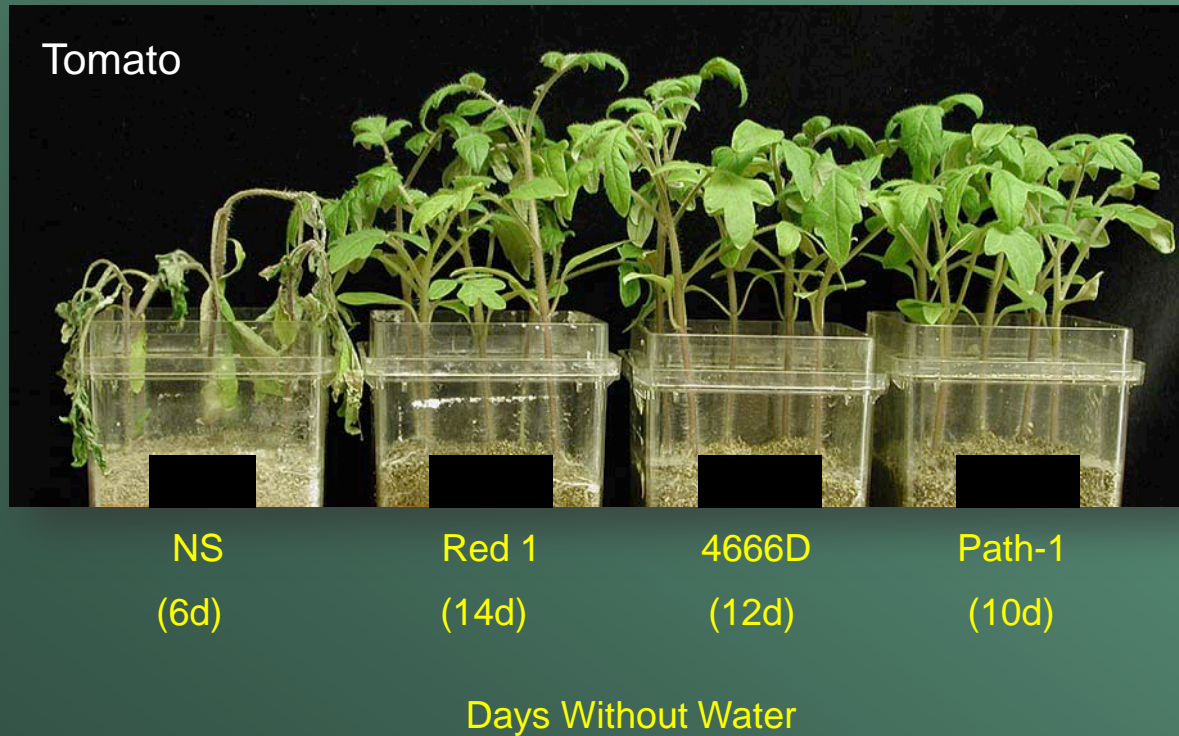


## TNT Tolerance



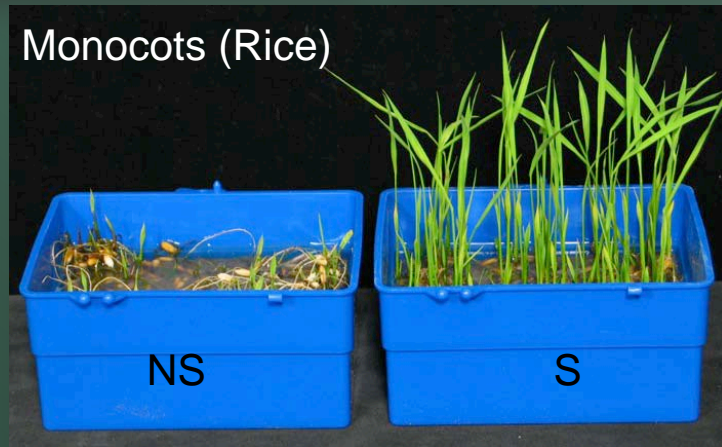


# Drought Tolerance Observed in Monocots and Dicots



0      12      15      18  
Days w/o Water

# Endophytes Regulate Plant Growth and Development: Nutritional Stress Tolerance





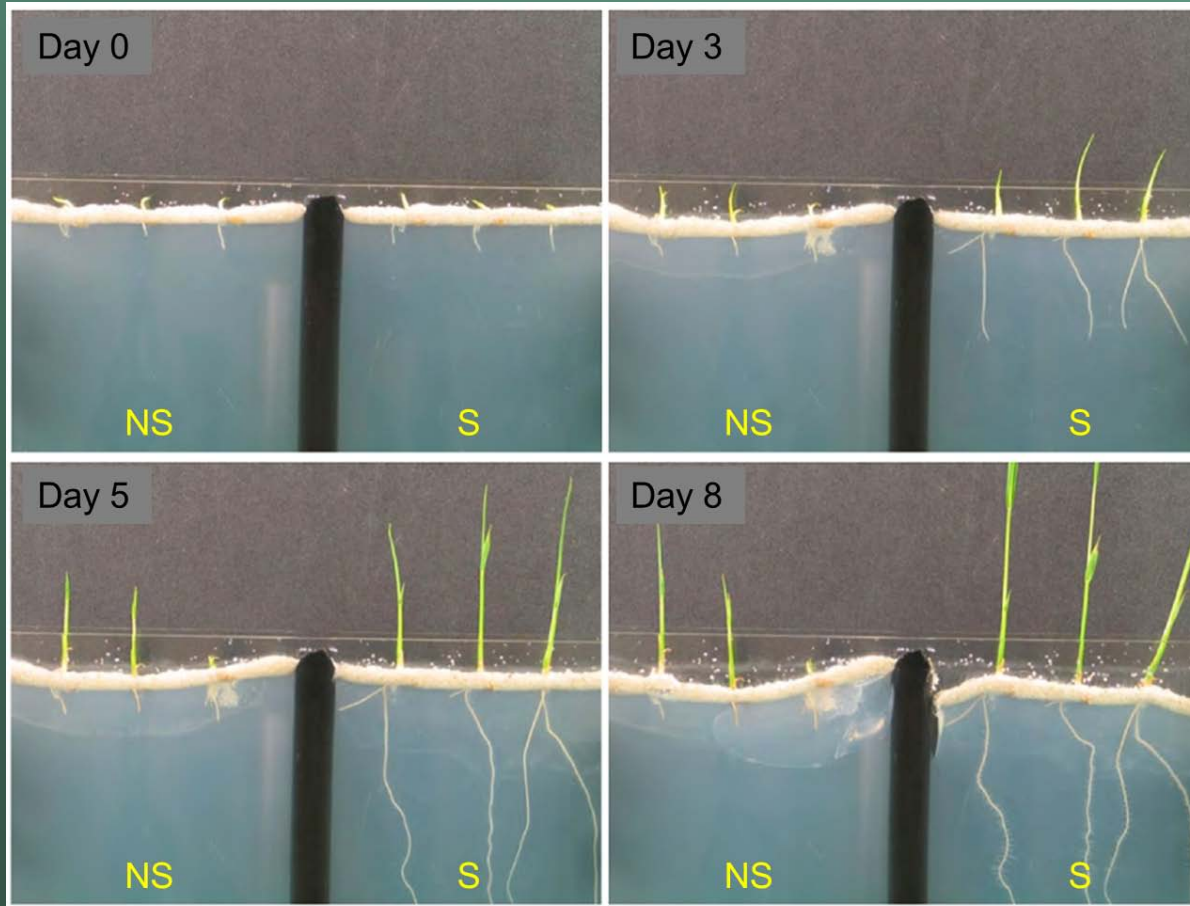
# Influence of Endophytes on Seedling Development

Rice



# Influence of Endophytes on Seedling Development

Rice



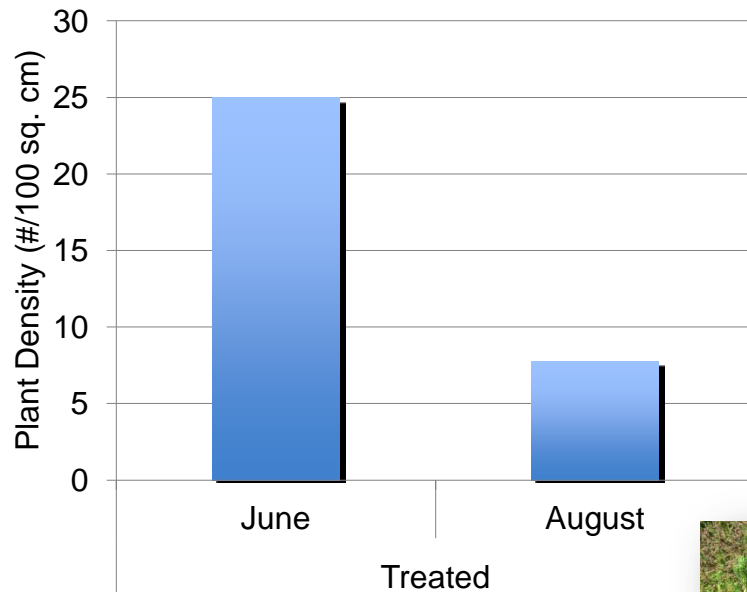


# Invasive Plants Analyzed for Fungal Endophytes

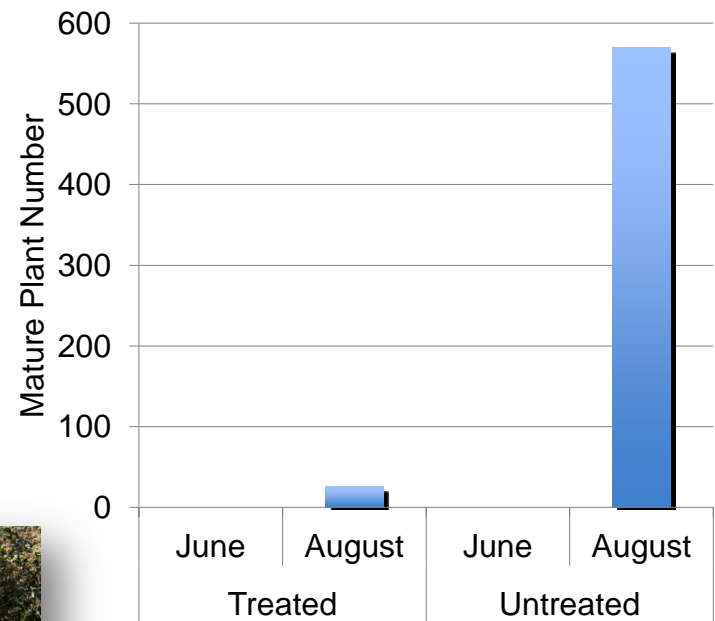
Invasive Plant	Genus-Species	Location	Role of Endophytes
Phragmites	<i>P. australis</i>	Great Lakes, WA	?
Spartina	<i>Spartina anglica</i>	WA	Salt tolerance
Halogeten	<i>H. glomeratus</i>	UT	Drought tolerance
Kochia	<i>Kochia scoparia</i>	WA	?
Diffuse knapweed	<i>Centaurea diffusa</i>	WA	?
Russian thistle	<i>Salsola kali</i>	UT, WA	?
Cheat grass	<i>Bromus tectorum</i>	UT, WA	?
Bulbous blue grass	<i>Poa bulbosa</i>	WA	?

# Preliminary Studies with Kochia Reveal the Importance of Endophytes for this Invasive Plant

Effect of Endophyte Eradication on Kochia Seedlings



Effect of Endophyte Eradication on Mature Kochia Plants





# Next Steps for Endophyte Research

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## 2011

- Test endophyte-eradication strategy on several invasive plants including *Phragmites*
- Test the effectiveness of different chemicals for eradicating endophytes
- Optimize chemical spray strategy for long-term management

## 2012 and beyond (no current funding)

- Expand strategy to include other invasive plants
  - Demonstrate the utility of this strategy in different areas
  - Identify potential non-target impacts
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# Gene Silencing

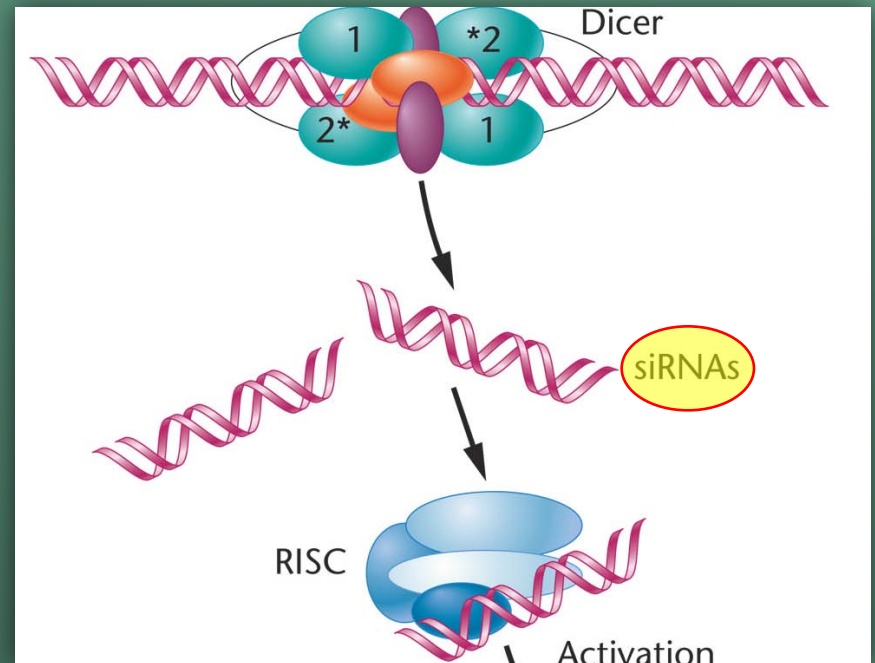
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# Gene Silencing

## Gene Silencing by RNA Interference (RNAi)

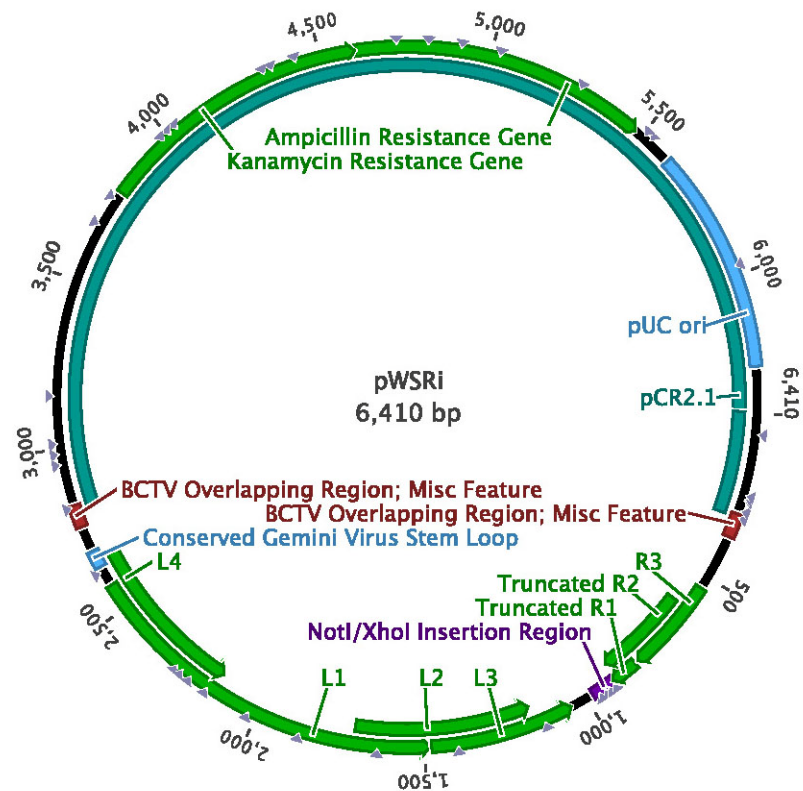
- Gene silencing mechanisms are used by plants and animals in normal anti-viral and developmental processes
- Double stranded RNA triggers the production of short interfering RNAs (siRNAs)
- siRNAs can repress gene expression in many ways





# Plant Gene Silencing Vector Developed at WSU

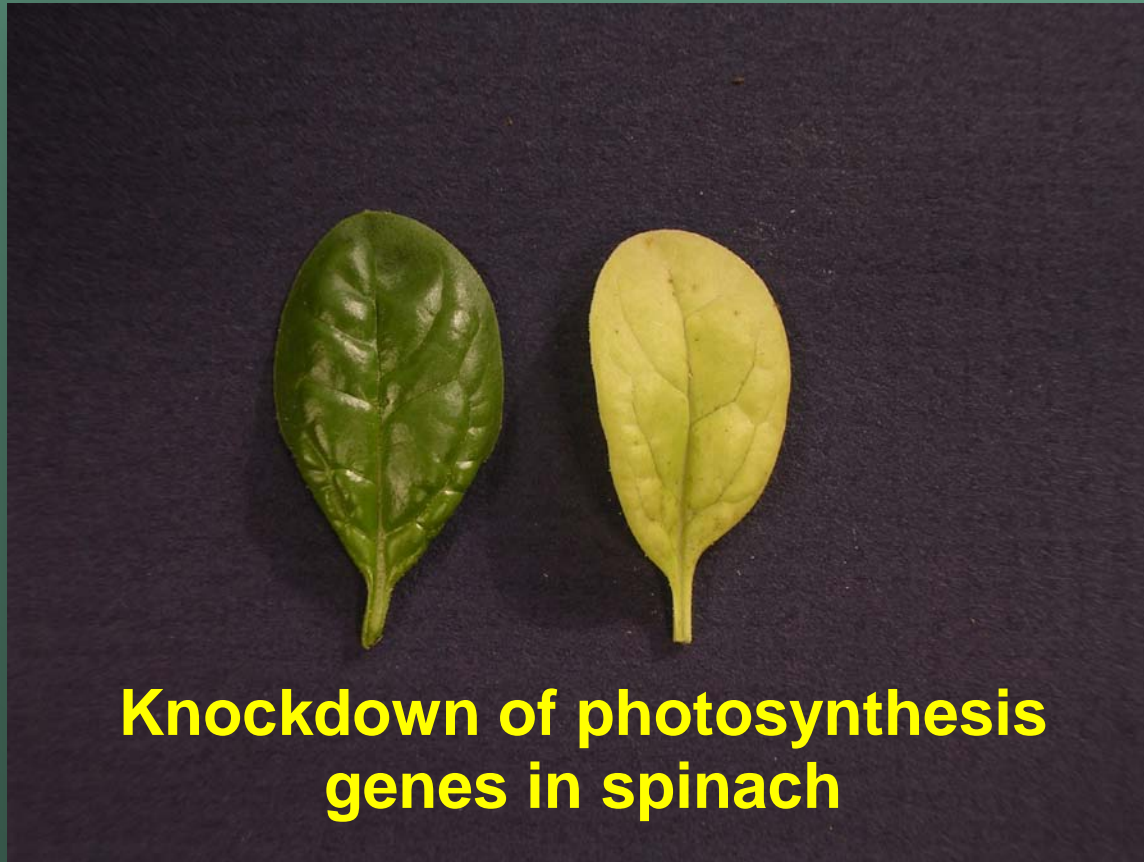
- **pWSRi**: plasmid Wayne State RNA interference
- Targeted gene attached to vector
- Vector introduced to plant cells -> siRNAs
- Result: Progressive yet transient silence
  - *Not incorporated into genome*



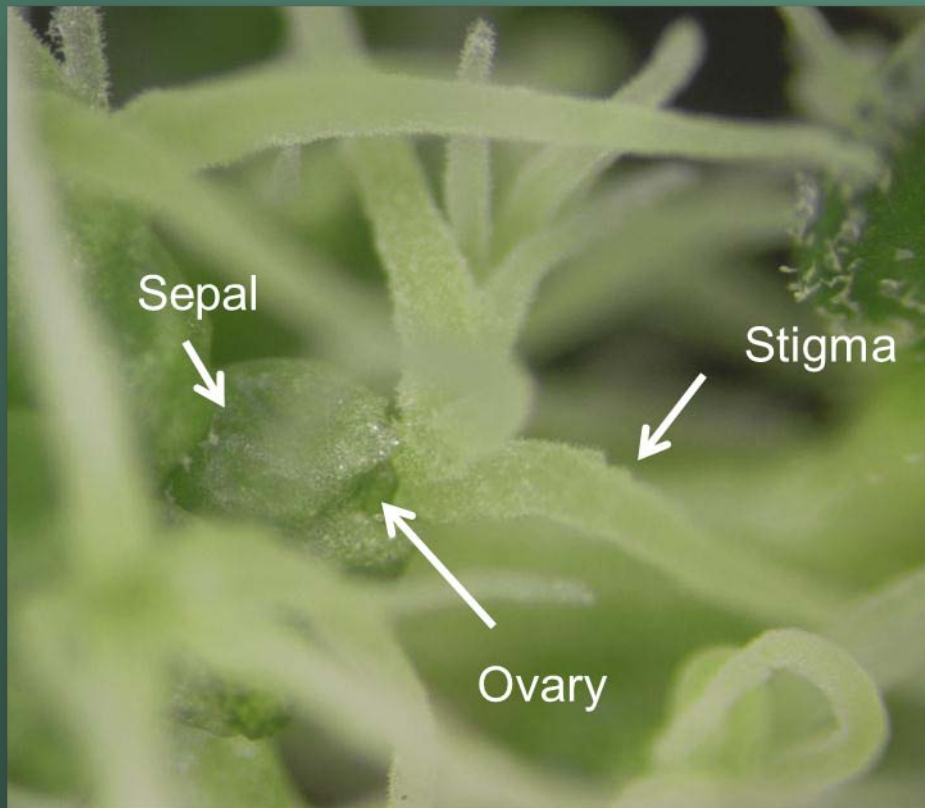
Golenberg et al. 2009. Plant Methods 5,9

# Gene Silencing Can Reduce Productivity

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# Gene Silencing Can Disrupt Flower Development



**Wildtype Female Flower**



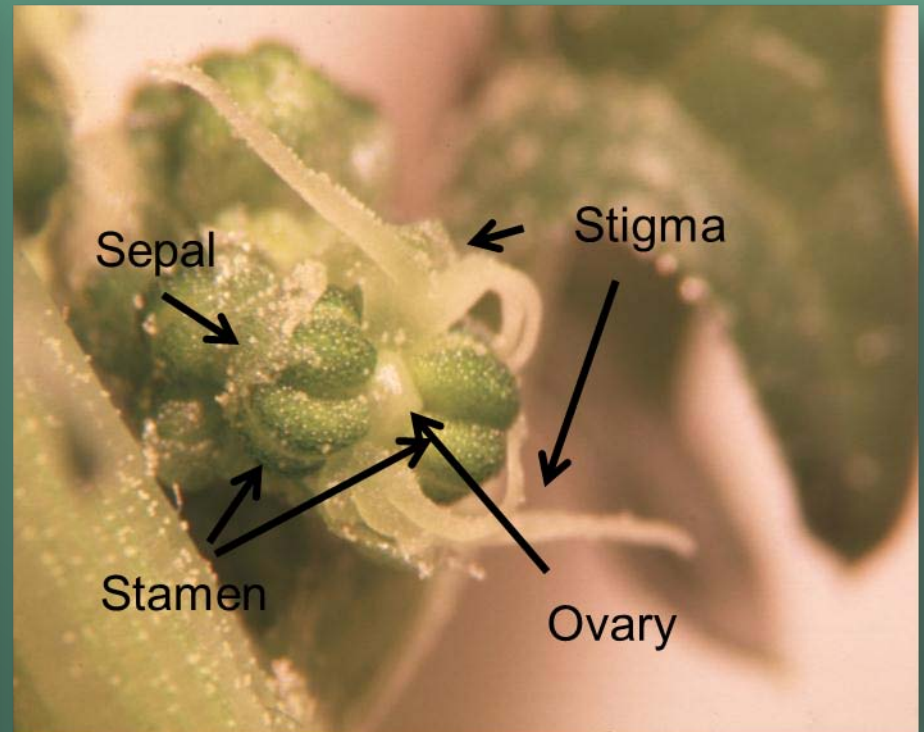
***SpAG* Silenced Female Flower**



# Gene Silencing Can Alter Organ Identity



**Wildtype Male Flower**



***SpPI* Silenced Male Flower**

# Gene Silencing and Habitat Restoration

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Invasive species achieve dominance by outcompeting native species through increased:

- productivity
- seed and sexual reproductive output
- vegetative reproduction

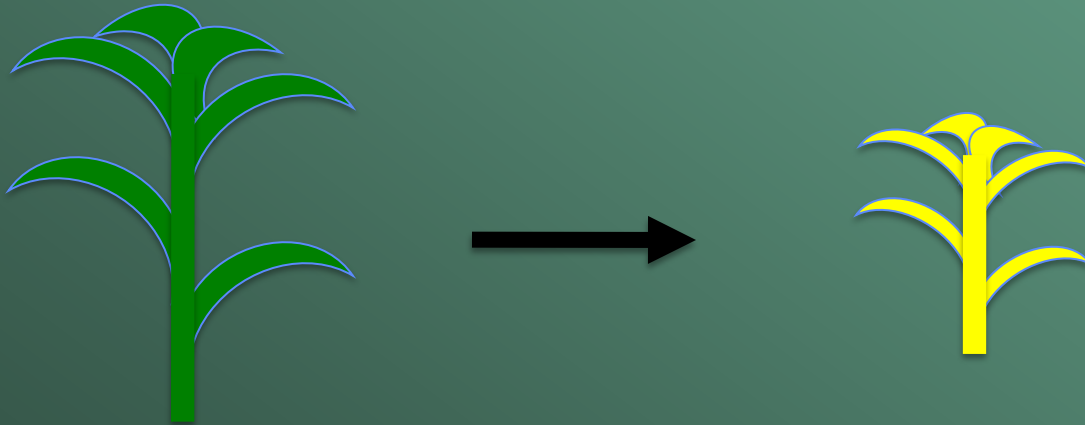


# Gene Silencing and Habitat Restoration

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Reduce competitiveness by:

- Reducing photosynthetic output by silencing photosynthesis machinery



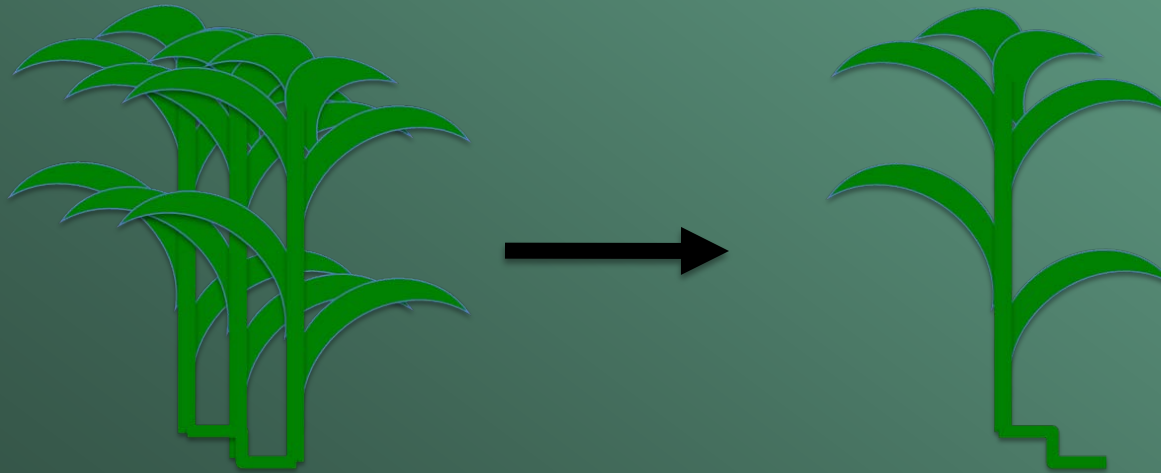


# Gene Silencing and Habitat Restoration

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Reduce competitiveness by:

- Reducing biomass by suppressing vegetative reproduction

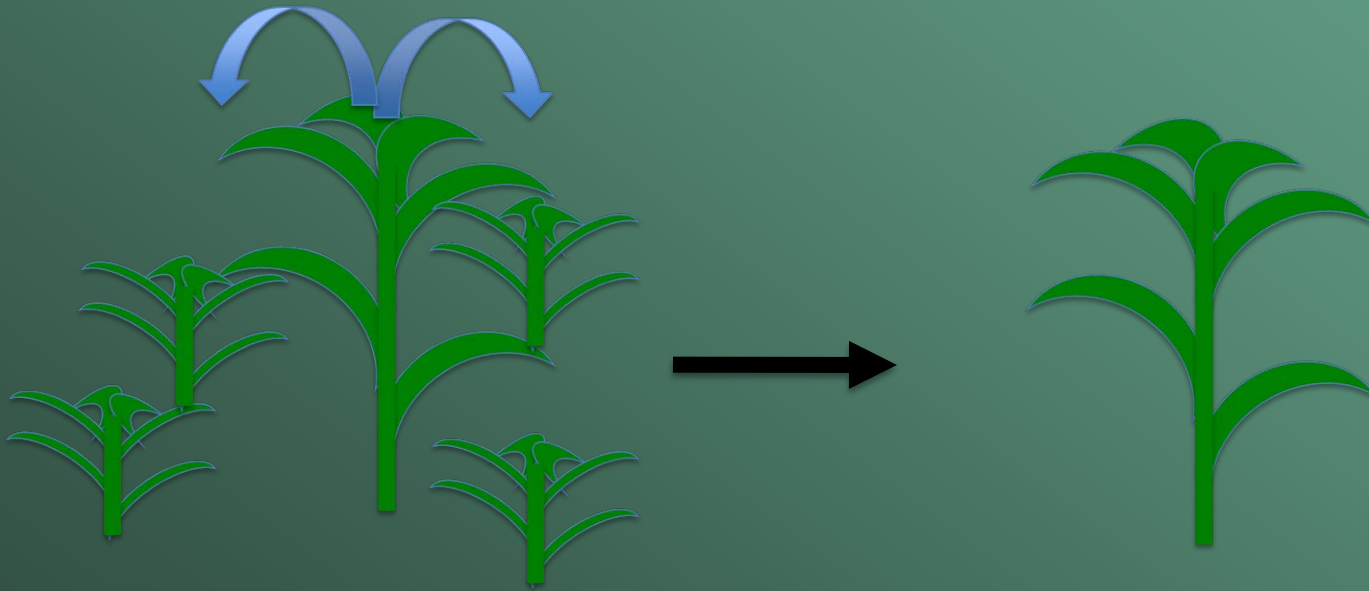


# Gene Silencing and Habitat Restoration

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Reduce competitiveness by:

- Reducing flower production and seed set



# Next Steps for Gene Silencing Research

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## 2011

- Identify additional floral or root developmental genes in *Phragmites*
- Test the ability of *pWSRi* to replicate in *Phragmites* leaf disks
- Test RNAi knockdown function of *pWSRi:PharbcS* constructs *in planta*

## 2012 and beyond (no current funding)

- Test and engineer RNAi knockdown effects for species specificity
- Conduct controlled competition experiments between RNAi treated *Phragmites* and native plants (e.g., *Typha*)
- Develop application technologies for field trials



# Innovative *Phragmites*-control strategies





