Innovative *Phragmites* Control Strategies for the Great Lakes Restoration Initiative

This project is funded by the Great Lakes Restoration Initiative (GLRI) an interagency program that addresses the most significant environmental problems in the Great Lakes ecosystem. Results from U.S. Geological Survey (USGS) scientific studies and monitoring are helping guide the restoration effort. The GLRI is made up of five focus areas that address these issues:

- Cleaning up toxic substances and areas of concern
- Combating invasive species
- Promoting nearshore health by protecting watersheds from polluted runoff
- Restoring and protecting habitats and wildlife
- Tracking progress and working strategically with partners

More information is available on the USGS GLRI Web page (*http://cida.usgs.gov/glri/*).

Project Background

Invasive species can disrupt natural ecosystems, be costly to manage, and consequently threaten native species. *Phragmites australis* (common reed), a tall wetland grass, has been a component of North American wetlands for many years. However, a strain from Europe, introduced in the early 19th century, is invading wetland habitats and aggressively displacing native vegetation. Invasive *Phragmites* alters the soil, produces copious seeds, and forms very dense stands that allow it to outcompete native plants for resources. As *Phragmites* invades an area, plant diversity often declines and critical habitat for fish and other wildlife is altered.

Native and invasive *Phragmites* (*GLPC)

Widely used management strategies include the use of herbicide, burning, and flooding to manage *Phragmites* and promote the growth of native plants. These methods are resource intensive and difficult to sustain indefinitely. Research into biological control (e.g., insect predation) techniques is ongoing, but we are investigating other innovative management tools to provide managers with a new, sustainable suite of control options.

We know that all plants form associations with microbes, some of which offer the plant a competitive advantage (e.g., increased growth rate). If associations between invasive *Phragmites* and its fungal microbes (i.e., endophytes) can be disrupted, its competitive advantage may be lost.

A second innovative control method lies in the potential to silence (i.e., turn off) genes that support the competitive abilities of *Phragmites* (e.g., seed set, photosynthesis). Technology exists to silence similar genes in spinach, tobacco, and tomato (see example at right). If key genes in *Phragmites* are successfully identified and silenced, the invasive properties of *Phragmites* could be minimized, and it could grow similarly to native species.

Normal spinach leaf (left) compared to leaf with a silenced photosynthesis gene (Ed Golenberg, Wayne State University)

This study represents a two-pronged approach by scientists from USGS, Wayne State University, and other agencies to explore new strategies to reduce invasive properties of *Phragmites* and minimize its competitive advantage.

Project Objectives and Approach

Phragmites seeds (*GLPC)

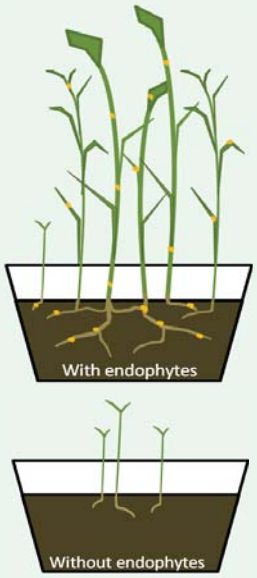
Objective 1: Microbial control

Define the role of symbiosis between *Phragmites* and its endophytic fungi and explore opportunities to disrupt those symbiotic relationships or enhance mutualistic symbiosis in native plant species.

Approach

We will investigate the relationships between *Phragmites*, endophytes, and chemical agents by:

- isolating and identifying fungal endophytes from lower plant sections (roots, crown and stem tissue) and seeds of *Phragmities*
  - The role of fungal endophytes in *Phragmites* plants will be examined by growing plants with and without these isolated endophytes to compare characteristics (*e.g.*, growth).
- treating *Phragmites* with fungicides and metals ions in an attempt to interfere with the relationship between the plants and their endophytic community



Example of plants grown with and without endophytes

Laboratory and field experiments will characterize sensitivity of *Phragmites* and native plants to commercial fungicide and other fungal inhibitors.

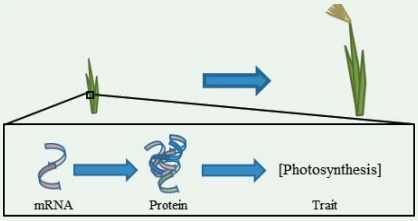
Stand of invasive *Phragmites* (*GLPC)

GLRI 67-2013

Objective 2: Gene silencing

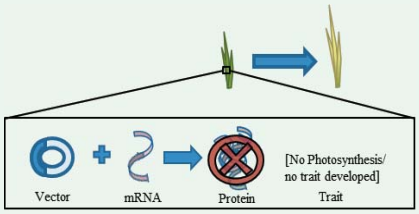
Explore opportunities to develop gene silencing technology that limits the competitive advantage *Phragmites* has over native species.

Simple diagram comparing normal plant growth and gene silencing



Normal plant growth:

Plant processes such as photosynthesis are controlled by genetic material within the plant cells. Specifically, genetic material (mRNA) codes for proteins that lead to particular plant traits. Healthy *Phragmites* plants are tall and robust, with abundant seeds, an aggressive root system, and exhibit high density growth.



Gene silencing:

The 'gene silencing' vector (containing RNAi) enters the plant. Infecting the plant with the RNAi vector disrupts protein synthesis and inhibits development of the targeted trait. Plants infected with the 'gene silencing' vector could have stunted growth, a yellow color, or no flowers (depending on which gene is silenced).

Approach

We are working to identify genes that, upon silencing, would impact:

- seed production
- root growth
- energy acquisition

Upon identification, we can create RNAi sequences that, when injected into the plant, silence the critical functions noted above.

Results of these experiments will be used to guide future proposals focused on implementing successful *Phragmites* control techniques and evaluating collateral impacts on native species.

Outcomes

This project is expected to:

- identify and characterize endophytes found in *Phragmites*
- characterize how *Phragmites* grows in the absence of endophytes
- test methods for controlling endophytes
- identify floral or root developmental genes in *Phragmites*
- describe the utility of several genetic vectors on *Phragmites*
- evaluate the effectiveness of gene silencing on flowering and root growth in laboratory experiments
- produce professional presentations and information products for managers and the public
- guide future proposals focused on implementation strategies for using successful techniques to control the invasive *Phragmites*

Phragmites dominating a coastal wetland (USGS)

This project addresses mission, vision, and multiple priorities identified in the USGS Strategic Plan (http://pubs.usgs.gov/circ/2007/1309/).

*GLPC photo credits: Great Lakes *Phragmites* Collaborative (http://greatlakesphragmites.net/)

Recent Highlights

The USGS and partners have made exceptional progress on a two-pronged approach to find more sustainable invasive species control strategies for landowners and resource managers since the start of this project.

Endophytes:

Scientists have identified and isolated endophytic fungi in invasive *Phragmites*, something not previously documented, and produced preliminary results suggesting that eliminating these endophytes can decrease the number of new *Phragmites* stems growing compared to control plots.

In 2013, a more comprehensive field study will be completed that is designed to provide proof of concept that controlling endophytes can decrease the growth of *Phragmites* in the field. Also, a Collaborative for Microbial Symbiosis and *Phragmites* Management was formed in 2013. This Collaborative brings together leading microbial ecologists to work collectively toward a broader *Phragmites*-management solution based on microbial symbiosis.

Gene Silencing:

The project team continues to make progress toward the application of this technology as a form of *Phragmites* management. Recent accomplishments include:

- the transcriptome (i.e., genetic roadmap) specific to *Phragmites* traits (e.g., photosynthesis, flowers) being analyzed to allow more targeted experimentation
- the completion of several 'proof of concept' studies, including silencing the photosynthetic ability of plants similar to *Phragmites* (see example at right)

Normal maize plant (left) and a plant with silenced photosynthetic gene (right)

These results and the overall direction of this project directly support the broader Integrated Pest Management (IPM) approach being developed for *Phragmites* and many other invasive plants. There are many opportunities to work with the existing partners to build on this work, integrate with IPM efforts, and target high priority sites.

Invasive *Phragmites* overtaking the shoreline (*GLPC)

Great Lakes RESTORATION

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