

**The Friends of Nachusa Grasslands
2020 Scientific Research Project Grant Report
Due June 30, 2021**

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2020 grant amount:

\$9000 awarded in 2019.

Research Project Topic: Identification of site-specific mycorrhizal fungi associates of the federally threatened Eastern Prairie Fringed Orchid (*Platanthera leucophaea*)

Research Project Purpose:

Platanthera leucophaea or the Eastern Prairie Fringed Orchid (EPFO) was once a common and widespread species in wet prairies, sedge meadows, bogs, and fens throughout the upper Midwestern United States. Conservation efforts aimed at its recovery have included habitat restoration, hand pollinations to promote genetic outcrossing/increased seed set, and sowing seeds into suitable habitats. Despite all of these efforts' seedling establishment leading to self-sustaining populations in situ has yet to be verified. Recent research suggests that all orchid conservation efforts should focus on understanding orchid fungal interactions. Orchids are critically dependent on fungi for seedling establishment and growth. The purpose of our research is two-fold. We want to examine fungal diversity across populations in EPFO in Illinois and Wisconsin using molecular markers (ITS and CETH primers) for identification. How fungal identities compare among populations of EPFO is unknown. This information will then allow us to perform seed germination experiments and potentially develop protocols to move seedlings into new or existing populations.

Research Project Outcomes to date:

In May of 2020, I delivered 9 EPFO seedlings to Nachusa Grasslands. Elizabeth Bach out-planted them because of COVID restrictions. Elizabeth continues to monitor the seedlings and updates me on their progress. In the interim, I have sequenced EPFO fungal associates representing the range in Illinois and a few populations from Wisconsin. I will continue examining fungal sequence diversity across the range of EPFO, including more populations from Wisconsin, Ohio and Michigan. Diversity across the range of the species has not been examined.

Describe how the grant funds you have received from the Friends of Nachusa Grasslands have been used in regard to the above topic, purpose, and/or outcomes:

The grant funds from the Friends of Nachusa Grasslands were essential in allowing me to travel to the four sites in Illinois and seven sites in Wisconsin to collect mycorrhizae from EPFO roots. The sampling from these sites provided the material used in the seed germination experiments to try and ascertain what strains of EPFO can be released between sites. Understanding fungal associates will be critical for conservation of EPFO. Your funding has also allowed me to sequence the fungal isolates, providing information on range-wide identity and similarity.

Describe how your project has benefited the work and goals of Nachusa Grasslands:

The Nachusa Grasslands organization is committed to reconnecting remnant prairie, woodlands, and wetlands through habitat restoration to create one of the largest and most biologically diverse grasslands in Illinois. This project specifically supports the Grasslands work in habitat restoration. Because all orchids are dependent on their fungal associates for seed germination the process of conservation starts with identifying the mycorrhizae the species uses. If EPFO uses the same mycorrhizae range-wide this information could open the door to other recovery projects.

Describe how your findings can be applied to challenges in management practices for restoration effectiveness and species of concern:

No one has yet been successful in growing this species from seed and having them survive when out-planted. Growing plants in a lab and then successfully out-planting them would be a significant step forward in the reintroduction and recovery of this species. When sowing EPFO seed in suitable habitat, if the seed is going to "take" it takes from 4 to 9 years for a blooming plant to appear. In an effort to cut down on this time, we are hoping to identify ways to inoculate seed with the necessary mycorrhizae before sowing in the field. If the fungi are similar across the range, seeds/seedlings can be exchanged over larger areas. Moving seed/ seedlings will improve levels of genetic diversity and possibly increase compatible mating types resulting in more viable seed production in populations.

Please list presentations/posters you have given on your research:

I presented a summary of fungal sequence diversity and methods for growing and out-planting EPFO seeds at the USFW EPFO Researchers meeting on March 20, 2020. I also presented a summary of EPFO mycorrhizal associate diversity at the Friends of Nachusa grasslands meeting in June, 2021.

Have you submitted manuscripts to scientific journals? If so, which ones? If not, do you anticipate doing so? (Please send digital copies of published articles to the Friends so that we can learn from your work.)

I need to finish collecting and sequencing fungi across the range of EPFO before the work is published.

What follow-up research work related to this project do you anticipate (if any)?

If the seedlings of EPFO survive out-planting it will be the first time they have reported to have done so. This makes this study significant for the conservation of native orchids. The seedlings will need to be checked periodically to monitor survival.