# The Kitty Todd Initiative

A pilot project to increase the population of the Endangered Terrestrial Orchid, Platanthera ciliaris – the orange fringed orchid.



#### A special needs plant:

The Kitty Todd Nature Preserve is the Nature Conservancy's 850 acre complex of open oak savanna and wet prairie lands located across the northern most portions of Ohio. Predominately porous acid sands, perched on top of a clay layer, the area historically has been an unforgiving environment for agriculture. Thus saved from the plow, the nutritionally poor lands remain mostly open wet prairie ecosystems with oak trees dotting the acreage. These acid sands are home to a wide population of rare and endangered plants, suited to these acidic conditions. Historically some twenty different populations of Platanthera ciliaris, commonly known as the orange fringed orchid were known across northern Ohio. Over the last 100 years of documentation, these twenty sites have now been reduced to one last known remaining colony. This initiative is an effort to help save the orange fringed orchid, native to these acids sands. With population numbers declining over recent years, a restoration plan using tissue culture techniques was started in 2011.

### The backstory:

Micropropagation techniques have evolved over the past decades and are now known collectively as "tissue culture". One of these approaches, in vitro asymbiotic seed germination is of particular interest to orchid propagators. The method has been previously demonstrated as highly effective in commercial tropical orchid propagation, and it is now widely practiced in native terrestrial orchid propagation. "*In vitro*" from the Latin definition – *in glass* – and "asymbiotic" referring to germination of the orchid seeds without the presence of a symbiotic fungus define this process. In nature, the orchid seeds must germinate symbiotically with a naturally occurring soil born fungus. In the asymbiotic approach, the fungus is replaced by a carefully controlled combination of chemicals and laboratory protocols.

The popularized term "cloning" is sometimes confused with "tissue culture" however distinct differences should be noted, as it is just one of many available techniques. In cloning, exact duplicate copies are generated in the laboratory. In certain applications this may be desirable. However, in asymbiotic germination, namely seed culture, this is not the case. Since the starting point is a seed, either mature or immature, each seed carries the genetic diversity of its combined parents, and thus each seed in the seed capsule is genetically distinct from each other. This is of great importance when one wishes to repopulate existing wild stands, or start new sites when outplanting seedlings into nature.

#### **Building on success:**

Over the last two decades, the asymbiotic seed culture approach has been effectively used with many of our native terrestrial orchids. Many researchers have added to this growing database of laboratory protocols over the years. Recent work within the genus *Cypripedium* is but one of many examples. Through precise manipulation of media, recipes and laboratory protocols, many *Cypripediums* can be reliably germinated in the 80-100% ranges from mature seed protocols. *Cypripedium reginae* was the first native terrestrial orchid to be placed in the "easy to germinate" category.



Cypripedium reginae – Native Lady Slipper Orchids

Others have followed. *Cypripedium acaule*, originally noted as very difficult to germinate, is now as easy and reliable as *Cypripedium reginae*. Using *in-vitro* asymbiotic seed germination protocols, *Cypripedium acaule* germinates in 6 weeks, with protocorms well developed and ready for re-plate transfers in 9 weeks from the initial laboratory sow date. Germination is 90- 100%, with all seeds germinating simultaneously thus providing for a commercially ready laboratory protocol. The subsequent re-plates are fully developed seedlings within 10 months.



Picture: Cypripedium acaule seedlings. Great Lakes Orchids LLC

From successful laboratory protocols, it is merely an issue of scale, to mass produce some of our most treasured native orchids.



Picture: 10,000 Cypripedium acaule seedlings –Great Lakes Orchids LLC.



Picture: Cypripedium acaule – adult

Successful plant-out protocols have been developed and *acaule* seedlings can bloom 3-4 years out of flask. Each specific cypripedium species requires its own unique recipe to obtain 90-100% germination, and, each in turn requires its own unique re-plate recipe to generate large, well developed seedlings. At our laboratory we do not have a generic multipurpose germination recipe; a one size fits all solution, for the cypripediums.

### Platanthera ciliaris:

*Platanthera ciliaris* seeds from native wild populations were secured and brought into the lab. *In-vitro* asymbiotic seed culture protocols have been applied and we have started to refine germination and re-plate media recipes to optimize germination and the subsequent seedlings. As with our previous experiences with *Cypripedium* we are finding that *P. ciliaris* when compared to five other *Platanthera* species that we have worked with all show a unique specificity concerning media formulations. This specificity is seen in the germination recipes and also the re-plate recipes.

### The Kitty Todd Preserve:

At the Kitty Todd Preserve, no adult or juvenile plants were removed or damaged in this study. Only seed pods are required to start the asymbiotic seed cultures in the laboratory. Pictured below, is one of several tagged P. *ciliaris* plants that were hand pollinated at the preserve. Later, these seed capsules were collected, and transported to the laboratory at Great Lakes Orchids LLC.



Picture: P. ciliaris - Kitty Todd Nature Preserve - Acid sand - Non Bog form.



Picture: Kitty Todd Nature Preserve –2011- Platanthera *ciliaris* – developing seed pods.

### Laboratory protocols:

In the photo below, a test tube of germinated seeds have developed to early stage protocorms:



Platanthera ciliaris- germinated protocorms.

These protocorms, grown on an agar slant, are now ready for individual needle transfer under sterile lab conditions to re-plate containers. These early stage protocorms transfers are preferable, because the individual corms will quickly knit together forming a dense, unusable matt of rhizoidal root hairs if left in the germination tube.

The replate vessel contains a modified agar recipe formulated to induce bud development while simultaneously encouraging roots to form.

## **Out-planting:**

The soil mix used for acclimating the seedlings from test tube to outdoor growing conditions is a sterilized blend. No soil borne mycorrhizae are used. In this way, we are not introducing non-resident organisms to the Kitty Todd Nature Preserve. The seedlings will naturally be exposed to native, on-site mycorrhizae upon relocation to the preserve. The seedlings and the subsequent adult plants apparently may not require any special soil mycorrhizae to develop into long lived established plants. It should be noted, that the special soil mycorrhizae may only be required for the germination of seeds in the wild. Once the seedlings have developed to the green leaf stage the soil borne mycorrhizae's role is in question. In controlled laboratory experiments, seedlings develop normally into full blooming adults without any mycorrhizae, returning year after year, increasing in size each season.

The long standing acceptance of the mutualistic, symbiotic fungal/orchid relationship is currently being reviewed by scientists. Several researchers now believe that the fungal mycorrhizae may indeed actually be acting more as a tolerated parasite in the adult orchid plants, then as a symbiotic partner.

The book is still open on this topic. We find that many folks in the field, botanists, site managers, professional gardeners, and hobbyists alike still hold to the current symbiotic fungi concepts. There is exciting research taking place around the world highlighting the revised parasitic relationships. Some of this work is from researchers in Australia. It seems that the leading research, and published science journal papers originate there.

The advancement in technical analysis in these areas has been driven by the application of advanced laboratory equipment that is now available to researchers. The sophistication of this new equipment and techniques has lead to breakthroughs at the biochemical, molecular, and genetic levels. These are exciting times for orchid researchers.



Picture: Platanthera ciliaris seedlings – acclimation to outdoor conditions. Early spring. Plastic humidity cap is in place for the first week. Additional shading is used on the tender seedlings. Outdoor acclimation bed. Great Lakes Orchids LLC.



Picture: Developing Platanthera ciliaris seedling – fully acclimated to outdoor conditions and ready for out-planting.

Careful optimizations of recipes and protocols have allowed germination and subsequent seedlings of good size and vigor with P. *ciliaris*. At this point in our experiments we have reached a reliable 90% germination rate. Our present re-plate recipe for *P*. ciliaris is consistently producing large seedlings with root diameters approaching 6 mm.



Platanthera ciliaris: 1<sup>st</sup> year seedling-early August – Great Lakes Orchids, LLC.



Platanthera ciliaris: Shade cloth over protective wire screen opened up for  $1^{st}$  year flower spike.

Platanthera ciliaris seedlings develop quickly upon outplanting. Acclimation protocols to soil conditions are routine and trouble free. Transplants will produce flower brackets and set blooms in the first or second season out of test tube. Current information points to approximately seven year adult life cycle.

The first out-planting of seedlings is scheduled for spring, 2012 at Kitty Todd.

This pilot study is under the direction of:

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Laboratory services for this project have been donated by Great Lakes Orchids, LLC.

You can follow this orchid restoration project at greatlakesorchids.com under tab listed **"One Test Tube at a Time",** special projects link. Great Lakes Orchids, LLC is a specialized tissue culture laboratory and nursery, licensed by the State of Michigan, for propagation and sale of terrestrial orchids. We are involved with several ongoing initiatives involving orchid restoration and invite you to log on, and meet the folks working behind the scenes on this, and other orchid projects at the laboratory.

File: Interim Report - 03-07-12 Great Lakes Orchids, LLC greatlakesorchids@gmail.com