Bridging the Gap Between Horticulture and Ecology: Integrated Conservation of Florida Orchids

Mike Kane
Plant Restoration, Conservation, and Propagation Biotechnology

Our diverse program integrates laboratory, greenhouse and field based research. We utilize the most advanced applications of in vitro plant culture and genetic analysis for the ecotypic selection, production and reintroduction of a range of plants—including aquatic and wetland plants, coastal dune grasses, orchids and other plants.
Members
Plant Restoration, Conservation, and Propagation Biotechnology
Wetland Plant *In Vitro* Propagation & Evaluation

- Plant selection
- Genetic analysis
- Surface sterilization
- Bud excision
- Culture establishment
- Clonal shoot multiplication
- Microcutting rooting
- Plantlet acclimatization
- Genotype early growth evaluation
- Field evaluation

- Culture indexing
- Low temperature germplasm storage

- Genotype early growth evaluation
- Field evaluation
Florida Native Orchids

- ~110+ species
  - 33 Epiphytic
  - 78 Terrestrial
  - ~3 Aquatic
- About 60% at risk, threatened or endangered
Threats to Orchids

- Deforestation
  - Urban development
  - Logging
- Habitat conversion
  - Agriculture
  - Urbanization
  - Road construction
- Drought
  - Hydrology
  - Non-beneficial fires
- Illegal collecting
Protecting Orchids

• Management
  – Identify orchids
  – Land usage
    • Invasive species
    • Prescribed burns

• How to manage
  – Study all aspects
    • Integrate disciplines
Florida Panther National Wildlife Refuge

- Created in 1989
  - Endangered species act
  - Protect panther and habitat
- 26,000+ acres
- Big Cypress Basin
- Habitat mosaic
  - Cypress strands and domes
  - Hardwood hammocks
  - Wet prairies
  - Pine flatwoods
- Number of orchids
  - 30+ species
Established in 2005
The Florida Orchid Restoration Partnership

• Utilize regional research to develop orchid conservation methods with international implications
• Understand the dynamic relationship between orchids and their habitats
• Implement management practices that promote the sustainability of Florida’s native orchids
• Encourage the protection of irreplaceable native orchid habitat throughout Florida and the world
New Orchid Conservation Model
Integrated Conservation Systems

Integrated Conservation – Utilization of multiple research methods to gain an as complete as possible understanding of a species, system, or landscape (Hopper 1997).
Integrated Orchid Conservation Research

Propagation Science

Genetic Diversity

Pollination Biology

Plant Reintroduction

Mycology

Ecology & Distribution

Species Recovery

(after Dixon & Batty 2003)
Research

Pollination Biology

Seed Germination/Propagation

Habitat Characterization

Field Transplant

T. Johnson
Orchid Seed Germination

• Propagation
  – Germination ecology
    • Environmental cues
    • Nutrient comparison
    • Fungal relationship
      – Identify mycobionts
  – Generate propagules for field transplant
Orchid Species Studied

- **Species**
  - *Calopogon tuberosus* (Grass Pink Orchid)
  - *Bletia purpurea* (Pinepink)
  - *Cyrtopodium punctatum* (Cigar Orchid)
  - *Dendrophylax lindenii* (Ghost Orchid)
  - *Dendrophylax porrectus* (Jingle Bell Orchid)
  - *Prosthechea cochleata* (Clamshell Orchid)
  - *Prosthechea boothiana* (Florida Dollar Orchid)
  - *Eulophia alta* (Wild Coco)
  - *Encyclia tampensis* (Florida Butterfly Orchid)
  - *Epidendrum nocturnum* (Night Scented Orchid)
  - *Spiranthes odorata* (Fragrant Ladies’ Tresses)
  - *Spiranthes floridana* (Florida Ladies’ Tresses)
  - *Habenaria macroceratensis* (Long-horned False Rein Orchid)
  - *Habenaria quinqueseta* (Michaux’s Orchid)
  - *Habenaria repens* (Water-spider Orchid)
  - *Sacoila lanceolata* (Leafless Beaked Orchid)
  - *Campylocentrum pachyrrhizum* (Ribbon Orchid)
  - *Pteroglossaspsis ecristata* (Giant Orchid)
Orchid Population Genetics

- *Bletia purpurea* (blue dots)
- *Cyrtopodium punctatum* (red dots)

Florida Panther National Wildlife Refuge

Genetic Analysis
In Vitro Ecology

The evaluation and use of in vitro culture techniques to identify, propagate, evaluate, and select plant genotypes and ecotypes for ecological purposes.
Spiranthes brevilabris
Mycobiont Preference

Developmental Stage
Stage 0 Stage 1 Stage 2 Stage 3 Stage 4 Stage 5

(%) 0 20 40 60 80 100

Sbrev-266
Sflo-305
Sflo-306
Sflo-308
Control

12 weeks

Stage 0
Stage 1
Stage 2
Stage 3
Stage 4
Stage 5

Developmental Stage
Calopogon tuberosus
Grass Pink Orchid

- Terrestrial orchid
  - Eastern North America
  - Corm forming
- Large distribution
  - Habitat differences
Photoperiod Effects

A. Michigan

B. South Carolina 1

C. South Carolina 2

D. South Carolina 3

E. North Central Florida

F. South Florida

Stage 1: Black
Stage 2: Red
Stage 3: Gray
Stage 4: Green
Stage 5: Blue
Stage 6: Orange
Does Growing Season Matter?

Growing Season Length (days between frosts)
- 125
- 210
- 270
- 365

Growing Degree Days (days with temps > 10C)
- 64
- 188
- 259
- 316
What Does Growing Season Influence?

- Biomass allocation
  - Rate
  - Allocation site
Reproductive Biology and Asymbiotic Seed Germination of *Cyrtopodium punctatum*

Dr. Daniela Dutra-Elliott
Species Information

Distribution: Florida, Cuba, Hispaniola, Puerto Rico, and the northwestern Caribbean coast of South America

Plants may take 15 years to flower

Capsules require 12 months to mature

Cyrtopodium punctatum
Species Information

- **Cyrtopodium punctatum** is endangered in Florida due to logging in the 1900’s
- It was extensively collected from the wild
Reproductive Biology

- Knowledge of a rare plant’s reproductive biology is essential for its conservation.

- It is important to know whether a species reproduces sexually and whether it requires the aid of pollinators.

- Sexual reproduction is the primary way organisms maintain genetic variability.
Pollination Syndrome

“A suite of floral traits associated with the attraction and utilization of a specific group of animals as pollinators”

- Flower morphology
- Flower color
- Fragrance
- Reward: nectar, oil
Research Objectives

1) Study the reproductive biology of *C. punctatum*
   a. Determine the breeding system
   b. Document floral visitors
   c. Determine pollination syndrome

2) Develop a propagation protocol for *C. punctatum*
   a. Determine the role of media and photoperiod on asymbiotic seed germination
   b. Investigate the role of photoperiod on seedling growth
   c. Acclimatize seedlings to greenhouse conditions
Reproductive Biology

• Determining the breeding system:
  – Capsule formation
  – Capsule size
  – Seed viability
  – Seed germination

• Determining the pollination syndrome:
  – Fragrance
  – Pollinator type
Breeding System Determination

- Six plants were used during two consecutive growing seasons
- Seven breeding system treatments were applied to five flowers of each plant
- A total of 245 flowers were used per year

<table>
<thead>
<tr>
<th>Condition</th>
<th>Bagging</th>
<th>Treatment</th>
<th>Pollen Source</th>
<th>Objective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Unbagged</td>
<td>Untreated</td>
<td>Open pollination</td>
<td>Evaluate fruit set under natural conditions</td>
</tr>
<tr>
<td>Agamospermy</td>
<td>Bagged</td>
<td>Emasculated</td>
<td>No pollination</td>
<td>Evaluate the rate of non-sexual reproduction</td>
</tr>
<tr>
<td>Spontaneous autogamy</td>
<td>Bagged</td>
<td>Untreated</td>
<td>The same flower</td>
<td>Measure the need for pollinators</td>
</tr>
<tr>
<td>Induced autogamy</td>
<td>Bagged</td>
<td>Emasculated</td>
<td>The same flower</td>
<td>Evaluate self-compatibility</td>
</tr>
<tr>
<td>Artificial geitonogamy</td>
<td>Bagged</td>
<td>Emasculated</td>
<td>Different flower on same plant</td>
<td>Evaluate self-compatibility</td>
</tr>
<tr>
<td>Artificial xenogamy</td>
<td>Bagged</td>
<td>Emasculated</td>
<td>Flower from a distant population</td>
<td>Evaluate outbreeding at long distances</td>
</tr>
<tr>
<td>Induced xenogamy</td>
<td>Bagged</td>
<td>Emasculated</td>
<td>Flower from same population, distant plant</td>
<td>Evaluate outbreeding at short distances</td>
</tr>
</tbody>
</table>
Breeding System Determination

- Pollination treatments applied to flowers
- Plants bagged and monitored until the first signs of capsule formation
- Capsules measured every sixty days for a year
- Capsules collected before dehiscence
Capsule Formation

Results

- Need for a pollinator vector
- Xenogamy
  - Induced: Crossing within the population
  - Artificial: Crossing between populations

![Graph showing % Capsule Formation across different pollination treatments](chart.png)
Tetrazolium Seed Viability Testing

**Methods**

- Seeds were dried over silica desiccant for 70 days at 23 ± 2°C, then maintained in cold storage (-10 ± 2°C)
- Three 5 mg seed subsamples from each capsule
- Seeds were treated for 15 min in 1.0 ml scarification solution (5.0 g of calcium hypochlorite, 1 ml of Tween 20, 99 ml of water)
- Rinsed 3 times in sterile dd water for 45 sec then soaked in sterile water for 24 hrs in dark at 22 ± 2 °C
- Water was replaced with 1% tetrazolium chloride (100 mL dd water and 1.0 g 2,3,5 triphenyl tetrazolium chloride; TTC; pH 7.0)
Tetrazolium Seed Viability Testing
Seed Germination Studies

Seed Sterilization
- 3 minutes in ethanol:NaOCl:sterile dd H2O (5:5:90 v/v)
- 3 rinses in sterile dd H2O

Media
- Orchid Seed Sowing Medium (P723)
- pH 5.7, autoclaved at 121°C, 116.67 kP
- 50 ml medium/plate dispersed into square 100x15 mm Petri plates

Photoperiod
- 16/8 h (L/D)
- Cool white fluorescent lights at 50 µM m⁻²s⁻¹
- 10 week incubation at 23°C ± 2°C
# Seed Viability and Germination

## Results

<table>
<thead>
<tr>
<th>Pollination Treatment</th>
<th>% Seed Viability</th>
<th>Total Germination</th>
<th>Seedling Development Stages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Agamospermy*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spontaneous Autogamy*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Induced autogamy</td>
<td>79.9 ab</td>
<td>2.9 bc</td>
<td>17.9 ab 1.2 b 1.7 a 0.35 a</td>
</tr>
<tr>
<td>Artificial geitonogamy</td>
<td>67.6 b</td>
<td>1.9 c</td>
<td>18.1 a 1.2 b 0.49 b 0.44 a</td>
</tr>
<tr>
<td>Artificial xenogamy</td>
<td>87.2 a</td>
<td>4.3 ab</td>
<td>17.6 b 2.7 a 1.9 a 0.55 a</td>
</tr>
<tr>
<td>Induced xenogamy</td>
<td>79.1 ab</td>
<td>4.7 a</td>
<td>17.5 b 4.0 a 1.1 ab 0.29 a</td>
</tr>
</tbody>
</table>
Pollination Observation

Methods

- Pollinator observations were conducted during the 2007 and 2008 flowering seasons
- Observations took place from 7:00 am to 6:00 pm for two days
- When peak visitation time was identified, observations took place from 10:00 am to 4:00 pm for seven days
- Visitors were captured, photographed, and identified
Visitors

<table>
<thead>
<tr>
<th>Insect Visitors</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Xylocopa micans</em></td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td><em>Xylocopa virginica</em></td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td><em>Apis melifera</em></td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td><em>Megachile xylocopoides</em></td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
Fragrance Analysis

Results

- Compounds identified are commonly found in floral fragrances
- Referred as “floral bouquet”
- Not specific to the Orchidaceae

![Graph showing various compounds and their abundances over time.](image-url)
Summary

• **Breeding System**: Xenogamy, species needs a pollinator

• **Pollination Syndrome**: Deceit pollination

• **Pollinator id**: Bees were not seen removing pollinia from flower, however; it is very likely that carpenter bees pollinate *C. punctatum* in southwest Florida
Study Objectives

1) Study the reproductive biology of *C. punctatum*
   a. Determine the breeding system
   b. Document floral visitors
   c. Determine pollination syndrome

2) Develop a propagation protocol for *C. punctatum*
   a. Determine the role of media and photoperiod on asymbiotic seed germination
   b. Investigate the role of photoperiod on growth and development of seedlings
   c. Acclimatize seedlings to greenhouse conditions
Asymbiotic Seed Germination

Methods

• Media
  – Knudson C
  – ½ Strength Murashige & Skoog
  – Orchid Seed Sowing Medium (P723)
  – Malmgren Modified Terrestrial Orchid Medium
  – Vacin & Went Modified Orchid Medium
  – 8 g L\(^{-1}\) TC agar, 1 g L\(^{-1}\) charcoal, 20 g L\(^{-1}\) sucrose
  – pH 5.7, autoclaved at 121°C and 116.67 kP
  – 50 ml medium/plate were dispersed into square 100x15 mm Petri plates

• Photoperiods
  – 0/24 and 16/8 h (L/D)
  – Cool white fluorescent lights at 50 μM m\(^{-2}\)s\(^{-1}\)
  – 10 week incubation at 23°C ± 2°C
Asymbiotic Seed Germination

• **Experimental Design**
  - 2 x 5 factorial
  - 8 replicates/treatment; 5 subreplicates/plate
  - Data collected every 2 weeks for 10 weeks
  - Seeds scored from Stage 1–5
  - Data were analyzed using general linear model procedures and least square mean separation at $\alpha = 0.05$

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intact testa</td>
</tr>
<tr>
<td>2</td>
<td>Embryo enlarged, testa ruptured (= germination)</td>
</tr>
<tr>
<td>3</td>
<td>Appearance of protomeristem</td>
</tr>
<tr>
<td>4</td>
<td>Emergence of two first leaf primordia</td>
</tr>
<tr>
<td>5</td>
<td>Elongation of shoot and further development</td>
</tr>
</tbody>
</table>
Asymbiotic Seed Germination

Results

![Graph showing percent germination across different media and light/dark cycles.](graph.png)

1. 1/2-MS
2. KC
3. MM
4. P723
5. VW

Light/dark cycles:
- 16/8 h L/D
- 0/24 h L/D

![Images of seed germination under different light/dark cycles.](images.png)
10 Weeks

- Stage 4 protocorms in dark
- Stage 5 protocorms in P723
## Photoperiodic Effects on Seedling Growth

### Results

<table>
<thead>
<tr>
<th></th>
<th>Shoot #</th>
<th>Leaf #</th>
<th>Shoot length (mm)</th>
<th>Leaf Width (mm)</th>
<th>Root #</th>
<th>Root length (mm)</th>
<th>Fresh wt (mg)</th>
<th>Fresh shoot wt (mg)</th>
<th>Fresh root wt (mg)</th>
<th>Dry wt (mg)</th>
<th>Dry shoot wt (mg)</th>
<th>Dry root wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>8/16 h L/D</strong></td>
<td>1.06b</td>
<td>4.51a</td>
<td>61.99a</td>
<td>2.26a</td>
<td>3.47b</td>
<td>67.19b</td>
<td>21.25b</td>
<td>7.29a</td>
<td>13.96b</td>
<td>2.16b</td>
<td>0.73a</td>
<td>1.39b</td>
</tr>
<tr>
<td><strong>12/12 h L/D</strong></td>
<td>1.23a</td>
<td>4.49a</td>
<td>59.31a</td>
<td>2.25a</td>
<td>3.63b</td>
<td>78.86a</td>
<td>22.31b</td>
<td>7.26a</td>
<td>15.06b</td>
<td>2.42b</td>
<td>0.72a</td>
<td>1.52b</td>
</tr>
<tr>
<td><strong>16/8 h L/D</strong></td>
<td>1.28a</td>
<td>4.33a</td>
<td>69.86a</td>
<td>2.15a</td>
<td><strong>4.45a</strong></td>
<td>87.07a</td>
<td><strong>28.98a</strong></td>
<td>9.26a</td>
<td><strong>19.71a</strong></td>
<td><strong>3.31a</strong></td>
<td><strong>0.91a</strong></td>
<td><strong>1.97a</strong></td>
</tr>
</tbody>
</table>

Measurements represent the mean of 90 seedlings/treatment. Numbers with the same letter are not significantly different at $\alpha = 0.05$. 
Greenhouse Acclimatization

- After 35 weeks culture, seedlings were potted in coconut husk in 38-cell plug trays
- Plug trays were covered with clear vinyl humidity domes
- Placed under shade (239 µmol m$^{-2}$s$^{-1}$) in the greenhouse
- 90% survivorship after 5 weeks
Conservation Implications

• Seeds can be germinated in the dark on P723 medium then transferred to light (16/8 h L/D) for further seedling development

• Seedlings successfully acclimatized to greenhouse conditions have been reintroduced
Conservation Implications

- Manual pollination should be conducted within populations to ensure that seeds are being produced for \textit{in situ} recruitment and \textit{ex situ} propagation for future reintroductions.

- Population genetic diversity sampling was completed to elucidate further \textit{in situ} management options.
So What’s New in Our Lab?
Seed Culture of *Dendrophylax lindenii* (Ghost orchid)
Dendrophylax lindenii
Asymbiotic and Symbiotic Seed Culture of *Dendrophylax lindenii* (Ghost orchid).

Asymbiotic/symbiotic seed culture of *Dendrophylax lindenii* (Ghost orchid).
Epiphytic Orchid Substrate Preference & Acclimatization Chamber

Vertical mesh substrate support
Water in bottom to maintain humidity
Acclimatization Chamber (side view)

Vertical mesh substrate support mount
Acclimatization Chamber (top view)

Vertical mesh substrate support with mount (2 per chamber)
Acclimatization Chamber (oblique view)
Epiphytic Orchid Substrate Preference & Acclimatization Studies
Native Orchid Seed Culture in Bioreactors

We are currently evaluating seed germination and uniformity of seedling development of Florida native orchid species directly in liquid culture using modified Belco Bell-Flo Spinner Flask Bioreactors (photo). We hope to develop an efficient method for mycorrhizal fungi inoculation en masse. This research is being conducted by Ben Hughes, doctoral student (right photo).
The Florida Orchid Conservation Conference

The Florida Orchid Conservation Conference was held December 2-3, 2011 at the Naples Botanical Garden, Naples Florida. The purpose of the conference was to share research advances on Florida orchid conservation with governmental officials and concerned citizens.

This was accomplished through presentations, hands-on activities and in situ field experiences. An effort was made to educate participants of the research conducted on Florida's diverse native terrestrial and epiphytic orchids.

However, emphasis was focused on the native orchid restoration and conservation research projects conducted on the Florida Panther National Wildlife Refuge, by the Plant Restoration Conservation and Propagation Biotechnology Program at the University of Florida and the Orchid Recovery Program at Illinois College.

Sharing this information with you

During the “town hall meeting” conducted at the end of the first day, it was suggested to have a medium that would allow the general public and other research facilities to have access to information related to the biology, propagation, and conservation of Florida native orchids.

The conference presentations, the orchid research review, and the current and future directions contained on this website represent the first step in providing governmental officials and the public the most up-to-date information on the orchid conservation areas with.

The researchers at the University of Florida and Illinois College invite you to use the information provided to obtain a wider view of our knowledge of Florida’s orchids. The orchid research review provides definitions and experimental overviews covered in the conference presentations.

- 2011 Conference Agenda (PDF)
- 2011 FOCC Flies (PDF)

http://hort.ifas.ufl.edu/plant-restoration/kane-lab/orchids/
Orchid Conservation Research Consortia

Photos courtesy of Ira Norwitz