

The Quarterly Journal of the Florida Native Plant Society

Palmetto



PROPAGATING & PRESERVING PAWPAWS *(and Other Rare Species) from Florida*



"Many plant species have disappeared from the earth forever, and many more face extinction. Battling this trend is the mission of the Endangered Species Propagation Program at CREW."

– Dr. Valerie C. Pence

ABOUT THE AUTHOR – Dr. Valerie C. Pence is the Head of the Plant Conservation Division at the Center for Conservation and Research of Endangered Wildlife (CREW) at the Cincinnati Zoo and Botanical Garden. Dr. Pence conducts groundbreaking research in the areas of plant propagation and cryopreservation.

She received her B.S. from Mount Holyoke College and her M.S. and Ph.D. degrees from Northwestern University in plant physiology and development.

Dr. Pence has received the Wilson Popenoe Award from the American Society of Horticultural Science and the 1998 Association of Zoological Horticulture Conservation Award, as well as grants from the Institute of Museum and Library Services, the Ohio Department of Natural Resources, the New England Wildflower Society, and the Ohio Orchid Society.

Her interests include adapting and applying the ever increasing number of techniques in plant science to problems facing endangered plant species and developing new techniques where needed.

Asimina tetramera in bloom. Photo by Marjorie Shropshire

To learn more about *Asimina tetramera* and endangered plant conservation, visit:
www.fnai.org/FieldGuide/pdf/Asimina_tetramera.PDF
www.fws.gov/endangered/i/q/saq3q.html
www.boksanctuary.org/conservation/plants
www.cincyzoo.org/Conservation/GlobalConservation/rareplantprogram/rareplant

Even on a cold winter's day in January, visitors to the Cincinnati Zoo & Botanical Garden (CZBG) can enjoy the Florida manatee, the American crocodile, or the four-petal pawpaw. The CZBG's Manatee Springs exhibit provides visitors with a taste of Florida's biodiversity, as well as its conservation challenges. While the Zoo's keepers are actively involved in the Manatee Rescue, Rehabilitation and Release Program, the Plant Research Division of CREW (Center for Conservation and Research of Endangered Wildlife) focuses on propagating and preserving rare plant species, many of which, like the four-petal pawpaw, come from Florida.

The Endangered Plant Propagation Program

The work on Florida endangered plants began in 1996 with the Plant Research Division's Endangered Plant Propagation Program (EPPP). The purpose of the EPPP is to use in vitro, or tissue culture methods for propagating and preserving endangered plants when traditional methods of plant propagation (seeds or cuttings) are not adequate. With funding from a grant from the Institute of Museum and Library Services and collaboration with the Center for Plant Conservation in St. Louis, Missouri, species are nominated as targeted species in the EPPP. One of the first to be targeted was *Asimina tetramera*, the four-petal pawpaw, nominated by Historic Bok Sanctuary (then, Bok Tower Gardens), in Lake Wales, Florida. The only known populations of this plant are found in Palm Beach and Martin Counties, Florida. It is listed as endangered by the State of Florida and by the U.S. government and is identified as globally endangered by the International Union for the Conservation of Nature and Natural Resources (IUCN). As with many rare species, *Asimina tetramera* is threatened by habitat loss, but also suffers from fire suppression and produces few seeds. About 30 of these seeds were sent to CREW to start the project.

The methods applied at CREW are all based on in vitro or tissue culture techniques. These procedures were developed in the middle of the 20th century, first for research and later for propagation of economically important species. Small pieces of plant tissue are surface sterilized and placed in a sterile container such as a petri plate or a test tube, with an artificial, nutrient medium. The sterile environment is important, since bacteria and fungi can kill the tissues. The tissues are washed with a dilute solution of bleach or alcohol before being put onto a medium that supplies minerals, sugar and plant hormones.

These hormones, also known as plant growth regulators, are important because they can stimulate shoot or root growth from plant tissues. A small shoot bud, leaf or stem piece placed in culture can be stimulated to produce multiple shoots. [Fig. 1] These shoots, in turn, can be isolated and moved to fresh medium to repeat the process, indefinitely. To produce plants, shoots are transferred to a medium with hormones that specifically stimulate the formation of roots. Once a shoot has roots, it can be moved out of tissue culture and acclimated to soil conditions.

Producing Roots From Shoots

CREW researchers germinated the *Asimina tetramera* seeds and used seedling shoot tips to initiate shoot cultures. Although tissue culture methods have been applied to many species worldwide, each species is different and may require some modification of standard procedures. Four-petal pawpaw shoot cultures were initiated within a year of receiving the seeds, but for several years the shoots did not produce roots, even though several protocols were tested. Interestingly, horticulturists have reported that root initiation in cuttings of *A. tetramera* and other pawpaw species has also been very difficult to achieve.



Fig. 1: Shoots of *Asimina tetramera* in nutrient medium. Once the shoots develop roots, they will be removed from tissue culture and acclimated to growing in soil conditions.

Photo by Dr. Valerie C. Pence

PROPAGATING & PRESERVING PAWPAWS



Fig. 2: Rooted shoots are slowly acclimated to lower humidity levels in a fogging tent before being moved to ambient conditions. Young plants can be seen in the pot at the upper left.



Fig. 3: In vitro collecting (IVC) allows researchers to collect plant samples in the field in vials of sterile medium. The vials are easily transported, and allow hundreds of collections to be made during a single collecting trip.

Photos by Dr. Valerie C. Pence

In 2001, a breakthrough came when a chemical known as silver thiosulphate (STS) was added to the medium. STS is known to inhibit the action of ethylene gas, a natural plant hormone. Typical of plant hormones, some ethylene is needed for root initiation, but too much can be inhibitory. The fact that STS stimulated rooting suggested that these plants were producing too much ethylene in culture and, thus, inhibiting root initiation. Without STS there was no rooting on the standard medium, but with STS, approximately 25% of the shoots made roots. This was dramatic, but still not ideal. If we wished to produce 100 plants for outplanting, we would need to begin with at least 400 shoots. These numbers were still a bit daunting.

As a result, CREW has done further research on rooting in *Asimina tetramera*. Studies of the production of ethylene in culture by *A. tetramera* tissues is underway using gas chromatography to measure ethylene levels. In addition, tests involving high doses of rooting hormones (auxins) have also proven effective in stimulating rooting to as much as 60% in some cases.

Once shoots are rooted, they are ready to be moved back into the “real world”, a process called acclimation. In order to slowly acclimate the shoots to lower humidity levels, the plantlets are placed in a fogging tent in the CREW greenhouse for several weeks before being moved to ambient conditions. [Fig.2] What is the fate of the plants that are produced? Some have been used for display at the CZBG’s Manatee Springs exhibit. Others are being prepared for a return to Florida where they will be part of outplanting experiments. The survival in the wild of plants produced in tissue culture will be compared with transplanted and natural seedlings that are also being monitored at several population sites.

Preserving Genetic Diversity in Plant Collections

The goal of conservation is to preserve as much genetic diversity within a species as possible, but when plants are propagated by tissue culture, they are clonal, or identical genetic copies of the original donor plant. Thus, when tissue culture is used for conservation, as many genetic lines as possible are propagated, in order to provide a mix of genetic individuals for outplanting. These genetic lines have been collected with the help of a technique known as in vitro collecting, or IVC. [Fig. 3]

IVC takes tissue culture to the field. Researchers take small pieces of shoot tips or young leaves from plants growing in the wild, wipe them with alcohol, and quickly place them into small vials of sterile medium. These vials are sturdy and easily transported, and hundreds of collections can be made during the course of a collecting trip. With the help of Dr. Anne Cox and Marjorie Shropshire, IVC collections have been made from dozens of different *A. tetramera* plants growing in several populations of the species. These samples were taken back to the lab at CREW, where they were transferred to plates of medium containing hormones to stimulate shoot growth. These collections are providing a diverse mix of genotypes, which are being documented using molecular fingerprinting techniques. These methods provide a picture of the genetic differences between individuals and the amount of diversity within the collection.

This genetic diversity is valuable, not only for plants being returned to the field, but also for material being preserved. Ex-situ, or off-site, conservation is an important back-up to the primary conservation efforts of maintaining habitat for populations in the wild. Seed banks are the most efficient methods for maintaining plant material ex-situ, should the wild populations be lost. But, for some plants, like the four-petal pawpaw, traditional seed banking is not an option, since four-petal pawpaw seeds are “recalcitrant”, or sensitive to drying, which is a necessary step in seed banking.

Endangered Plants in the Frozen Garden

CREW researchers have developed methods for freezing the tiny shoot tips from the *Asimina tetramera* cultures, and these can be kept in liquid nitrogen indefinitely. This process, known as cryopreservation, requires patience and a delicate hand to dissect out just the top 1 mm tip of each shoot. These shoot tips are then encapsulated in a sphere of gel and treated with special chemicals known as cryoprotectants, which help remove the water from the tissues and stabilize the remaining biochemicals. When this process is completed, the encapsulated shoots are plunged into liquid nitrogen, where they are rapidly frozen. Because the temperature of liquid nitrogen is so low (-320°F; -196°C), biological materials in liquid nitrogen are very stable and can remain viable for decades, or longer. Although the procedure is more involved than storing seeds, it is workable and provides the only method currently available for the long-term storage of the genetic diversity of *A. tetramera*. [Fig. 4]

Work with *A. tetramera* is also serving as a model for work with two other pawpaws, the beautiful pawpaw (*Deeringothamnus pulchellus*) and Rugel’s pawpaw (*Deeringothamnus rugelii*), which are also endemic to Florida. Like *A. tetramera* they have recalcitrant seeds. Other Florida endangered species in the EPPP include Avon Park harebells (*Crotalaria avonensis*), a small legume native to the central Florida scrub. Working with collaborators at Archbold Biological Station in Lake Placid, Florida, plants are being propagated for outplanting projects at several sites. Work is also being done on several other targeted species in collaboration with Historic Bok Sanctuary, Fairchild Tropical Botanic Garden, and Marie Selby Botanical Gardens.

While tissue culture methods cannot address all the issues of conservation, they are tools that can supplement conservation efforts with particular species. As in the case of the four-petal pawpaw, they can be applied to collecting, propagation, and long-term preservation and can help fill gaps when other methods are not adequate. The Cincinnati Zoo & Botanical Garden is testing ways in which laboratory research using tissue culture methods can combine with the work of field researchers to help increase the number of individuals available for research, education and reintroduction. By using all the tools available, both laboratory and field researchers are working ensure the survival of these endangered plants into the future. 🌱

While tissue culture methods cannot address all the issues of conservation, they are tools that can supplement conservation efforts with particular species.



Fig. 4: CREW’s “frozen garden” preserves plant material in liquid nitrogen, allowing for the long-term storage of the genetic diversity of the four-petal pawpaw.

Photo by Dr. Valerie C. Pence