A New Method of Propagation for *Ziziphus celata* (Florida ziziphus), a Florida Endangered Species

*Ziziphus celata* photos (above and right) by Shirley Denton
Florida ziziphus (Ziziphus celata Judd and D. Hall) is a federally endangered shrub found only in two counties in central Florida (USFWS, 1999; Weekley et al., 1999). This deciduous shrub grows to 2 m tall and produces solitary and axillary flowers bearing greenish sepals and five white petals (Delaney et al., 1989; Judd and Hall, 1984; Weekley et al., 1999). Leaves are alternate, oblong-elliptical to slightly ovate and less than 25 mm long (Judd and Hall, 1984). Fruits are ellipsoid drupes of 10-20 mm in length and turn yellowish when ripe (Delaney et al., 1989; Weekley et al., 1999). [Fig 1]

Florida ziziphus is one of an increasing number of rare plant species found only along the Lake Wales Ridge. The Lake Wales Ridge is a remnant sandbar isolated by water from what is now the United States landmass during the Pleistocene age (Myers, 1990; Webb, 1990). Due to its isolation, unique ecosystems have developed along this sandy ridge. The Lake Wales Ridge is comprised of mostly high pine ecosystems including sand pine scrub (Myers, 1990). Florida ziziphus is found in the southern portion of the Lake Wales Ridge in a matrix of high pine and sand pine scrub ecosystems (USFWS, 1999; Weekley et al., 1999).

Florida ziziphus may have a significant ecological role in the high pine and sand pine scrub community. Rabbits have been observed eating fallen fruits, which may also be a food source for gopher tortoises and small rodents. Evidence of herbivory has been seen on stems and leaves, which also act as cover for small animals and birds (T. Race, Curator of Endangered Plants, Historic Bok Sanctuary, personal communication, 2000; USFWS, 1999).

Florida ziziphus populations have been reduced to just five sites most of which consist of only a few individuals (USFWS, 1999; Weekley et al., 1999). Most individuals in the wild do not produce viable seed and populations are genetically isolated (Godt et al., 1997). Genetic analyses using both allozyme electrophoresis and RAPD techniques indicated that four of the five Florida ziziphus populations consist of a single genotype while the fifth population consists of seven genotypes (Godt et al., 1997; C. Weekley, Archbold Biological Station, personal communication, 2001). Breeding studies conducted at Historic Bok Sanctuary, using plants propagated from root cuttings, indicate that Florida ziziphus is self-incompatible and crossing within a genotype does not occur (Burkhardt et al., 1997).

Management tasks are currently undertaken by staff at Historic Bok Sanctuary (Lake Wales, FL) and Archbold Biological Station (Lake Placid, FL) to keep wild populations from declining further (Weekley et al., 1999). However, in order for stable populations to be restored, sexually reproducing populations must be established. Cross-compatible genotypes must be established within populations. Currently however, the only method for propagating a specific genotype of Florida ziziphus is by root cutting, which is destructive to the donor plant. Application of more efficient propagation methods needs to be explored. One such method is micropropagation.

Micropropagation is the rapid in vitro production of plants on a sterile defined culture medium under controlled conditions of light and temperature. This technology has been applied to the efficient production of many plant species. One key advantage is that production can
be initiated from very small pieces of initial plant material which results in little or no damage to the donor plants and produces plantlets that are genetically identical to the donor plant. In this study we explored the use of micropropagation as a potential method for generating specific genotypes of Florida ziziphus for use in producing sexually reproducing wild populations.

Successful plant micropropagation requires completion of several successive stages (Stages 0 - IV) (Kane, 2000b). The first stage (Stage 0) involves selecting and preparing the donor plant to increase the probability of establishment in culture. The plant material used to establish plant cultures varies. Excised embryos or seedlings are often used because frequently it is easier to remove potential bacterial and fungal contaminants from them that can affect plant culture growth. With *Ziziphus celata*, 1-year old seed produced in the Historic Bok Sanctuary Center for Plant Conservation’s ex situ collection of endangered plants were used. Seeds were cleaned of their fruit, dried and then stored in brown paper bags at room temperature until experimentation commenced. 

The next micropropagation stage (Stage I) requires establishment of aseptic (sterile) plant tissue in culture vessels on a defined medium. The culture medium usually consists of mineral salts, vitamins, and sucrose. Media are typically gelled with agar (a sea weed extract). Plant growth regulators are frequently incorporated into the medium. Many of these growth substances are naturally produced by plants and promote physiological responses like shoot growth or root growth when added to culture media.

We developed procedures to establish cultures of *Z. celata* using surface sterilized nodal sections excised from seedlings grown under greenhouse conditions. In March, 2001, 150 seeds were germinated in a soilless potting mix and maintained under greenhouse conditions until seedlings had produced approximately 10 nodes. The nodal sections were surface sterilized in dilute bleach (1.5% sodium hypochlorite), and then rinsed three times in sterile water. The nodal sections were placed on a sterile establishment medium consisting of Woody Plants Medium (WPM) mineral salts and vitamins (McCown and Lloyd, 1981), sucrose, supplemented with the plant growth regulator benzyladenine (BA) and solidified with 7g/L TC agar.

Shoot production occurred from axillary buds. These Stage I cultures were indexed for the presence of bacterial and fungal contaminants using Leifert and Waites sterility test medium (Phytotechnology Laboratories, cat. #L476, Shawnee Mission, KS) and procedures as described by Kane, (2000a).
After 28 days, indexed cultures determined to be contaminated were discarded.

The goal of the next micropropagation stage (Stage II) is rapid clonal shoot multiplication. Consequently, attempts were made to induce rooting in culture (Stage III). The medium was modified in an attempt to induce root formation. Various plant growth regulators called auxins were added to the medium. Although a few rooted plantlets were infrequently observed in vitro [Fig. 4], attempts to define a medium for in vitro rooting were unsuccessful. These results serve as the basis for future research including in vitro rooting by subculturing microcuttings onto a medium without growth regulators for several weeks prior to transfer onto rooting medium. Extremely infrequent rooting of microcuttings may be due to a number of factors, including genetic factors and should be evaluated further.

Establishment of cultures using nodal sections from seedlings proved to be an efficient method of culture initiation. Contamination rates were low as based on culture indexing procedures. Maximum shoot production (8 shoots/nodal section) was observed on medium containing 0.5 μM BA and 15 μM GA₃. Stage II cultures consisted of clusters of small axillary shoots. [Fig. 3] These shoot cultures were separated into individual unrooted shoots called microcuttings. These microcuttings are typically rooted in culture or, preferably, directly under greenhouse conditions.

Results of preliminary experiments indicated that shoot microcuttings of Z. celata could not be rooted ex vitro (Stage IV). Consequently, attempts were made to induce rooting in culture (Stage III). The medium components were modified in an attempt to induce root formation. Various plant growth regulators called auxins were added to the medium. Although a few rooted plantlets were infrequently observed in vitro [Fig. 4], attempts to define a medium for in vitro rooting were unsuccessful. These results serve as the basis for future research including in vitro rooting by subculturing microcuttings onto a medium without growth regulators for several weeks prior to transfer onto rooting medium. Extremely infrequent rooting of microcuttings may be due to a number of factors, including genetic factors and should be evaluated further.

Clearly, further experimentation needs to be completed to establish Stage II rooting and Stage IV acclimatization procedures for the micropropagation of Z. celata.

Although very high in vitro shoot multiplication rates were not achieved for Z. celata, the rates achieved on medium supplemented with the 0.5 μM BA (8-fold monthly increase) were acceptable to fulfill the objectives of this study. Mass production of the species on a scale required for most horticultural or agronomic crops is not required. With Z. celata, all that is required is that the generation of plant numbers of each genotype sufficient for restoration projects with little or no damage to the parent plant.

These experiments have provided important information about the challenges and potential to propagate Z. celata using micropropagation procedures. Currently, there are limitations

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**Fig. 3** – Stage II shoot production of *Ziziphus celata* cultured on media supplemented with 0.25 μM BA and 14.4 μM GA₃ after 28 days culture.

**Fig. 4** – *Ziziphus celata* microcuttings very rarely rooted. Microcutting shown rooted after 56 days culture in the presence of 5 μM indolebutyric acid (IBA), a plant growth regulator. Scale bar = 10 mm.
The Everglades Handbook – Understanding the Ecosystem

Reviewed by Suzanne Koptur

When The Everglades Handbook first came out, I liked it, and used it as a supplemental text for the general Ecology course we teach at Florida International University. It was ‘light-hearted, well-rounded, and highly readable’ (quoting from a 1995 review I wrote), but a little light on the treatment of plants and the historical literature. Professors in Environmental Studies used it for the textbook for the Ecology of South Florida course, as it provided a concise introduction to all the habitats as well as a brief history of the geology and climate of the area. I am happy to report that in its second edition, it has gotten even better!

The author, an independent ecologist, agreed to teach one semester’s offering of that course, and used his experience in teaching to guide the revision of his book. He has done a wonderful job. Though still highly readable, the book is now replete with references on every topic, so that interested readers can go to the sources he used, and learn more about every aspect of Everglades ecology. Each habitat has a plant list, and refers readers to relevant, up-to-date resources for plant distributions, conservation status, and illustrations. A new section on food webs helps the reader understand the importance of all the different habitats to the functioning of the ecosystem. The final section of the book reviews the influences of humans on the Everglades, including the impacts of specimen collecting and exotic introductions (plants and animals). Water and its movement determines what habitats exist and what organisms live there, and non-native humans transformed much of Florida over the last century with canals and draining flooded areas for agriculture and habitation. The author discusses Everglades restoration in a way everyone can understand, especially after his earlier explanations of geology and the aquifer system underlying the state.

Tom Lodge confides to his readers that he can understand the urge that many people have to collect things from nature, but says that once he learned to photograph things in nature, that urge subsided. The book has beautiful photos, mostly of animals and landscapes, but there are some distinctive plants, and the beautiful diagrams and maps convey the orderly complexity of habitat differentiation based on elevational

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cuttings are generally taken only when the parent plant is in severe decline. For these reasons, micropropagation technology still holds promise for the restoration of the species and should be examined further to determine viable rooting and acclimatization procedures.

Literature Cited:
The purpose of the Florida Native Plant Society is to conserve, preserve, and restore the native plants and native plant communities of Florida.

Official definition of native plant:
For most purposes, the phrase Florida native plant refers to those species occurring within the state boundaries prior to European contact, according to the best available scientific and historical documentation. More specifically, it includes those species understood as indigenous, occurring in natural associations in habitats that existed prior to significant human impacts and alterations of the landscape.