Propagation protocol for the

Endangered Crenulate Lead Plant

Amorpha herbacea var. *crenulata*

Julissa Roncal
Jack B Fisher
Meghan QN Fellows
Kristie S Wendelberger
Joyce Maschinski
Matthew W Fidelibus

ABSTRACT

Crenulate lead plant (*Amorpha herbacea* Walter var. *crenulata* (Rydberg) Isely [Fabaceae]) is a South Florida endemic that can be propagated sexually and asexually with success. We found that clean seeds (removed from pods) germinate best and can be frozen for storage. Softwood cuttings, treated with rooting hormone and stuck in perlite under periodic misting, rooted well but sometimes transplanted poorly. Mature plants can be salvaged with success, but because the plants grow on rocky soils the process can be very labor intensive. Establishing new populations of this plant, using these propagation techniques, is another tool for protecting this species.

Roncal J, Fisher JB, Fellows MQN, Wendelberger KS, Maschinski J, Fidelibus MW. 2006. Propagation protocol for the endangered crenulate lead plant, *Amorpha herbacea* var. *crenulata.* Native Plants Journal 7(1):89–93.

KEY WORDS
Cuttings, *ex situ* collection, outplanting, rescued population, seeds, seed storage, Fabaceae

NOMENCLATURE
USDA NRCS (2005)

Figure 1. Rescued population of the crenulate lead plant, *Amorpha herbacea* var. *crenulata* (Fabaceae), Miami, Florida. Photo by Kristie Wendelberger; detail photo by Adriana Muir

The genus *Amorpha* belongs to the plant family Fabaceae, a group of about 18000 species in 630 genera, a nearly cosmopolitan family and second only to grasses in economic importance (Judd and others 2002). *Amorpha* includes 15 species indigenous to North America (Mabberly 1997). *Amorpha herbacea* var. *crenulata* (Rydberg) Isely (Fabaceae), commonly known as the crenulate lead plant or the Miami lead plant, is endemic to South Florida (Figure 1).

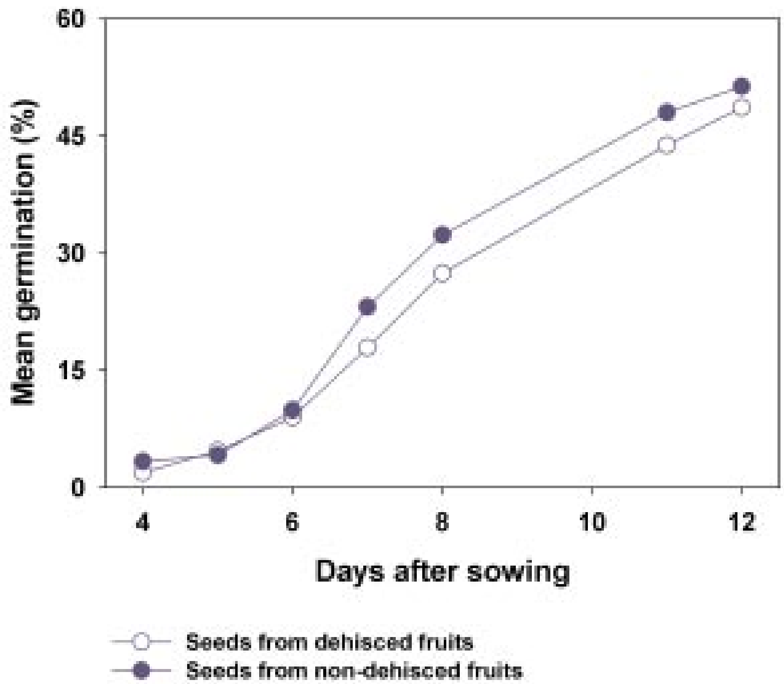

Figure 2. Germination percentage of seeds from naturally dehisced fruits and from non-dehisced fruits. Within 12 d, 50% of the seeds from both types of pods germinated.

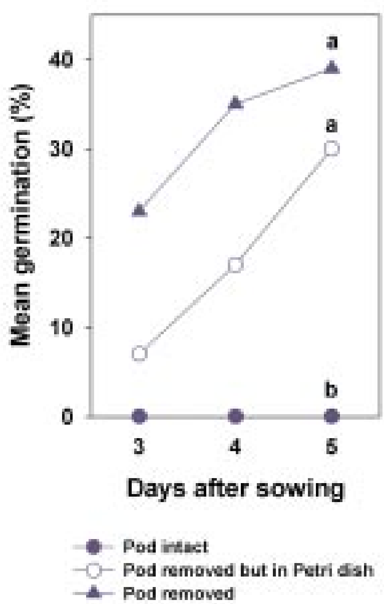

Figure 3. Effect of fruit pod on seed germination. Non-parametric Kruskal-Wallis one-way analysis of variance conducted for the fifth day after sowing. Test statistic = 6.214, df 2, *P* = 0.045. Numbers are mean germination percentages (N = 3 with 50 seeds per replicate) and different letters indicate significantly different germination percentages.

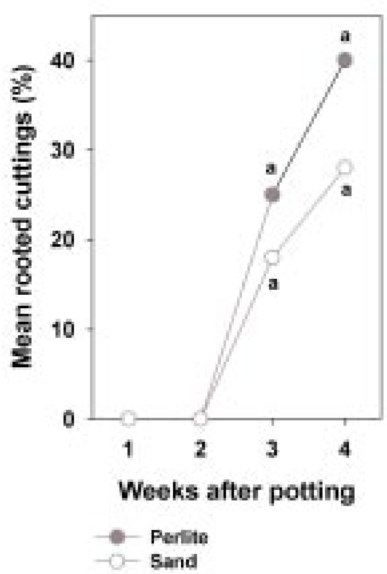

Figure 4. Effect of rooting substrate on percentage of rooted cuttings. Independent samples t-test conducted for each week after potting revealed no difference of substrate on percentage of rooted cuttings. For week 3, t = 0.62, df 18, *P* = 0.543. For week 4, t = 0.9, df 18, *P* = 0.378. Numbers are mean percentage of rooted cuttings (N = 10 with 40 or 70 cuttings per replicate) and letters indicate no significant difference within each week.

The crenulate lead plant is most common in ecotonal areas between pine rockland and marl prairie (Gann and others 2002), where it grows on poorly drained Opalocka sands or on Opalocka rock outcrop complex soils (USFWS 1999), at 2 to 7 m (6.5 to 23 ft) above sea level in full sun or partial shade. It is a perennial sprawling or erect glabrous shrub that grows to 2 m (6.5 ft) tall with new branches often having a reddish-purple color. Isely (1986) first separated this variety from the other forms of *A. herbacea* by the slightly crenulate margins of its leaflets. Leaves are imparipinnate with 21 to 45 elliptic-oblong leaflets, and midveins terminating in a sessile glandular knob.

It blooms from February to August–November (Coile 2000). Racemes are usually slender, 10 to 20 cm (3.9 to 7.9 in) long, commonly flexuous, and initially with a tapering rat-tail appearance (Figure 1). Flowers are visited by a nonnative honey bee from the Mediterranean, a metallic green sweet bee, a native leaf-cutting solitary bee, and small flies (Maschinski and others 2005). Fruits are indehiscent legumes, laterally compressed, asymmetrically erect or falcate, glandular-punctate, produced from February to August (Coile 2000) with most of the fruiting occurring in the fall. Up to 2 seeds (5 mm [0.2 in]) will be produced in a fruit, however, most fruits from wild plants have only one seed.

Currently, the crenulate lead plant has 4 natural and 2 reintroduced populations within its historic Miami-Dade County distribution range. An additional 4 natural populations were destroyed in the last decade because of urban development. Fewer than 2000 plants remain on fragments of pine rockland ecosystem on the Miami Rock Ridge. Habitat loss due to urbanization has rendered the crenulate lead plant as federally endangered by the United States Fish and Wildlife Service, endangered by the Florida Department of Agriculture and Consumer Services, and critically imperiled by the Florida Natural Areas Inventory.

The conservation of rare plants, such as the crenulate lead plant, may benefit from establishing an *ex situ* (off site; away from the plant's natural situation or location) collection that provides insurance against their extinction (Dixon 1994), plants for reintroduction or augmentation (Alley and Affolter 2004), and material to study their natural history (Conte and others 2004). Fairchild Tropical Botanic Garden (FTBG) has an *ex situ* collection of more than 100 rare taxa of South Florida and the Caribbean, including both live plants and seeds of the crenulate lead plant. Seeds can be an important component of an *ex situ* collection of rare plants because their storage is inexpensive, requires a relatively small space, and eases the pressure on wild populations. For these reasons, 28 500 seeds from 2 wild populations of crenulate lead plant and 6100 seeds collected from Fairchild's *ex situ* collection are stored at the National Seed Storage Laboratory (NSSL) of the National Center for Genetic Resources Preservation in Fort Collins, Colorado. Conservation research at FTBG has focused on determining seed storage behavior, germination requirements, dormancy characteristics, vegetative and whole plant propagation, and monitoring and ecological restoration of several rare South Florida species. This article compiles the results of experiments on the crenulate lead plant.

SEED PROPAGATION

The crenulate lead plant is readily propagated from seeds and was part of the horticultural trade until it was listed as federally endangered. Seeds for propagation should be collected from ripe fruit attached to the infructescence or from naturally dehisced fruit as similar germination percentages were obtained with both seed sources at each monitoring date (Figure 2). Ripe fruits easily detach from the infrucescence. They are plump, brown, and have a dry appearance with a flaky, transparent coating. Attaching a mesh bag to the inflorescence following the onset of fruit development can increase yield from a single plant. We tested viability of fresh seeds by sowing seeds on growth media (Metromix 360, VJ Growers Supply, Apopka, Florida) top-dressed with about 1 cm (0.4 in) of grit or sand in pint-size pots. The top-dressing of sand reduced compaction from watering, reduced moss and weed growth, and prevented seeds from washing out of the pot. We placed pots on a greenhouse bench under South Florida ambient conditions (25 to 35° C [77 to 95° F], 54% to 87% relative humidity, ~11–12 h photoperiod) and watered them 3 times a wk. Seeds can germinate year-round as described. After the seventh week, 60% to 84% of seeds germinated (Fellows and others 2001).

Because the fruits are single-seeded, and seed extraction is labor intensive, it is tempting to sow the whole fruits. Removing the pod, however, clearly increased germination rate (Figure 3). Additional experiments that monitored seed germination up to 12 d post-sowing also showed a higher germination success for clean seeds (results not shown). The pods appear to present a physical rather than a physiological germination barrier because, by the fifth day after sowing, germination of clean seeds was similar to that of seeds whose pods were extracted but left inside the Petri dishes (Figure 3). Fruits can float for 24 h in water, which suggests that the pods may have a dispersal function.

We have attained fast germination of freshly collected seeds by removing them from their pods, soaking them in a 10% bleach solution (0.6% sodium hypochlorite) for 15 min to reduce mold growth, rinsing them twice with clean water, and placing the clean seeds on damp filter paper in Petri dishes 9 cm in diameter. Evidently, germination was not inhibited by light as many seeds were germinated inside a glasshouse under South Florida ambient conditions. Depending on the evaporative potential of the germination area, sufficient water may need to be added to the dish to maintain a damp filter paper. A germination of 39% can be achieved in 5 d. Seeds can germinate while still attached to the infructescence (Maschinski and others 2002). Twenty seeds weigh 0.0518 g, so extrapolation suggests that 390 seeds can be found in 1 g (175 500 seeds/lb).

Crenulate lead plant has orthodox seeds but they are amenable to cryogenic storage. Germination of 30-mo-old, liquid nitrogen–stored seeds followed by a 72-h humidification yielded a 21% to 24% germination success within 15 wk (Fellows and others 2001).

VEGETATIVE PROPAGATION

Crenulate lead plant may be propagated from hard and softwood cuttings, which can be produced at the same time from a plant. We took cuttings 5 to 10 cm (2 to 4 in) long and had greatest success with those from stems with new leaves. Cuttings were collected in the morning from 8:00 to 10:00 and were transported in plastic bags with moist paper towels. Upon arrival at the nursery facility, the basal ends of the cuttings were immersed in a solution of 0.2% IBA and 0.01% NAA (Dip'n'Gro, 5X, Astoria Pacific Inc, Clackamas, Oregon). Then, the cuttings were inserted in 4-l (1-gal) containers filled with sand or perlite, placed on a mist bench in a greenhouse with 70% shade, and misted for 2 s every 10 min from 8:00 to 17:00 daily. Temperature ranged from 26 to 35 °C (78 to 95 °F). After 1 mo, 28% of cuttings in sand and 40% in perlite rooted, but rooting success was not significantly different between the 2 media (Figure 4). Thus, either sand or perlite is a satisfactory rooting substrate, but the roots of cuttings in sand may break when lifted whereas those in perlite seem to be less susceptible to breakage. This is probably because crenulate lead plant has fragile roots that do not easily support the

Photo by Kristie Wendelberger

Figure 5. Rescued crenulate lead plants 7 wk after potted at Fairchild Tropical Botanic Garden's nursery facility.

higher bulk density of saturated sand compared with that of perlite. Regardless of the rooting media selected, rooted cuttings should be carefully extracted, because cuttings that lost a substantial amount of roots generally died whether returned to the mist bench or potted. More than half of the rooted cuttings died after transplantation, probably due to root loss. The use of perlite, smaller rooting containers that can constrain root spread, and extra care at transplantation can probably improve survival of rooted cuttings. Softwood cuttings can yield the same rooting percentage as the lignified or hardwood cuttings when treated with the root hormone IBA. However, softwood cuttings tended to wilt on the mist bench and were more susceptible to injury during transplantation so proportionally fewer healthy potted plants resulted from softwood than hardwood cuttings (Maschinski and others 2002).

Root cuttings are not useful for vegetative propagation. Large roots (up to 10 cm [4 in] diameter) were dug from wild plants, cut into 20 to 30 cm (8 to 12 in) lengths, stored at 24 °C (75 °F) for 1 wk in paper bags, and laid horizontally in a tray of perlite on a mist bench in the greenhouse. No shoot buds formed and roots became soft and rotted after 4 wk (Maschinski and others 2002).

MYCORRHIZAL REQUIREMENT

Crenulate lead plants have arbuscular mycorrhizae when growing in native habitat. Seedling growth was promoted by addition of arbuscular mycorrhizal fungi (AMF) to pasteurized native soil in pots that had no additions of fertilizer (Fisher and Jayachandran 2002). Presumably, growth of rooted cuttings would also be promoted by AMF. However, seedlings were not obligate mycotrophs. They grew vigorously in AMF-free soil when phosphate or slow-release fertilizers were added to pots (Fisher and Jayachandran 2002). In the absence of AMF inoculum, healthy seedlings are grown in Fairchild standard potting mix (10% perlite, 40% Canadian peat, 20% composted bark, and 30% sand [by volume]) top-dressed with approximately 15 g (0.4 oz) of Nutricote (Florikam, Sarasota, Florida) controlled-release fertilizer (10N:10P_2O_5:17K_2O; 6 mo release rate at 25 °C [77 °F]) per 2.5 l of potting mix.

SALVAGING WHOLE CRENULATE LEAD PLANTS FOR OUTPLANTING

Transplanting whole plants had a high success rate but was labor intensive. Because roots of this plant grow between rocks and fissures in their native limestone substrate, it is very difficult to remove whole plants without root injury. Generally, it is too difficult and time consuming to dig more than 30 cm (1 ft) deep around most plants. At that depth, most plants have only one thick and woody taproot with few or no secondary roots. Though woody, the taproots are soft and brittle and the weight of the unsupported root and stems often cause root breakage before excavation is complete. Shovels or breakage injure most roots between several inches to one foot below the soil surface. Broken or damaged roots must be freshly cut before potting. Roots were kept moist for transportation from the wild population to the nursery facility. Before potting, any remaining broken roots should be cut off and half of each stem length should be removed. A few days after potting, plants might seem in poor condition, however, by the third or seventh week, plants show new shoots and inflorescence (Figure 5; Maschinski and others 2002). Rescued plants have been successfully outplanted and survival and growth is currently being monitored (Maschinski and others 2005).

ACKNOWLEDGMENTS

Research was funded by the Florida Department of Agriculture and Consumer Services and by the Institute of Library and Museum Services. The authors are grateful to Jennifer Possley, Sonia Thompson, Lauren Linares, Karen Griffin, Anne Frances, Ken Neugent, the Florida Native Plant Society, Miami Dade College students, and Miami-Dade Natural Areas Management for their help in different stages of this work. Susan Carrara and Dena Garvue contributed with seed-storage experiments.

REFERENCES

Alley H, Affolter JM. 2004. Experimental comparison of reintroduction methods for the endangered *Echinacea laevigata* (Boynton and Beadle) Blake. Natural Areas Journal 24:345–350.

Coile NC. 2000. Notes on Florida's endangered and threatened plants. Gainesville (FL): Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Bureau of Entomology, Nematology and Plant Pathology, Botany Section. Contribution No. 38, 3rd edition.

Conte L, Cotti C, Schicchi R, Raimondo FM, Cristofolini G. 2004. Detection of ephemeral genetic sub-structure in the narrow endemic *Abies nebrodensis* (Lojac.) Mattei (Pinaceae) using RAPD markers. Plant Biosystems 138:279–289.

Dixon KW. 1994. Towards integrated conservation of Australian endangered plants—the western Australian model. Biodiversity and Conservation 3:148–159.

Fellows MQN, Possley J, Lane C. 2001. An integrated conservation program for the protection of Florida's rare and endangered flora. Coral Gables (Miami, FL): Center for Tropical Plant Conservation, Fairchild Tropical Botanic Garden. Final report to Florida Department of Agriculture and Consumer Services. Contract #005619.

Fisher JB, Jayachandran K. 2002. Arbuscular mycorrhizal fungi enhance seedling growth in two endangered plant species from South Florida. International Journal of Plant Sciences 163:559–566.

Gann GD, Bradley KA, Woodmansee SW. 2002. Rare plants of South Florida: their history, conservation, and restoration. Miami (FL): The Institute for Regional Conservation. 1056 p.

Isely D. 1986. Notes about *Psoralea* sensu auct., *Amorpha, Baptisia, Sesbania,* and *Chamaecrista* (Leguminosae) in the Southeastern United States. Sida 11:429–440.

Judd WS, Campbell CS, Kellogg EA, Stevens PE, Donoghue MJ. 2002. Plant systematics, a phylogenetic approach. 2nd edition. Sunderland (MA): Sinauer Associates. 576 p.

Mabberly DJ. 1997. The plantbook. 2nd edition. Cambridge (UK): Cambridge University Press. 858 p.

Maschinski J, Fellows MQN, Possley J. 2002. Conservation of South Florida endangered and threatened flora. Coral Gables (Miami, FL): Center for Tropical Plant Conservation, Fairchild Tropical Botanic Garden. Final report to the endangered plant advisory council, Florida Department of Agriculture and Consumer Services. Contract #006466.

Maschinski J, Wright SJ, Wendelberger KS, Possley J, Fisher JB. 2005. Conservation of South Florida endangered and threatened flora: 2004–2005 program at Fairchild Tropical Botanic Garden. Coral Gables (Miami, FL): Center for Tropical Plant Conservation, Fairchild Tropical Botanic Garden. Final report to Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL. Contract #009064. 173 p.

[USDA NRCS] USDA Natural Resources Conservation Service. 2005. The PLANTS database, version 3.5. URL: http://plants.usda.gov (accessed 9 Nov 2005). Baton Rouge (LA): National Plant Data Center.

[USFWS] United States Fish and Wildlife Service, Southeast Region. 1999. South Florida multi-species recovery plan. A species plan . . . an ecosystem approach. Atlanta (GA).

AUTHOR INFORMATION

Julissa Roncal
Project Plant Ecologist
jroncal@fairchildgarden.org

Jack B Fisher
Senior Scientist
jfisher@fairchildgarden.org

Kristie S Wendelberger
Field Botanist/Permit Coordinator
kwendelberger@fairchildgarden.org

Joyce Maschinski
Conservation Ecologist/Team Leader
jmaschinski@fairchildgarden.org

Center for Tropical Plant Conservation
Fairchild Tropical Botanic Garden
11935 Old Cutler Road
Coral Gables (Miami), FL 33156

Meghan QN Fellows
Natural Resource Specialist
Fairfax County Park Authority
12055 Government Center Parkway
Fairfax, VA 22035
meghan.fellows@fairfaxcounty.gov

Matthew W Fidelibus
Assistant Cooperative Extension Specialist
Department of Viticulture and Enology
University of California, Davis
Kearny Agricultural Center
9240 South Riverbend Avenue
Parlier, CA 93648
mwf@uckac.edu