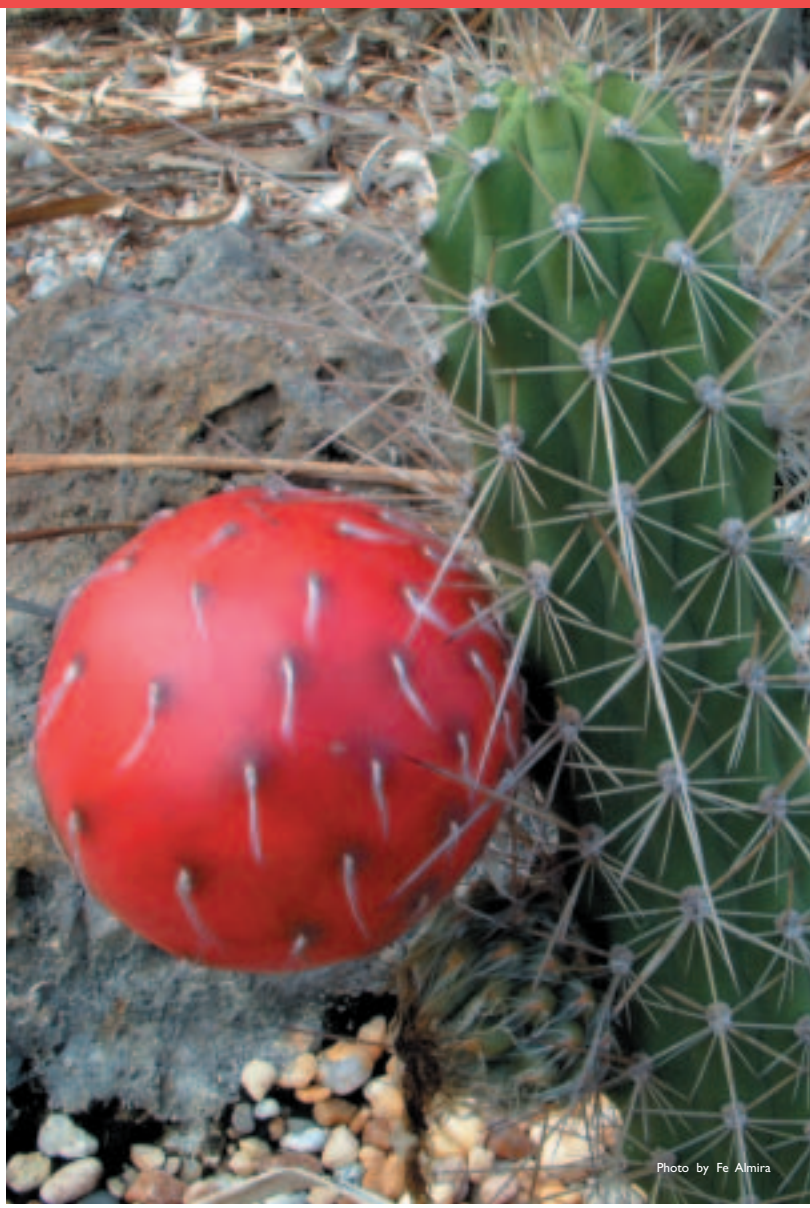

Photo by Fe Almira

Preliminary study shows germination of Caribbean applecactus (*Harrisia fragrans*) improved with acid scarification and gibberellic acid

| **Bijan Dehgan and Hector E Pérez**

ABSTRACT

To determine germination rate and final germination percentage of the endangered Florida endemic Caribbean applecactus (*Harrisia fragrans* Small [Cactaceae]), we treated seeds with sulfuric and gibberellic (GA_3) acids and germinated them under constant temperature regimes. Temperature effect was marginal at best, with only 2% of seed germinating in 50 d in 25 °C. Although there was improved germination with sulfuric acid (H_2SO_4) alone, significant increases in both germination rate and germination percentage were realized when seeds were scarified with 18 M H_2SO_4 for 15, 30, and 45 s, followed by soaking in 1000 ppm GA_3 for 24 h, with 68% germination in 120 d for the 45 s H_2SO_4 treatment. Emergence first occurred on day 18 for seed treated with 1000 ppm GA_3 but not until day 70 for the 500 ppm treatment. Control seeds failed to emerge during the observation period. These treatments represent a quick method of meeting Species Level Recovery Actions for *Harrisia fragrans* in the Multi-Species Recovery Plans for South Florida.

KEY WORDS

sulfuric acid scarification, conservation horticulture, seed propagation, dormancy

NOMENCLATURE

(North American cacti) USDA NRCS (2004); other cacti as cited

Caribbean applecactus (*Harrisia fragrans* Small [Cactaceae]; synonym *Cereus eriophorus* N.E. Pfeiffer & Otto var. *fragrans* (Small) L Benson), a critically endangered plant, is a 7- to 12-angled, shrubby, columnar erect or reclining cactus with showy nocturnal white flowers and large red berry (Figure 1). This Florida endemic was once found from Merritt Island (Brevard County) south to the St Lucie River in St Lucie County. As a result of habitat fragmentation, fire suppression, and exotic species invasion it now occurs in only 11 disjunct sites on a narrow band of the Atlantic Coastal Ridge (Figure 2). The present disjunct sites are segregated into 3 geographically isolated populations, all occurring on ridge habitat, 11 km (6.8 mi) long by 0.2 km (0.1 mi) wide, in coastal hammocks and shell middens from Volucia County south to St Lucie County (along east coast) and Monroe County (Wunderlin 1998; USFWS 1999; Wunderlin and Hanson 2004).

Since its original listing as an endangered plant in 1985 (USFWS 1999; FNAI 2000), a severe unknown stress on the Caribbean applecactus populations has resulted in high mortality (average 11%/y) and general recruitment failure (average 1%/y) (USFWS 1999). Preliminary, unpublished studies conducted at Fairchild Tropical Garden indicated that dormancy-induced delayed germination directly hinders reintroduction of the species into the wild (Garvue 2000).

Sulfuric acid (H_2SO_4) scarification to soften seed coat and/or remove chemical inhibitors from the testa is well-known in germination studies of many taxa (Baskin and Baskin 2001). Germination rate of *Echinocactus grusonii* Link and Otto, Hildman and Monats (Cactaceae) and *Echinocactus platyacanthus* Link and Otto seeds improved significantly when treated with concentrated sulfuric acid (De La Rosa-Ibarra and Garcia 1994). Godínez-Alvarez and Valiente-Banuet (1998) also found significant improvement in germination rate of *Pachycereus hollianus* (Weber) Buxb. seeds (Cactaceae) following sulfuric acid treatment.

Exogenous application of gibberellic acid has been shown to hasten growth of underdeveloped embryos and overcome morphological or physiological dormancies in a variety of species (Bewley and Black 1994; Baskin and Baskin 2001). Krulik (1981) points out that soaking seed of *Cereus* (P. Mill. spp.) for 30 m in 100 to 200 ppm GA_3 promoted germination. Similar results were obtained by De La Rosa-Ibarra and Garcia (1994) with *Echinocactus grusonii* and *Leuchtenbergia principis* Hooker (Cactaceae) when soaked in 0.1 ppm gibberellic acid after scarification. Moreover, the highest percentage seed germination for the endangered *Sclerocactus mariposensis* (Hester) N.P. Taylor (Cactaceae) was obtained when seeds were acid scarified or scarification was combined with soaking in 0.5 ppm gibberellic acid (Moreno and others 1991). In general, there is ample evidence in the literature illustrating that reduction of seed coat thickness by sulfuric acid scarification followed by gibberellic acid treatment enhances germination. This process has been

Figure 1. Harrisia fragrans (Caribbean applecactus) fruit. Photographed at Fairchild Tropical Garden. Photo by Fe Almira

Figure 2. Distribution map of *Harrisia fragrans* (Caribbean applecactus) in Florida. Redrawn from Wunderlin and Hansen (2004).

shown to enhance embryo development in many cases, resulting in significant improvement in germination time (Dehgan and Johnson 1982; Dehgan and Schutzman 1983, 1989; Keeley and Fortheringham 2000; Baskin and Baskin 2001).

Constant temperatures have been implicated as treatments to mitigate morphophysiological dormancy (Baskin and Baskin 2001). Much of the work on cactus seed germination has centered on temperature effects. Seeds of several Cactaceae (*Cereus, Coryphanta, Echinocactus, Lophocerus, Mammillaria*, and *Opuntia*, among others) had best germination between 17 °C and 34 °C (63 °F and 93 °F), with an optimum of about 25 °C (77 °F) (Rojas-Aréchiga and Vázquez-Yanes 2000). Rojas-Aréchiga and others (1998) found that 6 of 7 cacti species that were studied, germinated under a wide range of constant temperatures, although there was no significant difference in germination between constant or alternating temperature regimes. Seed of *Melocactus caesius* Went. (Cactaceae) germinated between 22 °C and 43 °C (72 °F and 109 °F) (Arias and Lemus 1984). Germination at constant or alternating temperatures did not differ for 5 of 8 cacti that were studied by Godínez-Alvarez and Valiente-Baunet (1998).

Objectives of our studies were to identify propagation protocols for reintroduction and augmentation of existing populations in protected sites. These objectives are in concert with Species Level Recovery Actions stated in the Caribbean applecactus recovery plan (USFWS 1999).

MATERIALS AND METHODS

Because of the critical endangered status of this species we decided not to collect seeds from wild populations. Seeds were obtained from the *ex situ* conservation collection at Fairchild Tropical Garden (Coral Gables, Florida). On 7 May 1999, semi-ripe fruit of Caribbean applecactus was collected from accession 87187E. On 3 June 1999, after the fleshy fruit (berry) had been determined to be ripe, approximately 2000 seeds were extracted and cleaned of pulp by using a wire mesh sieve under running water. These were disinfected in a 50% sodium hypochlorite solution for 15 min followed by rinsing under flowing distilled water for 5 min. Seeds were allowed to air dry after disinfecting. Cleaned seeds were kept at room temperature (24 °C [75 °F]) until experimentation commenced. For each experiment, unless stated otherwise, in a factorial arrangement, 4 replications of 25 seeds each were exposed to appropriate treatments and 3 replications of 25 seeds each were used for controls. The difference in number of replicates between treatments and controls was due to a shortage of seed and is considered in the statistical analysis.

Effect of Constant Temperature on Emergence

Effect of temperature on germination commenced on 22 June 1999. Seeds were sown in 9-cm (3.5-in) Petri dishes on

Whatman #1 filter paper. Experimental units were randomly assigned to germination chambers set at 25, 30, and 35 °C (77, 86, and 95 °F) with a 12 h light–12 h dark photoperiod. Light was provided by compact 15 watt soft white fluorescent bulbs (Westinghouse Electric Corporation, 2002 Angelo Brothers Company, Philadelphia, Pennsylvania). Petri dishes were irrigated as needed with 5 ml (0.17 fl oz) of deionized water for the duration of the experiment. Observations were made daily. Germination was considered complete after radicles protruded 2 mm (0.08 in).

Effect of GA_3 on Emergence

Beginning on 10 June 1999, 250 seeds were selected and separated into 2 lots of 100 seeds and 2 lots of 25 seeds. Seedlots of 100 were randomly assigned to either 25 ml (0.85 fl oz) of 500 ppm or 1000 ppm GA_3. Seeds were allowed to soak in solution for 24 h. Two replications of 25 seeds each were randomly assigned to 25 ml of distilled water to serve as controls.

Effect of Acid Scarification on Emergence

Beginning on 25 June 1999, we placed 100 seeds into a 200-ml (6.8-fl oz) beaker containing 25 ml of 18 M sulfuric acid for 15, 30, and 45 s and gently stirred with a glass rod. Following treatment, excess acid was removed with a 5 min rinse with distilled water. This was followed by 2 soaks in sodium bicarbonate ($NaHCO_3$) for 30 s each (Passam and Polyzou 1997). After rinsing, treated seeds were divided into appropriate replicates for placement in the greenhouse (see below).

Effect of Acid + GA_3 Soaks on Emergence

The combined effect of H_2SO_4 and GA_3 was examined on 25 June 1999 by first treating 100 seeds each for 15, 30, or 45 s with H_2SO_4 as described previously, followed by soaking in either 500 or 1000 ppm GA_3. Seedlots were then divided into 25 seeds each per replication.

Subsequent to acid scarification and gibberellic acid treatments, seeds were sown on a pre-moistened 3:1 (v:v) mixture of quartz sand and peat moss in 10-cm (4-in) plastic pots. Pots were randomly arranged on a bench within plastic trays inside a temperature-controlled greenhouse (night temperature = 18 °C +/- 3 [65 °F +/- 5]; day temperature = 24 °C +/- 3 [75 °F +/- 5]). To reduce heat load from solar radiation 30% shade cloth was erected over the bench. All trays were sub-irrigated once every 14 d. To prevent seeds from desiccation, plastic pots were covered with an 8.5-cm (3.3-in) Petri dish top. The tops were removed once seeds germinated. Observations were made daily until 120 d after sowing. Emergence of the cotyledons was taken as germination. Average emergence was calculated at 10 d intervals.

Several statistical methods were used to test the hypothesis that dormancy-breaking treatments would promote germination. When evaluating individual treatments against their controls, replicates were averaged and final average emergence at 120 d was ascertained. To analyze final average emergence within an experiment, irrespective of the average emergence of their controls, 2 tests were used. In experiments with only 2 treatments a z-test ($\alpha = 0.05$, $z = 1.64$) determined statistically significant results. When experiments consisted of 3 treatments a Chi-square test ($\nu = 2$, $\alpha = 0.05$) = 5.9914 was utilized. Finally, to compare the final average emergence at 120 d of treatments within an experiment to the final average emergence of the controls, a Chi-square test ($\nu = 1$, $\alpha = 0.05$) = 3.8415 was applied.

RESULTS AND DISCUSSION

Seeds exposed to constant temperature treatments yielded marginal germination. At 25 °C (77 °F), only 2% of the seeds germinated after 50 d; at 30 °C (86 °F) only 1% germinated in 40 d; at 35 °C (95 °F) none germinated at 50 d. Fungal contamination did not permit continuation of this trial; hence statistical analyses were not performed.

Although both the 500 and 1000 ppm GA_3 treatments resulted in increased emergence, only the 1000 ppm treatment had a significant effect on rate and final germination percentage. Emergence first occurred on day 18 for seeds treated with 1000 ppm GA_3 but did not commence until day 70 for the 500 ppm treatment. Control seeds failed to emerge during the observation period. Emergence for seeds treated with 1000 ppm GA_3 reached a maximum of 13% at day 80 and continued at this level until the experiment was terminated. By contrast, seeds treated with 500 ppm GA_3 never surpassed 1%.

Germination rate for all treatment times and final percentage of germination in the 15 and 45 s treatments was significantly enhanced by acid scarification. Final germination percentage for 15, 30, and 45 s scarification treatments was 8%, 33%, and 24%, respectively. Treated seeds emerged at day 55 (15 s), day 27 (30 s), and day 55 (45 s). Germination for 15, 30, and 45 s H_2SO_4 controls was 0, 52%, and 16% respectively at 120 d. Seedlings first emerged after day 90 and 102 for the 30 and 45 s controls, respectively.

Germination rate was significantly enhanced when seeds were treated with a combination of H_2SO_4 and GA_3. With 15, 30, and 45 s scarification and 500 ppm GA_3, seeds began to emerge after day 24, 24, and 18, respectively. At 120 days, 29%, 33%, and 31% emergence was observed (Figure 3). This was a significant enhancement as compared to seeds treated with H_2SO_4 alone, where 16%, 8%, and 8% germination occurred at 111, 101, and 111 d, respectively.

Seeds germinated significantly earlier and in greater numbers when treated with H_2SO_4 followed by 1000 ppm GA_3 (Figure 4). Seeds scarified for 15, 30, and 45 s emerged after day 17, 24, and 11, respectively. Germination percentage after 120 d was 50%, 30%, and 68%, respectively. By contrast, seeds treated with H_2SO_4 alone for 15 and 30 s control seed emerged after

101 and 118 d. Forty-five s control seeds failed to emerge. Final percentage of emergence of 15 and 30 s H_2SO_4 reached 20% and 4%, respectively. Results for H_2SO_4 treatments followed by either 500 ppm or 1000 ppm GA_3 were a significant enhancement compared to seeds treated with H_2SO_4 alone (Table 1).

The final germination percentage of acid scarification + GA_3 treatments and controls is presented in Figure 5. Note that final percentage of emergence in the 15 s + 1000 ppm GA_3 treatment was 1.7X greater than 15 s + 500 ppm GA_3. Seeds treated for 45 s + 1000 ppm GA_3 was more than 2X greater than that of 45 s + 500 ppm GA_3. In each of those instances, final germination percentage was significantly greater for the 1000 ppm treatment. Such significant improvement in germination when H_2SO_4 treatment is followed by GA_3 soak is usually indicative of morphophysiological seed dormancy (Baskin and Baskin 2001); where sulfuric acid softens or reduces thickness of the seed coat and GA_3 enhances embryo development.

SUMMARY

The principal objective of the Caribbean applecactus recovery plan is to conserve and increase existing populations and prevent further extinction. Strategies to meet this goal include conducting experimental outplantings, reintroduction of plants into protected sites, and conservation of germplasm. At the heart of these strategies is the identification of germination protocols. These experiments demonstrated that by using a combination of acid scarification followed by soaking in 1000 ppm GA_3 significantly higher germination rates and final germination percentages are attainable than with other treatments. These results represent preliminary steps toward developing germination protocols for Caribbean applecactus. Future work should focus on mechanisms that would improve seedling survival.

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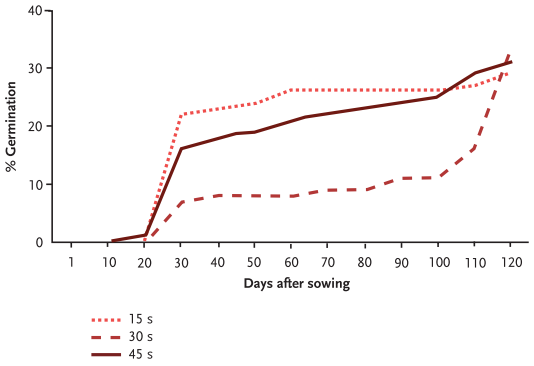

Figure 3. Germintion over 120 d of Caribbean applecactus seed after acid scarification for 15, 30, or 45 s followed by 24 h soak in 500 ppm GA_3.

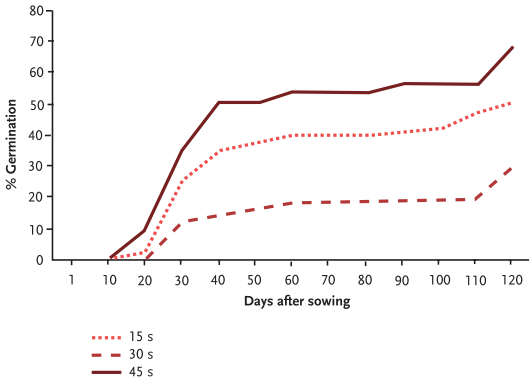

Figure 4. Germintion over 120 d of Caribbean applecactus seed after acid scarification for 15, 30, or 45 s followed by 24 h soak in 1000 ppm GA_3.

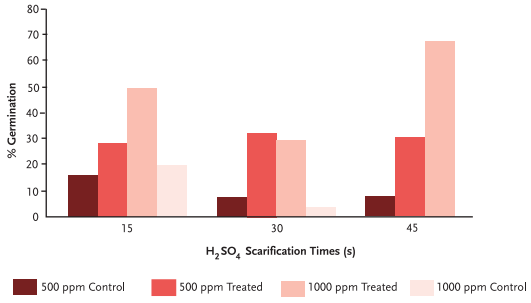

Figure 5. Final germination percentage at 120 d of Caribbean applecactus seed exposed to combinations of acid scarification and GA_3.

TABLE 1

Days until emergence of Caribbean applecactus seed after 24 h soak in 500 or 1000 ppm GA_3, acid scarification for 15, 30, or 45 s, and combinations of those treatments.

Treatment	Final germination (%)	Days until emergence
GA_3		
500 ppm	1	70
1000 ppm	13	80
Control	0	0
Acid scarification		
15 s	8	55
30 s	33	27
45 s	24	55
Acid + 500 ppm GA_3		
15 s + 500 ppm	29	24
30 s + 500 ppm	33	24
45 s + 500 ppm	31	18
Acid + 1000 ppm GA_3		
15 s + 1000 ppm	50	17
30 s + 1000 ppm	30	24
45 s + 1000 ppm	68	11

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