Sexual and Asexual Propagation of Wild Lime (*Zanthoxylum fagara* L. Sarg.), a Native Florida Plant with Ornamental and Ecological Value[1]

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Abstract

A series of four propagation studies were implemented to better understand the seed biology of wild lime (*Zanthoxylum fagara* L. Sarg. [Rutaceae]) and its adventitious rooting response to auxin treatments. Plant origin (north central vs south Florida ecotypes) did not affect initial seed viability but significantly influenced germination response to seasonal temperatures. Fifty-five days after sowing north central Florida seeds, germination was similar among spring, summer and fall treatments (28.9 to 41.1%), but was reduced by the winter temperature (10.7%). South Florida seeds showed greatest germination under the fall temperature (71.2%), and the least germination under the summer temperature (30.2%). Additional seed treatments including applications of gibberellic acid (GA_3) with kinetin nominally improved germination by 1.2 times compared to non-treated seed. Seeds tolerated cryopreservation treatments, including combinations of a plant vitrification solution, liquid nitrogen, phloroglucinol and precooling, suggesting long-term storage capability. As an alternative to seed propagation, cutting propagation was found to be a reliable means of reproducing wild lime with 91.0% rooting success when softwood cuttings were treated with 8,000 mg·kg^{-1} (0.13 oz·lb^{-1}) indole-butyric acid (IBA), compared to 3,000 mg·kg^{-1} (0.05 oz·lb^{-1}) IBA (86.3%) or the non-treated control (71.2%).

Species used in this study: Wild lime, *Zanthoxylum fagara* (L. Sarg).

Chemicals used in this study: 2,3,5-triphenyl-2H-tetrazolium chloride (TZ); sodium hypochlorite solution (Clorox bleach); gibberellic acid (GA_3); kinetin (kinetin), plant vitrification solution 2 (PVS2); liquid nitrogen (LN); phloroglucinol (phloroglucinol); glycerol (glycerol); dimethyl sulfoxide (DMSO); MS media (Murashige and Skoog media), sucrose (sucrose); 15N-5P-15K liquid fertilizer (Peters Excel Cal-Mag Special); indole-3-butyric acid (IBA); 14N-14P-14K slow-release fertilizer (Osmocote).

Index words: seeds, viability, dormancy, cryopreservation, cuttings, vegetative, Rutaceae.

Significance to the Horticultural Industry

The demand for attractive native plants for commercial and residential landscapes is rising. In the past five years, the nursery industry in Florida reported a critical need for reliable and efficient propagation systems to produce a diverse palette of native species that support ecologically friendly gardening. The results presented herein show that wild lime (*Zanthoxylum fagara)* can be efficiently propagated by seed or cuttings, supporting its wider use in Florida and beyond. As a host plant to several swallowtail (*Papilio*) butterfly species, wild lime could possibly be a candidate for micropropagation along with other important pollinator species. Ongoing studies are underway to investigate other underutilized native species with potential for introduction to the ornamental industry. Propagation and production research along with associated marketing will remain key to their availability and demand.

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Introduction

When used correctly, native plants can naturally offer desired aesthetic attributes such as color and form, while bringing biodiversity and function for ecologically friendly landscaping. The use of native plants in residential and commercial landscapes is gaining momentum (Rihn et al. 2022). This increase can be attributed to both municipal mandates (Larson et al. 2020) and the genuine interest of homeowners and consumers wanting to create ornamental gardens with purpose. With the right plant choices, such gardens offer various benefits such as pollinator attraction (Kalaman et al. 2021a), provision of nectar and pollen resources (Kalaman et al. 2021b), support for native wildlife biodiversity (Berthon et al. 2021), and reduced water consumption (Stacy et al. 2021). Despite interest, the limited production of native plants may not be able to meet consumer demands (Hall et al. 2020), which could have resulted from low availability as only 841 native plant vendors were reported in the United States in 2018 (White et al. 2018). These vendors produce only 26% of the vascular native plant taxa, partially due to limited propagation knowledge of hard-to-cultivate species (Camhi et al. 2019). In response, propagation practices have been explored to optimize production of natives by seed, cuttings, and tissue culture (Wilson et al. 2022).

Progress has been made towards the propagation and landscape evaluation of a variety of native species that are

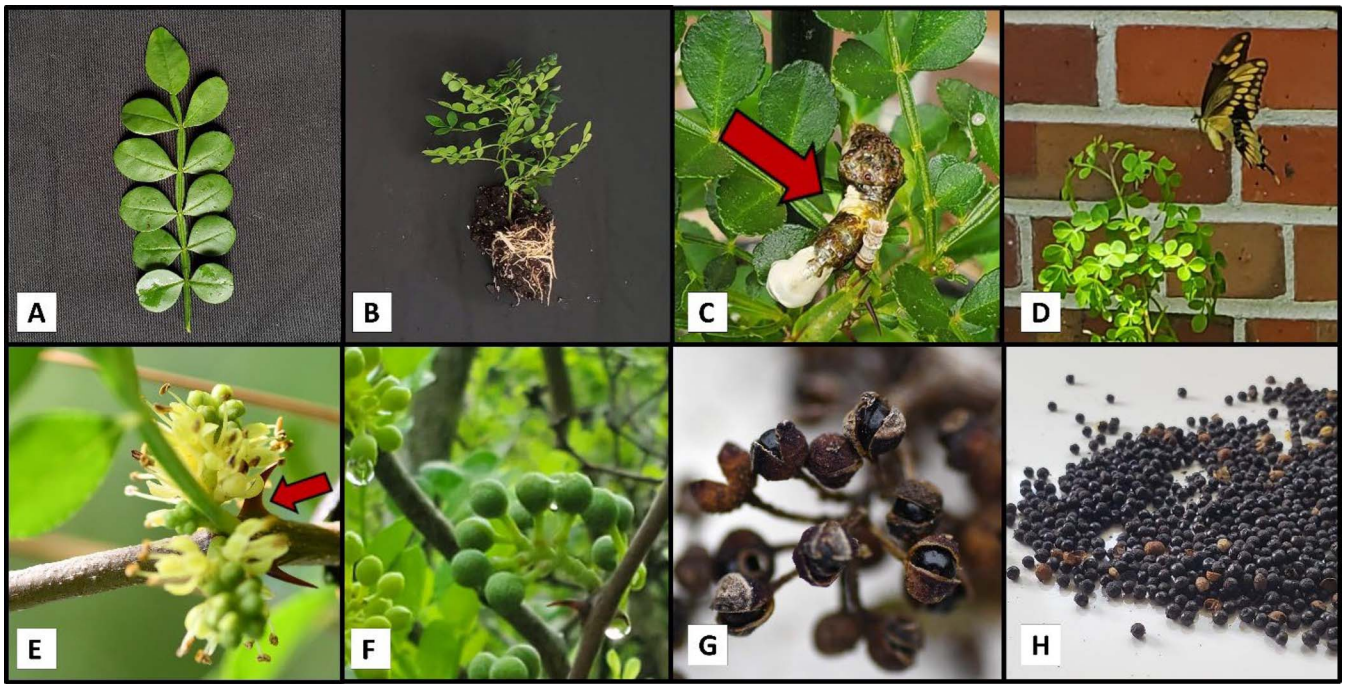

Fig. 1. Ornamental and reproductive traits of wild lime (*Zanthoxylum. fagara* L. Sarg.) featuring: (A) glossy, odd pinnately compound leaf with winged rachis, (B) woody growth habit, (C) giant swallowtail (*Papilio cresphontes*) larva (indicated by arrow), (D) giant swallowtail butterfly visiting host plant, (E) inflorescences in yellow axillary panicles and stems with short, recurved stipular spines (indicated by arrow), (F) immature, glandulous fruit, (G) mature seeds in dehiscent follicles, and (H) globose, black seeds.

either 1) attractive in their natural areas and have potential for the ornamental industry; or 2) are already in limited cultivation, but merit widened use for landscapes and gardens. This includes species such as squareflower [*Paronychia erecta* (Chapm.) Shinners] (Campbell-Martınez et al. 2022), coastalplain honeycombhead [*Balduina angustifolia* (Pursh) B. L. Rob.] (Campbell-Martınez et al. 2021), wireweeds [*Polygonum* spp. (Thetford et al. 2012)], goldenasters [*Chrysopsis* spp. (Graves et al. 2021)], woody goldenrod [*Chrysoma pauciflosculosa* (Michx.) Greene] (Miller et al. 2018), wild coffees [*Psychotria* spp. (Young et al. 2022)], and sweet acacia [*Vachellia farnesiana* (L.) Wight & Arn.] (Smith et al. 2022, Xu et al. 2023), as examples. The main challenges encountered for seed propagation of these species and others is the narrow collection windows, limited seed availability, rapid decline in seed viability, low seed vigor, and complex dormancy, which prevent an efficient and reliable production schedule (Davies et al. 2018). Cutting propagation can alleviate some of these challenges and offers other advantages such as enhanced uniformity, faster flowering, and year-round stock plant availability (Singh 2021). Yet, even for landscape plants not intended for restoration purposes, there is a valid concern for the lack of genetic diversity inherit in clonal propagation (Basey et al. 2015). Thus, knowledge of both asexual and sexual propagation techniques for native ornamentals will be useful for their expanded nursery production and use.

The goal of this study was to develop sexual and asexual techniques for the propagation of an attractive, small native tree, wild lime, that exhibits a suite of ornamental and ecological traits meriting its expansion in commercial nursery production. This species has a neotropical distribution including Florida (FL), Texas (U.S. Department of Agriculture Natural Resources Conservation Service 2024), Central and South America, and the Caribbean (Reynel 2017). In Florida's hammocks, wild lime frequently occurs in the central and southern peninsula (Wunderlin et al. 2024) where it grows in filtered understory shade.

Of the citrus family (Rutaceae), wild lime has great morphological interest. This short-trunked species features multi-stemmed, irregularly shaped branches armed with recurved spines under each leaf node (Fig. 1, F), and compound leaves that are alternate and imparipinnate (odd-pinnately compound) with obovate (largest at apex), elliptic, or oblanceolate leaflets (Reynel 2017). Leaflets have crenate margins (Fig. 1, A), are less than 2.5 cm (0.98 in) long, bright green, and glabrous with prickles underneath. The rachis and petiole of leaves are winged, and like other citrus, the foliage has pellucid (semi-translucent) punctate glands containing essential oils. When crushed, these leaves are highly fragrant, having a lime-like aroma (Setzer et al. 2005). Attractive, yellow flowers are dioecious, occurring in axillary clusters (Fig. 1, E) and remaining closed for extended periods of time (Tomlinson 2001). Fruit occurs as follicles that split open when mature to reveal globose seed (Fig. 1 G-H). Plants are frost tolerant [USDA cold hardiness zones 9-11 (U.S. Department of Agriculture 2024)] but can experience extensive damage when exposed to freezing temperatures [< 0 C (32 F)] for longer than 6 hours (Lonard and Judd 1991). Typically reaching 4.6 to 6.1 m (15.1 to 20.0 ft) tall with a similar spread, wild lime is versatile in its landscape use. This densely foliated species is quite adaptable to growing in a variety of soils, has a high drought tolerance, moderate tolerance to salt spray, and can tolerate short periods of brackish-water inundation (FNPS 2024). It can be trained as a shade tree for patio gardens, used as a

specimen in the landscape or in road medians, or easily incorporated into designed wildlife and pollinator gardens.

Despite its many aesthetic and ecological attributes, wild lime has limited commercial availability (FANN 2024) with propagation knowledge lacking. Prior research with the closely related winged prickly ash (*Z. armatum* DC.) by Datt et al. (2017) and Purohit et al. (2015) indicated seeds possess a combination of physical and physiological dormancy. Germination of other *Zanthoxylum* species was also reported to be problematic (U.S. Department of Agriculture Forestry Service 2008). Specific to wild lime, Luera and Gabler (2022) reported seed germination to be low. Commercial nurseries in Florida describe germination to be sporadic, with usage of fresh seeds essential. Alternative methods such as cutting propagation may have merit for this species, but rooting responses are not well known (Phuyal et al. 2018). Thus, to better understand the seed biology and propagation potential of wild lime, a series of four experiments was conducted with specific objectives to: 1) determine the effects of seed treatments (origin, temperature, stratification, and cryopreservation) on germination and 2) determine the optimal auxin concentration required to maximize rooting responses and plant quality.

Materials and Methods

Four experiments were conducted to evaluate the propagation of wild lime. The first three addressed seed dormancy, germination, and preservation, while the fourth experiment explored rooting performance of cuttings treated with and without auxin.

Pre-germination seed viability and effects of seed origin and temperature on germination. On 31 August 2021 and 27 September 2021, wild lime fruit were harvested from a natural population in south Florida (Elliot Key, FL) and a cultivated population located in the butterfly garden of a local business in north central Florida (Gainesville), respectively. Trees from both locations were maturely established, absent of fertilizer or irrigation inputs. Collected follicles were allowed to naturally dehisce to release seeds in paper bags at room temperature [22-25 C (71.6-77 F)] for 2-3 weeks prior to experimentation. A subsample of seeds was examined using an Ultra Focus x-ray system with embryo fill calculated using Faxitron Vision software (U.S. Forest Service National Seed Laboratory, Dry Branch, Georgia, U.S.). Two replicates of 100 seeds were then cut laterally and stained overnight at 37 C (98.6 F) in a 1.0% TZ (2,3,5-triphenyl-2H-tetrazolium chloride) solution in accordance with the Association of Official Seeds Analysts (AOSA) rules for TZ testing (AOSA 2010). Seeds were considered viable when firm embryos stained evenly red under 10× magnification. An additional four replicates of 100 seed from south FL, and 50 seeds from north central were placed in temperature-controlled incubators. Seeds were surface sterilized with a 0.8% sodium hypochlorite solution (10% v:v bleach: deionized water) for 10 minutes, triple rinsed with water, and soaked overnight in sterile deionized (DI) water prior to placing in 10.9 × 10.9 cm (4.29 × 4.29 in) transparent polystyrene germination boxes (Hoffman Manufacturing, Albany, Oregon, U.S.) lined with two 10.16 × 10.16 cm (4 × 4 in) sheets of white blotter paper and one sheet of 10.16 × 10.16 cm (4 × 4 in) unbleached crepe germination paper (Hoffman Manufacturing, Albany, Oregon, U.S.) moistened with approximately 15 mL (0.51 oz) of sterile deionized water (DI). Four replicates of germination boxes were then randomly placed into each temperature-controlled incubator (Percival I30VL, Percival Scientific, Perry, Iowa, U.S.) set to mimic spring [29/19 C (84.2/66.2 F)], summer [33/24 C (91.4/75.2 F)], fall [27/15 C (80.6/59 F)] or winter [22/11 C (71.6/51.8 F)] alternating temperatures in Florida (Perez and Kettner 2013). Light was provided in a 12-hour photoperiod by cool white, fluorescent lights (General Electric Co., Boston, Massachusetts, U.S.) delivering 62.28 $\mu mol \cdot m^{-2} s^{-1}$ (428.2 ft-c) for 12 hours at shelf level. Approximately 5 mL (0.17 oz) of sterile deionized (DI) water was added to each germination box as needed to maintain moisture. Germination was checked once a week for 8 weeks and recorded as the first sign of radical emergence.

Seed imbibition. Using the same seeds collected from north central FL, a seed imbibition study was conducted using four replicates of 25 seeds subjected to one of three treatments. The first subsample of seeds was mechanically scarified using coarse grit sandpaper, weighed, and then soaked overnight in DI water. The second set of seeds was also mechanically scarified but soaked in 500 $mg \cdot L^{-1}$ (0.07 $oz \cdot gal^{-1}$) gibberellic acid (GA_3) for 24 hours. The third set of seeds served as the control and was only soaked overnight in DI water. Dry mass (M_0) of each replicate was recorded prior to soaking and then in 12-hour increments ($M_{1)}$ until the experiment ended after 336 hours (2 weeks). Increase in fresh mass was calculated using the following equation: $[(M_1 - M_0)/M_0] \times 100$ according to Heather et al. (2010).

Effects of GA_3 and kinetin on seed germination. Using the same seeds collected from north central FL, on 21 Oct 2021 four replications of 50 seeds were surface sterilized with a 0.8% sodium hypochlorite solution (10% v:v bleach: deionized water) for 10 minutes, and triple rinsed with water. Seeds were placed in GA_3 and kinetin at one of two different rates [200 $mg \cdot L^{-1}$ (0.03 $oz \cdot gal^{-1}$) GA_3 + 100 $mg \cdot L^{-1}$ (0.01 $oz \cdot gal^{-1}$) kinetin, or 400 $mg \cdot L^{-1}$ (0.05 $oz \cdot gal^{-1}$) GA_3 + 200 $mg \cdot L^{-1}$ (0.03 $oz \cdot gal^{-1}$) kinetin] or left as a non-treated control and placed in sterile deionized water. Seeds were soaked overnight in their respective solutions prior to placement in germination boxes as explained in the first experiment, then randomly placed into a temperature-controlled Percival incubator set to mimic spring [29/19 C (84.2/66.2 F)] with light provided in a 12-hour photoperiod delivering 62.28 $\mu mol \cdot m^{-2} s^{-1}$ (428.2 ft-c) for 12 hours at shelf level. Germination was checked once a week for 6 months and recorded as the first sign of radical emergence.

Seed cryopreservation. Seeds from the north central Florida origin (7 weeks post collection) were placed in a desiccator with silica gel desiccant beads on 12 November 2021 for 72 hours to determine initial moisture content. After 72 hours, four replicates of 50 seeds each were subjected to five different cryopreservation treatments and included a non-treated control. The control was not immersed in a plant vitrification

solution 2 (PVS2) nor liquid nitrogen (LN). Cryopreservation treatments consisted of seed 1) immersed in PVS2 but not LN, 2) immersed in LN [-90 C (-130 F)] but not PVS2, 3) immersed in PVS2 followed by LN, 4) pre-cooled with ice prior to immersion in PVS2 and then LN, and 5) pre-cooled with ice, immersed in a solution of PVS2 plus 1.0% phloroglucinol (PG) followed by LN. Both the control and the PVS2 only (treatment 1) were held at room temperature [24 C (75.2 F)] for 72 hours. PVS2 was comprised of 30 mL (1.01 oz) glycerol (Thermo Fisher Scientific Corp. Waltham, Massachusetts, U.S.), 15 mL (0.51 oz) ethylene glycol (Thermo Fisher Scientific Corp.), 15 mL (0.51 oz) dimethyl sulfoxide (DMSO, Sigma-Aldrich Corp. St. Louis, Missouri, U.S.), in 40 mL (1.35 oz) ½ strength MS media (Murashige and Skoog 1962) with 14% sucrose at ~pH 5.7 as described by Vendrame et al. (2007). After 72 hours in LN (treatments 2-5) cryotubes were removed and rapidly reheated in a water bath held at 39.7 C (103.46 F). All seeds were rinsed, surface sterilized with a 0.8% sodium hypochlorite solution (10% v:v bleach: DI water) for 10 minutes, and triple rinsed with water. Seeds were then soaked overnight in DI water prior to placement in germination boxes as explained in the first experiment, then randomly placed into a temperature-controlled Percival incubator set to mimic spring [29/19 C (84.2/66.2 F)] with light provided in a 12-hour photoperiod delivering 59.58 $\mu mol \cdot m^{-2}s^{-1}$ (409.6 ft-c) for 12 hours at shelf level. Germination was checked once a week for 6 months and recorded as the first sign of radical emergence.

Effects of IBA concentration on rooting performance of softwood cuttings. A cutting experiment was conducted on 31 Aug 2022 using wild lime stock plants finished in 11.4 L (3 gal) plastic containers. Stock plants were maintained in full sun and fertigated regularly (typically 3 times a week) with a 150 $mg \cdot L^{-1}$ (0.02 $oz \cdot gal^{-1}$) 15N-5P-15K liquid solution (Peters Excel Cal-Mag Special; IC Specialty Fertilizers Co., Dublin, Ohio, U.S.). Softwood cuttings with a semi-hardwood base were collected from the terminal branches of the stock plants in the morning and stored in a cooler prior to sticking. Cuttings with 4-6 nodes [about 15 cm (5.91 in) long] were wounded below the basal node with 3-4 leaves kept towards the apex. The basal end of cuttings was dipped in DI water only (control) or water and then treated with a commercial talc-based auxin rooting hormone (Hormex, OHP Inc., Mainland, Pennsylvania, U.S.) containing IBA in preformulated concentrations of 3,000 $mg \cdot kg^{-1}$ (ppm) or 8,000 $mg \cdot kg^{-1}$ (ppm). Four cuttings of each treatment were randomized and stuck into each of five cell trays [containing 48 cells 5.0 cm (1.97 in) wide, 5.0 cm (1.97 in) long, 7.0 cm (2.76 in) deep (Dillen Products, Middlefield, Ohio, U.S.)] filled with Promix BK 55 media containing 55% pine fines, 30% peat and 15% perlite (Premier Tech, Quebec, Canada). Each tray (block) was randomly placed along a greenhouse bench where overhead mist was provided every five minutes for 5 seconds (Onset Computer Corp., Bourne, Massachusetts, U.S.). Photosynthetically active radiation (PAR) in the greenhouse at the level of the cuttings was recorded as 241.80 $\mu mol \cdot m^{-2}s^{-1}$ (1,213 ft-c) with an LI-250A Light Meter (LI-COR Biosciences, Lincoln, Nebraska, U.S.). The mist house was set to cool at 23.9 C (75.0 F) and heat at 18.3 C (64.9 F).

Each of the 60 cuttings (4 cutting replicates × 3 treatments × 5 blocks) were observed once a week for the presence or absence of roots. When root initiation began, liquid fertilizer was applied twice a week [150 $mg \cdot L^{-1}$ (0.02 $oz \cdot gal^{-1}$) Peters Special 15N-5P-15K]. At 5 weeks, overhead misting was reduced to 5 seconds every thirty minutes and cuttings were top-dressed with 0.61 g (0.02 oz) of slow-release fertilizer [Osmocote 3–4-month 14N-14P-14K (ICL Specialty Fertilizers Co., Dublin, Ohio, U.S.)]. Nine weeks after sticking, cuttings were removed from the mist house and the overall root quality was visually assessed using a scale from 0 to 4 as described by Smith et al., (2022) where 0 = dead; 1 = alive but no roots; 2 = roots present and holds little to no media; 3 = holds some but not all media, and 4 = well-formed root ball that holds most of the plug medium when removed from the tray. Roots were then gently washed, final rooting percentage was calculated per treatment per block, and the length and mean of the longest two roots were recorded.

Statistical analysis. Seed germination data from the origin and cryopreservation experiments was analyzed using generalized linear model procedures with a binomial distribution function and a logit link function as implemented in SAS® PROC GLIMMIX (SAS/STAT 14.1; SAS Institute, Cary, North Carolina, U.S.). Seed origin, Season, Time (DAS) and all two-and three-way interactions were treated as fixed effects.

Seed germination data from the pre-hormone and cryopreservation studies were analyzed using Generalized Non-Linear Model procedures in SAS® PROC NLMIXED (SAS/STAT 14.1, SAS Institute, Cary, North Carolina, U.S.) through a 3-parameter logistic growth model:

$$Proportion\,germinated = \frac{c}{\left(1 + Exp(-a \cdot (DAS - b))\right)}$$

where a = growth rate, b = inflection point, c = asymptotic final germination, and DAS = days after the start of the experiment. Monthly means were predicted from the fitted curve and treatments and regression parameters compared using pairwise t-tests. For the cryopreservation experiment, treatment, month after treatment and their interactions were the fixed effects. Least squares means were compared using the Least Significant Difference (t-test) approach without any adjustment for multiple comparisons based on the recommendations made by Milliken and Johnson (2009) and Saville (2015). Germination proportions (95% CI) were transformed to percentages post analysis.

For the cutting experiment, treatment effects of rooting quality, primary root number, and root length were analyzed using an analysis of variance (ANOVA) performed within SAS. If significant, means were separated using appropriate tests. Root quality was assessed on a Likert-type scale and thus the multinomial distribution with a cumulative logit link as implemented in SAS® PROC GLIMMIX (SAS/STAT 14.1) is appropriate.

Results and Discussion

Results presented herein describe the use of two seed origins, four temperature treatments, two pre-hormone treatments,

Table 1. **Initial embryo fill prior to germination tests was calculated using x-ray software to non-destructively determine the presence or absence of embryos in the seed; viability was based on 24 hours tetrazolium (0.1%) staining of laterally cut seeds using a subsample of wild lime (*Zanthoxylum fagara* L. Sarg.) seeds collected from north central and south Florida. Least squares germination means are presented with 95% confidence limits in parentheses. They were generated from a generalized linear mixed model analysis using a binomial distribution function with a logit link 35, 45 and 55 days after the start of the experiment. Experiments were conducted in incubators set to mimic summer [33/24 C (91.4/75.2 F)], spring [29/19 C (84.2/66.2 F)], fall [27/15 C (80.6/59.0 F)] and winter [22/11 C (71.6/51.8 F)] seasons.**

	Percent germination Days after start (DAS)		
	35	**45**	**55**
North central FL			
Spring	23.9 (16.9, 32.8)[z]a	28.2 (19.2, 39.3)a	28.9 (20.9, 38.4)a
Summer	26.0 (18.0, 35.9)a	37.6 (26.5, 50.2)a	32.9 (23.8, 43.5)a
Fall	24.0 (16.7, 33.3)a	34.1 (23.8, 46.2)a	41.1 (31.4, 51.6)a
Winter	7.6 (4.5, 12.7)b	8.9 (4.9, 15.7)b	10.7 (6.5, 17.0)b
	Embryo fill = 90%	Initial viability = 86%	
South FL			
Spring	56.0 (50.7, 61.2)b	61.3 (56.6, 65.7)b	62.0 (56.7, 67.0)b
Summer	21.3 (17.1, 26.2)c	26.5 (22.3, 31.1)c	30.2 (25.1, 35.9)c
Fall	67.5 (61.4, 73.0)a	70.3 (65.0, 75.0)a	71.2 (65.4, 76.4)a
Winter	52.4 (47.2, 57.6)b	56.2 (51.6, 60.7)b	59.3 (54.1, 64.2)b
	Embryo fill = 98%	Initial viability = 87%	
Source	P > F		
Origin	<0.0001		
Season	<0.0001		
Origin*Season	<0.0001		
DAS	<0.0001		
Origin*DAS	0.3891		
Season*DAS	0.6921		
Origin*Season*DAS	0.2954		

[z]Means within origin and time (DAS) followed by the same letter are not statistically different at $\alpha = 0.05$.

and four long term storage treatments used to propagate wild lime by seed, and the use of three auxin formulations for production of wild lime cuttings.

Seed viability and effects of origin and temperature on seed germination. Seeds from both origins (north central and south FL) had similarly high embryo fill (90-98%) and pre-germination viability (86-87%), revealing that both seed populations were of similar quality at the start of experimental treatments (Table 1). Significant effects of origin ($P \leq 0.0001$), season ($P \leq 0.0001$), and time [Days after start (DAS)] ($P \leq 0.0001$) were observed for seed germination of wild lime (Table 1) as well as their origin * season interaction ($P \leq 0.0001$). The interactions of origin * DAS, season * DAS, and origin *season *DAS were not significant, revealing that seeds from each origin responded similarly to season and time. Seeds collected from north central Florida had similar germination responses to spring, summer and fall temperatures 35, 45, and 55 DAS. At 35 DAS, mean seed germination in these seasons ranged from 23.9 to 26.0%, that was 3.2 times greater than germination in the winter season (Table 1). At 45 DAS, mean seed germination in spring, summer and fall ranged from 28.2 to 37.6%, that was 3.7 times greater than germination in the winter season. At 55 DAS, mean seed germination in spring, summer and fall ranged from 28.9 to 41.1%, that was 3.2 times greater than germination in the winter season (Table 1). Seeds collected from south Florida also had similar responses to temperature at 35, 45, and 55 DAS, but greatest germination was observed in the fall season and least germination was observed in the summer season (Table 1). At 35 DAS, 67.5% germination was reached in the fall that was 1.3 times greater than spring or winter, and 3.2 times greater than summer. At 45 DAS, 70.3% germination was reached in the fall that was 1.2 times greater than spring or winter, and 2.7 times greater than summer. At 55 DAS, 71.2% germination was reached in the fall that was 1.2 times greater than spring or winter, and 2.4 times greater than summer.

Effects of a pre-hormone treatment on germination. One month after soaking with two different concentrations of GA_3 and kinetin, germination ranged from 10.1% to 26.7% among treatments (Table 2). The greatest germination response was observed with seeds treated with 400 mg·L^{-1} (0.05 oz·gal^{-1}) GA_3 + 200 mg·L^{-1} (0.03 oz·gal^{-1}) kinetin, followed by the 200 mg·L^{-1} (0.03 oz·gal^{-1}) GA_3 + 100 mg·L^{-1} (0.01 oz·gal^{-1}) kinetin seed treatment. After an additional month, germination of non-treated seeds was low (23.2%), whereas the germination of GA_3 plus kinetin treated seeds were similarly improved (30.4 to 31.1%) (Table 2). After 3 months, germination percentage continued to be similar between hormone treatments (30.4 to 32.8%) compared to non-treated seeds (26.7%). This trend remained for an additional 3 months, where final germination of treated seeds was 1.2 times greater than germination of non-treated seeds.

The number of days it took for seeds to reach 50% of their final germination (T50FG) ranged from 21 to 38 days (Table 2). Seeds treated with the higher 400 mg·L^{-1} (0.05 oz·gal^{-1}) GA_3 + 200 mg·L^{-1} (0.03 oz·gal^{-1}) kinetin solution took the least amount of time (21 days) to reach T50FG compared to

Table 2. **Mean germination percentage with 95% confidence intervals in parentheses, and rate (T50FG) of hormonally pre-treated [seeds were soaked for 24 hours in gibberellic acid (GA_3) and kinetin at two rates] and a non-treated control for wild lime (*Zanthoxylum fagara* L. Sarg.) seeds placed in incubators set at 29/19 C (84.2/66.2 F) with a 12-hour photoperiod for 6 months.**

	Germination percentage (%)		
Month after treatment	**Control (non-treated)**	**200 mg·L^{-1} GA_3 + 100 mg·L^{-1} kinetin**	**400 mg·L^{-1} GA_3 + 200 mg·L^{-1} kinetin**
1	10.1 (8.6, 11.5)[z]c	13.5 (11.5, 15.6)b	26.7 (24.1, 29.3)a
2	23.2 (21.2, 25.1)b	31.1 (29.6, 32.5)a	30.4 (29.2, 31.5)a
3	26.7 (25.4, 28.0)b	32.8 (31.4, 34.2)a	30.4 (29.2, 31.6)a
4	27.1 (25.5, 28.7)b	32.9 (31.4, 34.2)a	30.4 (29.2, 31.6)a
5	27.1 (25.5, 28.8)b	32.9 (31.4, 34.2)a	30.4 (29.2, 31.6)a
6	27.2 (25.5, 28.8)b	32.9 (31.4, 34.2)a	30.4 (29.2, 31.6)a
	Number of days until 50% of final germination (T50FG) was reached		
	38b	34b	21a

[z]Means within a row followed by the same letter are not statistically different at $\alpha = 0.05$.

other treatments, followed by the 200 mg·L^{-1} (0.03 oz·gal^{-1}) GA_3 + 100 mg·L^{-1} (0.01 oz·gal^{-1}) kinetin and control treatments that took 34-38 days to germinate (Table 2).

Effects of cryopreservation on seed germination. Within the first month, germination ranged from 8.5% to 15.0%. From month 2 to 6, germination of seeds treated with Ice+PVS2+PG+LN and Ice+PVS2+LN was similar (Fig. 2) and remained significantly higher than all other treatments, including the non-treated control (Fig. 2). No significant difference was found between the control and seed that were placed in PVS2 only for 72 hours, LN for 72 hours, or PVS2 then LN for 72 hours.

Effects of talc IBA concentration on rooting of softwood cuttings. After 9 weeks under mist, cutting survival was 80% [0 mg·kg^{-1} (ppm) IBA], 95% [3,000 mg·kg^{-1} (ppm) IBA] and 90% [8,000 mg·kg^{-1} (ppm) IBA]. Among auxin treatments, the percentage of softwood cuttings that rooted was 71.2% [0 mg·kg^{-1} (ppm) IBA], 86.3% [3,000 mg·kg^{-1} (ppm) IBA], and 91.0% [8,000 mg·kg^{-1} (ppm) IBA] with a nonsignificant difference ($P = 0.3076$) (Table 3). Significant auxin responses were found for the number of primary roots ($P = 0.0005$) and the average root length ($P = 0.0104$). The greatest number of primary roots was observed for cuttings treated with 8,000 mg·L^{-1} (1.07 oz·gal^{-1}) IBA (6.57), followed by 3,000 mg·L^{-1} (0.40 oz·gal^{-1}) IBA (4.78), and then

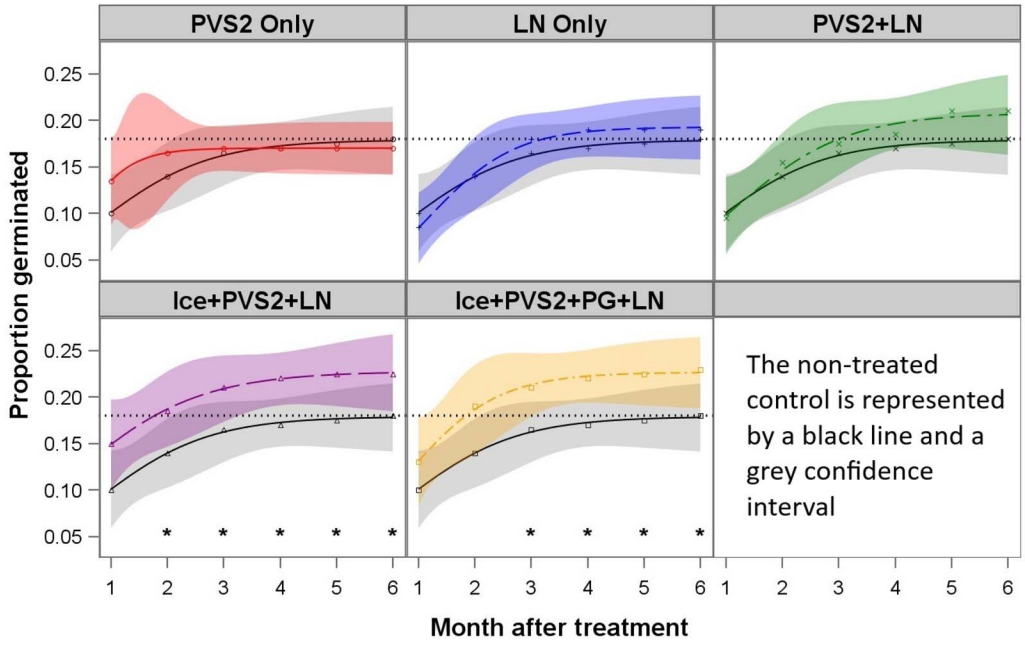

Fig. 2. **The effects of cryopreservation treatments on seed germination of wild lime (*Zanthoxylum fagara* L. Sarg.) compared to the non-treated control. The experiment consisted of a non-treated control and five treatments. The control was not immersed in plant vitrification solution 2 (PVS2) nor liquid nitrogen (LN). Treatments consisted of 1) immersion in PVS2 but not LN, 2) immersion in LN but not PVS2, 3) immersion in PVS2 then LN, 4) pre-cooled on ice, immersed in PVS2 and then LN, and 5) precooled on ice, immersed in a solution of PVS2 plus 1.0% phloroglucinol (PG) then LN. Following treatments, seeds were placed in germination boxes at 29/19 C (84.2/66.2 F) for 6 months. Mean germination is presented and approximate 95% prediction intervals estimated from a 3-parameter logistic growth mixed model as implemented in SAS® PROC NLMIXED (SAS/STAT 14.1; SAS Institute, Cary, NC). The dotted horizontal reference line indicates the maximum germination for the non-treated control. Asterisks indicate treatment means differ statistically meaningful from the non-treated control at $\alpha = 0.05$.**

Table 3. **Effect of talc-based rooting hormone indole-3-butyric acid (IBA) at 0, 3,000, or 8,000 mg·kg^{-1} (ppm) on the primary root number, root length (mm), rooting percentage, and rooting quality of softwood cuttings of wild lime (*Zanthoxylum fagara* L. Sarg.) stuck on 31 Aug 2022 and placed on a mist bench for 9 weeks. Confidence intervals for means are provided in brackets. For each response, means within a column followed by the same letter are not statistically different at $\alpha = 0.05$.**

IBA conc. (mg·kg^{-1})	Primary root [z] number	Root [y] length (mm)	Percentage with roots	Percentage with root quality rating of 4[w]
0	3.21 (2.33, 4.39)c[x]	89.55 (59.69, 119.40)b	71.2 (61, 89)a	17 (5, 46)b
3,000	4.78 (3.73, 6.14)b	119.15 (101.02, 137.27)a	86.3 (56, 97)a	56 (29, 80)a
8,000	6.57 (5.25, 8.22)a	130.40 (112.25, 148.56)a	91.0 (61, 98)a	71 (41, 90)a
DenDF	46	21.3	8	3
Fvalue	9.05	5.71	1.37	8.97
ProbF	0.0005	0.0104	0.3076	0.0542

[z]Only primary roots originating from the basal end of the cutting were counted and included in the analysis, secondary roots were excluded.

[y]Rooting lengths of the 2 longest roots were averaged. Root length can only be measured for cuttings with roots, hence, live stems without roots were not included in analysis.

[w]Proportion of cuttings with a maximum root quality rating of a 4 indicates roots completely held the medium and were ready for transplanting.

[x]Means within a column followed by the same letter are not statistically different at $\alpha = 0.05$.

0 mg·L^{-1} (0 oz·gal^{-1}) IBA (3.21) (Table 3). Root length was similar among 3,000 and 8,000 IBA treatments, and 1.4 times longer than non-treated cuttings. A small amount (17%) of the control cuttings were able to reach the maximum root quality of 4. More than half of cuttings (56%) treated with 3,000 mg·kg^{-1} (ppm) IBA produced sufficient roots that completely held the medium (designated by a root quality rating of 4). Almost three quarters of the cuttings (71%) treated with 8,000 mg·kg^{-1} (ppm) IBA achieved the maximum root quality rating of 4. While this study was limited to a 9-week production time, it is probable that higher visual quality ratings would be reached with more time.

In summary, our results suggest that *Z. fagara* seeds imbibe normally (data not shown) and are not physically dormant, but a portion of the seeds do possess physiological dormancy. The first experiment determined that seeds of this species were capable of germinating at rates that would meet commercial standards (≥70%) when sourced from the south Florida population and sown in the fall [27/15 C (80.6/59.0 F)]. However, when seeds were sourced from a cultivated population in north central Florida, much lower germination was reached, despite seeds having moderate pre-germination viability (potential for germination). The effect of temperature (season) on germination was also less dramatic in seeds collected from north central FL, where only the winter treatment resulted in a significant reduction of germination. Population effects on germination of native species are not uncommon (Baskin and Baskin 2014). As examples, Campbell-Martınez et al. (2022) collected seeds from three different squareflower [*Paronychia erecta* (Chapm.) Shinners] coastal ecotypes and found one population to have 11% less germination than the other two populations. Likewise seeds of coastalplain honeycombhead [*Balduina angustifolia* (Pursh) B. L. Rob.] had 28% higher germination from one population than the other (Campbell-Martınez et al. 2021). In the current study, it is probable that the south Florida natural population was more fit than the north central cultivated population (i.e., robust number of plants with long term survival, growth and reproductive success). It is also noteworthy to mention that while vouchered in 29 counties in Florida with an emphasis in the southern region (Wunderlin et al. 2024), wild lime does not naturally occur in the northern part of the state, and thus the north central population was in the periphery of its growing range.

Although outside of the scope of this study, photoperiod may or may not have affected germination of this species. We used a standard 12-hour photoperiod to germinate seeds of wild lime, consistent with AOSA guidelines and our former studies. Other species of Rutaceae such as mountain rue [*Ruta montana* (L.)], pink lime-berry [*Clausena excavata* (Burm f.)], and white confetti bush [*Coleonema album* (Thunb.) Bartl. & H.L. Wendl)] illustrate varying germination responses depending on temperature and photoperiod, or the combination thereof. Mountain rue (*Ruta montana*) for example, germinates best in continuous darkness (81.66%) compared to alternating light and darkness (60%) (Bendahoua et al. 2023). However, photoperiod showed no significant effect on germination (∼98% between both light regimes) of pink lime-berry (Vieira et al. 2010), and white confetti bush displayed higher germination in dark conditions at a constant temperature of 15.0 C (59.0 F), but germination improved significantly when seeds were exposed to a 16-hour photoperiod with varying temperatures (Fajinmi 2012).

Results from the second experiment revealed seed germination of wild lime responded favorably when pretreated with gibberellic acid plus kinetin, suggesting seeds of this species possess non-deep physiological dormancy. The positive response of hormonal pre-treatments is consistent (but less pronounced) with findings from the closely related winged prickly ash (where seeds treated with 200 mg·L^{-1} (0.03 oz·gal^{-1}) GA_3 + 100 mg·L^{-1} (0.01 oz·gal^{-1}) kinetin had nearly 6 times higher germination than non-treated seeds (Datt et al. 2017).

The last seed experiment revealed initial moisture of wild lime seeds to be less than 10%, suggesting they are tolerant of maturation drying and orthodox, a benefit to commercial propagation (Davies et al. 2018). Moreover, seeds were tolerant of various cryopreservation treatments, indicating their suitability for long term germplasm storage. For application to the nursery industry, future studies are warranted to determine the effects of timed cold and

room temperature storage on seed viability to offer more precise guidelines on seed storability.

Our results from the cutting experiment indicate that cutting propagation of wild lime may be a worthwhile alternative to seed propagation when needed. Softwood cuttings with a semi-hardwood base treated with 3,000 or 8,000 $mg \cdot kg^{-1}$ (ppm) talc-based IBA will have better rooting percentages (86.3-91%) and quality than non-treated cuttings (71.2%). These positive rooting responses are within the minimal 70-80% rooting percentages considered acceptable by some specialty native nurseries (Lubell and Brand 2018) and consistent with that reported for a closely related Chinese pepper tree (*Z. beecheyanum* K. Koch) where 89-100% rooting was achieved with only 1500 $mg \cdot kg^{-1}$ (ppm) IBA (El-Banna et al. 2024). It should be noted that the authors observed significant rooting failure (<29%) in prior experiments sticking cuttings of different maturity, length, season, auxin type and stock plant source. Thus, cutting propagation of this species is not as versatile as others that require less specific methodology. Further, since there was a population effect on seed germination, future cutting experiments that utilize stock plants of different populations as a main effect may be worthwhile.

Results presented herein suggest wild lime to be an ideal candidate for nursery propagation by seeds or cuttings. Seeds can be collected in the fall and germinated soon after or stored for early spring. Depending on the population, seeds may experience reduced vigor and/or non-deep physiological dormancy, which can be naturally overcome in dry storage or by use of gibberellic acid and kinetin. Maximum germination can be achieved between one and two months. When seeds are not available or a shorter production cycle is needed, cutting propagation is possible. Optimal rooting percentage and quality can be achieved by collecting softwood cuttings with a semi-hardwood base from healthy stock plants, wounding the basal end, and applying talc based IBA at moderate to high concentrations. Finished liners can be obtained within a 7 to 9-week production cycle under mist.

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