Effects of temperature, seed provenance, and substrate on germination of the endemic and threatened Paper nailwort (*Paronychia chartacea* ssp. *chartacea*)

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Abstract Paper nailwort (*Paronychia chartacea* ssp. *chartacea*) is an endemic and threatened herb restricted to central Florida. There is limited information on seed propagation for this species. We tested the effects of temperature [22/11 °C (winter), 27/15 °C (spring), 29/19 °C (fall), and 33/24 °C (summer)], substrate (filter paper vs native soil), provenance (two collection locations), and time in dry storage on seed germination in three experiments. Germination was generally higher in cooler temperatures (∼50-70%; winter or spring) compared to warmer temperatures (∼0-25%; fall and summer), with overall germination highest at the spring temperature. We also found that germination increased up to 94% for seeds at fall and summer temperatures when they were transferred to winter temperatures at 32 days (i.e., warm stratification improved germination), and increased from 1% to 24% when seeds were sown at 15/5 °C and transferred to 27/15 °C. No significant differences were found between substrate or provenance. Overall germination rates increased over time in dry storage. These data indicate seeds of paper nailwort are non-dormant, non-deep physiologically dormant, or a combination of the two, prefer cooler to warmer temperatures for germination overall, prefer a warm pretreatment, and can be dry stored for at least four months.

Keywords Lake Wales Ridge, dormancy, seed propagation

Introduction

Paper nailwort (*Paronychia chartacea* ssp. *chartacea*) is a gynodioecious annual to short lived perennial herbaceous groundcover species (Hawkes 2004, Schafer et al. 2013). It is endemic to 5 counties in central-Florida along the Lake Wales Ridge and is listed as a state-endangered and federally-threatened species (Christman and Judd 1990, USDA NRCS 2021, Wunderlin et al. 2021). Paper nailwort occurs in open gaps within dry (xeric) ecosystems including Florida rosemary scrub, cleared sandhills, and in disturbed roadside areas of scrub (Hawkes 2004, Schafer et al. 2013) and occurs at higher densities in recently burned sites via seed recruitment compared to unburned sites (Johnson and Abrahamson 1990). Paper nailwort is

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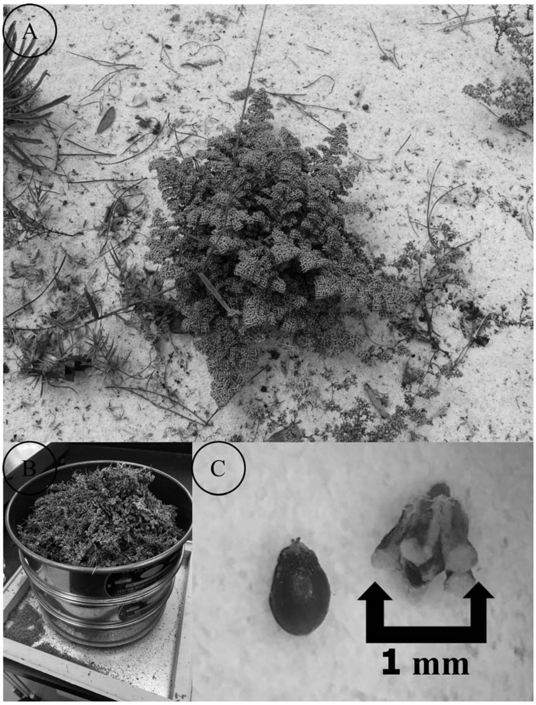

Figure 1A-C. Whole plant of Paper nailwort (*Paronychia chartacea* ssp. *chartacea*) during peak flowering (Figure 1A). When fruits were mature, plant material was collected and processed to isolate fruit from debris (Figure 1B). Single fruit (utricle containing a single seed; left) and utricle covered with persistent calyx (right) is shown (Figure 1C).

under threat due to >80% of its habitat loss to conversion to residential development and citrus groves (Peroni 1985a, 1985b).

Paper nailwort flowers profusely in the summer and early fall (Figure 1A). Highly divided terminal cymes have many, small 1.5 to 2.0 mm, white flowers. The fruit is an utricle ovoid in shape. Seeds of paper nailwort are approximately 0.5 mm wide and 1mm long and are covered by a persistent calyx (Figure 1B and C). Seeds mature during the fall and are dispersed by gravity and possibly by wind, rain, and ants (Petru and Menges 2003, Hawkes 2004, Stephens et al. 2012). In a one-year field experiment, emergence of seedlings in the wild primarily occurred in recently burned (<8 years) areas more than in areas with a longer burn frequency, away from shrubs compared to near shrubs, and survival of emerged seedlings was ~50% after a one-year field experiment (Hawkes 2004). Survival of emerged seedlings was similar in another study within scrub gaps (Petru and Menges 2003). In greenhouse experiments, emergence was improved by the presence of organisms in soil biocrusts (~80%) compared to the absence of organisms in soil biocrusts (~30%) and emergence was higher under wet conditions compared to dry conditions for seeds collected in the fall and sown shortly thereafter (Hawkes 2004). Seedling recruitment was higher in roadsides compared to Florida rosemary scrub but had higher seed production in Florida rosemary scrub compared to roadsides (Schafer et al. 2013). Others have reported low germination (11%) after 90 days when seeds were placed at 23 °C under a 16-hour photoperiod (McKently and Adams 1994). Application of GA (5, 50, and 100 ppm) did not increase germination compared to a control (~60% germination) when placed in a growth chamber with day/night temperatures of 29/21 °C for seeds collected in the fall, dry stored in a fridge (4 °C) and sown in the spring (Stephens et al. 2012). However, it remains unclear whether seeds of paper nailwort possess non-deep physiological dormancy which may have been alleviated after several months in storage (Baskin and Baskin 2014) within the Stephens et al. (2012) study.

Seed dormancy characteristics and the effects of temperature and seed provenance remain largely unknown for paper nailwort. Seeds collected from 3 provenances in northwest Florida of the closely related squareflower (*P. erecta*) were non-dormant, displayed high germination (94-99%) at 22/11, 27/15, and 29/19 °C but this was reduced to 27% at 33/24 °C (Campbell-Martínez et al. 2021). Here we test the effects of temperature, seed pretreatments (e.g., cold and warm stratification), and substrate on germination for seeds collected from 2 provenances of paper nailwort. We hypothesized a reduction in germination at higher temperatures compared to lower temperatures, seeds do not require pre-treatments, different germination by substrates, and similar germination across provenances.

Materials and Methods

Fruits (utricles) of Paper nailwort (*Paronychia chartacea* ssp. *chartacea*) were randomly collected on 9 Oct. 2018 from two wild provenances in rosemary scrub areas located in the Lake Wales Ridge of central Florida. The first provenance of about 321 plants was located within the Hickory Lake Scrub Preserve (HLSP). About 6 miles east-northeast of HLSP, the second provenance of about 300 plants was located within the Lakes Wales Ridge State Forest (LWSF). Fruits were collected from plants with yellow to brown fruiting stems and were harvested from <20% of floral material present per plant. Fruits were commercially cleaned at the Forest Service Seed Extractory (Bend, OR). There seeds were passed through sieves, sorted on a single deck vibratory table, and further separated by a continuous seed blower which sorted the material by weight. Seed viability was determined via X-ray analysis and the number of seeds per gram were calculated using standard procedures at the Forest Service Seed Extractory.

A series of three germination experiments were conducted (Experiment 1, 2, and 3) 3.4,13.1, and 19.1 weeks after seed collection, respectively. Seeds were dry stored under ambient laboratory conditions (approximately 24°C) prior to each experiment. Seeds were placed in 11x11x4 cm transparent polystyrene germination boxes (Hoffman Manufacturing, Corvallis, OR) with lids and containing either filter paper or native soil. The native soil was collected in each provenance near where plants were present and was characterized as St. Lucie or Archbold series soils >200cm deep sandy, xeric, upland soils occurring on ancient dunes. Boxes with paper contained one sheet of germination paper on top of one sheet of blotter paper (Anchor Paper Company, St. Paul, MN) moistened with 15 mL of autoclaved, distilled water. Boxes containing native soil were filled to 1 cm and moistened to saturation prior to seeds being placed on the soil surface. Each germination box containing 25 seeds were placed in growth chambers (Percival Scientific, Model I30VL, Perry, IA) with a 12-hr photoperiod [76.08 ± 7.54 μmol ($m^{-2}s^{-1}$)] where warm temperatures corresponded with light. Experimental design was a randomized complete block design for all three experiments. Each rack in the chamber was treated as a block. Germination and disease were monitored 3 times a week and boxes were watered as needed with autoclaved, distilled water. Germination was counted as radicle emergence outside of the calyx. Seeds that germinated or turned soft and mushy or had visible hyphal growth (due to contamination) were removed from the experiment as they occurred.

Experiment 1. On 2 Nov. 2018 an experiment was initiated with a 4 (temperature) × 2 (substrate) × 2 (provenance) full factorial arrangement of treatments using a subset of the seeds (3.4 wks after collecting). Temperatures included Florida seasonal temperatures of 22/11 (winter), 27/15 (spring), 29/19 (fall), and 33/24 °C (summer) described by Pérez and Kettner (2013), and substrate included native soil and filter paper. For each treatment, a total of four replicates of 25 seeds were used for a total of 400 seeds. After 32 days all germination boxes were transferred to the 22/11 °C (winter) growth chamber for an additional 15 days.

Experiment 2. On 9 Jan. 2019 an experiment was initiated with a 2 (temperature) × 2 (provenance) full factorial arrangement of treatments using dry stored seeds (13.1 weeks after collecting). Temperatures included 22/11 and 27/15 °C. For each treatment, a total of four replicates of 25 seeds were used for a total of 100 seeds. Seeds were placed in germination chambers for 28 days on top of filter paper.

Experiment 3. On 20 Feb. 2019 an experiment was initiated with a 3 (temperature) × 2 (provenance) full factorial arrangement of treatments using dry stored seeds (19.1 weeks after collecting). In accordance with the Association of Official Seed Analysts (AOSA, 2003) standard testing procedures, temperatures included 15/5, 27/15, and 35/25 °C rather than the simulated seasonal temperatures of Florida (described by Heather et al., 2010) used in Experiments 1 and 2. For each treatment, a total of four replicates of 50 seeds were used for a total of 300 seeds. After 32 days all germination boxes containing seeds on top of filter paper were transferred to the 22/11 °C growth chamber for an additional 15 days.

Statistical analysis. Main effects and their interactions were analyzed using generalized linear mixed models procedure (PROC GLIMMIX in SAS 9.4). A Kenward-Rogers approximation was used for computing the denominator degrees of freedom for the fixed effects tests. Position in the growth chamber were considered a random effect. Differences between means for significant main effects and interactions ($P \leq 0.05$) were computed using the ilink option of the LSMEANS statement.

Results

X-ray analysis demonstrated that there was high seed fill (87% and 89%) in both provenances s. There were 8,709 and 7,661 live seeds/g for HLSP and LWRSF.

Provenance ($F = 4.24$, $p = 0.0452$), temperature ($F = 139.97$, $p = <0.0001$), and the three-way interaction between provenance × temperature × substrate ($F = 3.85$, $p = 0.0139$) affected final germination of seeds that were sown 3.4 weeks after collection (Figure 2A) in Experiment 1. Seed germination ranged from 0% to 73% among treatments. There was a general trend for higher germination in cooler temperatures of winter and spring, (22/11 and 27/15 °C, respectively) compared to warmer temperatures of fall and summer (29/19 and 33/24 °C, respectively) (Figure 2A). Germination was >50% in cooler temperatures for all treatments except LWRSF seeds placed in soil under winter temperatures (46%) and HLSP seeds placed in soil under spring temperatures (41%). A maximum of 73% germination was achieved with LWRSF seeds (on paper and spring conditions) that was greater than LWRSF seeds (germinated on soil in winter), greater than HLSP seeds (germinated on soil in spring), and greater than HSLP and LWRSF seeds germinated on paper or soil in fall and summer. Regardless of provenance or substrate, germination was similarly low (14 to 25%) for seeds placed under fall conditions and absent (0%) for seeds placed in summer conditions.

Temperature ($F = 41.22$, $p = <0.0001$), substrate ($F = 5.05$, $p = <0.0296$), and the three-way interaction between provenance × temperature × substrate ($F = 3.04$, $p = 0.0383$) affected seed germination when all seeds were subsequently placed in winter temperatures for an additional 15 days (Figure 2B) in Experiment 1. While there was little change for seeds placed initially in cooler temperatures to winter

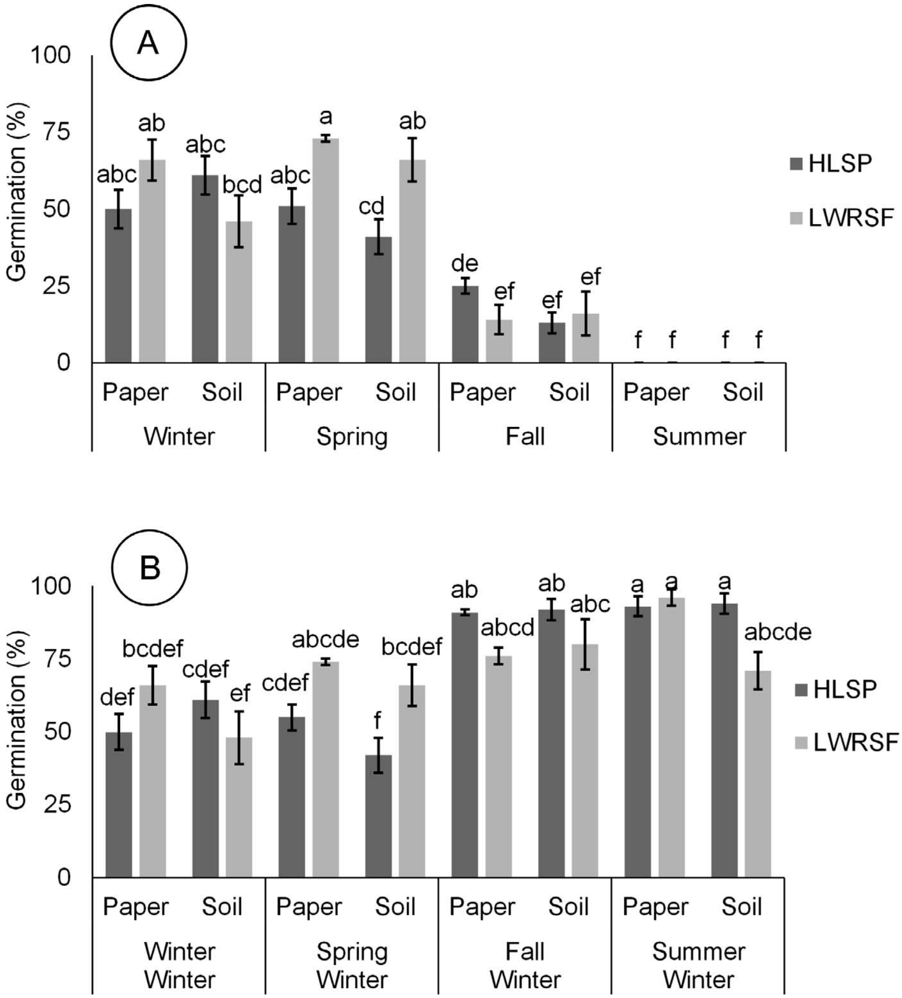

Figure 2A-B. Percent germination (SE) of Paper nailwort (*Paronychia chartacea* ssp. *chartacea*) seeds collected from two central-Florida provenances (HLSP = Hickory Lake Scrub Preserve and LWRSF = Lakes Wales Ridge State Forest). In Experiment 1 seeds (3.4 wks after collecting) were placed on filter paper or native soil in either winter (22/11 °C), spring (27/15 °C), fall (29/19 °C) or summer (33/24 °C) temperatures for 32 days (Figure 2A). Seeds were then transferred to winter chambers for an additional 15 days (Figure 2B). Seeds were placed in a growth chamber set at day/night temperatures with a 12-hr day photoperiod of cool-white fluorescent light corresponding to high temperatures. Significant differences (at $\alpha = 0.05$) for the significant three-way interaction (provenance × temperature × substrate) within each day after seeding ($P = 0.0139$ at 32 days and $P = 0.0383$ at 47 days) are indicated using lowercase letters.

temperature, seeds placed initially in warmer temperatures to winter temperature had increased germination (71 to 96%; Figure 2). Germination was higher for all treatments in summer then winter temperatures (93 to 96%; except for HLSP seeds in soil which had 71%) for seeds in all cooler to winter temperatures (42 to 66%),

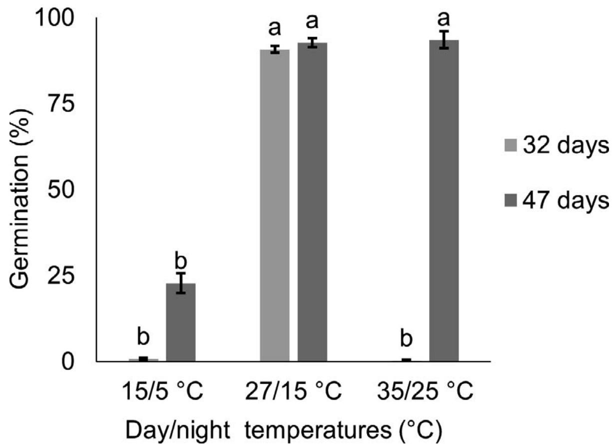

Figure 3. Percent germination (SE) of Paper nailwort (*Paronychia chartacea* ssp. *chartacea*) seeds collected from two central-Florida provenances (HLSP = Hickory Lake State Park and LWRSF = Lakes Wales Ridge State Forest). In Experiment 3, dry stored seeds (19.1 weeks after collecting) were placed in either 15/5, 27/15, or 35/25 °C for 32 days and then transferred to winter chambers for an additional 15 days (47 days). Seeds were placed in a growth chamber set at day/night temperatures with a 12-hr day photoperiod of cool-white fluorescent light corresponding to high temperatures. Germination was compared only within significant differences (at $\alpha = 0.05$) for the significant temperature factor within each day after sowing ($P = <0.0001$ at 32 days and $P = <0.0001$ at 47 days) are indicated using lowercase letters.

except for LWRSF seeds placed on paper in spring followed by winter temperatures (74%; Figure 2).

In Experiment 2, where seeds were dry stored for 13.1 weeks, only temperature affected seed germination ($F = 27.78$, $p = 0.0002$). Germination was higher at simulated winter temperatures compared to spring temperatures (83 ± 3 vs. 58 ± 4) 28 days after sowing (data not shown).

In Experiment 3, where seeds were dry stored for 19.1 weeks, temperature dramatically affected germination ($F=369.17$, $p = <0.0001$) 32 days after sowing (Figure 3). Germination was higher for seeds sown at 27/15 °C (91%) than for seeds sown at 15/5 (1%) or 35/25 °C (1%). However, once all seeds were transferred to 27/15 °C for an additional 15 days (i.e., 47 DAS), germination of initially-treated 35/25 °C seeds was as high (94%) as the germination of the initially-treated 27/15 °C seeds (93%). Seeds initially placed in 15/5 °C still had low germination (23%) after being placed in 27/15 °C.

Discussion

High germination percentages (up to 73%) of untreated (e.g., not stratified or scarified) seed dry stored for 24 days achieved here indicate a large proportion of seeds of paper nailwort are most likely non-dormant or have non-deep physiological dormancy when shed from the mother plant (Baskin and Baskin 2004), confirming our initial hypothesis. This is consistent with this and several other species in Caryophyllaceae (Maschmeyer and Quinn, 1976; Murru and others, 2015; Stephens et al 2012). Likewise, non-dormant or non-deep physiologically dormant seeds have been documented for paper nailwort's congener, squareflower, which do not require seed pretreatments to achieve high germination (94-99%) after 21 days in dry storage (Campbell-Martínez et al. 2021). However, when seeds of paper nailwort were initially placed in warm temperatures (e.g., were warm stratified) and then transferred to more optimal winter temperature conditions, germination was improved greatly. This indicates thermoinhibition of seeds and a small percentage (~20%) of seeds may have non-deep physiological dormancy which can be overcome with warm stratification (Baskin and Baskin 2004).

Germination percentages of paper nailwort were consistently higher at simulated spring and winter temperatures and reduced at warmer temperatures (simulated fall and summer) conforming our initial hypothesis that germination would be reduced at warmer temperatures. This germination pattern is similar to squareflower except that squareflower also had higher germination under simulated fall temperatures and was only reduced at summer temperatures (94 to 99% compared to 27%; Campbell-Martínez et al., 2021). Data from our results suggests seeds of paper nailwort may germinate more readily during the winter or spring after seed dispersal in the fall. This is supported by observations in Stephens et al. (2012) who reported emergence during spring and winter field trials in intact scrub, though higher emergence was observed during the spring trail than the winter field trial (25 vs. 1%). With the exception of the native soil, spring temperature treatment, the effects of provenance on germination were nominal for paper nailwort.

We found high germination without the use of native soils. This contrasts with a study conducted by Hawkes (2004) that found native soils with biocrust increased germination compared to autoclaved soil (~30% vs. ~80% germination after 3 months in a greenhouse) for seeds cold stored for a few months in a refrigerator. It is possible that storage conditions and length in the Hawkes (2004) study induced secondary dormancy in seeds (Baskin and Baskin 2020), that factors related to autoclaving soil inhibited germination, or genetic factors and/or environmental conditions of the mother plants' population resulted in seeds with different dormancy characteristics and/or germination requirements. Future germination studies are needed to understand the role of soil biocrusts on seed dormancy and germination of paper nailwort under natural conditions and to consider the role that gynodioecy may have in seed viability and dormancy.

There was a general trend of higher final germination for seeds initially exposed to warmer temperatures (simulated fall and summer temperatures) and then placed under optimal germination temperatures compared to seeds which remained under optimal germination temperatures throughout the duration of the experiment. As such, warm stratification could prove an effective pre-treatment to maximize seedling performance during restoration projects involving direct seeding or during greenhouse propagation when seed supply is limited (Pedrini et al. 2020). More research should be investigated as to whether this is feasible at scale (e.g., treatment of large amounts of seed at once) and whether increased germination after seed pre-treatment is observed under field and greenhouse conditions.

In summary, our findings suggest that seeds of papery nailwort are orthodox and do not require seed pre-treatments. Seeds prefer cooler temperatures (simulated winter and spring) for germination across provenance and do not require native soils for high germination. Exposure of seeds to warm temperatures (simulated fall and summer) is not detrimental to seeds and may enhance final germination percentages. This finding may be important in light of climate change and suggests the need to further study the influence of temperature fluctuations on seed germination of this and other Lake Wales Ridge endemics. Germination percentages may also be increased by after ripening in dry storage. Data here indicate seeds of paper nailwort should be sown during the winter to spring during greenhouse production and that seeds remain viable in dry storage for at least 4 months.

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