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Genetic diversity & germination of *Amsonia tharpii*, a rare plant species

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Introduction

- Deserts are are diverse ecosystems that are increasingly threatened due to land use changes. Endemic desert plants with small population sizes may go extinct in the near future without proper management.
- *Amsonia tharpii* (Tharp's Bluestar, Apocynaceae) is a herbaceous perennial plant, with narrow heteromorphic leaves, white salverform corollas, fruits that are follicles, and brownish-red, corky cylindrical seeds. Only five populations exist; four from southwestern New Mexico, and one from west Texas (Fig. 1A). The entire distribution where *A. tharpii* occurs has undergone significant land use changes from increased oil and gas well development (Fig. 1B). Population sizes are small for *A. tharpii* and seedling recruitment has not been observed. Because of threats to populations and unknown recruitment this species is a conservation concern.
- *Amsonia fugatei* was described after *A. tharpii* and is another narrowly distributed species from central New Mexico (Fig. 1A). *Amsonia fugatei* and *A. tharpii* are morphologically similar, but differences exist in floral tube length and trichome cover density. To date neither species has been placed into a phylogenetic context raising questions about the validity of *A. fugatei*. Determining species boundaries will help to inform conservation planning.
- This work will help determine how to identify seedlings and germination procedures for *Amsonia tharpii*.

Hypotheses

- Germination will be highest for larger seeds at higher temperatures, specifically 25℃.
- We expect low genetic diversity for *A. tharpii* due to land use change and small population sizes. In addition, we hypothesize that the lone population from Texas will suffer from inbreeding depression.
- We hypothesize *Amsonia fugatei* and *A. tharpii* will be genetically distinct due to differences in floral tube size, which may have an impact on pollinator attraction and effectiveness.

Methods

Germination Experiment

- A total of 100 seeds of each of the 5 extant populations (10 seeds from each of 10 maternal lines) were included. We measured the mass (g) and size (length and width to the nearest mm using digitial calipers) of each seed. Seeds were surface sterilized in a 1% bleach solution for 5 minutes prior to being plated on agar petri dishes, which were then placed into incubators at three day/night (12hr/12hr) temperature treatments (15°C/5°C, 20°C/10°C, and 25°C/15°C).
- Germination was scored daily as the radical emerged.

Genetic Analyses

- A modified CTAB extraction (Doyle and Doyle 1987) was used to extract genomic DNA from silica dried leaf samples. DNA was visualized via gel electrophoresis and quantified with a qubit.
- A modified ddRADSeq library was prepared with 96 samples and sequenced at Northwestern University.

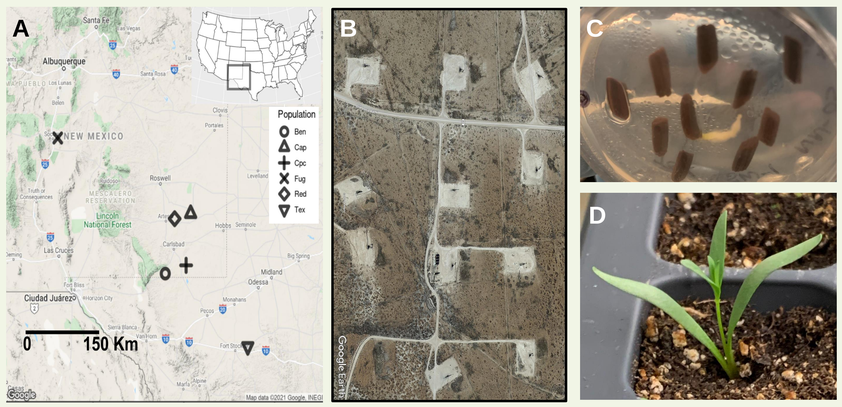


Fig. 1 A) Distribution of *A. tharpii* in NM and west TX. B) Aerial photograph of land use change from oil wells. C) *A. tharpii* seeds on petri dish. D) Seedling.

Results

Fig. 2. Proportion of seeds germinated for each treatment.

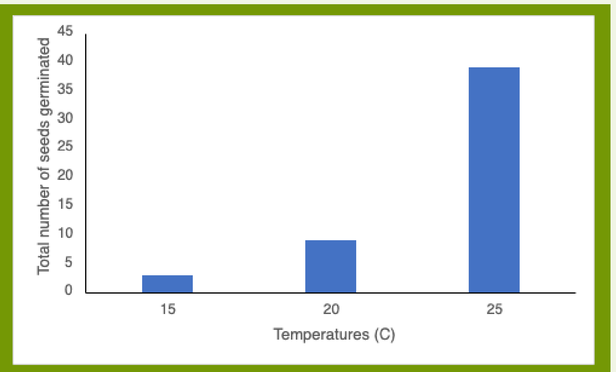


Fig. 3. Between population variation in seed germination at 25℃. Number of samples/population = Ben = 13, Cap = 3, Cpc = 1, Red = 13, Tex = 8.

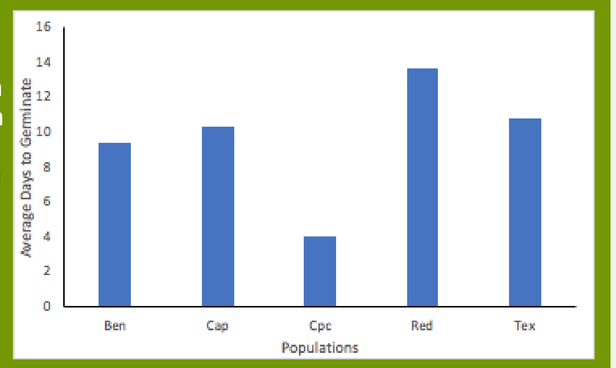
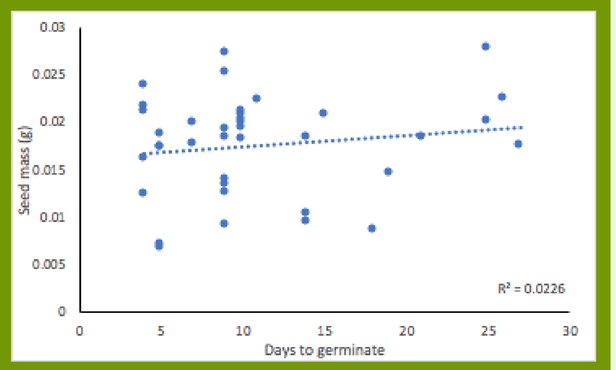


Fig. 4. Scatter plot for seed mass (g) and time to germination (Days). There was a weak correlation (r = 0.15, p = 0.36).



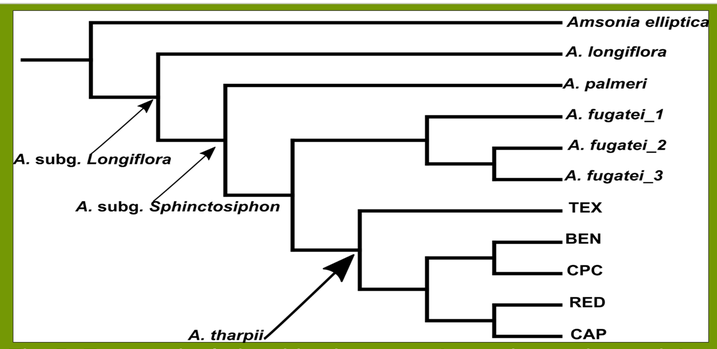


Fig. 5. Expected relationships between *Amsonia* species and *A. tharpii* populations.

Discussion and Conclusion

- Overall germination was low and appears to require high daytime temperatures. Only 51 of 500 (10%; Fig. 2) seeds germinated, and of those, 39 germinated in the 25℃ treatment (Fig. 2).
- The Ben Slaughter population had the highest average seed mass and germination rate, while the Texas population has the second highest germination and third highest average seed mass (Fig. 3). There was no significant differences between germination rate ($p = 0.42$, $F = 0.99$). Texas, although the smallest of populations still produces viable seeds.
- Overall, we found that larger seeds have higher germination rates, but this was weakly correlated (Fig. 4).
- Although *A. tharpii* and *A. fugatei* have similar morphologies, they are not conspecific, but sister species (Fig. 5).

Conservation Implications

- These data and ongoing studies of genetic diversity will help inform species management. Based on the data we can infer that max germination requires seeds to be plated on agar at 25℃ for two weeks, then planted in a two millimeters deep soil bed.
- The genetic data will inform how land use change affects populations over time and will help select which populations are the best for breeding programs because they will be able to tell which is the most genetically diverse.
- Our work will help the Fish and Wildlife Service determine if *A. tharpii* should be listed under the Endangered Species Act.

Acknowledgements

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