



THE UNIVERSITY OF ARIZONA

Cooperative Extension

Maricopa County

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Southwest Vegetation Management Association
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Funded Project Final Report

INVESTIGATING TEMPERATURE TOLERANCE OF STINKNET SEEDBANKS University of Arizona Proposal #50295

Project Summary:

Stinknet (*Oncosiphon pilulifer*) is a cool season growing annual forb recently listed as an Arizona Class B Noxious Weed. Stinknet competes with Arizona's native vegetation. It is unpalatable to wildlife and livestock, and it causes allergies in some people. Dried stinknet fuels wildfire and supports a fire regime in the Sonoran Desert, an ecosystem not adapted to fire.

This study will examine artificial stinknet soil seedbanks placed at Cooperative Extension sites outside the current established range of stinknet in Arizona. The test seedbanks will be exposed to natural weather conditions at the sites. They will be monitored for winter temperature and resulting germination. Controlled germination experiments with stinknet seeds will seek to determine the temperature range suited for stinknet germination, as well as the temperatures required for sterilizing a stinknet seedbank. The study will seek to determine the potential upper elevation limit for stinknet colonization in Arizona through a study of seedbank temperature tolerance.

The project involves:

- Test planters planted with stinknet seed and placed at various Cooperative Extension sites in locations colder and/or higher than Sonoran Desert locations.
- Controlled germination studies.



Test Planter Methods:

In 2023, test planters were seeded with stinknet seed and placed at nine Cooperative Extension sites around Arizona. The test planters were placed in northern and eastern Arizona locations with climates cooler than Phoenix and other stinknet-invaded regions. Control planters were placed in Phoenix, AZ, where stinknet is rampant. Planters were designed to contain stinknet plants and seeds and prevent their escape into the surroundings, while also designed with a screen cover to expose the growing space inside the planter to the ambient environmental conditions at the site.

Stinknet seed used in the planters was collected from sites in the northern Phoenix metro area; SE of Happy Valley Road and N. 19th Avenue (June 2022), Anthem Way (July 2022), and near the I-17 Transfer Station (October 2022). Seed from the three sites was mixed.

Approximately 0.04 grams of stinknet seed (plus chaff) was sprinkled into each planter and watered in as each planter was established.

Each planter was provided with an Elitech RC-5+ Temperature Data Logger Reusable Recorder. Each data logger was set to record for the maximum allowable duration (180 days = 6 months) with a temperature record each 9 minutes. The data logger was enclosed in a ziplock bag and placed in a plastic receptacle, buried slightly below the soil in each planter.

Planters received little or no irrigation after setup. The screen tops allowed precipitation at the site to enter. Planters were monitored for stinknet germination and growth.

Test planters were placed at nine locations plus a control location (Phoenix).

LOCATION	ELEVATION	COORDINATES
Dos Cabezas	4933 ft	N32 09.561 W109 36.954
Duncan	3698 ft	N32 44.088 W109 05.934
Flagstaff, site 1	7056 ft	N35 10.314 W111 40.670
Flagstaff, site 2	6997 ft	N35 13.312 W111 36.855
Holbrook	5096 ft	N34 54.376 W110 09.283
Kykotsmovi	5615 ft	N35 52.819 W110 36.395
Page	4309 ft	N36 54.971 W111 28.015
Phoenix (control)	1125 ft	N33 24.370 W111 59.508
Safford	2959 ft	N32 48.889 W109 40.853
St. Johns	5763 ft	N34 30.726 W109 22.687



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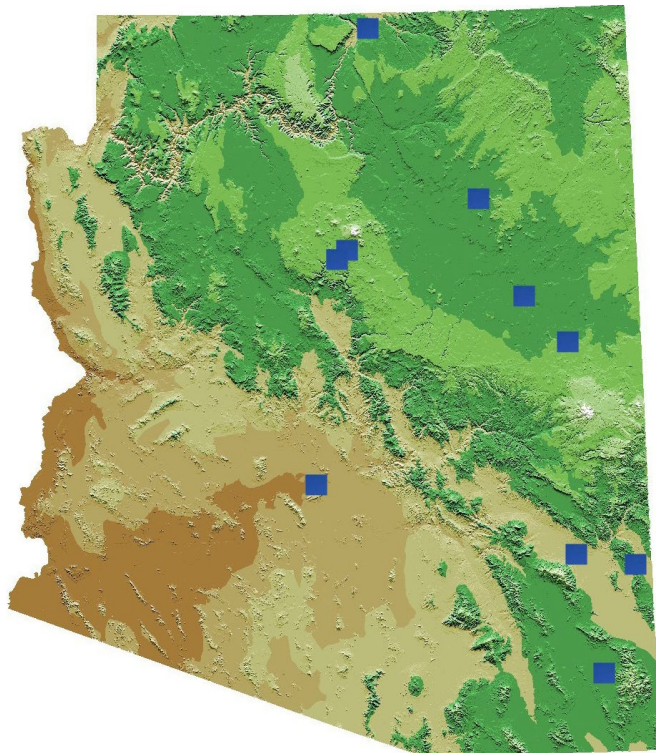
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Map of planter locations:



2023 Test Planter Results:

LOCATION	ESTABLISHMENT	# PLANTERS	GERMINATION	DECOMISSION
Dos Cabezas	1/23/2023	2	NO	12/8/2023
Duncan	1/24/2023	2	NO	12/8/2023
Flagstaff, site 1	3/31/2023	2	NO	10/16/2023
Flagstaff, site 2	3/31/2023	2	NO	10/16/2023
Holbrook	3/8/2023	2	NO	9/5/2023
Kykotsmovi	3/7/2023	1	NO	7/20/2023
Page	3/7/2023	1	NO	10/16/2023
Phoenix (control)	2/17/2023	2	YES	4/3/2023
Safford	1/23/2023	2	NO	12/8/2023
St. Johns	3/8/2023	2	NO	9/5/2023



Early 2023 was characterized by widespread precipitation (arriving as snow in most of the test planter locations). Snow and weather delayed the establishment of planters at higher elevations, therefore stinknet seed was not exposed to an entire winter in these sites. The widespread precipitation resulted in stinknet germination in desert locations in and around Phoenix.

Stinknet germination was observed in the control planters in Phoenix. Stinknet germination was not observed in any of the experimental planters either in the spring of 2023 or summer of 2023. Because the planters were not intensely monitored, it is possible that stinknet germination might have occurred and might not have been noticed if the tiny seedling died from freezing or drying. However, no stinknet plants grew to any noticeable size in any of the 2023 experimental planters.

Stinknet seedlings in the control planters germinated well, but did not grow to maturity. The control seedlings died with drying conditions in April of 2023 corresponding with warming and dry weather at the Phoenix site.

Temperature sensors in the test planters did not operate as desired, but they suggest the planter design may have elevated the daytime temperatures experienced within the planters, and this could have contributed to drying out of the soil in the planters.

2024 Test Planter Results:

Test planters established in 2023 were decommissioned from all northern Arizona locations. Test planters were maintained in the three southeastern Arizona sites, as well as the control planters in Phoenix.

LOCATION	REESTABLISHMENT	# PLANTERS	GERMINATION	DECOMISSION
Dos Cabezas	12/8/2023	2	YES	1 + 1 Ongoing
Duncan	12/8/2023	2	YES	1 + 1 Ongoing
Phoenix (control)	11/30/2023	2	YES	4/30/2024
Safford	12/8/2023	4	YES	1 + 3 Ongoing

Test planters in Dos Cabezas, Duncan, and Safford were re-seeded with stinknet seeds (sourced from Table Mesa Exit on I-17, collected October 2023). Two additional planters were established in Safford.

On February 14, 2024 one planter from each southern Arizona experimental site was retrieved and transported to Tucson. Stinknet germination was observed in each of these planters later in the spring. Additionally, planters retrieved from Kykotsmovi and Flagstaff the prior summer



showed germination of stinknet seed which had been exposed to the previous winter and summer at these sites.

Test planters remaining in Dos Cabezas, Duncan, and Safford showed stinknet germination later during the spring of 2024. Southeast Arizona reportedly experienced more precipitation and milder weather during the spring of 2024 than in the spring of 2023.

Test Planter Discussion:

Stinknet seed was not successful in growing during the spring or summer in northern Arizona test planters during 2023. Stinknet seed was not successful in growing during the spring or summer in southeast Arizona planters during 2023, but did successfully grow in southeast Arizona planters during the spring of 2024.

Stinknet seed was able to grow in planters exposed to winter conditions in Flagstaff, Kykotsmobi, and southeast Arizona when these planters were transported to the Sonoran Desert. Additionally, freezing experiments later during this study showed stinknet seed can survive and germinate after one week at -40°F in a freezer. This indicates stinknet seed can withstand extreme cold and remain viable.

The failure of stinknet germination in many of the test planters is apparently not due to sensitivity of the seed to cold. However, stinknet plants are not as tolerant as seeds. Stinknet seed is tiny, at less than 0.5 mm in length. Seedlings are correspondingly small, and vulnerable to drying out. Late-germinating stinknet seedlings may be killed in dry warm conditions, as demonstrated in the 2023 control planters. Arizona characteristically enters a drying weather pattern in April and May, at the time temperatures at higher-elevation sites in Arizona are approaching the mild temperatures at which stinknet typically germinates. Stinknet seedlings are potentially vulnerable to hard freeze events at higher elevation sites with colder winters than experienced in the Sonoran Desert.

Controlled Germination Methods:

Stinknet seeds collected from different sites around the Phoenix metro area were germinated in petri dishes. Seed was placed on moistened filter paper in a petri dish. Ten seeds were placed in a petri dish, and ten petri dishes were used in each experiment. The petri dishes were placed in clear plastic enclosures containing a thin layer of water, to maintain a saturated atmosphere and prevent drying of the petri dish contents. A 41.5W LED lamp with a 4000K spectrum illuminated the petri dishes over an 18 hour day photoperiod (6 hour night). The lamp was held approximately 10cm above the seeds, providing intense illumination. Air temperature was between a low of 72°F and a high of 82°F.



Controlled Germination Results:

The initiation of stinknet seed germination in petri dishes was observable within three days. Germination was allowed to continue for approximately two weeks. Seedlings were then counted.

SEED SOURCE	GERMINATION PERCENTAGE
Seed mixture used to seed the 2023 test planters (untreated)	42%
Seed mixture used to seed 2023 test planters (frozen 48 hours at 4°F)	76%
Ahwatukee, AZ	43%
Queen Creek, AZ	70%
Reach 11 Recreation Area (untreated)	67%
Reach 11 Recreation Area (frozen at -40°F for two weeks)	89%
Reach 11 Recreation Area (frozen at -40°F for two weeks) Repeated	38%
Table Mesa Road Exit along I-17	81%
Early seed set (April 11, 2023), South Phoenix 48 th Street Quick Stop	13%
Early seed set (April 13, 2024), South Phoenix 48 th Street Quick Stop	26%
Seed from stinknet plants isolated from cross-pollination	1%

Controlled germination discussion

Untreated stinknet seeds exhibited germination rates from 42% to 81%.

Stinknet seed from Reach 11 germinated at 67% (untreated). Seed from the same site was frozen for two weeks at -40°F. Two germination tests of this seed germinated at 38% and 89%, showing this seed remains viable. There is potential for variation in germination rates when variables are kept constant.

Freezing treatments did not correlate with a reduction in viability.



Seed collected from stinknet plants immediately after seed set in early spring showed germination rates of 13% and 26%, indicating that seed shows viability immediately after seed set and without exposure to summer heat or dormancy. However, germination rates were observed to be lower than in seed which had spent more time on the plant. Seed remaining on the plant into summer would possibly allow for more seed maturation and/or a longer exposure to summer heat in the field.

Seeds were collected from six stinknet plants grown in pots kept in screened enclosures at least six feet apart. This separation of plants was intended to limit or prevent cross-pollination of plants by insects or wind. Seeds appeared sunken and poorly formed. The “best” looking hundred seeds were selected from this batch and given the petri dish germination test. Only one seedling (1% germination rate) was observed. This suggests stinknet plants require outcrossing and do not self-pollinate reliably.

Stinknet Seedling Cold Tolerance Test Methods

Petri dishes containing young stinknet seedlings from the germination experiments were frozen for differing lengths of time. Petri dishes containing varying numbers of seedlings were placed in a freezer at 14°F, then were placed under LED grow lights as in the germination studies. After two weeks, the survivorship of seedlings was observed. Seedlings had been grown from seeds from the Reach 11 site.

Stinknet Seedling Cold Tolerance Test Results

- Seedlings frozen at 14°F for one hour showed some damage to the first true leaves and some cotyledons were killed.
- Seedlings frozen at 14°F for three hours showed some damage to the first true leaves and apical meristems. No damaged cotyledons were observed.
- Seedlings frozen at 14°F for eighteen hours were killed.



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Stinknet Seedling Cold Tolerance Results

Stinknet seedlings showed vulnerability to freezing at 14°F. Exposure for one hour produced some tissue damage, while exposure for eighteen hours killed all seedlings. This suggests that environments in Arizona which experience prolonged hard freeze conditions in winter may not kill stinknet seeds on the site but may kill stinknet seedlings growing during a freeze event of sufficient duration. However, conditions in outdoor environments will likely differ from petri dishes.

Project Budget:

Awarded Amount: \$1308

Year 1, First Installment: \$654

Mileage southeast AZ	125.94
Filter paper	17.36
Petri dishes	156.30
Mileage northern AZ, March	343.10
TOTAL	642.70

Year 2, Second Installment: \$654

Mileage southeast AZ	320.87
Calibrated mini-freezer: ADT Chest Freezer bd100cdw100black	336.65
TOTAL	657.52



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Additional funds from Cooperative Extension were utilized to establish two planters at most of the trial sites (one per site had been originally budgeted). These funds were also needed to cover mileage through later parts of the project.

Matching funding from Cooperative Extension account:

Mileage northern AZ	481.88
HDX 27 Gallon totes	77.70
HDX 27 Gallon totes	143.76
Fiberglass window screen	12.00
Spray paint	15.09
Spray paint	22.76
Petri dishes	37.12
Temperature sensors	441.84
TOTAL	1232.15

Results of this research have been interpreted in a poster session at the NACAA Western Regional Meeting in Hurricane, Utah in September 2023; the ALVSCE poster session at the University of Arizona on 2 April 2024; and at the Southwest Vegetation Management Association annual conference in October 2024.

Acknowledgements:

I would like to thank Arizona Cooperative Extension personnel, Hattie Braun, Mike Hauser, Kim McReynolds, Susan Sekaquaptewa and Kristi Schoppmann for assistance in placing and monitoring stinknet planters. Financial support was provided in part by funding from the Southwest Vegetation Management Association's Vegetation Management Grant Program.

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