Ecoculture Reforestation Network, LLC
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Final Report

Evaluation of Screwbean Mesquite Ecosystem

Project # 2021-ECOCULTURE-2070A

Revised July 3, 2025

Executive Summary

Introduction

- Screwbean mesquite (*Strombocarpa pubescens*) is an important tree for wildlife and was once a major food source for Indigenous communities. Since 2005, the species has been declining in many areas, affecting riparian habitat restoration efforts.
- Earlier work by Ecoculture confirmed the presence of fungal disease called branch canker (caused by *Neoscytalidium dimidiatum*), which was linked to widespread die-offs across the Southwest and recommended testing treatments and identifying disease-resistant trees.
- This project was conducted from July 2023 to June 2025 and looked to understand how the disease is impacting trees locally and regionally, test treatments to reduce decline due to disease and establish the genetic basis of observed resilient phenotypes for future restoration.
- To achieve these objectives, we implemented the following three project components
    1. Surveys of tree health in previously monitored areas, DCP parcels and nearby properties
    2. Testing of management techniques like burning and coppice-cutting to promote resprouting
    3. Establishing a provenance garden grown from seeds collected across the Southwest to study disease resistance

Methods

- In June 2023, we surveyed 14 DCP parcels and six regional sites to document screwbean mesquite presence and health. At each site, we estimated tree density and assessed at least 10 trees for canopy area, dieback percentage, and branch canker symptoms. In June 2025, we established 10 survey transects at 9 DCP parcels with screwbean stands.
- Seven regional sites monitored in 2022 were revisited in Fall 2023 to assess changes in dieback and disease. Each tree surveyed was scored for dieback and branch canker and results compared to the previous year.
- Symptomatic trees at three sites were coppiced in early 2023. Resprouting and growth were monitored to assess treatment response.

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- Trees in two previously burned areas were compared to unburned controls to assess whether low intensity burning suppresses dieback and disease.
- 525 Seedlings from 55 mother trees and 24 populations were planted at a DCP Parcel near Mesquite, NV in February 2025. Heights and survival were recorded one month later.

Results

- Screwbean mesquite were present on 11 of 14 DCP parcels with large variation in tree density. Branch canker and dieback were found at several sites but at low intensity. 2025 transect surveys revealed that disease was found on trees at 8 of 9 parcels but that dieback was minimal, with the exception of the southernmost parcels close to Lake Mead.
- At revisited regional sites, disease progression from 2022 through 2023 was minimal for most cites, with several notable exceptions such as Ash Meadows NWR in Nevada. However, dieback and/or disease incidence are above 50% at four of seven sites, suggesting that these stands are not healthy, despite appearing stable during the study year.
- Coppicing resulted in disease-free resprouting for most study plants and trees in burned areas showed significantly less dieback than unburned controls, hinting that fire may reduce disease severity or reset host-pathogen dynamics. We believe both management techniques have potential to stimulate declining stands.
- Planting of the provenance garden was successful but root development in the greenhouse was low and may contribute to low survival going forward. We found variation in growth between populations, indicating potential genetic variation. Continued study of this garden has potential to yield insights into variation in genetic resistance to causes of decline.

Conclusions

- The results suggest that screwbean mesquite is declining unevenly across the region, with branch canker likely working in combination with other environmental and/or biotic causative mechanisms.
- Experimental treatments showed promise: coppicing and fire may help reduce disease and dieback progression and promote regrowth.
- Future provenance gardens should allow more time for root development prior to outplanting.
- Long-term monitoring of natural and planted populations, ongoing experimental assessment of remedial techniques and exploration of novel mechanisms are needed to better understand the causes of widespread decline in the species and to identify and evaluate effective management strategies to protect the species going forward.

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Introduction

Project description

This project was carried out between July 2023 and February 2025 and represented an attempt by the Clark County Desert Conservation Program (DCP) to understand the status of the screwbean mesquite (*Strombocarpa pubescens*) on DCP properties and to take steps to identify effective management strategies for the species. The focus of the project centered on understanding the role of the fungal disease sooty or branch canker (*Neoscytalidium dimidiatum*) in regional declines and die-offs of the species and exploring mitigation approaches for managing the disease.

The project consisted of three components:

1. baseline population health surveys of screwbean mesquites on DCP properties and follow-up surveys of regional populations
2. evaluation of the efficacy of potential mitigation techniques (low-intensity burning and coppice-cutting) for stimulating resprouting, which has been observed to slow disease spread within populations
3. propagation and planting on DCP property of a 525-tree screwbean mesquite provenance garden grown from seeds collected from 55 mother trees growing in 23 populations across five states

Background and motivation for conducting the project

The Clark County Multiple Species Habitat Conservation Plan (MSHCP) and the associated Incidental Take Permit requires that the DCP acquire, restore, and manage riparian property along the Muddy and Virgin Rivers to preserve, enhance, and protect habitat for several rare and/or listed birds. To date, the DCP has acquired and manages approximately 117 acres along the Muddy and 665 acres along the Virgin River.

Screwbean mesquite *(Strombocarpa pubescens)* is an important tree species of desert riparian systems in the Southwest and is found on many DCP properties within riparian corridors. Screwbean mesquite provides wildlife habitat, supports a diversity of insect pollinators, and was a significant dietary staple for Indigenous communities of the Sonoran, Chihuahuan, and Mojave Deserts.

Since approximately 2005, screwbean mesquite trees have been experiencing die-offs, often affecting large patches, in the western portion of the species' range. Die-offs have been found to be associated with disease symptoms. The symptoms occur on the trunk and branches and include cankers, bark lesions, discoloration of xylem tissues, longitudinal wood necrosis, and extensive gumming. Recovery of threatened and endangered avian species relies upon improving riparian habitat. The screwbean mesquite die-off threatens these recovery efforts, and it is important to understand the extent and causes of the die-offs.

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In 2022, U.S. Fish and Wildlife Service (USFWS) and Nevada Division of Wildlife (NDOW) partnered to contract Ecoculture LLC (Ecoculture) to discover the causes of the screwbean decline, identify areas for monitoring, and develop a monitoring protocol. Ecoculture conducted surveys throughout the region and collected samples from the symptomatic tissues of individuals exhibiting cankers, oozing sap, and branch mortality. Disease symptoms were observed on the screwbean mesquites of the lower Virgin River during surveys in July and August 2022. Ash Meadows NWR and Shoshone, CA are showing a steady increase in die-back that also seems to be associated with the fungal pathogen. Clark County Wetlands Park has some stands exhibiting large-scale die-off, while others, especially those that have resprouted from burned areas, are apparently healthy.

Findings from tissue collections identified the fungus, *Neoscytalidium dimidiatum,* as the likely causative agent of disease symptoms (Cowan et al. 2024). This fungus causes the diseases branch canker and sooty canker, which have caused die-offs of native tree species in other ecosystems where they have sometimes spread from agriculture crops into wild populations. The symptoms exhibited by screwbean mesquite most closely match those of branch canker and this name will be used for the disease symptoms for the remainder of this report. Recommendations from those findings were to identify and test treatments that mimic disturbance to provide land managers with treatment options with potential to mitigate the effects of the pathogen. One goal is to induce a resprout response like those found from fire, flooding, and mechanical thinning. Identifying disease-resistant populations or individuals for use in future restoration efforts was also suggested.

Goals and objectives of the project

The goals of this project were twofold: 1) to better understand the role that the disease, branch canker, is playing in driving die-offs of screwbean mesquite both across the southwest and locally at DCP properties, 2) to identify and assess potential remediation techniques that might be employed by land-managers to slow or stop the decline of the species locally and 3) To establish a screwbean mesquite provenance garden that would provide an opportunity to identify genetic traits related to disease-resistance and resilience to climate shifts. To achieve these goals, we identified three primary objectives. First, we set out to re-survey sites across the region that we had been monitored in the previous year (Cowan et al. 2024), allowing us to assess multi-year patterns. This effort included visiting previously unsurveyed sites at both DCP properties and in California and southern Arizona, expanding our understanding of the extent of decline in screwbean mesquite populations regionally. Second, we wanted to assess mitigation options by collecting data on resprout response to both wildfire and to our own coppice-cutting, which we hoped would mimic natural disturbance. Third, we wanted to establish a common garden experiment using collected seeds from mother trees across the region to identify disease-resistant genotypes and populations. To maximize the likelihood of identifying disease-resistant genotypes, we attempted to collect seeds from healthy individuals in diseased populations and from healthy populations which are adjacent to diseased populations.

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Methods

Baseline surveys

2022-2023 Surveys

Baseline surveys were conducted at 14 DCP parcels (A-H, 2L-M, 2F, 5A, 6A-B) from June 23-24, 2023. Locations of survey sites are included in the spatial file "screwbean-mesquite-project-locations.kml", included with project data. Surveys from six sites California and Arizona which were visited in December 2022 are also included here. Surveys consisted of a thorough walkthrough to identify screwbean mesquite trees. When the species was found, we estimated the number of trees per acre and the following protocol was followed to assess stand health: We walked a transect through the center of each patch and surveyed every tree within 10 m of the transect line. If dense vegetation made this impossible, we walked along paths and selected visible trees opportunistically. At least 10 trees per parcel were included when possible. We recorded the following:

- Tree canopy area (length x width in feet of horizontal area covered by the tree canopy)
- Percentage dieback (i.e. dead tissue; estimated for the whole tree)
- Branch canker symptoms
    - Present/Absent

2025 DCP Parcel Transect Establishment and Surveys

To establish a repeatable survey protocol that will allow monitoring of specific sites for screwbean health going forward, we established 10 recorded transects at nine DCP parcels from June 16-18, 2025. Transects were opportunistically located in known screwbean mesquite stands and were of varying length and shape (rarely was it possible to walk straight through the dense stands). Where stands were larger than a few acres, multiple transects were established. Transect tracks were recorded as straight lines but all trees within 10 meters of the line were surveyed. We recorded the same variables as for the 2022-2023 surveys (though canopy area is reported in meters for 2025 data) with the addition of noting presence/absence of boring beetle damage (found to be mostly *Chrysobothris octocola* during previous surveys; Cowan et al. 2024). To calculate percentage mortality, trees were marked as dead only when we were confident that the snag was a screwbean mesquite, making it unlikely that older snags were included.

Regional monitoring

To assess whether dieback and/or disease had progressed through 2023, we revisited seven sites that had been intensively monitored (surveyed periodically throughout the growing season) in 2022 (Table 1). These included one DCP property (3B). Locations of regional monitoring sites are included in the spatial file "screwbean-mesquite-project-locations.kml", included with project data. Site visits took place in 2023 between September 20 and November 14, which was near the end of the growing season for screwbean mesquite in the region. A subset of the trees that had been included in the previous year's study was revisited and surveyed for dieback and disease symptoms. To facilitate comparison between years we used the protocol from 2022. We scored the following:

- Size class (diameter of largest branch or main stem at just above ground level)
  - 1 = < 1 in.
  - 2 = 1-2 in.
  - 3 = 2-5 in.
  - 4 = 5-10 in.
  - 5 = > 10 in.
- Dieback (estimated for the whole tree):
  - 0 = 0-1% dieback
  - 1 = 2-25% dieback
  - 2 = 26-50% dieback
  - 3 = 51-75% dieback
  - 4 = 75-99% dieback
  - 5 = Dead
- Branch canker symptoms
  - Present/Absent

Table 1. Regional Monitoring Sites visited in both 2022 and 2023.

Site Name	State	Number of trees surveyed 2023
Ash Meadows NWR Point of Rocks	NV	6
Ash Meadows NWR - Wash	NV	26
Beatty	NV	3
Clark County Wetlands	NV	20
DCP Parcel 3B	NV	7
Shoshone	CA	34
Virgin Riverside	NV	23

Mitigation Treatments

Coppice Study

To determine whether coppicing (cutting all stems of just above ground level) would trigger resprouting in diseased and dying trees with subsequent increased vigor and disease resistance, we selected 24 trees with branch canker symptoms at three sites (Clark Co. Wetlands, Shoshone, Cocopah Nursery) for inclusion in the study. Locations of coppice study sites are included in the spatial file “screwbean-mesquite-project-locations.kml”, included with project data. Trees at Clark Co. Wetlands and Shoshone were natural recruits, whereas Cocopah Nursery trees were planted. We chose trees from a variety of size classes and with a range of branch canker-driven

dieback percentages. Prior to cutting, stem diameter at root collar and percentage dieback was recorded for each tree. All stems of each tree were cut just above the root collar from January-February 2023. Trees were checked periodically through the growing season of 2023 to determine if resprouting had occurred. When resprouting from the root collar was observed, the length of the longest sprout was recorded.

Burn Study

To determine whether screwbean mesquites that have resprouted after experiencing low intensity burning are less susceptible to branch canker infection than unburned trees, we identified two burn sites at Clark County Wetlands where extensive resprouting of burned trees had occurred and the time-since-burn was known. One site had been burned three years prior to this study (in summer 2020) and the other nine years prior (summer 2014). These sites were compared with each other and with adjacent unburned "control" sites. Locations of burn study sites are included in the spatial file "screwbean-mesquite-project-locations_2021-ECOCULTURE-2070A.kml", included with project data. Trees at all sites were natural recruits that were 12-14 years old. We visited 50 trees at the 3-year-old burn, 29 at the 9-year-old burn and 169 at the unburned sites. We measured the following variables for each tree: percent dieback per tree and disease presence/absence, using protocols described above. All data was collected from October-November 2023.

Provenance Garden

Seeds for the provenance garden were collected from across the southwest in September and October 2023. This was the ideal time for seed collection as seed-production for the species occurs in late summer. Seeds were collected from 55 mother trees found in 24 populations (one to seven mother trees per population). Metadata for all mother trees (GPS coordinates, population, presence/absence of branch canker symptoms, whether the tree was planted or wild) is included in the data file "screwbean-mesquite-project-data_2021-ECOCULTURE-2070A.xlsx", tab "Provenance seed source data". The spatial file "screwbean-mesquite-mother-trees.kmz" contains the locations of all mother trees. Germination, propagation and grow-out were conducted by the Northern Arizona University Research Greenhouse from December 2023 to March 2025. Details of the grow-out process may be made available upon request. Seedlings were grown in 4" x 14" pots for 14 months. A total of 525 seedlings were included in the provenance garden, representing eight to 11 replicates per mother tree (actually siblings grown from different seeds). Seedlings were planted at least 3.5 meters apart into DCP parcel 2-J from February 25-27, 2025, and watered twice with at least a gallon of water for each plant within 46 hours of planting. All seedlings were planted with a unique tag number (metal tag with engraved number on a U-stake) that can be used to ascertain the unique tree ID code for each seedling (found in "screwbean-mesquite-project-data_2021-ECOCULTURE-2070A.xlsx", tab "Provenance tag & height data"). Tree ID codes indicate seed source information (population, mother tree number and replicate). Locations of individual trees were taken with ~0.6 meter accuracy using a Trimble DA2 model GPS antenna. The height of each seedling was measured approximately one month later (March 31, 2025).

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Statistical Analysis

All analyses were performed in JMP (JMP®, Version 18.0.2. SAS Institute Inc., Cary, NC, 1989–2023). All significance testing was performed with an alpha level of 0.05.

Baseline Sites

For each 2022-2023 baseline site, we calculated the following metrics:

- Average tree canopy area and standard deviation
- Average percent dieback (dead tissue) per tree and standard deviation
- Percentage of trees exhibiting branch canker symptoms (henceforth "diseased")

For 2025 transect surveys, we calculated the following:

- Trees per acre using the area of the transect (length x 20 m) and the total number of trees recorded in the transect area.
- Percentage of dead trees
- Percentage of diseased trees
- Percentage of trees with visible boring beetle damage
- Median tree canopy area and quartiles (75%/25%)
- Median percent dieback (dead tissue) per tree and quartiles (75%/25%)

For both 2022-2023 and 2025 data, we explored the relationship between tree size (a continuous variable for baseline data) and percent dieback using bivariate fit modeling. Tree size and percent dieback were compared between diseased and non-diseased trees (2022-2023 & 2025 data) and trees with and without beetle damage (2025 data) and using Wilcoxon rank-sum tests. For 2025 data, we explored the relationship between disease occurrence and likelihood of exhibiting beetle damage using a chi-square test.

Regional Monitoring

Dieback scores were converted to percentage values to ease analysis and visualization. Each dieback score was coded as the midpoint of the bin range. So, a score of "1" (2 – 25% dieback) was changed to 11.5% and a score of "2" was changed to 38%, etc. Regional monitoring dieback percentage values from the end of the 2022 monitoring study (recorded October/November 2022) were subtracted from the 2023 dieback percentages to generate a change in dieback percentage for each tree. A high score indicated a tree that had much more dieback by the end of 2023 than measured in 2022, a negative score was a tree that had less and a neutral score ("0") had no additional dieback by the end of 2023. These percentages were then compared to zero within each of the seven sites using Wilcoxon Rank-Sum tests (data were nonparametric) to determine whether net progression of dieback was positive, neutral or negative. Percentage progression of dieback was compared amongst size classes and pairwise comparisons were performed using Wilcoxon rank-sum tests. Note that tree size for regional monitoring data was binned and is therefore categorical, whereas tree size for baseline data is described by canopy area and is continuous.

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Disease status (branch canker symptoms present/absent) for individual trees was compared between 2022 and 2023 and they were categorized as follows:

- "no disease": the tree exhibited no branch canker symptoms in either year
- "diseased": the tree exhibited branch canker symptoms in both years
- "acquired": the tree showed no branch canker symptoms in 2022 but was symptomatic in 2023
- "lost": the tree showed branch canker symptoms in 2022 but was asymptomatic in 2023

Mitigation Treatments

Coppice Study: Maximum recorded length of the longest resprout was used with the interval between cutting and final growth measurement to calculate weekly growth rates for individual trees. The relationships between weekly growth rate and both stem size (cm) and percentage dieback prior to cutting were explored using bivariate fit modeling. The relationships between likelihood of resprouting and growth rate and stem size were explored using logistic regression.
Burn study: Percentage dieback was compared amongst the three treatments (3-year-old burn, 9-year-old burn, unburned) using Wilcoxon Rank-Sum tests. The proportion of diseased to non-diseased trees was compared between the three treatment types using a Contingency Analysis.

Provenance Garden

Variation in seedling height at one month post-planting was compared between seed source populations and between mother trees using one-way ANOVAs.

Results

Baseline surveys

2022-2023 Surveys

Table 2 shows site data for all baseline properties with at least two trees surveyed. Data file "screwbean-mesquite-project-data _2021-ECOCULTURE-2070A.xlsx", tab "Provenance height data" contains data for all 66 surveyed trees (tree size, % dieback, branch canker presence/absence). Of the 14 DCP parcels surveyed for the first time (3B was first visited in 2022 and is included with regional monitoring), six were found to have screwbean mesquite stands. Parcels A-H (the Muddy River parcels) had no screwbeans. Parcel 5A was very difficult to access due to dense tamarisk and no screwbeans were observed in the limited areas that we visited. In general, dieback on screwbean mesquite was low and little disease was found. The site with the highest incidence of disease (2L & 2M) also had the highest percentage dieback but both values were low. However, just west of 5A is a dense stand of screwbeans with substantial regrowth and many young trees (tree sizes: 40-150 ft^2), approximately 25% of which showed signs of branch canker (n=20). Across the river we observed several large screwbean mesquite snags and large living trees (canopies >550 ft^2) that showed 50-90% dieback and disease symptoms. One large, branch canker-free tree with less than 5% dieback was a striking exception.

All non-DCP properties showed high dieback, and branch canker was observed at Sonny Bono NWR and Afton Canyon, CA. Across all baseline sites, we found no linear or polynomial correlation between tree size and percent dieback (lowest p-value was 0.12). However, the median size of diseased trees was larger than that of non-diseased trees (336 vs. 105.5 ft^2; $z = -2.12$, $p = 0.034$). Diseased trees also had higher median dieback than non-diseased (30 vs. 7%; $z = 2.25$, $p = 0.025$).

Table 2. Baseline sites surveyed in 2022/2023 for dieback and disease. Canopy area is the length x width horizontal area covered by the tree canopy. Percent diseased is the percentage of trees exhibiting branch canker symptoms. *Sites in italics are DCP properties*.

Site	# of trees surveyed	Estimated trees per acre	Average canopy area in square feet (standard deviation)	Average percent dieback (standard deviation)	Percent diseased
2F & 2G	4	5	540 (136)	3.5 (4.0)	0
2L & 2M	10	20	303 (245)	3.8 (4.1)	10
6A & 6B	10	50-100	283 (243)	0.7 (2.2)	0
Morongo Valley	4	17	268 (188)	46.2 (33.0)	0
Quitobaquito Spring, AZ	2	2	90 (0)	37.5 (3.5)	0
Sonny Bono NWR, CA	10	40	76 (37)	41.0 (25.6)	100
Afton Canyon, CA	10	9	549 (100)	23.6 (23.1)	70
Vallecito SP, CA	10	50-100	632 (248)	76.5 (16.3)	0

2025 DCP Transect Surveys

Table 3 shows transect data for the 10 transect surveys. No linear or polynomial relationships existed between trees size and percent dieback (lack-of-fit p-values were 0.022-0.029). The median canopy size of trees with beetle damage was more than double that of non-damaged trees (27.75 vs. 12 m^2; $z = 4.24$, $p < 0.001$). Beetle-damaged trees had higher median dieback than non-damaged trees (10 vs. 0%; $z = 4.70$, $p < 0.001$). A relationship between the likelihood of exhibiting disease symptoms and beetle damage was found, indicating that trees with beetle damage were more likely to exhibit disease symptoms ($\chi^2 = 25.33$, $p < 0.001$).

Table 3. DCP parcel transects surveyed in 2025. Trees per acre was calculated only for the transect area (length x 20 m). Canopy area is the length x width horizontal area covered by an individual tree canopy. Percent diseased is the percentage of surveyed trees exhibiting branch canker symptoms. Quartiles within parentheses are 75% and 25%, respectively.

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Transect Name	# of trees surveyed	Trees per acre	Median canopy area in m^2 (quartiles)	% dead	% diseased	% with beetle damage	Median % dieback (quartiles)
Parcel 2J	26	170	9 (20/1)	4	12	32	5 (10/0)
Parcels 2F/2G	16	44	20 (34.5/1)	0	6	12	0 (5/0)
Parcels 2L/2M-1	15	36	25 (37/10.5)	0	20	93	0.05 (0.05/0.05)
Parcels 2L/2M-2	5	24	36 (67.5/15.5)	0	0	60	5 (12.5/0)
Parcel 3A	24	78	12 (21/4)	0	0	12	0 (0/0)
Parcel 5A-1	20	65	7 (16.75/4)	0	50	45	28 (60/10)
Parcel 5A-2	15	98	39 (80/21)	0	80	40	30 (50/15)
Parcel 5A-3	23	91	30 (45/15.5)	9	76	81	30 (50/10)
Parcel 6A	15	72	42 (65/22)	0	13	47	0 (0.05/0)
Parcel 6B	15	74	20.5 (55.87/13.87)	60	100	83	60 (82.5/37.5)

Regional Monitoring

The amount of dieback observed in 2023 varied widely between sites, from a median of 0.5% at DCP parcel 3B to 87.5% at Beatty and Ash Meadows Wash (Figure 1). Dieback change also showed large variation between sites, from a median of -11% at DCP parcel 3B to 76% at Beatty (Figure 2). Most sites also showed high within-site variation, for example, dieback change at Shoshone ranged from -87% to 88.5% with a 25-75% quartile spread of 35.6%. Four of the seven sites had a median dieback change of 0% (Ash Meadows Point of Rocks, Shoshone, Clark Co. Wetlands and Virgin Riverside). Disease status change also varied widely (Figure 3). Across all sites, observation of new branch canker symptoms in 2023 was low and the proportion of trees showing loss of symptoms was generally higher (e.g. Clark Co. Wetlands).

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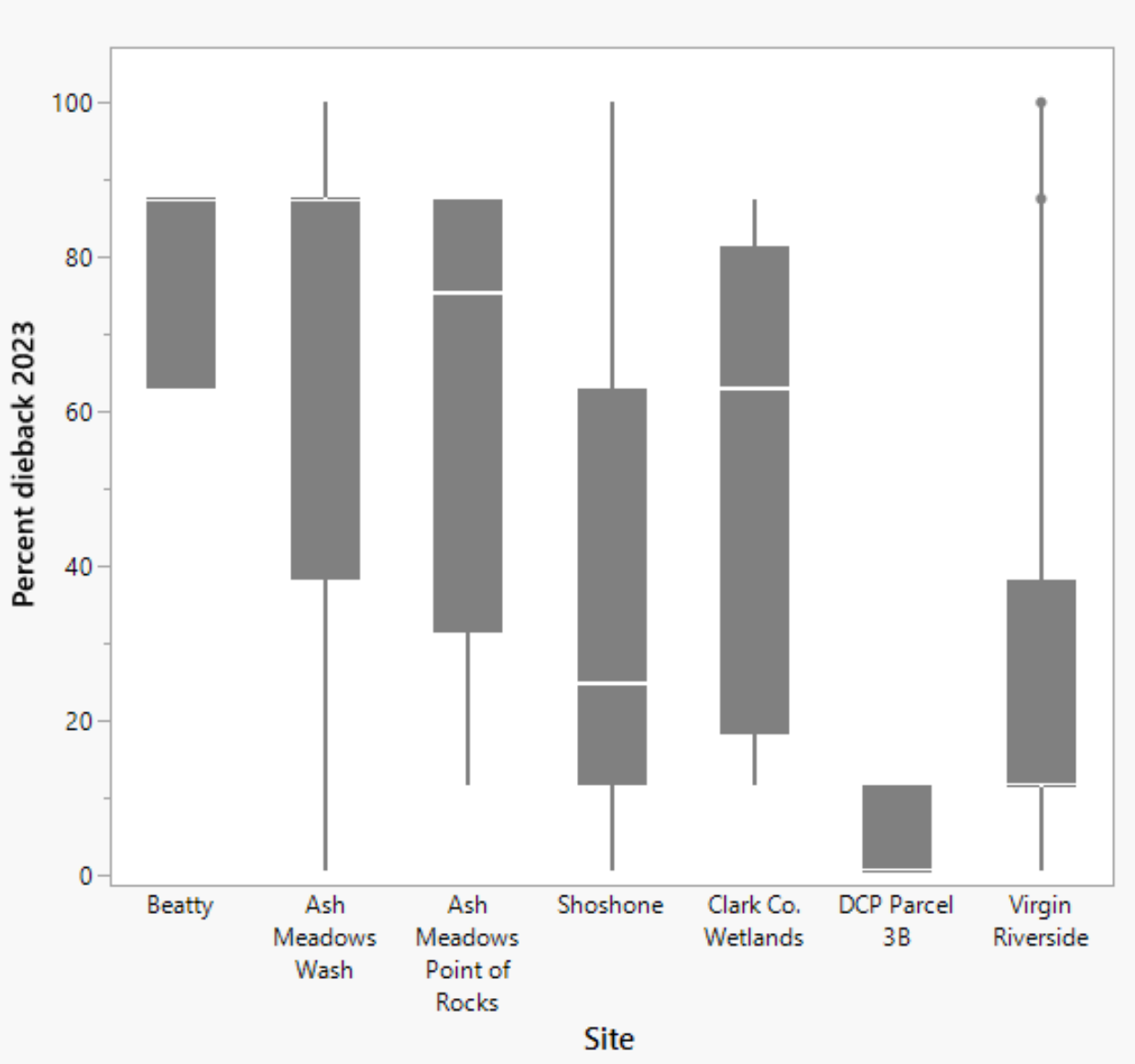

Figure 1. Percent dieback (tissue death) measured on individual trees at the seven regional monitoring sites in 2023 (September-November). These percentages were generated from binned scores (0-5; described in Methods). White lines show medians, boxes show 25-75% quartiles and lines indicate range.

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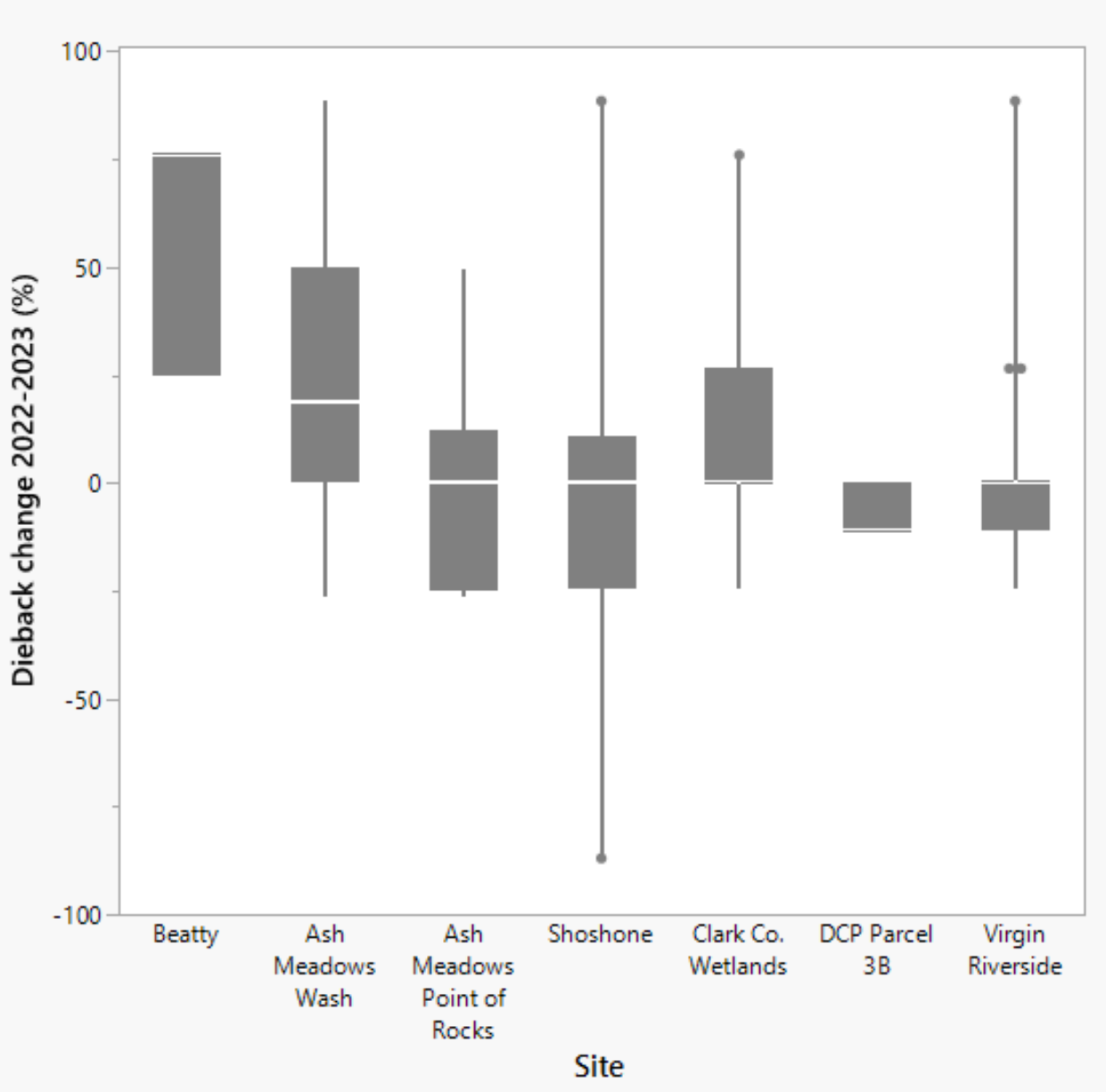

Figure 2. Change in percent dieback (tissue death) on screwbean mesquite trees measured at each monitoring site between October-November 2022 surveys and September-November 2023 surveys. These percentages were generated from binned scores (0-5; described in Methods). White lines show medians, boxes show 25-75% quartiles and lines indicate range.

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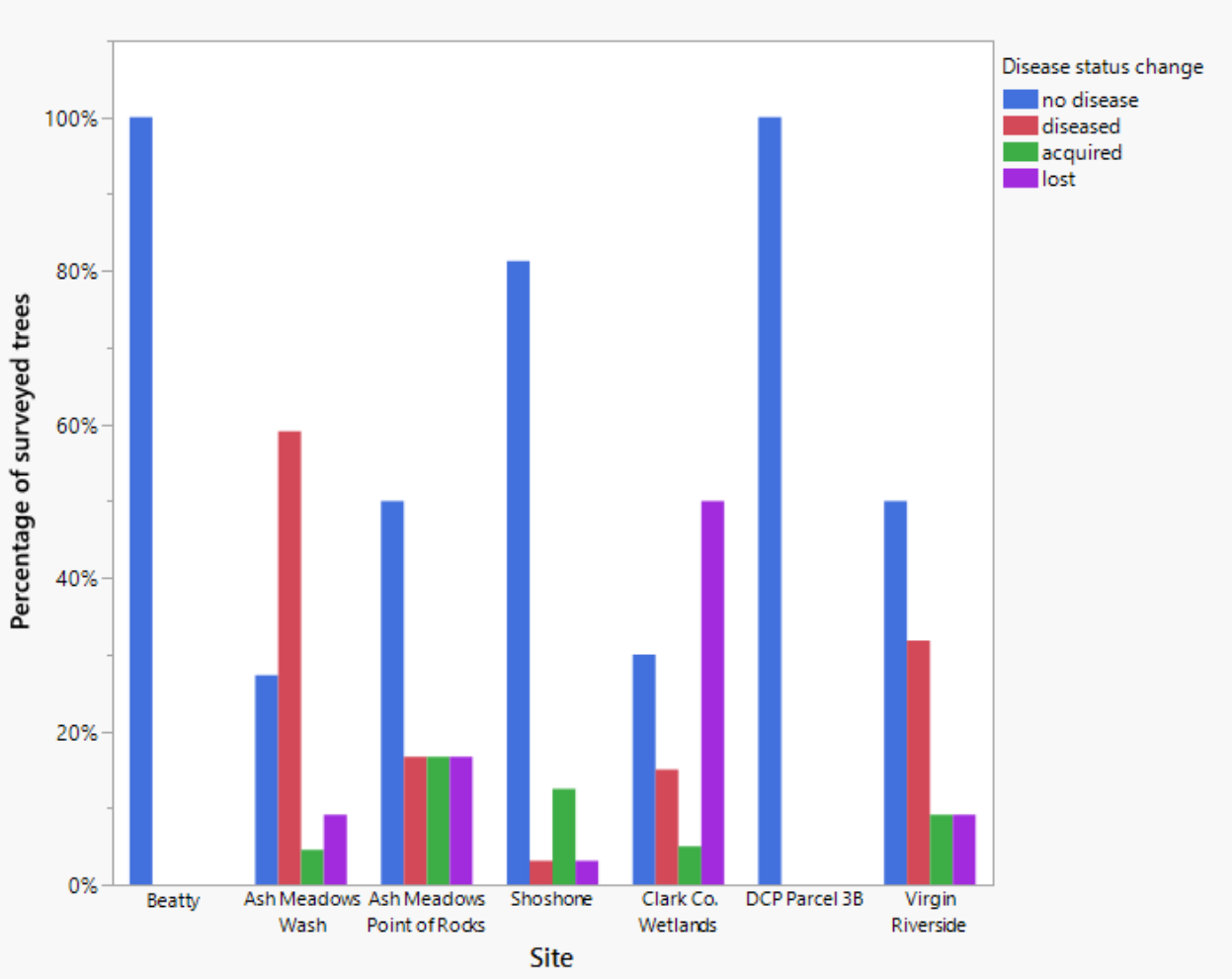

Figure 3. Change in disease status for the seven regional monitoring locations. See Methods: Regional Monitoring for a description of disease status change categories.

Mitigation Treatments

Coppice study

Of the 24 screwbean mesquite trees that were coppiced, only 17 were included in the study. Seven trees were buried in floods in Clark Co. Wetlands and Shoshone. The remaining trees showed a 71% resprout rate (12 of 17). Dieback on trees prior to cutting varied widely, from 0% to 95%, with a median of 50%. Likelihood of resprouting had no relationship with percentage dieback at time of cutting, however ($p = 0.55$). Stem size (diameter at root collar) of coppiced trees varied widely, ranging from 4.6 to 56.5 cm with a median of 9.8 cm. Larger trees may have been more likely to resprout than small trees, with the p-value approaching significance ($\chi^2 = 3.76$, $p = 0.053$). Neither stem size nor percentage dieback at time of cutting showed a relationship with resprout growth rate (p-values for linear and polynomial correlations ranged

from 0.24 to 0.90). Growth rate was based on measurements taken between 159 and 233 days post-coppicing.

Burn Study

Percentage dieback per tree differed significantly amongst the three treatments (3-y.o. burn, 9-y.o. burn, unburned; n = 248, $\chi^2 = 76.73$, $p < 0.001$), with median percent dieback highest at the unburned site, intermediate at the 9-year-old site and lowest at the 3-year-old site (63%, 38% & 11.5%, respectively). The percentage of trees showing disease symptoms was highest at the 9-year-old site, intermediate at the unburned site and lowest at the 3-year-old site (59%, 43% & 6%, respectively; n = 248, $\chi^2 = 34.96$, $p < 0.001$).

Provenance Garden

All 525 seedlings were successfully planted in DCP parcel 2-J. The shapefile folder "Screwbean_Mesquite_Provenance_Garden_shapefiles" and the spatial file "screwbean-provenance-garden.kmz" contain all planted tree locations, tag numbers and spatial metadata. One month after planting, 6.5% (34 of 525) of seedlings appeared dead (root collar not green). The 491 living seedlings averaged 41.22 cm in height with high variation (standard deviation = 19.43). Seedling height varied significantly amongst the 23 populations (n = 491, F = 3.53, $p < 0.001$) and amongst the 55 mother trees (F = 2.03, $p < 0.001$). Seedling height data is found in data file "screwbean-mesquite-project-data_2021-ECOCULTURE-2070A.xlsx", tab "Provenance tag & height data", along with basic metadata for each seedling (unique code, tag #, population of mother tree).

Discussion

Baseline Surveys & Regional Monitoring

Taken as a whole, the results of our new 2023 surveys and our multi-year regional monitoring do not show a picture of strong decline driven by disease. In fact, when the relatively high number of trees exhibiting loss of branch canker symptoms (see fig. 3) is accounted for, net infection seems to have declined in 2023. For example, at Clark Co. Wetlands, though 5% of surveyed trees showed new symptoms after the 2023 growing season, fully 50% showed loss of symptoms entirely. Further, tissue dieback observed on individual trees is roughly stable for most regional monitoring sites (fig. 2). Numerous sites with high progression of dieback and/or high total dieback (e.g. Beatty, Morongo Valley, Vallecito SP) are apparently disease-free, suggesting that other drivers of decline exist. Sites such as Ash Meadows Wash and Clark Co. Wetlands certainly show high dieback associated with branch canker and, in the case of the former, with dieback on individual trees increasing towards an average of 90%, the likelihood of stand loss seems high. Taken in the aggregate, however, we find that disease is only sometimes associated with dieback and decline and that the underlying mechanisms making one stand susceptible to branch canker and another resistant are unknown. This is exemplified by Virgin Riverside and DCP Parcels 3A and 3B, sites which are only half a mile apart yet show very different disease and dieback patterns (see figs. 1 & 3). Considering that the disease is found affecting trees at

sites both up and downstream of Parcels 3A and 3B, which appear disease-free, it is very likely that these stands are exposed to the spores of *N. dimidiatum* but no trees are developing disease symptoms. Considering that all screwbean stands along this reach are apparently natural recruits, we find no reason to believe that genetic variation in disease susceptibility should exist in this area. We find it likely, therefore, that localized unidentified stressors are driving disease incidence.

The worldwide increase in *N. dimidiatum* outbreaks is worth noting, and it is certainly possible that canker infections by this pathogen may have driven rapid local extinctions of screwbean mesquite stands across Arizona (Cowan et al. 2024). The fact that we have not observed such rapid mortality during the two years that we have been monitoring these populations does not mean future die-offs will not occur. In fact, Shoshone, a site with little progression of disease or dieback during 2022-2023, is reported by local land managers to have lost most of its older trees during a striking die-off between 2012 and 2014. Indeed, trees at Shoshone are mostly small (the lowest median size amongst regional monitoring sites) and numerous large screwbean mesquite snags can still be found in the area. As younger trees are apparently disease-resistant (Cowan et al. 2024), the trees being monitoring at this site likely represent natural recruitment after a die-off event. Ash Meadows Wash, the monitoring site with the clearest progression of dieback, the highest incidence of disease and the bleakest outlook is also the nearest monitored stand to Shoshone (~32 miles away). While we have been monitoring it for two years and the rate of tree mortality is apparently much lower than that experienced at Shoshone, the progression of median dieback from 38% to almost 90% in 2023 is striking. If, as has been suggested, a shift towards a hotter, drier climate is leading to increased activity of *N. dimidiatum*, it seems likely that infections by this pathogen are at least worsening regional loss of screwbean mesquite stands, even if there are other mechanisms of decline in play.

Our findings from DCP properties along the Virgin River suggest that the screwbean mesquite stands on Clark County parcels are largely healthy at the upstream sites near Bunkerville (e.g. but increasingly unhealthy further downstream (e.g. parcels 5A and 6B). However, relatively unhealthy stands (e.g. Virgin Riverside, a regional monitoring site with ~36% diseased trees) exist within a mile of DCP properties near Bunkerville. Considering our lack of understanding of the drivers of disease susceptibility we suggest that future decline of currently healthy screwbean mesquite on DCP properties is certainly possible and should be monitored going forward.

Mitigation Treatments

The finding that most diseased screwbean mesquite trees will resprout after coppicing regardless of the extent of branch canker-driven dieback is encouraging. We observed no infection on resprout tissue and, considering that the resprout stems may be less susceptible to branch canker for several years, this approach has the potential to “reset” disease progression. In other words, a tree with 95% dieback, which is likely on the cusp of dying, may resprout vigorously after coppicing and the new growth will be disease-free for several years. Further monitoring of coppiced trees would be necessary to determine how long this delay in infection lasts but it

seems likely to be a useful mitigation tool for delaying mortality due to aggressive branch canker infections.

Although it is often a challenge for land managers to implement controlled burning as a management tool, the limited data presented here suggest that the approach has strong potential to postpone or even obviate disease-driven mortality. Three years after a fire, resprout tissue from burned trees showed low tissue dieback, despite being surrounded by diseased and dying trees. The intermediate level of dieback at the 9-year-old burn suggests that the solution is temporary and that trees will eventually be reinfected. In fact, disease incidence was higher at this burn than at unburned sites. This is an interesting pattern with no clear explanation though we suspect it may have to do with the visibility of disease symptoms being lower on very unhealthy trees. For example, a tree with 95% dieback often appears as largely consisting of dead tissue, with few or no active disease symptoms.

Provenance Garden

Seed collection, propagation and planting were successful for this portion of the project. However, although our survey of planted trees found high survival one month after planting (93.5% in late March), qualitative observations suggested that much of the above ground tissue was already dead or dying. Root development at the time of planting was much lower than we had hoped for; grow-outs longer than 14 months are probably necessary for this species, especially if it will be planted into natural environments without irrigation. The planting site was chosen due to the presence of wetland plants across the site, suggesting that the area has wet soil for much of the growing season. If water returns to the planting site soon, we expect that many of the seedlings will rebound, sprouting from the root collar even when above-ground tissue is dead. Variation in seedling height amongst populations and mother trees is largely due to performance in the greenhouse. This variation may indicate genetic differences but how this will translate into phenotypic variation in a natural environment remains to be seen.

Conclusions & Recommendations

Although the results of this project seem to paint a picture of stable populations with little change in disease or dieback incidence, we advise a cautious response. To fully understand the patterns and mechanisms of decline in screwbean mesquite would take a much larger collaborative effort than what we have accomplished here. The fact that we observe rapid dieback progression in at least one sizeable population at Ash Meadows and that anecdotal evidence and comparisons of current and historical distributions suggest recent large-scale decline of the species across Arizona and parts of California should indicate that vigilance will be necessary if we want to help this species continue to play a vital role in riparian forests of the southwest US. We think that a disturbance-based management approach has great potential. Sites along the Virgin River, with its natural flood regimes, are relatively healthy compared to old, seemingly decadent stands such as those at Ash Meadows NWR and Vallecito SP in California, where natural disturbance in the form of wildfire and large floods is largely suppressed. Mitigation techniques that mimic natural disturbance, such as coppicing and low-intensity burning, have the potential to be valuable tools for land managers to cultivate stand-level resilience. Ultimately, further research

into underlying stressors such as climatic shifts and groundwater decline would help us to develop a more comprehensive understanding of the drivers of regional screwbean mesquite decline, allowing land managers to develop more effective management approaches.

We believe that the provenance garden approach deserves further effort. Seedlings need more time in the greenhouse and may benefit from at least one summer in an irrigated outdoor nursery. Screwbean mesquite seedlings observed at Song Dog Nursery appear robust and would likely show high survival when planted without irrigation at appropriate wetland sites in the area. We currently have several hundred “left over” seedlings at our greenhouse in Flagstaff and copious seed stores remain from most of the mother trees included in this project. Future efforts could focus on growing provenance seedlings to an adequate size prior to planting and/or providing irrigation for plantings.

Literature Cited

1. Cowan J, Hu J, Haubensak K, Grady KC. Range-wide population decline of a foundational riparian species tree is linked to an endemic fungal pathogen in the western United States. Biol Conserv. 2024 Aug;296:110704.

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