OAHU ARMY NATURAL RESOURCES PROGRAM
MONITORING PROGRAM

GERMINATION RESULTS OF A *TETRAMOLOPIUM FILIFORME* VAR. *POLYPHYLLUM* SEED SOW TRIAL

INTRODUCTION

In order to meet IP stability goals, the OANRP five-year plan for the Kalena MFS PU for *Tetramolopium filiforme* var. *polyphyllum* was to locate and establish a new reintroduction site within Lihue or adjacent MU using plants or seeds from SBW-B greenhouse collections (OANRP 2014). It was presumed that several attempts would be necessary to establish a successful site and meet stability goals. This taxon generally grows on sparsely vegetated exposed rocky ridges and nearly vertical cliffs, and may hybridize with other *Tetramolopium* taxa. Two sites with appropriate habitat, that lack other *Tetramolopium* taxa, and have feasible access for management were located, including a site near Puu Hapapa (SBS-A), and a site near Kamaohanui ("Skeet Pass") (SBW-D) (Figures 1 - 4). A trial was conducted to explore if OANRP can establish reproductive populations via seed sowing at these sites.

Figure 1. Locations of *T. filiforme* var. *polyphyllum* seed sowing trials at Hapapa (SBS-A) and Skeet Pass (SBW-D), and the Puu Kalena in situ site (SBW-B).

Figure 2. Habitat selected for *T. filiforme* var. *polyphyllum* seed sowing at Hapapa.

Figure 3. Habitat selected for *T. filiforme* var. *polyphyllum* seed sowing at Skeet Pass.

Figure 4. A memorable day in the field sowing seeds at Hapapa with Daniel Sailer. Note the big smile despite chilly and challenging conditions.

METHODS

Seeds were sown at Hapapa on November 30, 2016, and at Skeet Pass on December 20, 2016. Fifty 30 cm x 30 cm seed sow plots were established at each site, with an estimated 520 viable seeds per plot. Plots were located on terrain ranging from gentle slopes to cliffs requiring rappel use (categorized by degree of slope as <30°, 30-60°, and >60°), and in varying substrate (moss, lichen, crumbly eroded rock, rock crevice, rock ledge, and soil). Plots were separated by at least one meter, and marked using pre-numbered write-on aluminum tags along with a small amount of flagging nailed into the substrate (Figure 5). Because of concerns over seeds blowing away during or after sowing due to the lightweight, wind-dispersed seed structure, and the steep and windswept nature of the site, tackifier (Turbo Tack) was experimentally applied with squirt bottle in a thin layer on the substrate in half of the plots to help seeds adhere to the substrate. Tackifier is an additive commonly used in hydroseeding to enhance adherence to substrate. Seeds were generally sown on unmodified ground surfaces, though small weeds were occasionally hand-pulled prior to sowing, and any obstructive debris was removed. Seed used in the trial were from bulk collections of hand pollinated OANRP greenhouse SBW-B stock stored in the OANRP seed bank for less than one year.

Viability assays were conducted under laboratory conditions both with and without tackifier for seeds used at each site to examine if tackifier affects germination, and to estimate the number of viable seeds per plot. Seeds were sown on agar in petri dishes, including 50 seeds per sample (200 seeds total).

Figure 5. Scattered *T. filiforme* var. *polyphyllum* seeds and marker for Plot 22 at Hapapa. Yellow circles are shown around a few of the seeds for reference.

Petri dishes were stored in a Percival Controlled Environment Chamber (with diurnal light and temperature settings matching average monthly temperatures for the Nike missile installation at Pahole, at approximately 2100 feet elevation), and examined weekly for germination.

A subset of plots were monitored to assess germination at Hapapa on January 4, 2017 (5 week post-sowing), and at Skeet Pass on March 6, 2017 (11 weeks post-sowing). The monitoring was intended to provide a rough approximation of germination among plots, as well as to assess the utility of tackifier. It was anticipated that germination would likely be spread out over several weeks, and at the time of monitoring, some seeds might have already germinated and died, and others might not have germinated yet, depending on weather conditions. Seedlings of *T. filiforme* var. *polyphyllum* are very small, and it would be difficult to walk around or rappel to all plots for close inspection without damaging seedlings from stray seeds that ended up outside of plots. To minimize disturbance to the sites, only plots accessible off-rappel were monitored, and seed numbers were approximated. At Hapapa, 76% of the plots were monitored, and 66% were monitored at Skeet Pass. Seedling counts per plot were categorized as 0, 1-50, 50-100, 100-200, and >200 at Hapapa, and as 0, 1-25, 25-100, 100-200, and >200 at Skeet Pass.

Statistical tests included chi-square to compare germination with and without tackifier, and ordinal regression to assess the effects of tackfier use and slope on counts of seeds. All statistical analyses were performed in IBM SPSS Statistics Version 24.

RESULTS

Germination rates were similarly high under laboratory conditions for seeds sown with (87% germination) vs. without (94% germination) tackifier (chi-square: p = 0.091, X = 2.85) (Figure 6). Most seeds germinated within 1-2 weeks. Any observed differences in the field between plots with or without tackifier would be presumably due to differences in adherence rather than an inherent capacity for tackifier to enhance or inhibit germination.

Figure 6. *Tetramolopium filiforme* var. *polyphyllum* seedlings germinating in the OANRP seed lab with (left) and without (right) tackfier. Both treatments had similarly high germination.

All monitored plots at Hapapa had germinated seedlings. Among the observed plots, 29% had 1-50 seedlings, 34% had 50-100 seedlings, 34% had 100-200 seedlings, and 3% (a single plot) had >200 seedlings. Most plots had small seedlings, comprised of cotyledons with emerging true leaves, though 8% had larger seedlings with cotyledons and expanded true leaves (Figure 7).

Figure 7. Examples of *T. filiforme* var. *polyphyllum* seedlings observed at Hapapa, showing larger seedlings with expanding true leaves (left), as well as smaller ones consisting primarily of cotyledons (right).

Seedlings were present in 61% of the monitored plots at Skeet Pass. Among plots with seedlings, 75% had less than 25 seedlings, 20% had 25-100 seedlings, and 5% (1 plot) had 100-200 seedlings.

Tackifier use did not influence the number of germinated seedlings within plots at either site (Hapapa: p = 0.876; Skeet Pass: p = 0.344) (Figure 8), nor did the degree of slope (Hapapa: p = 0.425; Skeet Pass: p = 0.210) (Figure 9).

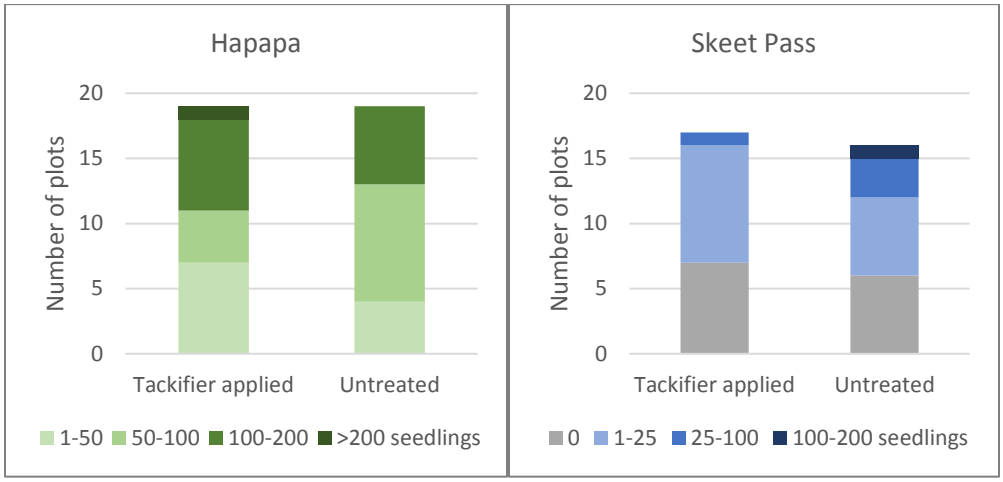

Figure 8. Number of germinated *T. filiforme* var. *polyphyllum* seedlings observed during monitoring in plots with and without the application of tackifier during seed sowing at Hapapa and Skeet Pass. Tackifier use did not impact germination results.

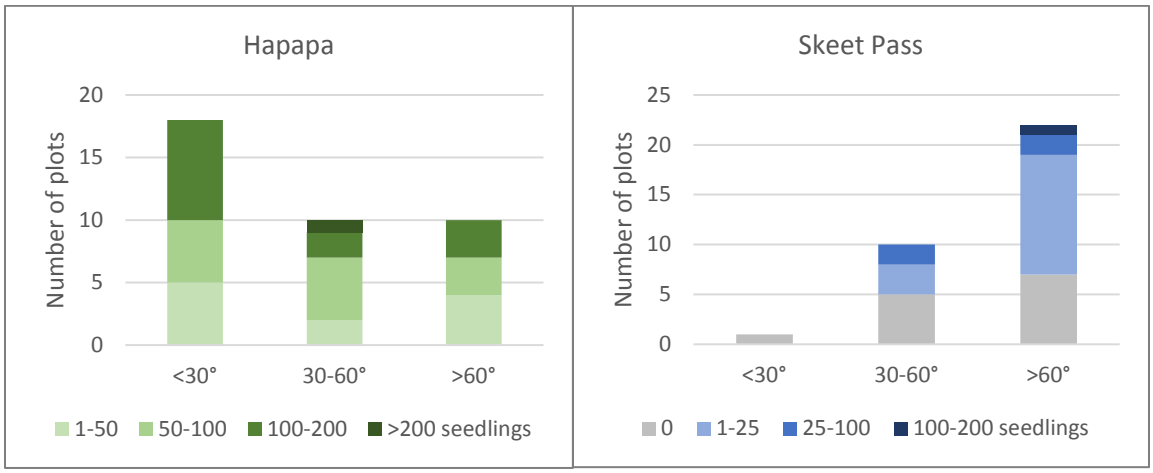

Figure 9. Number of germinated *T. filiforme* var. *polyphyllum* seedlings observed during monitoring in plots on gentle (<30°), moderate (30-60°), and steep (>60°) slope at Hapapa and Skeet Pass. The vast majority of available substrate at Skeet Pass was on moderate to steep slope, and as such the gentle slope category was only minimally represented. Slope did not impact germination results.

DISCUSSION AND FUTURE DIRECTIONS

While more seedlings were observed at Hapapa than at Skeet Pass, differences in germination between the sites cannot be compared, as the amount of time between sowing and monitoring differed considerably between the two sites. Regardless, it is apparent that germination does occur at both sites in the majority of seed sow plots, and as such both sites hold the potential for the formation of reproductive

populations. Future monitoring of survivorship will provide a better indication of the ability to successfully establish reproductive populations via seed sowing.

Tackifier is unnecessary for sowing *T. filiforme* var. *polyphyllum* seeds. While the degree of slope did not affect the number of seedlings among observed plots, the plots requiring rappel were not monitored, and their relative influence could not be assessed. The ultimate influence of slope will be evaluated in accordance with survival to reproduction during more thorough monitoring. Similarly, substrate type will also be assessed in association with survival.

Survivorship monitoring will occur annually at each site (all plots will be monitored). An estimated 52,038 viable *T. filiforme* var. *polyphyllum* SBW-B seeds remain in storage, which may be used for future sowing attempts at these or other sites, as needed.

REFERENCES

Oahu Natural Resources Program. 2014. Chapter 2: Five Year Rare Plant Plans *in* Status Report for the Makua and Oahu Implementation Plans.