

Appendix 1: Lyon Arboretum Seed Storage Trials

Written by Lauren Weisenberger, Lyon Arboretum Seed Storage Facility

1. *Alectryon macrococcus* var. *macrococcus*

None tested - ~30 seeds stored at 4C/ 20% RH

ALSINIDENDRON

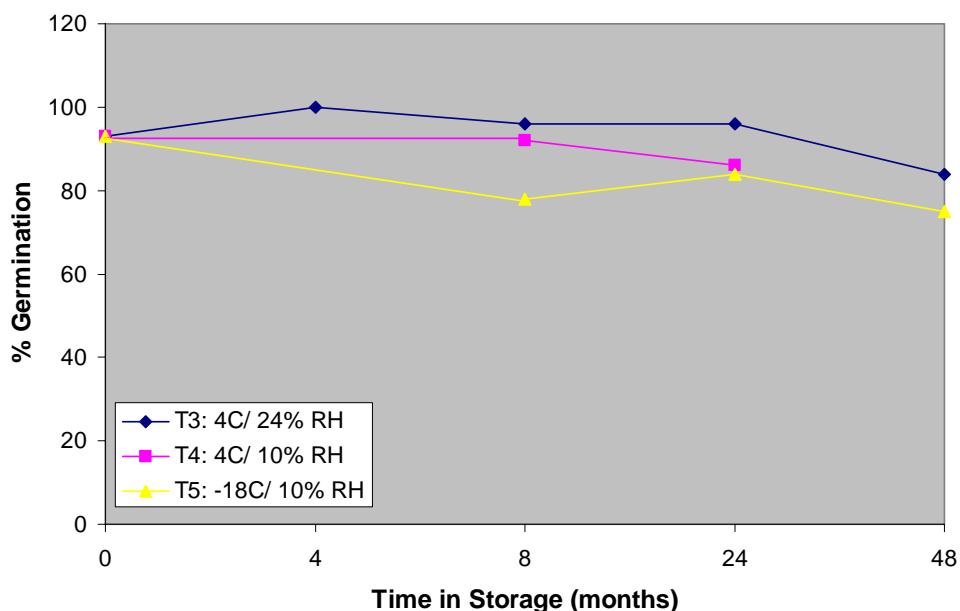
Collection: Fruits are best collected when they become less fleshy and dry out and begin to open on their own. Seeds can be squeezed and scraped out from the fruit. Seeds should be black, and unripe seeds are tan. Seeds without embryos are smaller and thinner. Fruits, especially ones still fleshy, will stain skin purple.

2. *Alsinidendron obovatum* (Caryophyllaceae) – ma‘oli‘oli

Collection Dates: 2 Mar 1999; 9 May 2000

Summary: (ND) The percent of seeds germinated on agar and also on paper wetted with H₂O₂ are significantly higher than the percent of seeds germinated on paper wetted with water or KNO₃. Storage results are for treatments 3, 4 and 5. Only one 2-year test was conducted. Viability was the same for all three, with refrigerated seeds having only slightly higher % germination. Recommended storage: 4C at 20% relative humidity (RH).

Storage Potential for Seeds of *Alsinidendron obovatum* - 2 Mar 1999



3. *Cenchrus agrimonioides* var. *agrimonioides* (Poaceae) – kamanomano

Collection Dates: 31 Oct 2000; 4 May 2004

Collection: Seeds are brown, large (2.6mm long) and bristly; easily removed from the plant when mature.

Summary: (PD) – no significant germination recorded due to lack of an adequate amount of seeds to test. Fresh seeds were tested only on agar (60% germination). Stored seeds (treatments 2, 3b and 5a) tested after 4 mos. had 20-80% germination, 20-40% germination after 14 mos. All tests used only 10 seeds. Seeds initially are germinating more successfully on agar and paper+GA3 than KNO3 or H2O for the 2004 collection. Recommended storage: 24C at 20% RH

CHAMAESYCE

Collection: Fruits in drupes containing 1- 3 grayish seeds (1-3mm long) each. Fruit should be dry and open on own to release seed when mature. Recommended storage: 4C at 20% RH

4. *Chamaesyce celastroides* var. *kaenana* (Euphorbiaceae) – ‘akoko

Collection Dates: 1 Nov 2001

Summary: (PD) Two of 10 fresh seeds germinated. Only 34 seeds stored in treatments 2 & 5a. One of 10 seeds from T2 and 2 of 10 from T5a germinated after 6 months of storage; none of the remaining seeds germinated.

5. *Chamaesyce herbstii* (Euphorbiaceae) – ‘akoko

Collection Date: 18 Jan 2001

Summary: (PD) Twelve seeds total were received. 1 of 3 germinated as fresh seeds. 2 of 3 T2 seeds and 1 of 3 T5a seeds germinated after 4 months of storage. Seeds that did not germinate appeared rotten or hollow. It is uncertain if seeds were initially collected hollow or decayed in storage.

CYANEA

Collection: Seeds are round or oval and slightly flattened. Ripe seeds are light tan to black. Fruits are spherical berries 5-10 mm in diameter for *C. longiflora*, and 16-30 mm for *C. grimesiana* & *C. superba*. Ripe fruits are orange or purple, with soft, orange pulp. Seeds can be extracted by scraping out inside pulp and seeds and mashing through a small-size sieve into a large beaker of water (wet-sieving). Seeds usually sink and pulp can be gently poured and

stirred off. Seeds that float may be oily and will sink if misted with a spray bottle before pouring off pulp. Dry the seeds on filter paper or a coffee filter. Recommended storage: 4C at 20% RH

6. *Cyanea grimesiana* subsp. *obatae* (Campanulaceae) – haha

Collection Dates: 1 Dec 2000

Summary: (ND) This collection is very small; larger collections are necessary to substantiate results. Germination for all treatments tested is not high, but GA3+ agar or paper is recommended. Only storage treatments 2 and 5a have been tested, and only T2 showed any germination after two years.

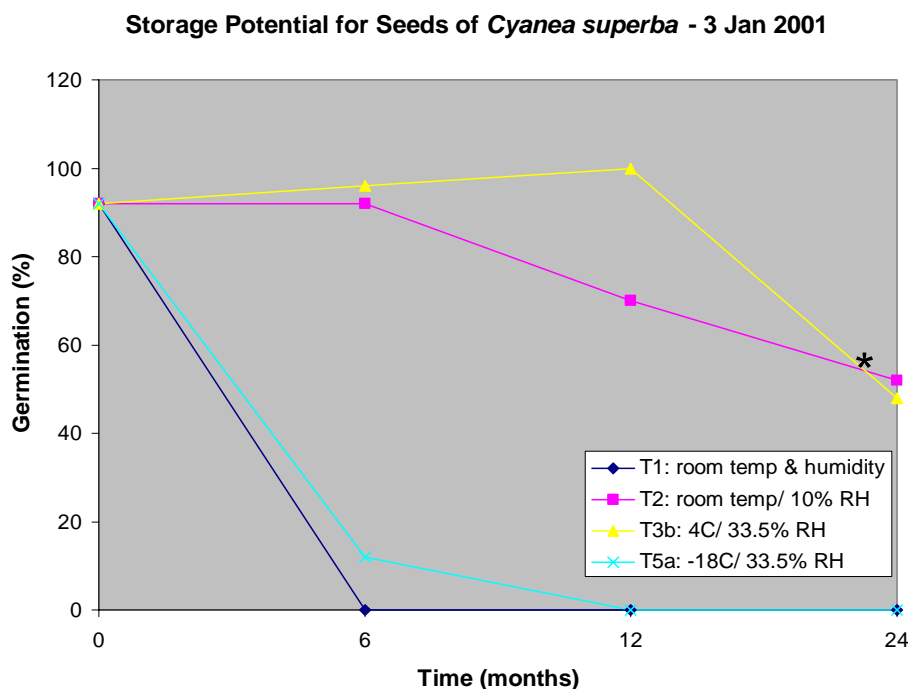
7. *Cyanea longiflora* (Campanulaceae)

Summary: Seed Bank species only; no storage tests were conducted. Initial viability varies among collections, though most have 55-85% germination. Seeds must be mature (flesh of fruit should not be all green, seeds should be dark brown) for good germination. Seeds appear to germinate best on agar and GA3 may increase germination or at least speed to which seeds germinate.

8. *Cyanea superba* (Campanulaceae) – haha

Collection Dates: 11 Jan 1998; 1 Apr 1998; 11 Jan 1999; 3 Jan 2001; 20 Dec 2002

Summary: (PD/ND) Seeds germinate best with GA3 on paper, though agar + GA3 may yield even higher germination. Storage treatments 1, 2, 3b, and 5a have been tested for two years. T1 and T5a yield 0% germination while T2 and 3b yield similar germination, around 50%. Seeds may be intolerant to freezing. Storage potential beyond two years has not yet been established.



9. *Cyrtandra dentata* (Gesneriaceae)

Collection Dates: 8 Oct 2003; 10 Mar 2004; 21 July 2004-10-04

Summary: Fresh germination on agar is 100%. Seeds have only been tested after 6 months of storage. After 6 months, refrigerated seeds have the highest germination, ~ 90%.

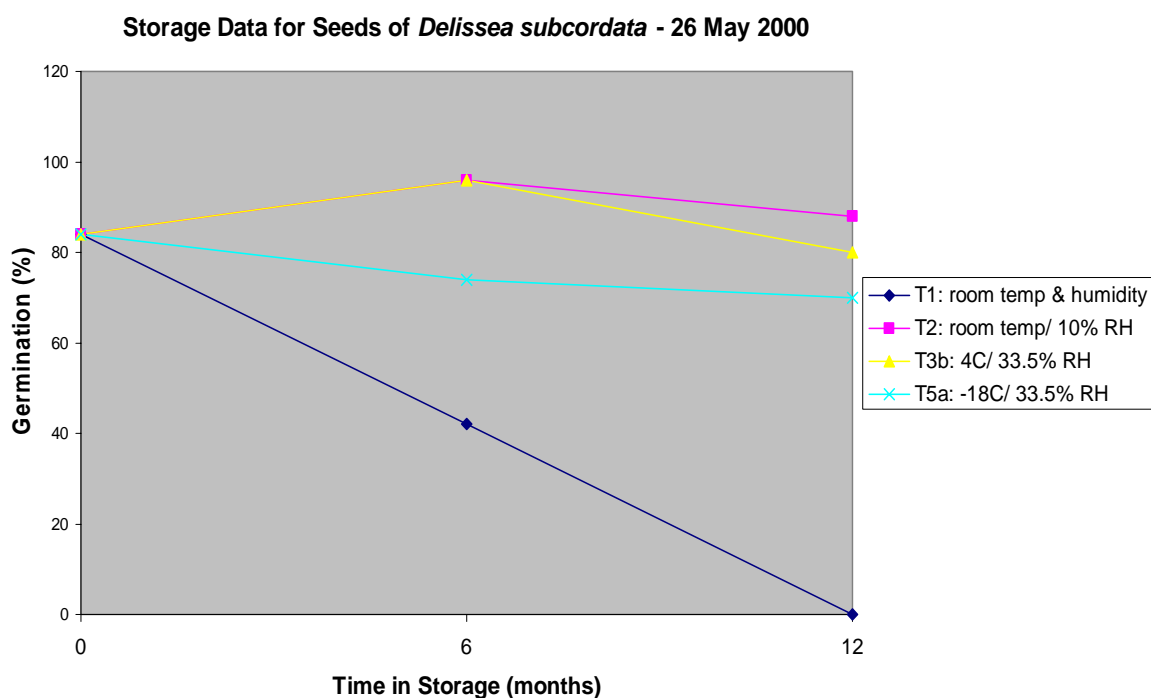
10. *Delissea subcordata* (Campanulaceae)

Collection Dates: 25 May 2000; 18 Jan 2001

Collection: Fruit are purple and fleshy yet may be still firm at ripening. Fruit can be removed individually or as an inflorescence. Seeds are light in color, slightly tan/yellow and can easily be scraped out of cut fruit. Seeds can then be air-dried to more easily sift/pick out remaining pulp.

Summary: (PD/ND) Seeds germinate best on agar and GA; the agar+GA3 treatment should be tested. Seeds stored imbibed in the dark germinate well when exposed to light (treatment T8), suggesting good potential to form soil seed banks. Storage treatments 1, 2, 3b, and 5a have been tested for one year. After one year, seeds stored in treatment T1 show no viability, but a lack of difference between 2, 3b, and 5a indicates that the best storage temperature and drying relative humidity have yet to be determined. Another collection has tested seeds for only T5 and T5a for

two years. Both -18C treatments yielded low germination after two years. Recommended storage: 4C at 20% RH



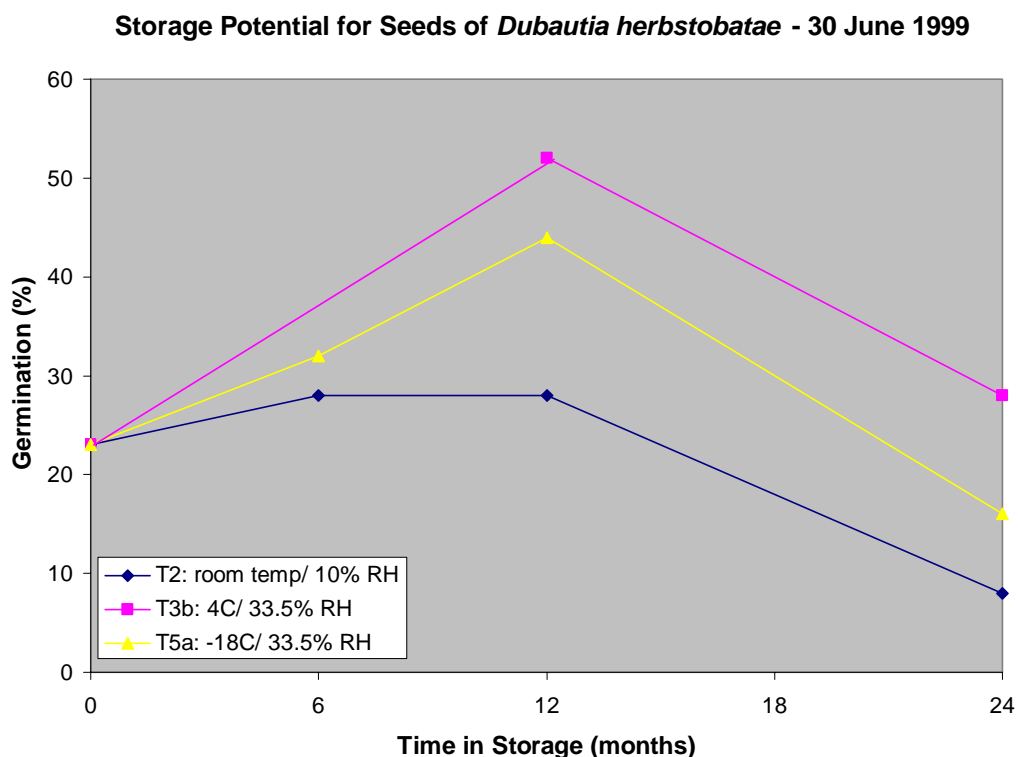
DUBAUTIA

Collection: Collect dried flower heads. Seeds are long and thin with bristles (hairs) on one end.

11. Dubautia herbstobatae (Asteraceae)

Collection Dates: 16 Jun1999; 30 Jun 1999

Summary: (PD) Seeds only germinated on agar. Storage results for T2, 3b and 5a have been recorded for two years. T3b shows best results but not significantly different from 5a and 2. The five-year test is currently running, with no T2 germination and equal yet still low T3b and T5a germination.



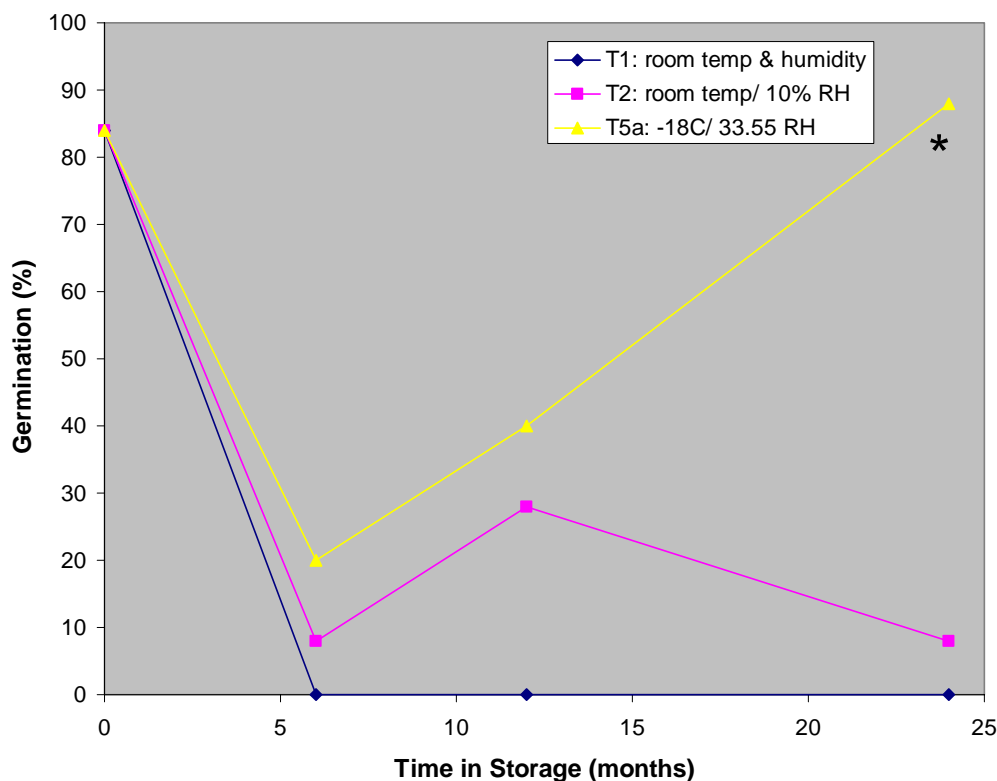
12. *Flueggea neowawraea* (Euphorbiaceae) – mehamehame

Collection Dates: 8 Jan 2002

Collection: Fruit is dry and capsules pop open when seeds are mature. Fruit must therefore be collected around time of dehiscence. Seeds are ~1mm long and tan. Soak seeds in water to remove floating seeds prior to sowing.

Summary: (PD) Seeds germinate on a wide range of treatments except seeds on paper with water gminated significantly less than the other treatments. Storage treatments 1, 2, and 5a have been tested for two years. T5a yielded significantly higher germination (88%) than the other two treatments (T1 (0%); T2 (8%)). Seeds need to be stored at -18C for successful long-term storage.

Storage Potential for Seeds of *Flueggea neowawraea* - 8 Jan 2002



HEDYOTIS

Collection (for all species of *Hedyotis* except *H. terminalis*): Fruits should dry out and dehisce when seeds are mature. Seeds are small and numerous in each capsule. Seeds are black and have various shapes.

13. *Hedyotis degeneri* var. *degeneri*

Only in seedbank. Except for one collection that was not fully mature, initial viability ranges from 65% to almost 100%. Seeds are stored at 4C with 20% RH.

14. *Hedyotis parvula*

Only in seedbank. Initial viability has a very wide range. Reasons for poor germination for some collections are unknown.

15. *Hesperomannia arbuscula* (Asteraceae)

Only in seedbank for germination. ~30 viable seeds received; seeds just started to germinate. No storage data.

HIBISCUS

Collection: Seeds are brownish, 3-5mm long. Trichomes are numerous and piercing to skin. Collect at time of dehiscence. If fruit are not open, squeeze to open and scrape seeds out with a pick.

16. *Hibiscus brackenridgei* subsp *mokuleianus* (Malvaceae) – ma’o hau hele

Collection Dates: 24 Apr 2000; 3 Mar, 21 Apr, 17 May 2004

Summary: (PY) Only tested on agar after scarification. T5 seeds tested after 3 years yielded 40% germination (2000 collection). GA3 tends to speed up germination. Seeds from 2004 collections are mostly inviable. There was very little germination and seeds became very moldy over the first week of being imbibed. Seeds may germinate slightly more when sanded as opposed to clipped, and this may be true for most seeds of Malvaceae, where three species of *Abutilon* also show better germination when sanded instead of clipped.

LIPOCHAETA

Collection: Collect flower heads when completely dry and show no green. Rub heads to separate seeds from debris and sift out seeds.

17. *Lipochaeta tenuifolia* (Asteraceae) – nehe

Collection Dates: 8 May 2000; 23 May 2000; 7 May 2001; 25 May 2001; 1 June, 6 June 2004

Summary: (PD) Weak fresh germination with agar and GA3. Agar+GA3 needs to be properly tested. No germination with other standard treatments. No germination from stored seeds except from T2 after the 1-year test. T1, 5, and 5a have also been tested. Hot water soaks have also been tested with none to minimal results. Initial seed viability is unknown for all collections except from 2004, where embryos appear healthy and viable. Both 2004 collections will be used for a scarification test (seed coat versus seed coat removed) and a dormancy test (Move-along Experiment).

18. *Neraudia angulata* var. *angulata* (Urticaceae) – ma’aloa

Collection Dates: 27 Feb 2003; 17 March 2004

Collection: Fruit are dry and reddish when mature; one seed per fruit. Seeds are 1 to 3.5 mm long and pale tan. Seeds that are white are probably immature.

Summary: (PD) Seeds germinate best on agar and significantly more than other germination treatments except GA3. 1-year storage tests are ongoing; seeds may store best at T2, but refrigeration treatments need to be tested. All tests have low germination. The 2004 collection has much higher fresh germination (50% and still ongoing).

NOTOTRICHUM

Collection: Fruit (2mm long) dry when seeds are mature; one seed per fruit.

19. *Nototrichium humile* (Amaranthaceae) – kulu’i

Collection Dates: 14 July 2000; 2 Nov 2000

Summary: (PD) One fresh seed (out of 50) germinated in KNO₃ treatment. No other seeds germinated in other germination treatments, as well as the 1, 2 and 5a storage treatments.

PHYLLOSTEGIA

Collection: Nutlets have fleshy outside and turn black when ripe, 3-6 mm long.

20. *Phyllostegia kaalaensis* (Lamiaceae)

Collection Dates: 28 Oct 2002

Summary: No seeds (only 12 tested) germinated on the four (agar, paper, KNO₃, GA3) treatments tested.

21 .*Plantago princeps* subsp *princeps* (Plantaginaceae) – manene

Collection Dates: 11 Jan 2000; 29 Dec 2000

Collection: One to 2 seeds, black to brown or brown/red, are in each dry capsule.

Summary: (PD) None of the 7 seeds from the first collection germinated. They were tested on agar. Seven seeds were also collected for the second collection. Two out of 2 germinated initially on agar. The one seed placed in T1 storage did not germinate at 6 months. One of the 2 seeds placed in T2 germinated after 6 months and both of the seeds placed in T5a treatment germinated at this time. Initial viability for seeds in the seed bank is typically high (75-100%).

22. *Pritchardia kaalae* (Arecaceae)

One collection received in 2000 for testing. All seeds were given to Ray Baker and Nellie Sugii.

23. *Sanicula mariversa* (Apiaceae)

Collection Dates: 25 May 1999; 26 May 1999; 29 June 1999; 23 May 2002

Collection: Fruit are 2-6mm long and densely covered in prickles. Fruit are in heads and can easily be collected when dry.

Summary: (MPD) Numerous germination and scarifications treatments have been used with no success. The only time seeds germinated were when they were plated on agar and then re-sown on agar. Others have successfully germinated them on potting mix in pots.

SCHIEDEA

Collection: Fruits are tan, papery capsules when fully mature. Numerous seeds are in each capsule. Squeeze the capsule and pick out seeds. Seeds are small, hard, and black.

24. *Schiedea kaalae* (Caryophyllaceae) – ma'oli'oli

Collection Dates: 7 June 2002

Summary: Germination is very low overall. GA3 is recommended and agar+GA3 should be properly tested. Paper+H2O yielded significantly lower germination than the other treatments. Seeds do not germinate in T7 and T8 results are unknown. Storage treatments 1, 2 and 5a were tested at 6 months. T1 showed no germination and T2 and 5a showed low germination similar to the initial test on fresh seeds.

25. *Schiedea nuttallii* (Caryophyllaceae)

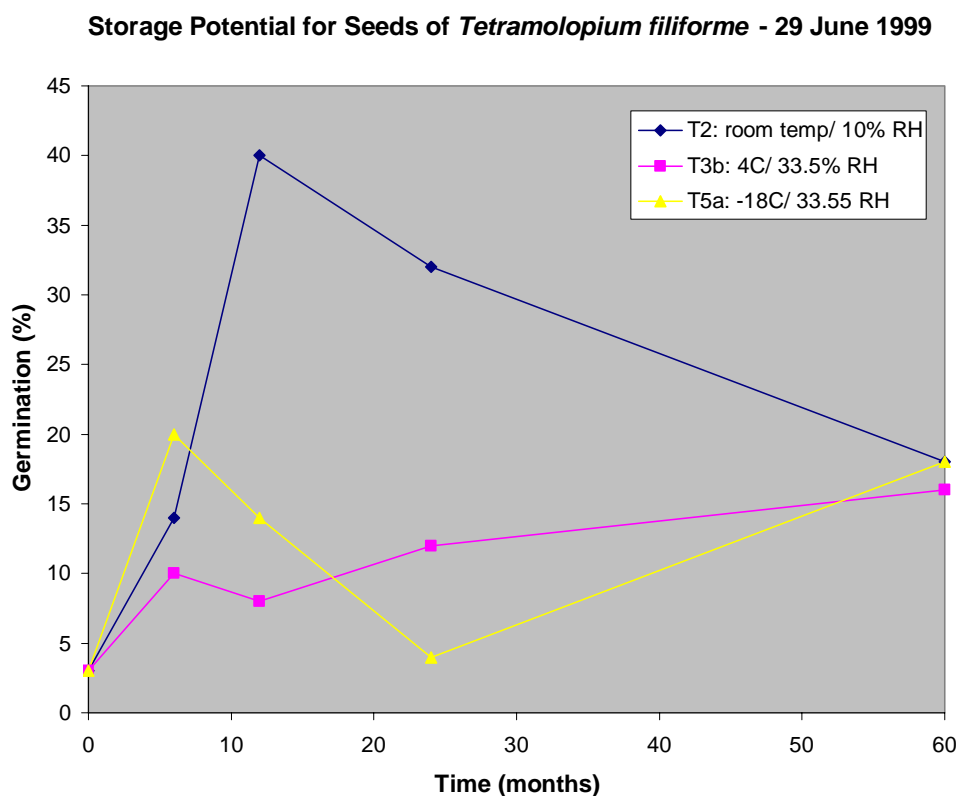
Only in seedbank. Initial germination ~50% or lower for most collections. All collections very small.

26. *Tetramolopium filiforme* (Asteraceae) – pamakani

Collection Dates: 6 Apr, 16 Jun, 29 Jun, & 30 Jun 1999; 7 Jan 2000

Collection (for all *Tetramolopium* spp.): Collect dried flower heads. Seeds are yellowish tan and flattened in appearance with long bristles at one end.

Summary: (PD) Low germination from all initial germination treatments. Untreated agar or paper in Petri dishes is recommended. Storage treatments 2, 3b and 5a have been tested for five years (5-year test ongoing). Results are similar for all three treatments, overall germination is moderate to low but T2 is recommended. Stored seeds have higher germination than fresh seeds, reflecting the seeds' physiological dormancy.

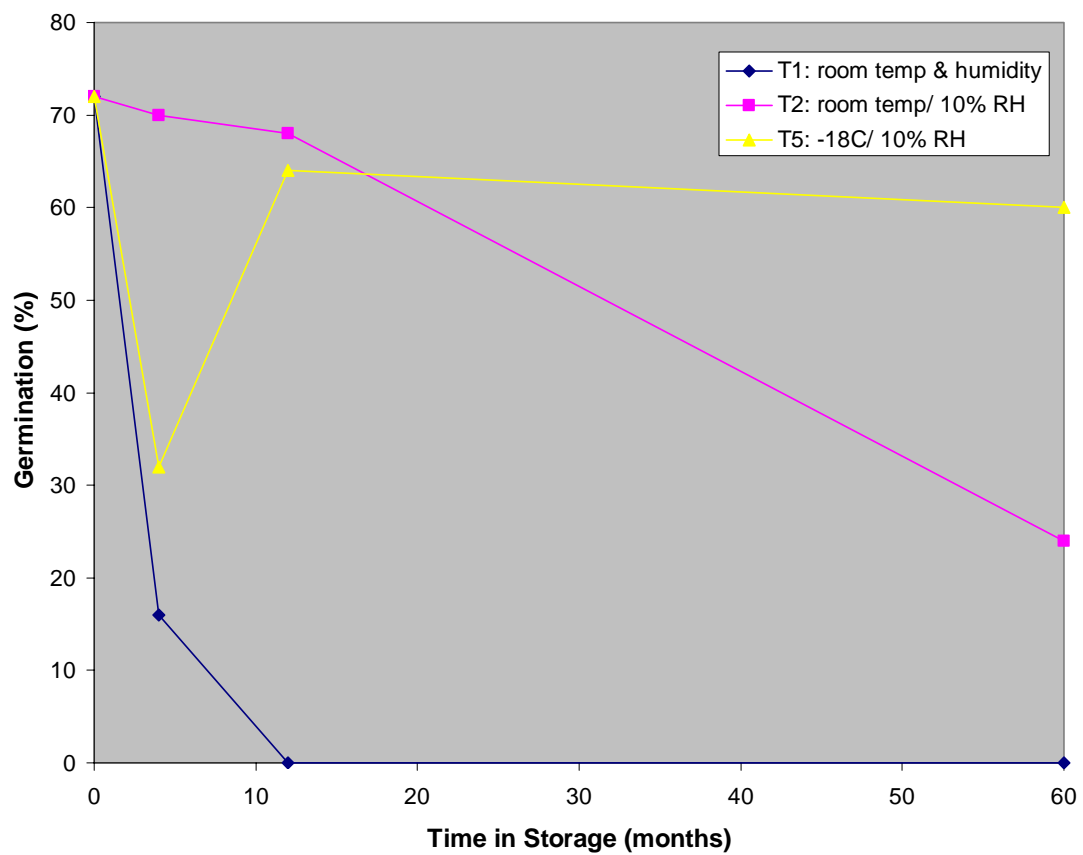


27. *Viola chamissoniana* ssp. *chanissoniana* (Violaceae) – pamakani

Collection Dates: 29 June 1999

Collection: Numerous black seeds (~2 mm long) are in each capsule (~ 15mm long).

Summary: (PD) Seeds germinate significantly more on agar than on paper + H₂O, KNO₃, or H₂O₂. Agar + GA₃ should be properly tested. No T1 germination by 1 year. T2 and T5 have been tested for 5 years. After 5 years, T5 seeds (60%) have significantly higher germination than T2 seeds (24%). Another collection is necessary to test other treatments.

Storage Potential for Seeds of *Viola chamissioniana* ssp. *chamissioniana* - 29 June 1999

6.0 MANAGEMENT UNIT STATUS

Based on the April 29, 2004 meeting of the Makua Implementation Team (MIT), management unit (MU) boundaries were revised. At this meeting Natural Resource Staff (NRS) proposed removing severely degraded habitat from the original MU boundaries. In addition, since the U.S. Army (Army) has decided to focus management on fewer and more viable populations, associated MU level work is centered on better habitat where success is more attainable. In general, MU level work consists of ungulate control and weed control across large areas.

Ungulate management

Where ungulate control is necessary a combination of monitoring, fencing, hunting, and snaring is employed. Most of the Makua Implementation Plan (MIP) MUs require a fence, but there are some that do not. The need for a fence is indicated via shading per specific MU in the Table 6.1 below. The status of ungulate control and fences is also included in this table. For more details about on-going projects related to the MIP see Chapter 1 of the PCSU annual report to the Army for 2004.

Fencing

Fencing projects are very slow to implement because of the associated paperwork. In the next year the Army is going to prepare an Environmental Assessment to cover all the actions in the MIP. Additionally, an umbrella Conservation District Use Permit (CDUP) will be sought for management actions planned over the next three years (CDUPs are only issued for three-year time periods). This will speed the process of fence construction significantly. Until that time, fence planning is proceeding at one large-scale fence per year. This year, we focused on getting one large fence constructed in Makaha, in addition to many small population fences. We scoped the Makaha Sub-Unit I fence in February of this year. Army Archaeologists conducted a review of the fence line and consulted with the State Historic Preservation Office in April 2004. Funding was secured and the fencing contract awarded at the end of the fiscal year 2004. Construction may begin as early as the spring of 2005. The small population fences NRS also worked on this year are described in the relevant species reports (Chapter 5: Rare Plant Stabilization Status), as well as in Chapter 3 of the PCSU year-end report.

Weed management

Weed control is conducted across MUs with priority placed on the habitat that is the most intact. In many of the MIP MUs there is a significant amount of alien-dominated forest. It makes sense, then, to focus reintroduction and weed control efforts on those areas where success is most likely. This is not to say that managing weed-dominated areas cannot be done, but it is certainly more time consuming with less guarantee of success. Extensive weed control is conducted in areas within MMR and has begun in offsite MIP MUs. The need for weed control is indicated via shading per specific MU in Table 6.1 below. The weed control conducted over the last year in each of the MIP MUs is also summarized in the table. For detailed specific weed control project discussion see Chapter 2 of the PCSU year-end report to the Army for 2004.

Landowner Issues

Offsite work has been slow to begin, as it requires time to become oriented to projects and new areas. NRS have been working with other conservation organizations on Oahu, including the Nature Conservancy (TNC), the Honolulu Board of Water Supply and the State of Hawaii. Each of these agencies has their own set of management goals and varying management approaches.

For these landowners to coordinate with the Army's Natural Resource Program requires a great deal of their limited time. This has lead to some challenges in initiating management actions. NRS are working to establish reliable and efficient means of communication about projects. Overall, agencies that have a conservation mandate are more than willing to provide guidance to NRS because of the significant workforce we can provide. We have hired a coordinator/liason for the Nature Conservancy of Hawaii who works full-time at Honouliuli Preserve. This arrangement is working well.

Table 6.1 Management Unit Status

Management Unit	Fenced	Ungulate Control	Weed Control
Alaiheie to Palikea Gulch	No	Goat control is underway in Lower Kaala NAR nearby.	None
Central and East Makaleha	No	Limited goat control is underway in Central and East Makaleha.	None
Ekahanui	Partial	TNC conducts fence maintenance and pig control outside this enclosure.	31.5 person hours were spent controlling weeds around rare plant reintroductions.
Haili to Kawaihapai	No	None	None
Kaena and Keawaula	No	None	253 person hours were spent conducting weed control over less than two acres.
Kahanahaiki	Yes	Transects monitored 4x/year. Exclosure is maintained as ungulate free. Fence maintenance is on going.	686 person hours were spent controlling weeds over 21 acres.
Kaluaa and Waieli	Partial/ Upcoming	Central Kalua'a is already fenced and an additional fence will be built this year in upper Waieli near Puu Hapapa.	The Army's liason has conducted weed control in Kaluaa and Waieli mainly focused around a core reintroduction site. NRS have also conducted about 50 hours of weed control.
Kaluakauila	Yes	Unit is ungulate free. Transects are monitored 4x/year. Fence repairs were conducted this year after heavy rains. Hunting conducted around the perimeter of fence 2x this year.	101 person hours were spent controlling weeds over 56 acres.
Kamaileunu	No	None	None
Keaau and Makaha	No	The State of Hawaii manages a public hunting program in this area.	None

Management Unit	Fenced	Ungulate Control	Weed Control
Lower Kahanahaiki	No	Snaring is on-going in this unit mainly to keep pig pressure off of the Kahanahaiki fence line and to protect the native resources in the MU.	None
Lower Ohikilolo	Yes	A goat fence is maintained and monitored to ensure no goats from adjacent population get in.	659 person hours were spent controlling weeds over 7 acres.
Lower Opaepala	Upcoming	The Koolau Mountains Watershed Partnership acquired funding construct a fence. Construction will begin after EA approved.	90.5 person hours were spent controlling weeds over less than an acre.
Makaha	Upcoming	None	None
Mt. Kaala NAR	No	Goat control via hunting and snaring is on going.	None
Ohikilolo	Yes	Goat control via hunting and snaring is on going. Monitoring along three transects is conducted to guide management.	55 person hours were spent controlling weeds over 17 acres.
Pahole	Yes	Fence is maintained by the State. Unit is ungulate free.	17 person hours were spent controlling weeds over 5.5 acres.
Palikea	Partial	TNC conducts snaring outside the perimeter of the existing fence.	4 person hours were spent controlling weeds over 7 acres.
Puu Kumakalii	No	None	None
Upper Kapuna	Upcoming	Construction is pending the State acquiring final funding.	48 person hours were spent controlling weeds over 7 acres.
Waianae Kai	No	None	None
Waiawa	No	None	None
West Makaleha	Partial	NRS assisted the State in controlling an incipient goat population 4x this year.	92 person hours were spent controlling weeds over 5 acres.

Shading in the table above indicates that this type of management is needed for the MU.