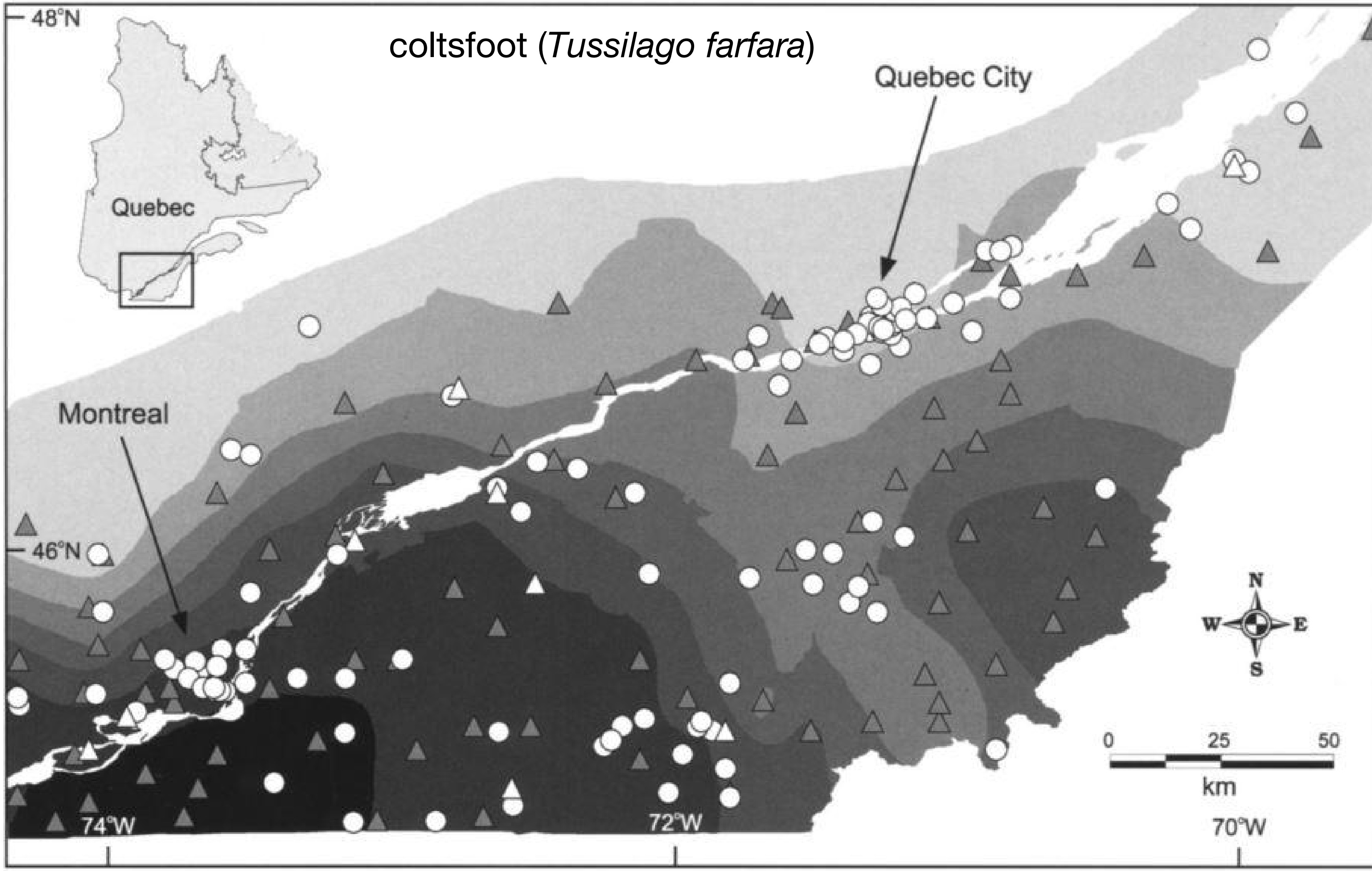
Picking from the Past in Preparation for a Pest: Assessing the Potential for Herbaria to Serve as Novel Sources for Tropical Seed Preservation

Dustin Wolkis & Susan Deans

National Native Seed Conference
February 16, 2017


Celebrating Ferns



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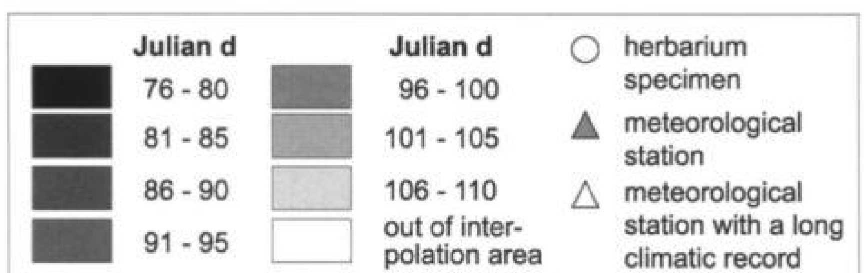
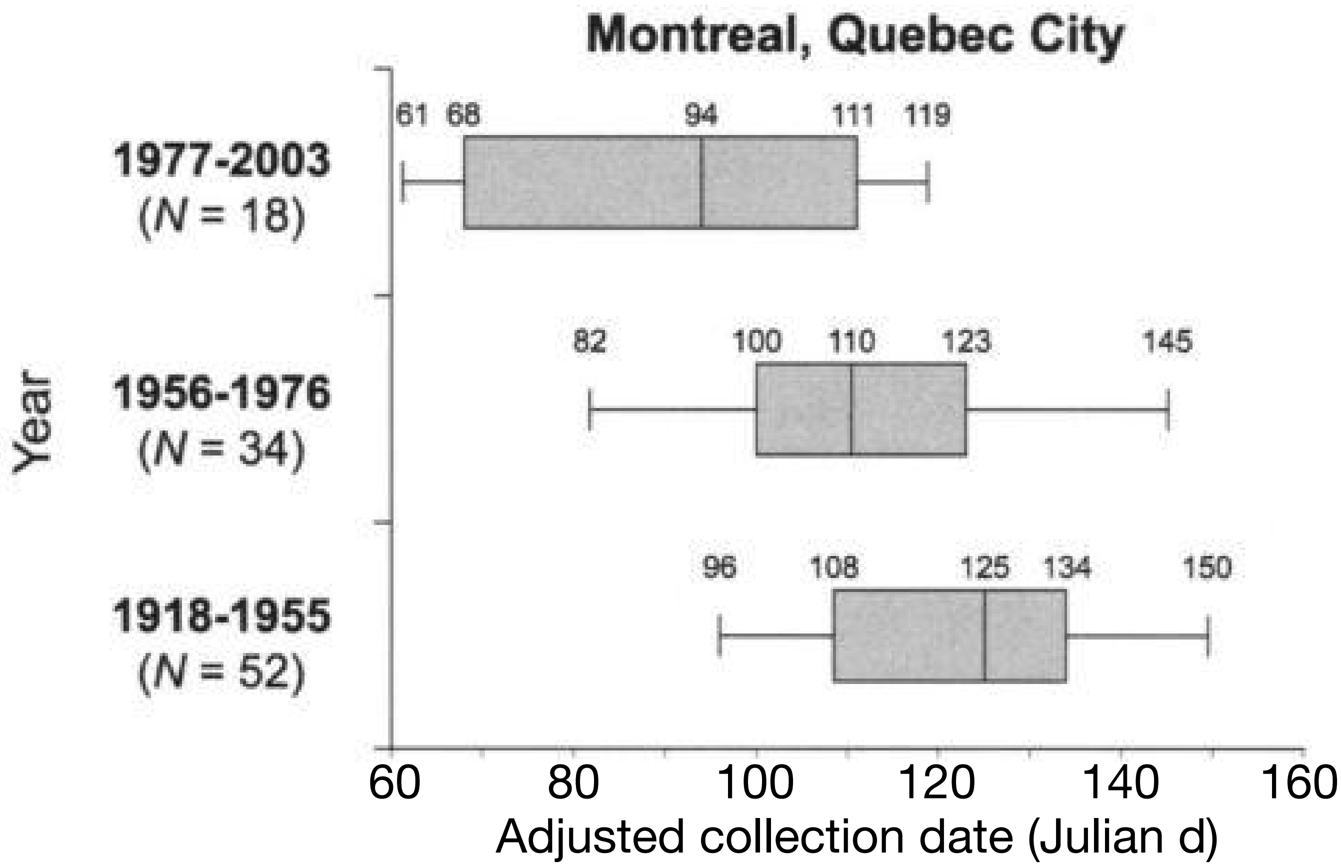


coltsfoot (*Tussilago farfara*)



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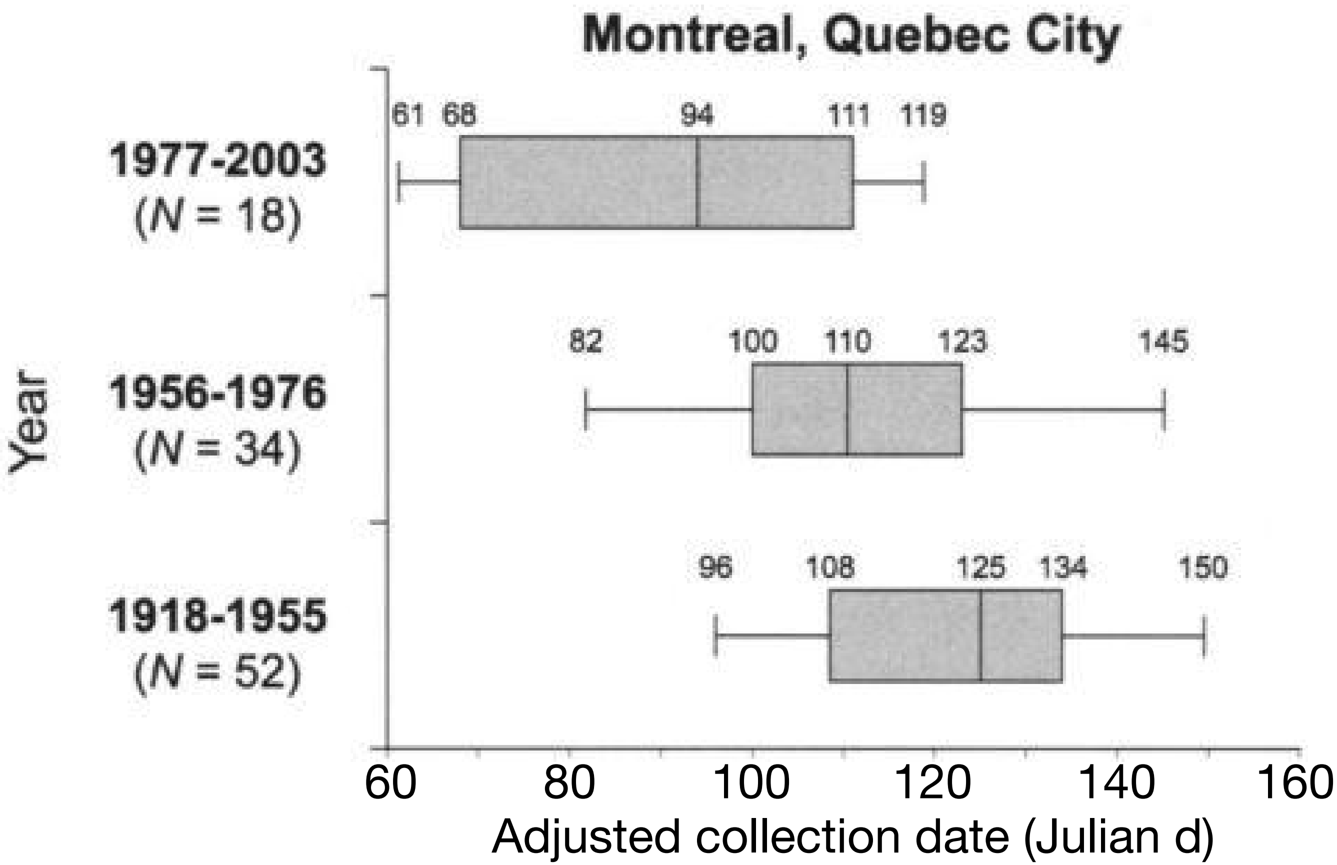

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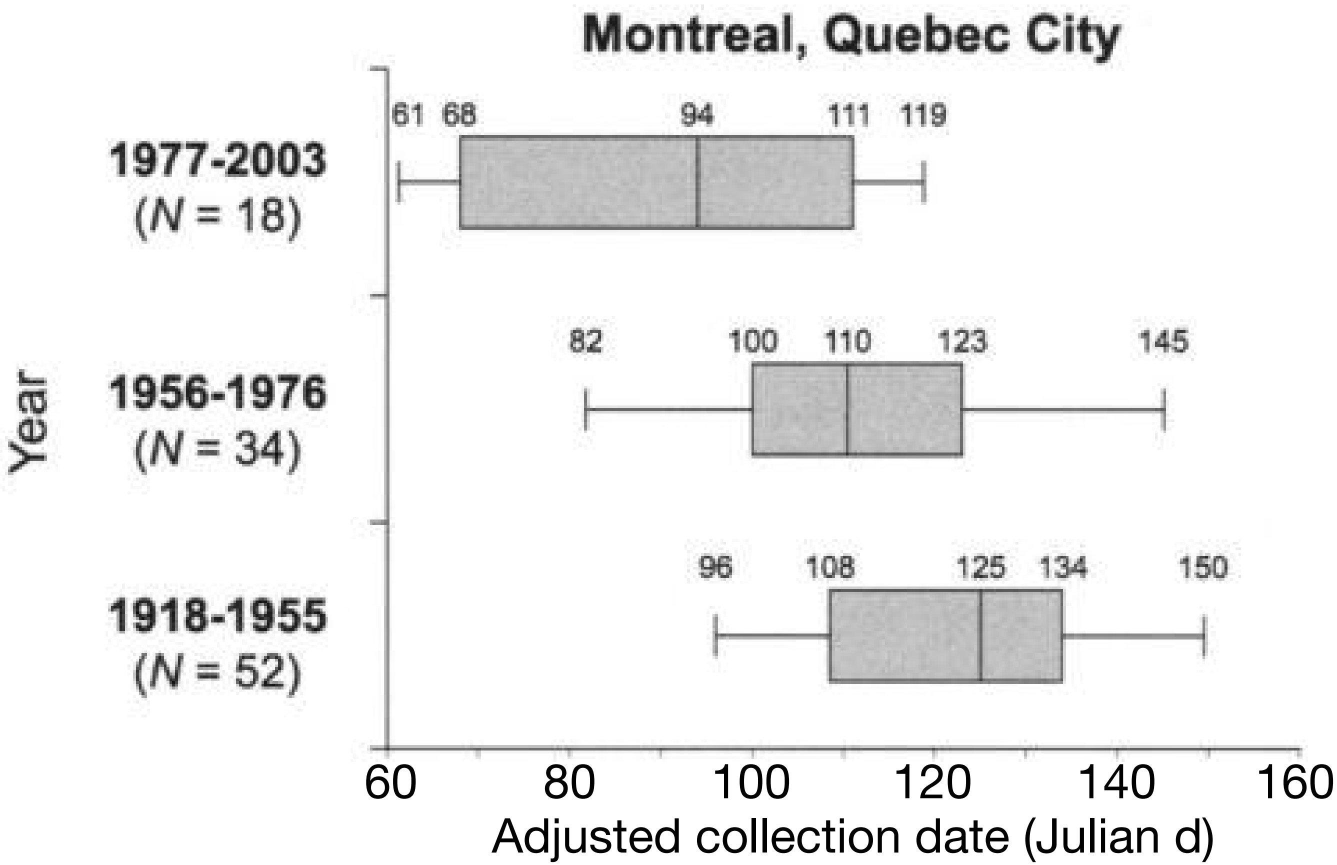

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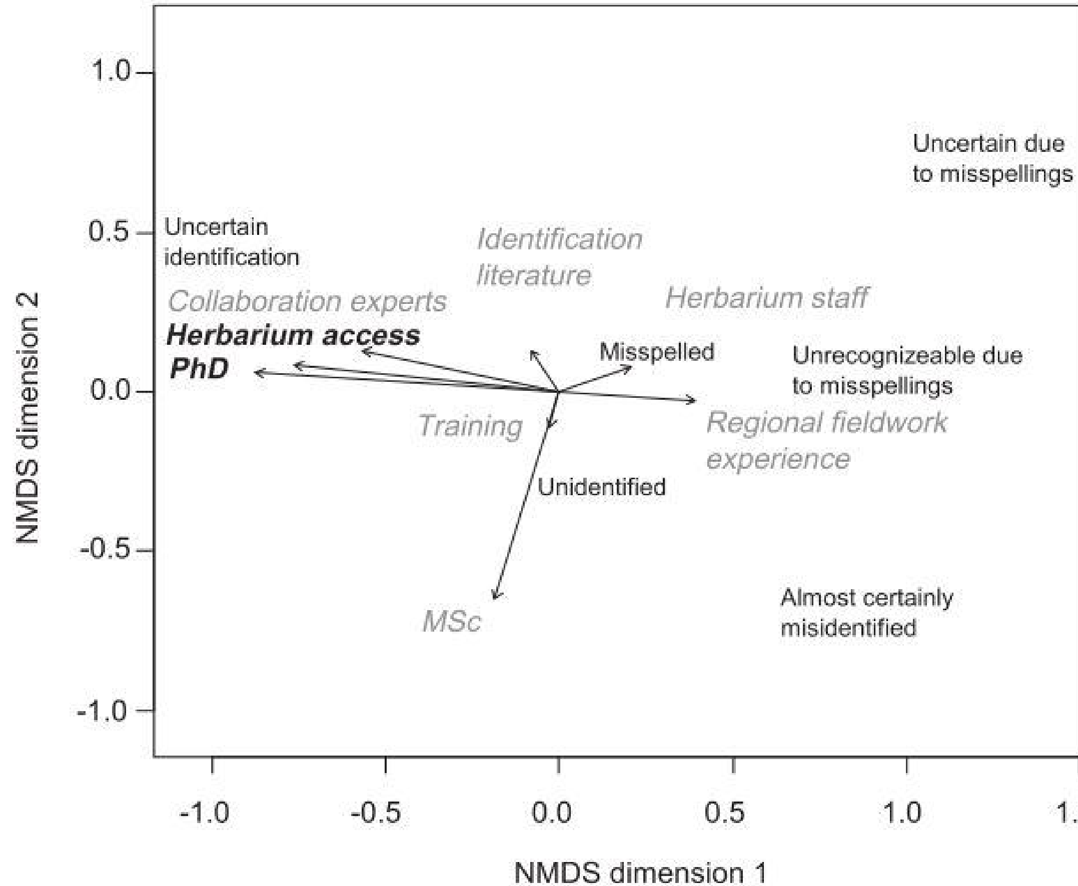
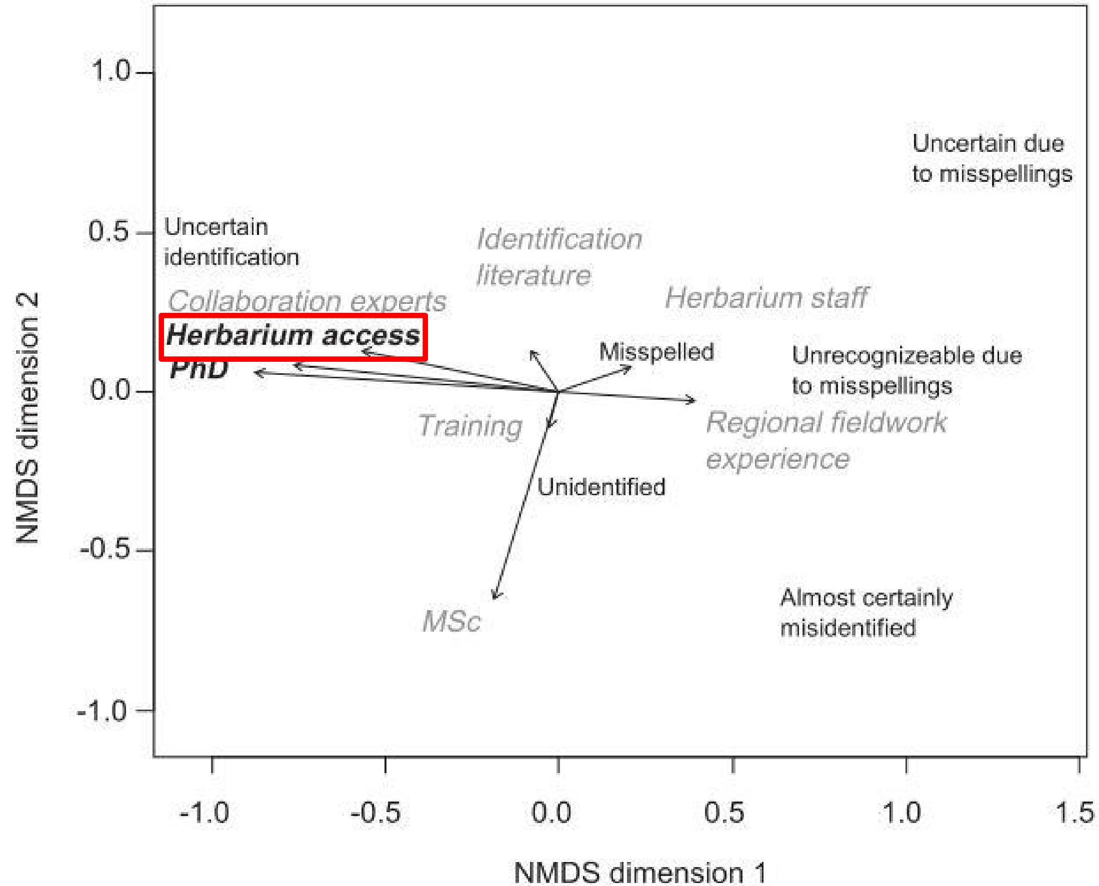
“Herbarium specimens are useful phenological indicators”

INTRODUCTION



	NMDS dimension 1	NMDS dimension 2	R^2	P
Regional fieldwork experience	1.00	-0.07	0.11	>0.1
MSc	-0.28	-0.96	0.33	>1.0
PhD	-00.1	0.07	0.56	<0.01
Training	-0.26	-0.97	0.01	>0.1
Herbarium access	-0.99	0.11	0.43	<0.05
Herbarium staff	0.93	0.36	0.04	>0.1
Access to identification literature	-0.54	0.84	0.02	>0.1
Collaboration with taxonomic experts	-0.97	0.23	0.25	>0.1

Ahrends A, Rahbek C, Bulling MT, et al (2011) Conservation and the botanist effect. Biol Conserv 144:131–140. doi: 10.1016/j.biocon.2010.08.008



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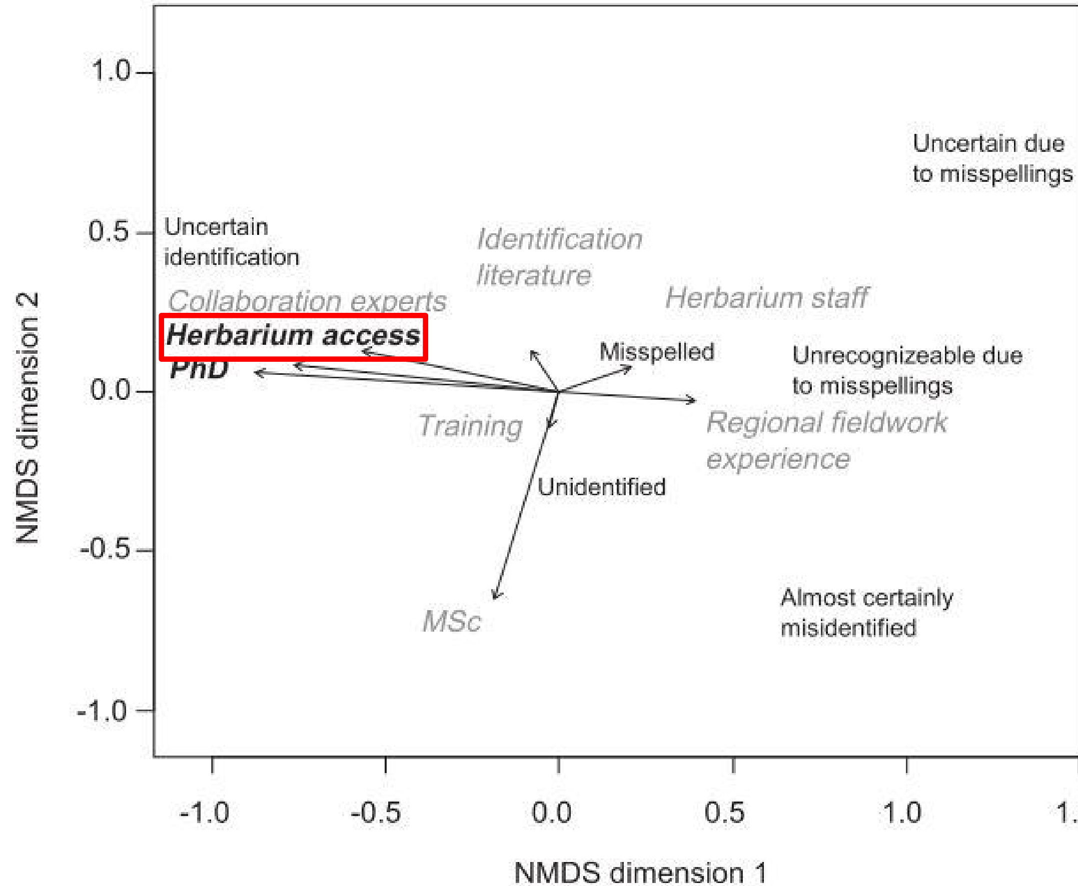

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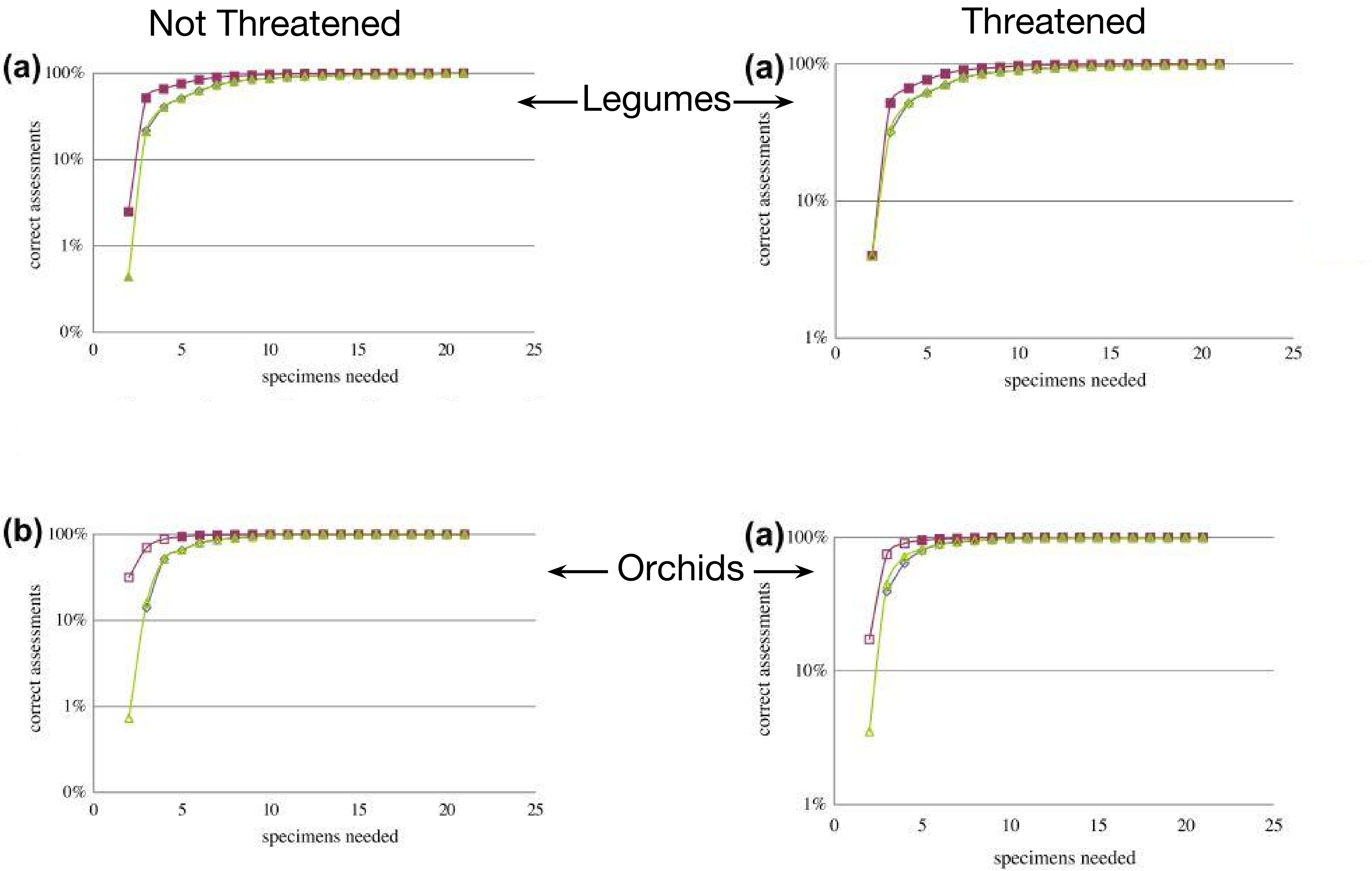

Rivers MC, Taylor L, Brummitt NA, et al (2011) How many herbarium specimens are needed to detect threatened species? Biol Conserv 144:2541–2547. doi: 10.1016/j.biocon.2011.07.014

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Nakahama N, Hirasawa Y, Minato T, et al (2015) Recovery of genetic diversity in threatened plants through use of germinated seeds from herbarium specimens. Plant Ecology 216:1635–1647. doi: 10.1007/s11258-015-0547-8

Seed Storage in Herbaria Conditions

Species	Period (years)
Cassia multijuga	158
Albizzia julibrissin	149
Cassia bicapsularis	115
Leucaena leucocephala	99

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Seed Storage in Herbaria Conditions

Species	Conditions of Storage	Pre-storage germination (%)	Post-storage germination (%)	Period (years)
Prosopis juliflora	Dry atmosphere of herbarium in S.W.	?	60	50
Acacia aneura	Closed containers at room temperature (20–25°C)	56	60	13
A. hemsleyi	Closed containers at room temperature (20–25°C)	96	96	13
A. holosericea	Closed containers at room temperature (20–25°C)	95	84	14
A. leptopetala	Closed containers at room temperature (20–25°C)	73	72	18
A. victoriae	Closed containers at room temperature (20–25°C)	80	60	18

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Species	Herbarium and Collection Date	Seed age yrs/Date of Germination	Germination Success
Asclepias lanuginosa	DEK 23 Aug 1976	12/1989	0/7 (0%)
Asclepias meadii	KANU 10 Aug 1987	3/1990	9/15 (60%)
	23 Aug 1987	3/1990	6/23 (26.1%)
	9 Sep 1987	3/1990	5/6 (83%)
	14 Jul 1987	5/1992	0/15 (0%)
	10 Aug 1987	5/1992	0/10 (0%)
	10 Jul 1988	4/1992	0/11 (0%)
	13 Jul 1988	4/1992	0/16 (0%)
Astragalus neglectus	F 1868	121/1989	(−)
	14 Aug 1978	111/1989	(−)
	Jul 1884	105/1989	(−)
	18 Jul 1911	78/1989	(−)
	25 Jul 1933	56/1989	(−)
	7 Jul 1936	53/1989	(−)
Astragalus neglectus	NYS 1884	105/1989	(−)
	1 Sep 1926	63/1989	(−)
	7 Sep 1931	58/1989	(−)
	10 Aug 1941	48/1989	(−)
Astragalus neglectus	WIS Aug 1882	97/1989	(+)
	2 Aug 1938	51/1989	(−)
	27 Sep 1941	48/1989	(+)
	15 Aug 1947	42/1989	(−)
	29 Jul 1949	40/1989	(−)
	1 Aug 1961	28/1989	(+)
Astragalus tennesseensis	M Sep 1982	4/1986	(+)

Bowles ML, Betz RF, Demauro MM (1993) Propagation of rare plants from historic seed collections: Implications for species restoration and herbarium management. Restor Ecol 101–106.

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Table 1. Germination results of seeds from herbarium specimens of 26 taxa extinct in Belgium.

Taxon	Seed age (yr)	qty-test	qty-ger	qty-emp	qty-mou	qty-fre	Pretreatment	Treatment	Duration (days)
Adonis aestivalis	112	20	0	0	20	0	C(34d)	10°C; 8/16; KNO_3	25
Adonis aestivalis f. *aestivalis*	90	18	0	0	18	0	C(34d)	10°C; 8/16; KNO_3	122
Adonis aestivalis f. *aestivalis*	72	13	0	0	13	0	C(34d)	10°C; 8/16; KNO_3	122
Adonis annua	45	12	0	0	12	0	C(34d)	10°C; 8/16; KNO_3	99
Adonis annua	74	12	0	0	12	0	C(34d)	10°C; 8/16; KNO_3	122
Adonis annua	144	22	0	0	22	0	C(34d)	10°C; 8/16; KNO_3	122
Adonis flammea	31	43	0	0	43	0	C(34d)	10°C; 8/16; KNO_3	99
Adonis flammea	77	26	0	0	26	0	C(34d)	10°C; 8/16; KNO_3	122
Anthericum ramosum	122	9	0	0	9	0	C(83d)	16°C; 12/12; GA_3	105
Anthericum ramosum	105	6	0	0	6	0	C(83d)	16°C; 12/12; GA_3	105
Anthericum ramosum	122	17	0	0	17	0	C(83d)	16°C; 12/12; GA_3	209
Anthericum ramosum	150	12	0	0	12	0	C(83d)	16°C; 12/12; GA_3	209
Asperula arvensis	133	13	0	0	13	0	C(56d)+NK	25°C; 8/16	23
Asperula arvensis	40	11	0	0	11	0	C(56d)+NK	25°C; 8/16	23
Asperula arvensis	110	15	0	0	15	0	C(56d)+NK	25°C; 8/16	23
Asperula arvensis	102	15	0	0	15	0	C(56d)+NK	25°C; 8/16	28
Bupleurum tenuissimum	125	33	2	0	31	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	?	50	0	0	50	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	101	50	5	0	45	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	158	50	0	0	50	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	144	50	0	0	50	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	144	50	0	0	50	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	144	50	1	0	49	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	144	50	0	0	50	0	–	23/9°C; 12/12	62
Bupleurum tenuissimum	144	50	0	0	50	0	–	23/9°C; 12/12	62
Bupleurum tenuissimum	144	50	0	0	50	0	–	23/9°C; 12/12	62
Calendula arvensis	69	15	0	0	15	0	–	21°C; 12/12	55

Godefroid S, Van de Vyver A, Stoffelen P, et al (2011) Testing the viability of seeds from old herbarium specimens for conservation purposes. Taxon 60:565–569.

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Adonis aestivalis f. *aestivalis*	90	18	0	0	18	0	C(34d)	10°C; 8/16; KNO_3	122
Adonis aestivalis f. *aestivalis*	72	13	0	0	13	0	C(34d)	10°C; 8/16; KNO_3	122
Adonis annua	45	12	0	0	12	0	C(34d)	10°C; 8/16; KNO_3	99
Adonis annua	74	12	0	0	12	0	C(34d)	10°C; 8/16; KNO_3	122
Adonis annua	144	22	0	0	22	0	C(34d)	10°C; 8/16; KNO_3	122
Adonis flammea	31	43	0	0	43	0	C(34d)	10°C; 8/16; KNO_3	99
Adonis flammea	77	26	0	0	26	0	C(34d)	10°C; 8/16; KNO_3	122
Anthericum ramosum	122	9	0	0	9	0	C(83d)	16°C; 12/12; GA_3	105
Anthericum ramosum	105	6	0	0	6	0	C(83d)	16°C; 12/12; GA_3	105
Anthericum ramosum	122	17	0	0	17	0	C(83d)	16°C; 12/12; GA_3	209
Anthericum ramosum	150	12	0	0	12	0	C(83d)	16°C; 12/12; GA_3	209
Asperula arvensis	133	13	0	0	13	0	C(56d)+NK	25°C; 8/16	23
Asperula arvensis	40	11	0	0	11	0	C(56d)+NK	25°C; 8/16	23
Asperula arvensis	110	15	0	0	15	0	C(56d)+NK	25°C; 8/16	23
Asperula arvensis	102	15	0	0	15	0	C(56d)+NK	25°C; 8/16	28
Bupleurum tenuissimum	125	33	2	0	31	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	?	50	0	0	50	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	101	50	5	0	45	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	158	50	0	0	50	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	144	50	0	0	50	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	144	50	0	0	50	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	144	50	1	0	49	0	–	23/9°C; 12/12	309
Bupleurum tenuissimum	144	50	0	0	50	0	–	23/9°C; 12/12	62
Bupleurum tenuissimum	144	50	0	0	50	0	–	23/9°C; 12/12	62
Bupleurum tenuissimum	144	50	0	0	50	0	–	23/9°C; 12/12	62
Calendula arvensis	69	15	0	0	15	0	–	21°C; 12/12	55

Godefroid S, Van de Vyver A, Stoffelen P, et al (2011) Testing the viability of seeds from old herbarium specimens for conservation purposes. Taxon 60:565–569.

Photo: University of Hawaii

RAPID ʻŌHIʻA DEATH

ʻŌhiʻa-Lehua Legend

| By Leilehua Yuen

ʻŌhiʻa-lehua flowers and seed capsules in the rain at Volcano

Long, long ago on the island of Hawaiʻi in the district of Puna, there lived a beautiful girl. Lehua was her name. She had a face as round and glowing as the moon with eyes that glimmered like starlight, a back as straight as the pali—the great sea cliffs—and hair that rippled down it like a waterfall. Her heart was as kind and generous as her face and form were beautiful, and all who knew her loved her.


Photo: Dustin Wolkis

Photo: Dustin Wolkis

Photos: Dustin Wolkis


Photo: Dustin Wolkis


Photo: Dustin Wolkis

RAPID ʻŌHIʻA DEATH

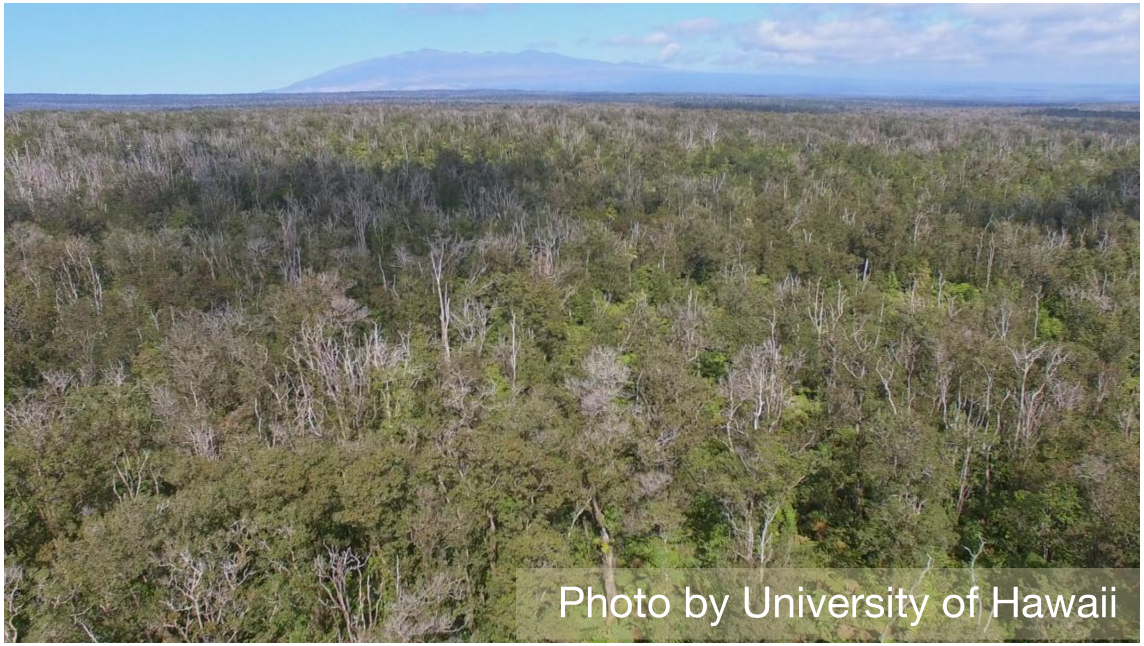
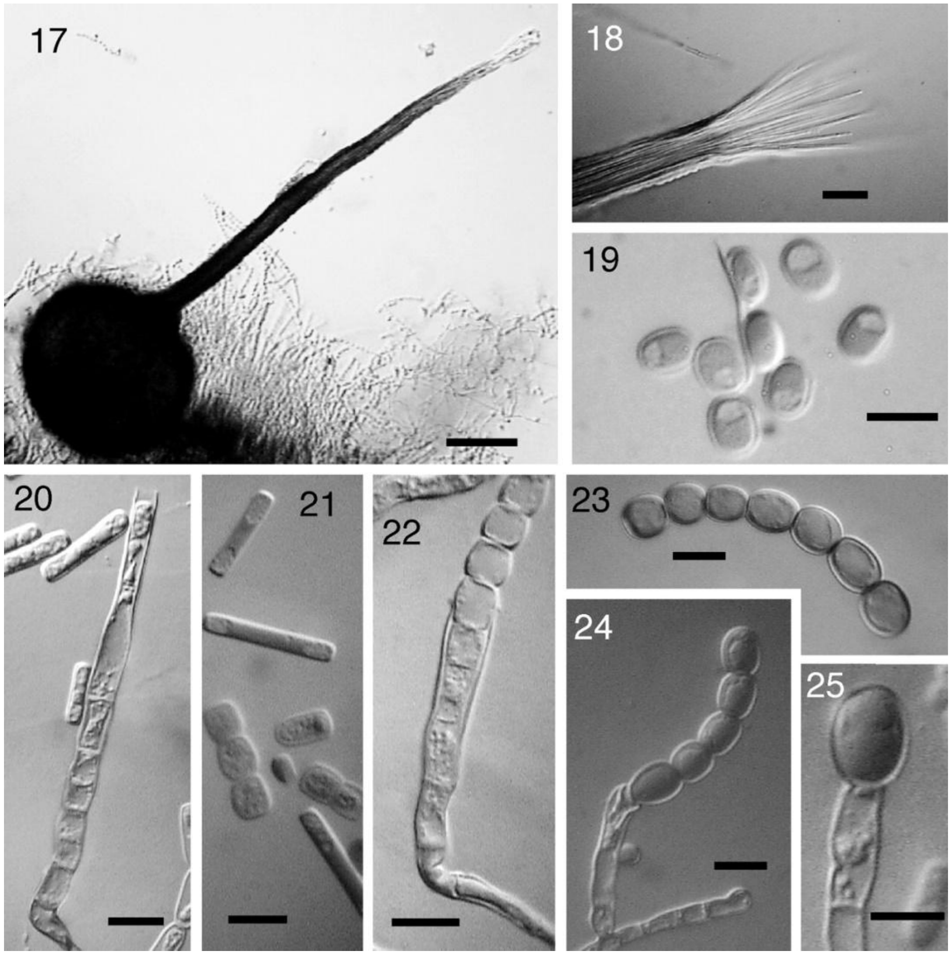


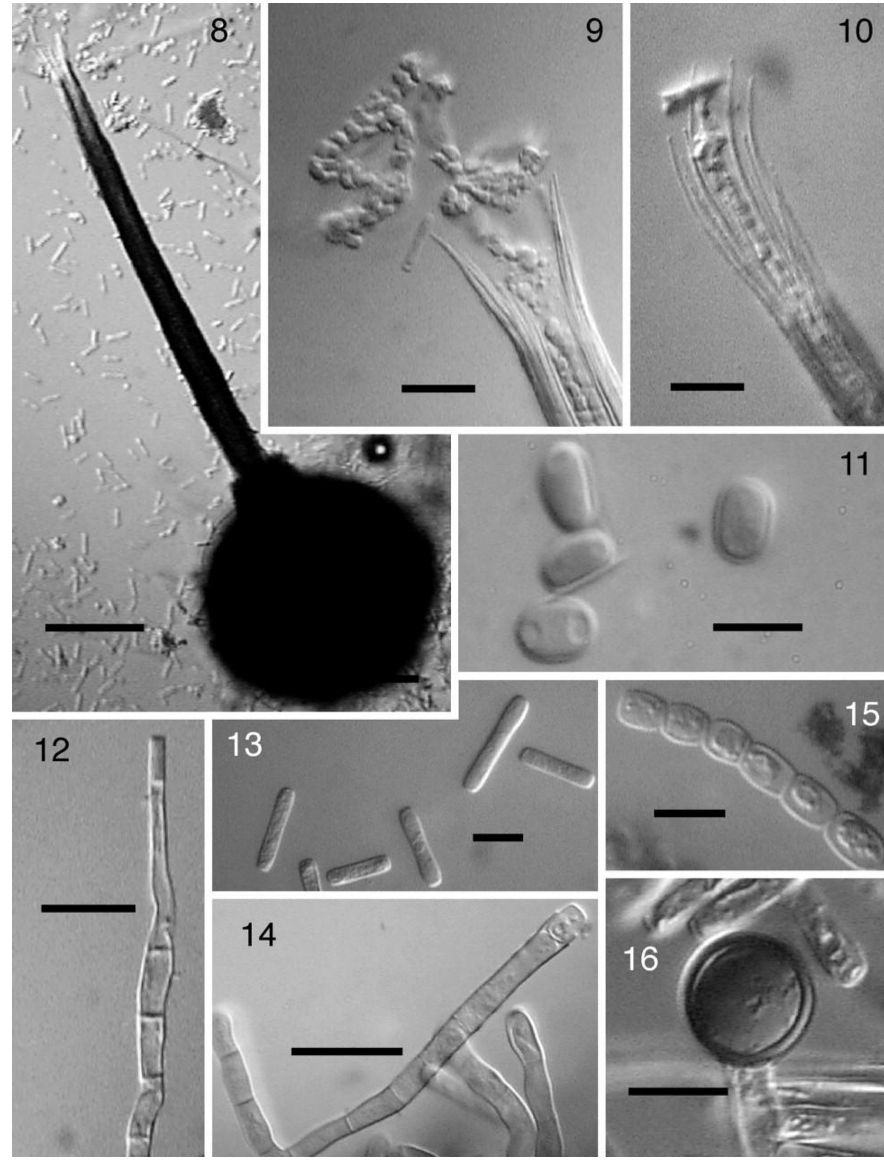
Photo by JB Friday. Lowland wet forest of Puna District, Hawaiʻi Island.

Photo By Molly Solomon

RAPID ʻŌHIʻA DEATH

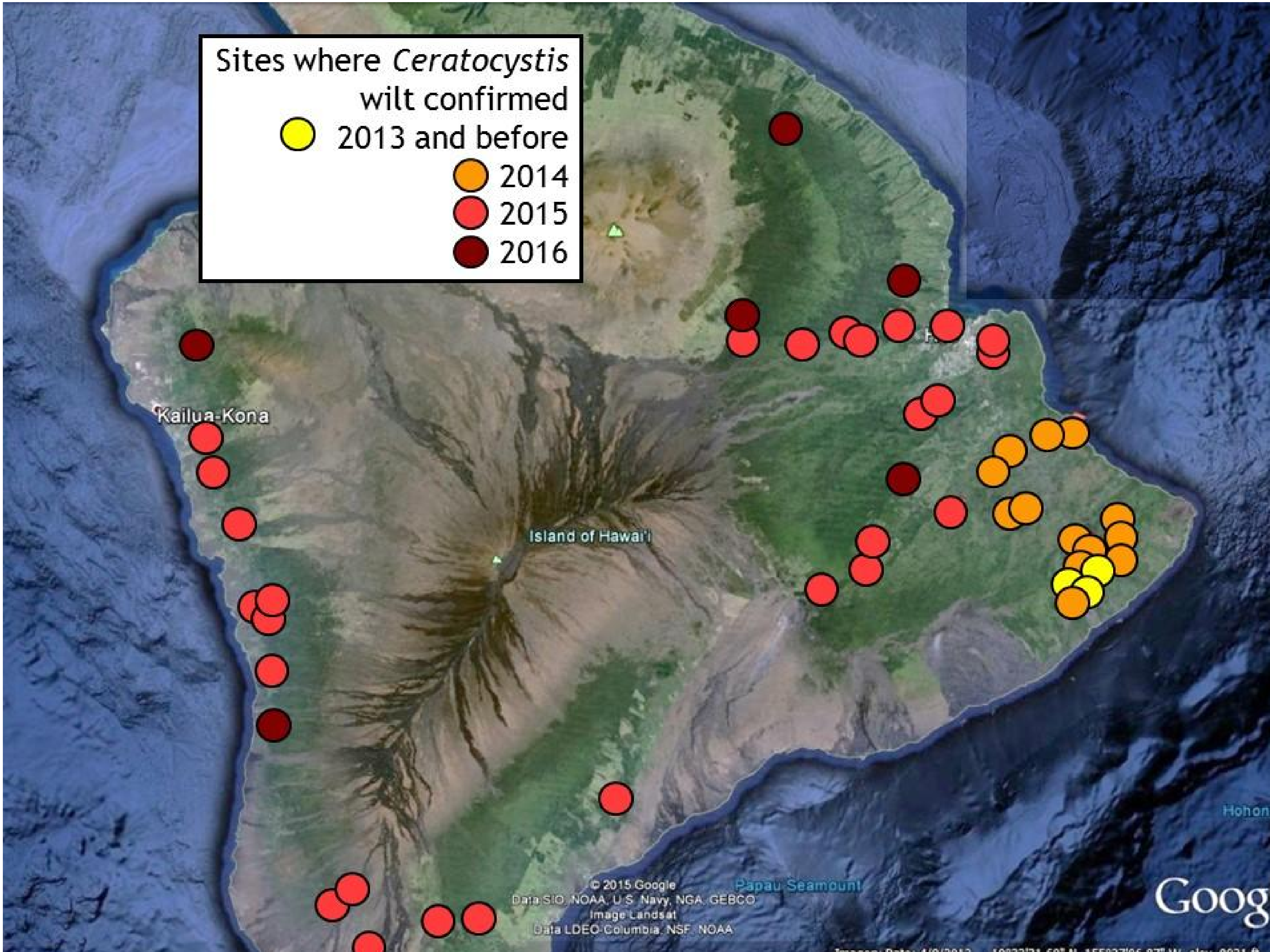


Ceratocystis populicola



Ceratocystis variospora.

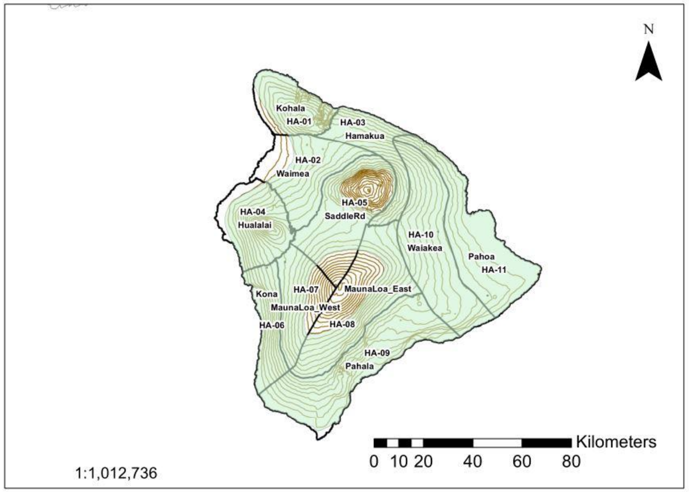
Johnson J A, Harrington TC, Engelbrecht CJB (2005) Phylogeny and taxonomy of the North American clade of the *Ceratocystis fimbriata* complex. Mycologia 97:1067–1092. doi: 10.3852/mycologia.97.5.1067


Sites where *Ceratocystis* wilt confirmed
2013 and before
2014
2015
2016
Kailua-Kona
Island of Hawai'i
© 2015 Google
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Image Landsat
Data LDEO-Columbia, NSF, NOAA
Papau Seamount
Imagery Date: 4/9/2013 19°33'21.60" N 155°27'06.87" W elev 9031 ft

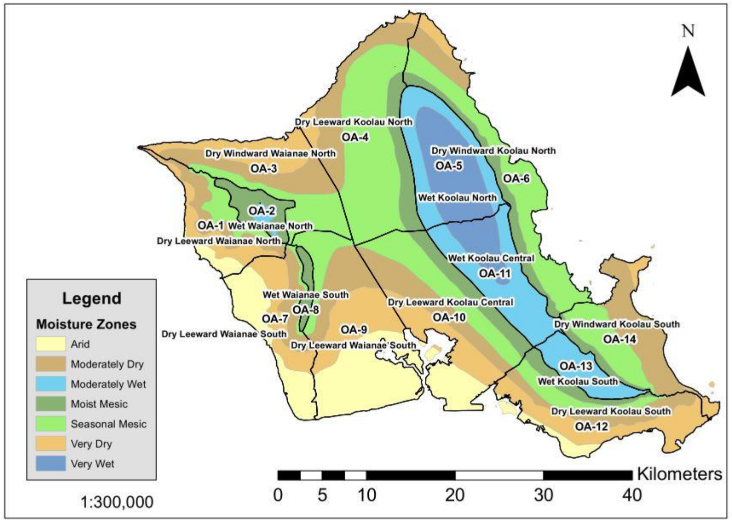
friendsoflyon.com/ohialov

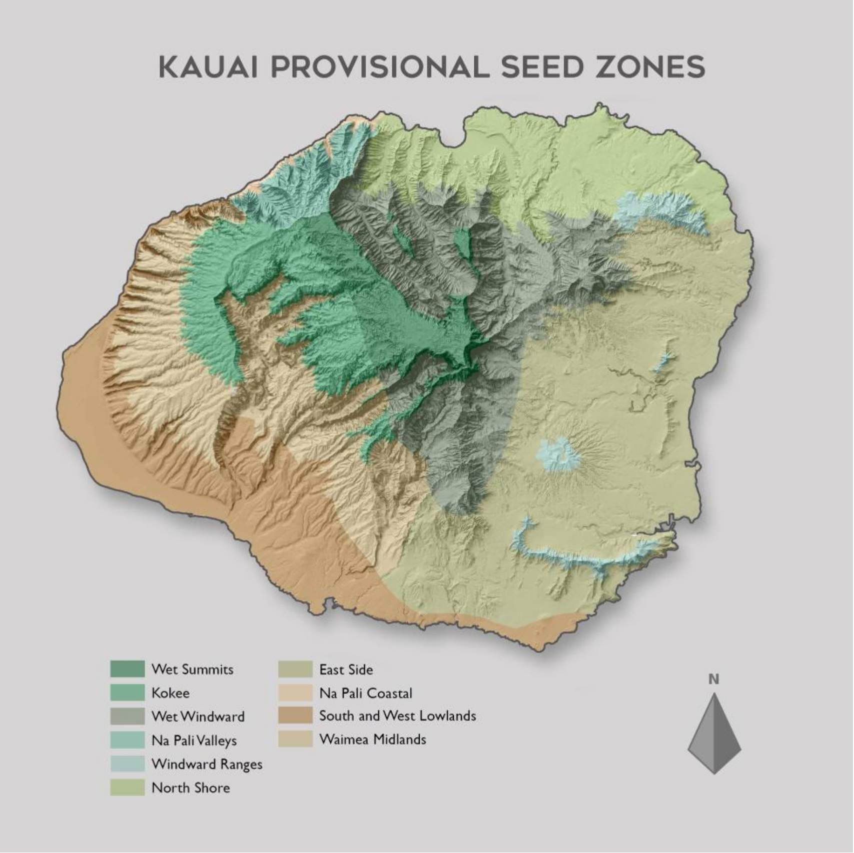
Provisional Seed Zones

Metrosideros Seed Zones



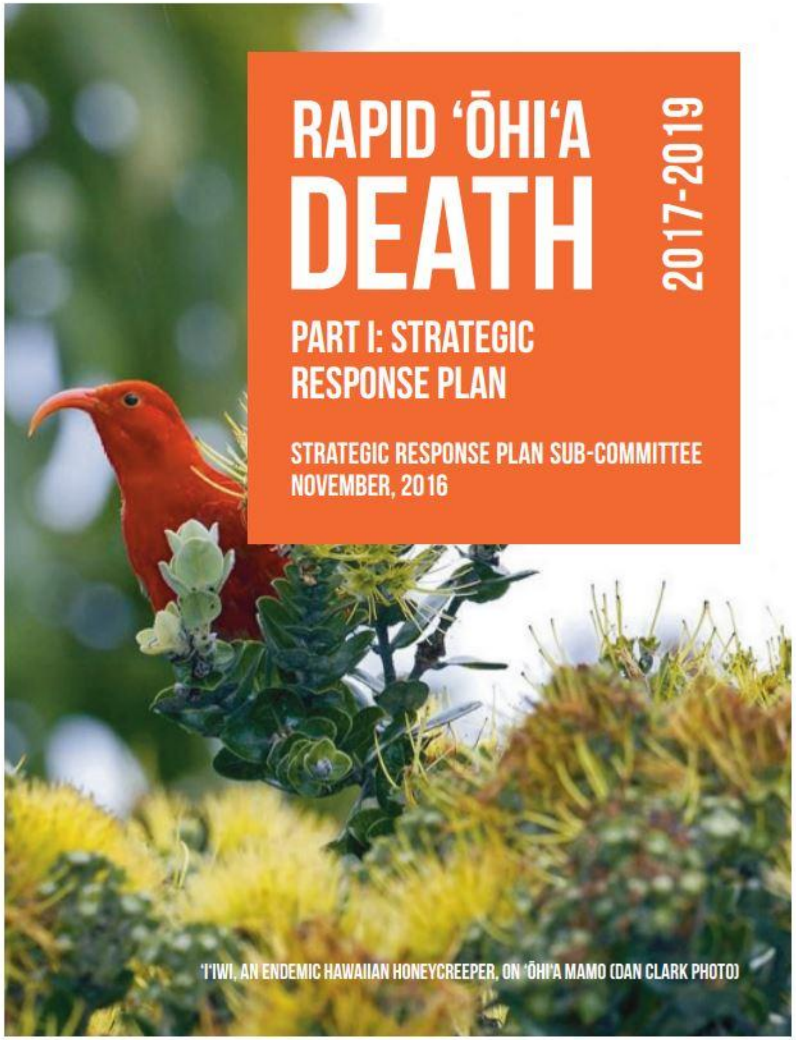
Oahu MetPol Seed Zones



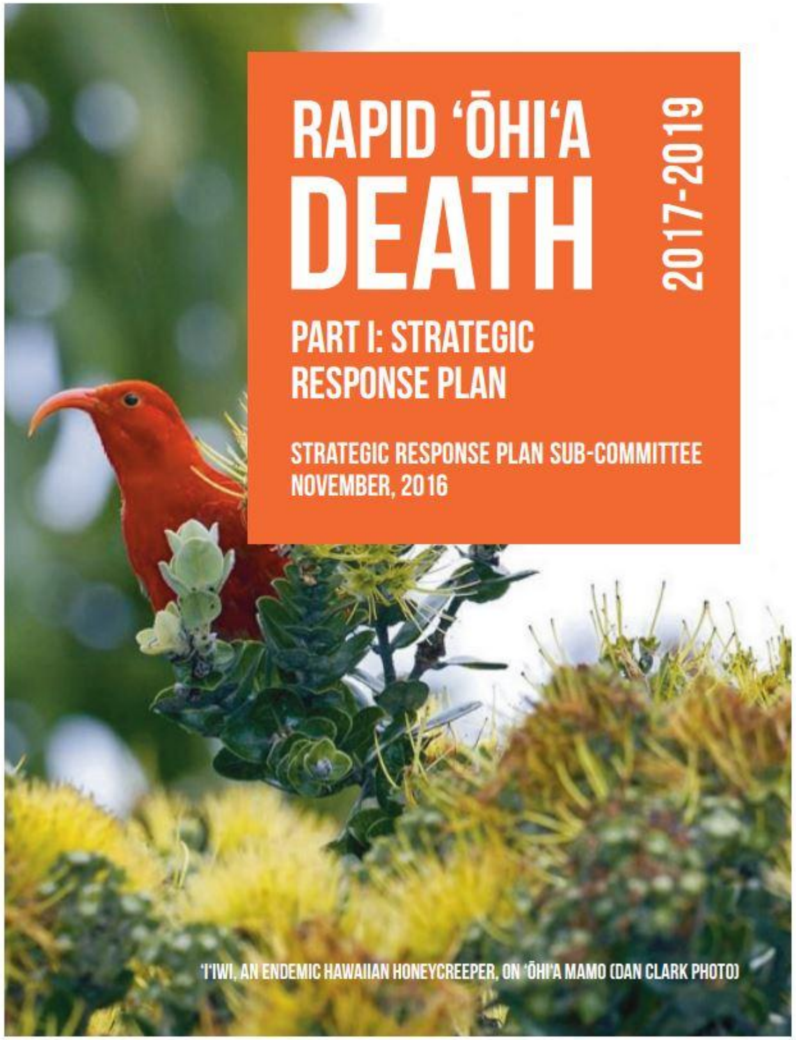


laukahi.org/ohia

“An important tool in preventing the extinction of threatened flora is the gathering and storage of seeds as a “genetic safety net.” This approach is commonly referred to as seed banking, and is especially important when the source of the threat is poorly understood or cannot yet be mitigated, as is the case with ROD.”

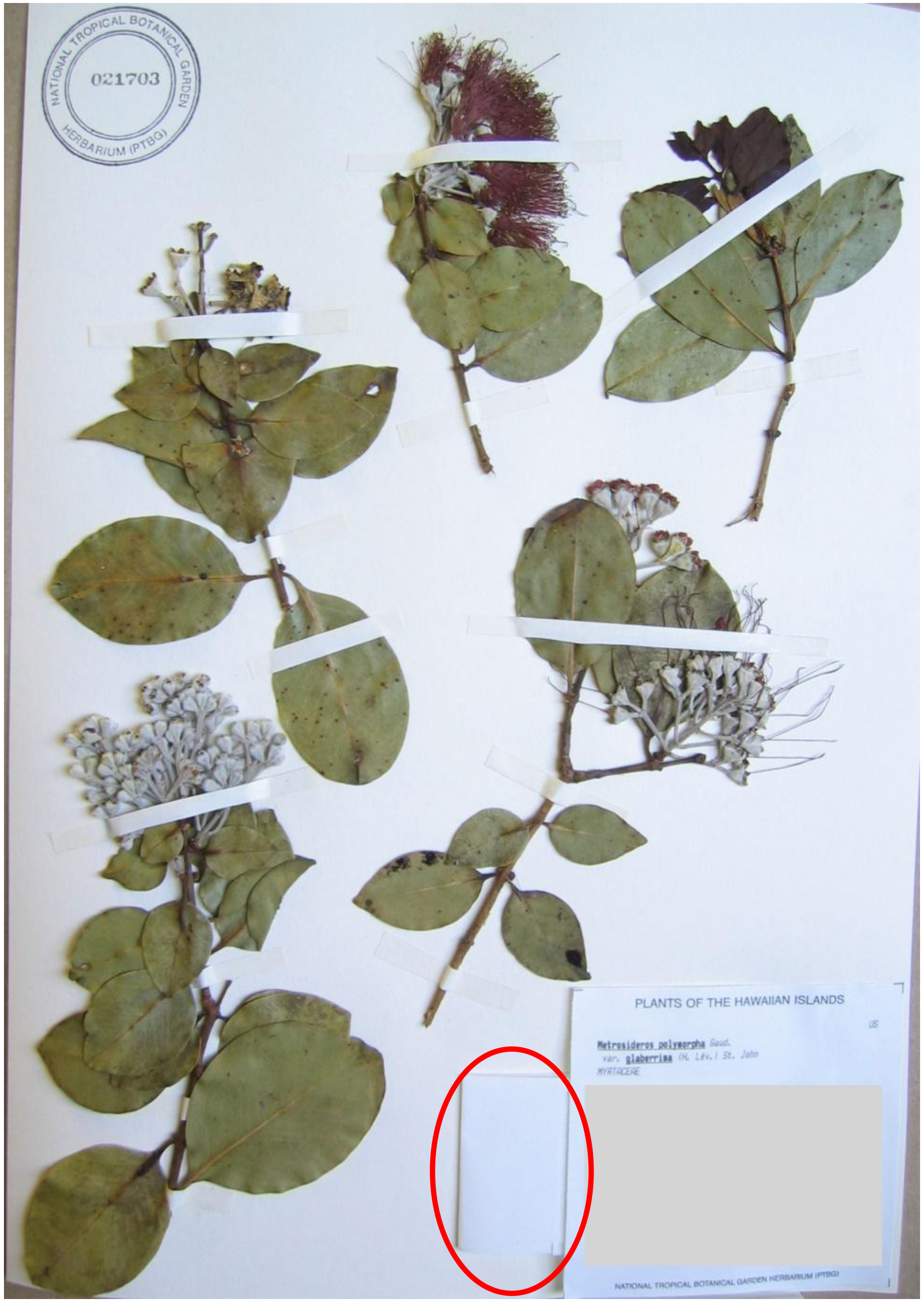


Item	Personel/item	2017	2018	2019	Total for 3 yrs
Resource needs for Cultural Engagement					
1	Hawaiian cultural specialist	$100,000	$100,000	$100,000	$300,000
2	Operational budget for workshops, travel	$20,000	$20,000	$20,000	$60,000
	Cultural Engagement Subtotal	**$120,000**	**$120,000**	**$120,000**	**$360,000**
Resource needs for Research					
Item	Personel/item	2017	2018	2019	Total for 3 yrs
3	Forest pathologist post-doc	$100,000	$100,000	$100,000	$300,000
4	Molecular biology post-doc	$100,000	$100,000	$100,000	$300,000
5	Molecular biology technician	$60,000	$60,000	$60,000	$180,000
6	Pathology post-doc	$100,000	$100,000	$100,000	$300,000
7	Pathology technician	$60,000	$60,000	$60,000	$180,000
8	Pathology supplies & equipment (growth chambers, vehicles)	$241,000	$100,000	$100,000	$441,000
9	Entomology post-doc	$100,000	$100,000	$100,000	$300,000
10	Field technicians (2 FTEs)	$120,000	$120,000	$120,000	$360,000
11	Laboratory technicians (2 FTEs)	$120,000	$120,000	$120,000	$360,000
12	Field and lab supplies & equipment	$30,000	$30,000	$30,000	$90,000
13	Forest ecology technicians (2 FTEs)	$120,000	$120,000	$120,000	$360,000
14	Remote-sensing flights	$250,000	$250,000	$250,000	$750,000
15	Remote-sensing post-doc	$100,000	$100,000	$100,000	$300,000
16	Remote-sensing technicians (2 partial FTEs)	$75,000	$75,000	$75,000	$225,000
	Research Subtotal	**$1,576,000**	**$1,435,000**	**$1,435,000**	**$4,446,000**
Resource needs for Response to Threat					
Item	Personnel/item	2017	2018	2019	Total for 3 yrs
17	Decontamination stations for vehicles/heavy equip.	$250,000	$50,000	$50,000	$350,000
18	Aerial and survey work (helicopter, plane, and ground)	$120,000	$120,000	$120,000	$360,000
19	Statewide survey crew (4 FTEs)	$255,000	$255,000	$255,000	$765,000
20	GIS/Data manager (1 FTE)	$100,000	$100,000	$100,000	$300,000
21	Survey equipment and supplies	$50,000	$50,000	$50,000	$150,000
22	Control ground crew (3 FTEs)	$190,000	$190,000	$190,000	$570,000
23	Equipment, supplies, contractor for operations, vehicle, etc.	$168,000	$100,000	$100,000	$368,000
24	Seed banking and restoration planner	$150,000	$150,000	$150,000	$450,000
	Response Subtotal	**$1,283,000**	**$1,015,000**	**$1,015,000**	**$3,313,000**
Resource needs for Outreach and Engagement					
Item	Personnel/item	2017	2018	2019	Total for 3 yrs
25	Outreach/Education personnel (5 FTEs)	$450,000	$450,000	$450,000	$1,350,000
26	Outreach materials, signage, media, etc.	$136,000	$90,000	$90,000	$316,000
	Outreach Subtotal	**$586,000**	**$540,000**	**$540,000**	**$1,666,000**
Resource needs for Response Coordination					
Item	Personnel/item	2017	2018	2019	Total for 3 yrs
27	Coordinating group staff (1 FTE, travel, operating costs etc.)	$90,000	$90,000	$90,000	$270,000
	Grand Total	**$3,655,000**	**$3,200,000**	**$3,200,000**	**$10,055,000**

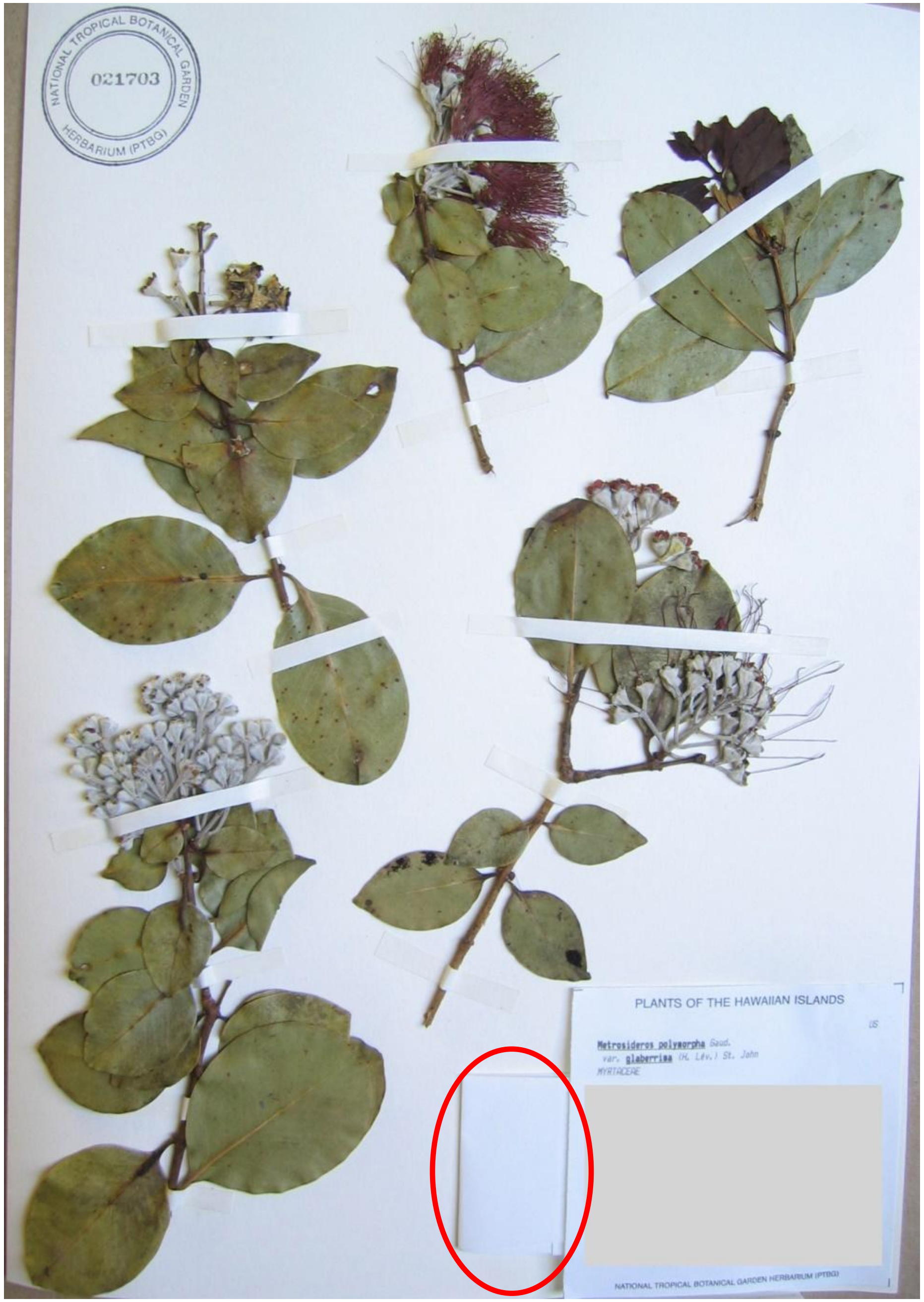


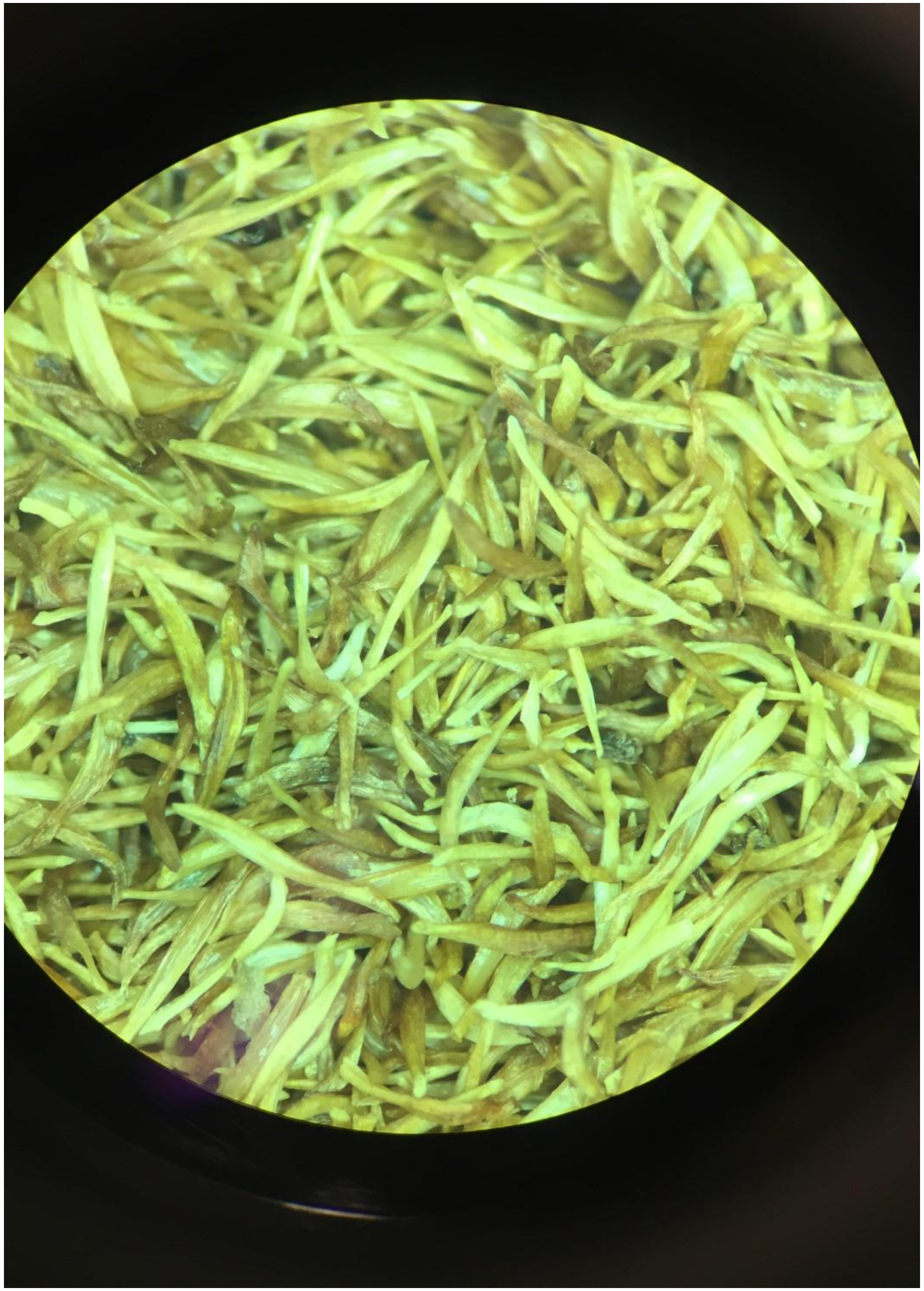
RAPID ʻŌHIʻA DEATH

RAPID ʻŌHIʻA DEATH



RAPID ʻŌHIʻA DEATH





QUESTIONS

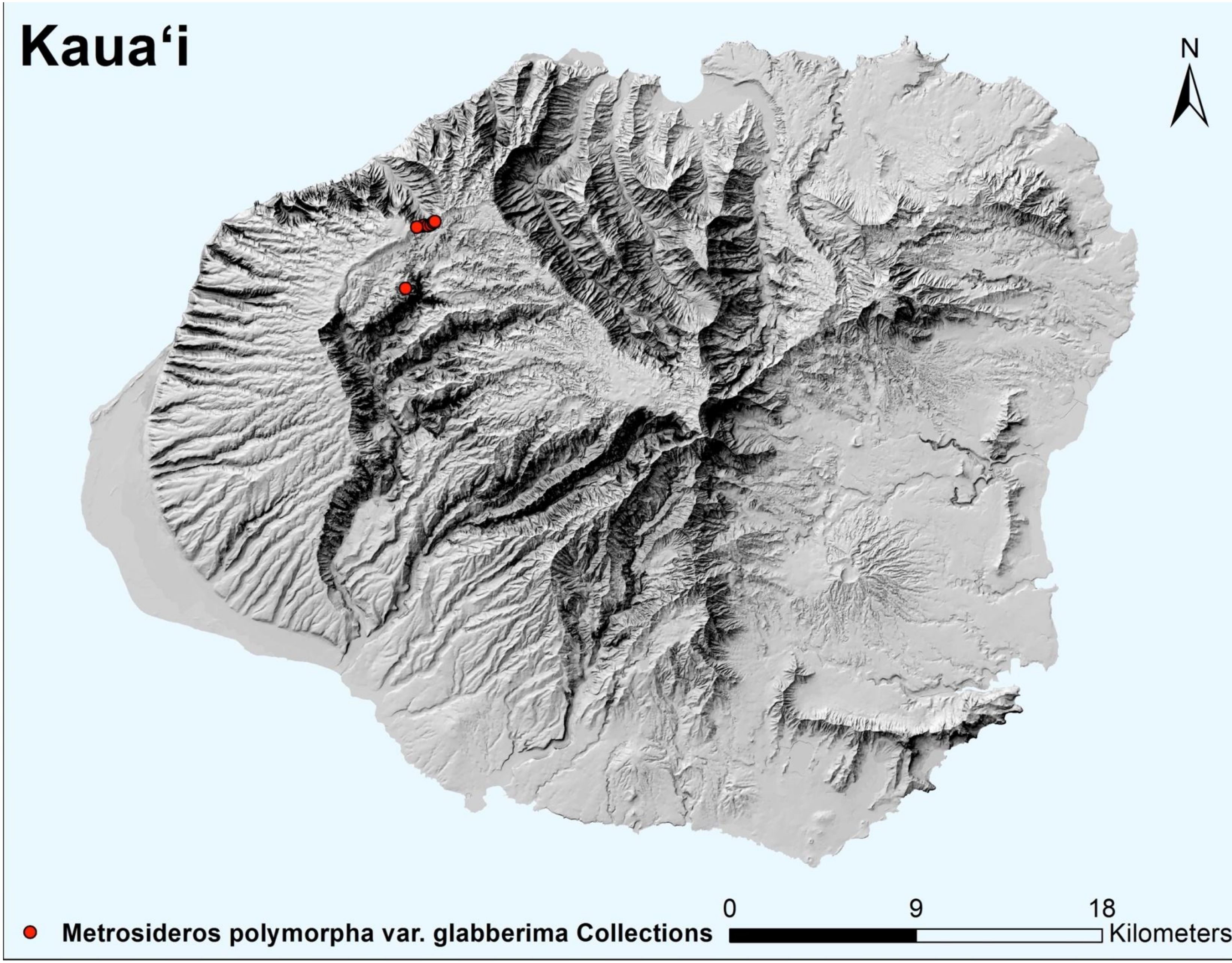
1. How do seeds of ‘ōhi‘a respond after entry into herbarium (PTBG) using current curation protocols?

2. What is the long term viability of ‘ōhi‘a seeds stored in herbarium conditions?

3. How long do ‘ōhi‘a seeds survive in optimal *ex situ* storage conditions?

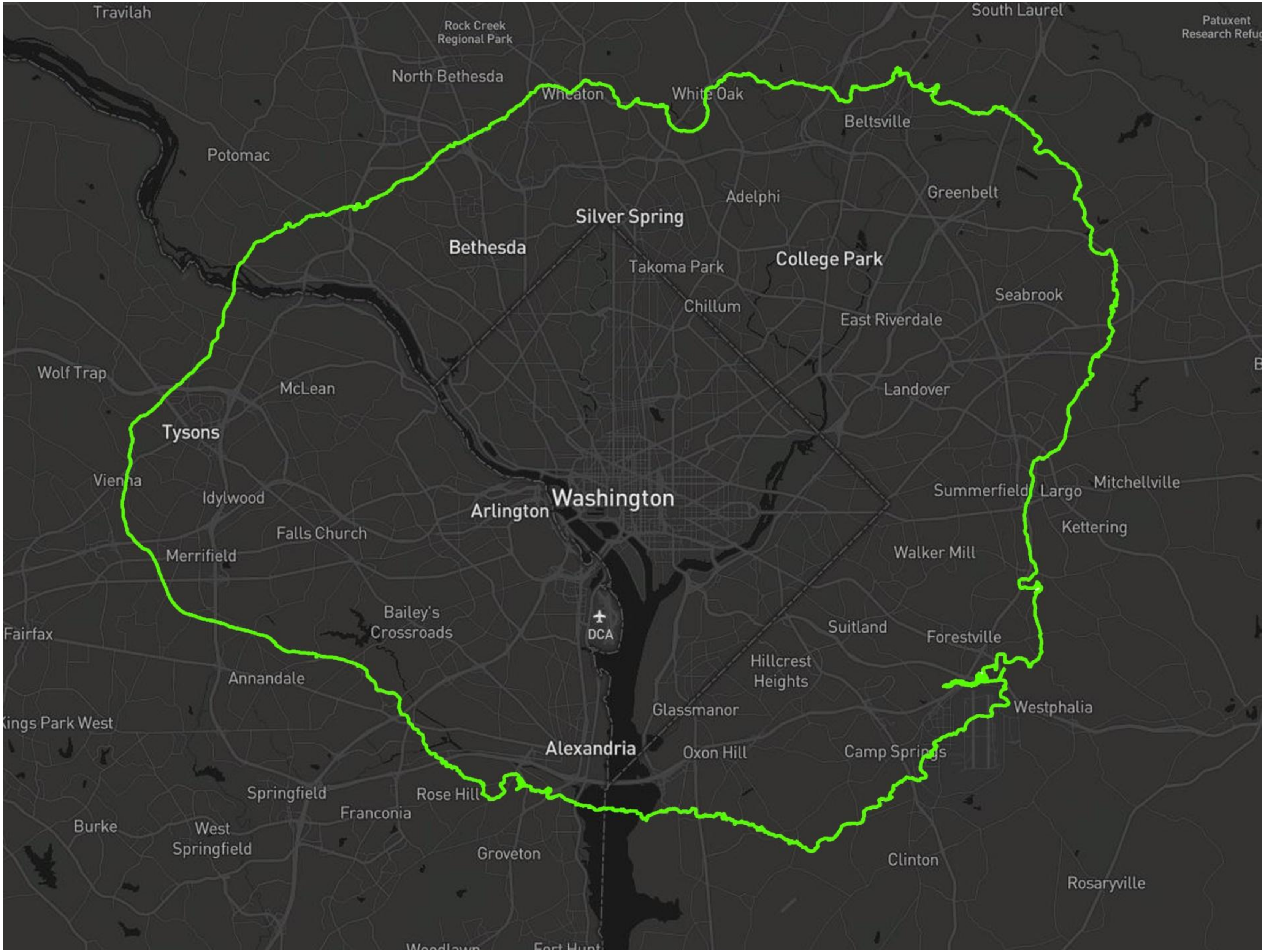
METHODS

Initial seed survival after herbarium entry



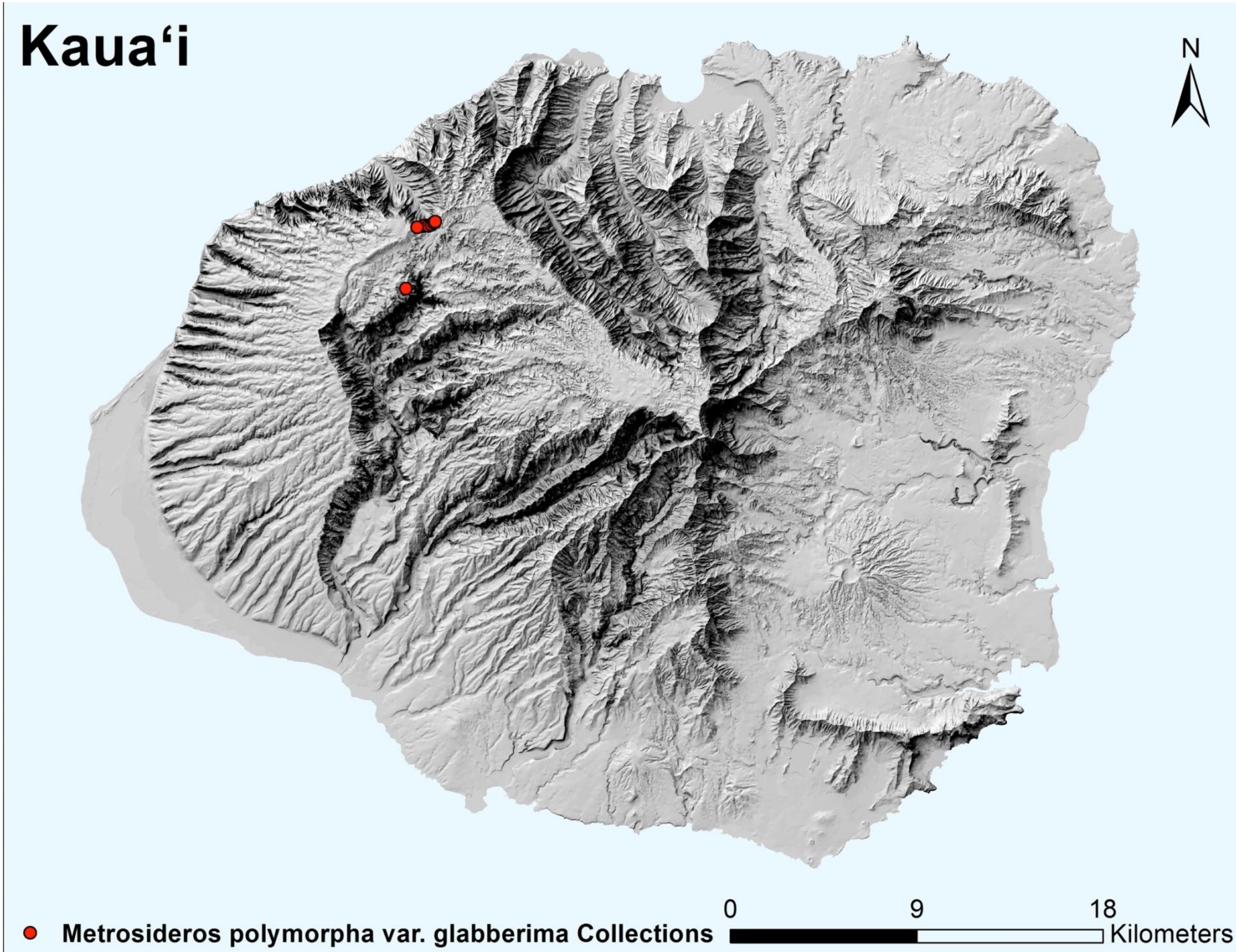
METHODS

Initial seed survival after herbarium entry



METHODS

Initial seed survival after herbarium entry



METHODS

Initial seed survival after herbarium entry

PTBG

BISH

US

METHODS

Initial seed survival after herbarium entry

3 replicates of
n = 50 seeds

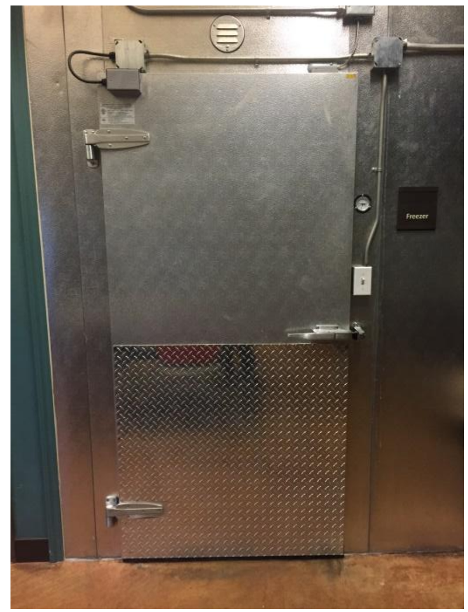
PTBG Current Curation Protocol

fresh
(control)

or

dry heat (57°C/5% RH) in the herbarium specimen drier for five days

or



frozen for two weeks (-20°C/ 63% RH)

or

dry heat (57°C/5% RH) in the herbarium specimen drier for five days

+

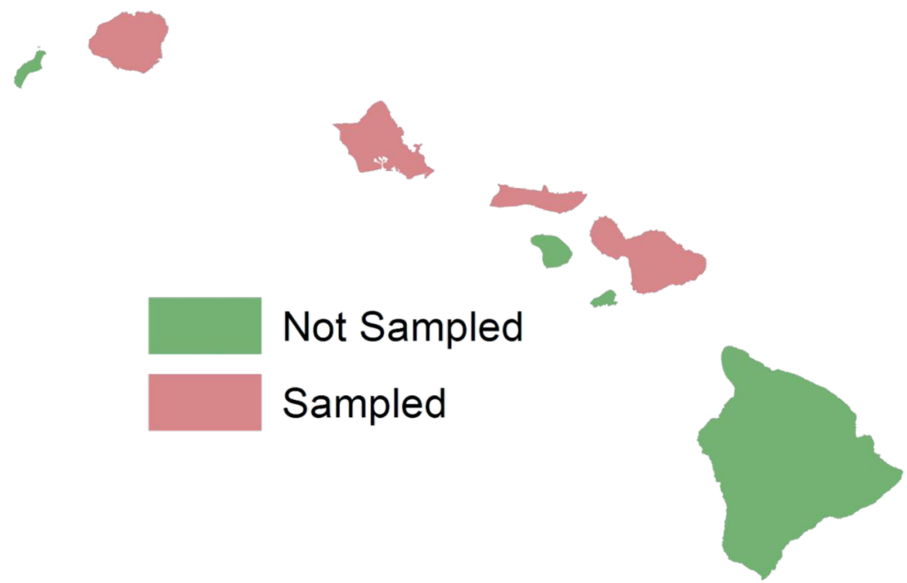
frozen for two weeks (-20°C/ 63% RH)

METHODS

Long term seed viability at herbaria conditions

n = 36-78 seeds

Specimen ID	Island	Originating Herbarium	Collection Year
17061	Kauai	MO	1978
017060	Kauai	A	1985
045640	Kauai	PTBG	1986
017068	Kauai	PTBG	1987
001939	Maui	PTBG	1989
012984	Kauai	PTBG	1991
014109	Kauai	PTBG	1992
012958	Kauai	PTBG	1992
018005	Kauai	PTBG	1993
018852	Molokai	PTBG	1994
018919	Hawaii	PTBG	1995
033861	Kauai	PTBG	1996
033716	Maui	PTBG	1996
061649	Kauai	PTBG	2004
044537	Molokai	PTBG	2005
059925	Oahu	PTBG	2008
070747	Oahu	US	2011
070687	Oahu	US	2011
070672	Oahu	US	2011
070744	Oahu	US	2011



METHODS

Long term seed viability banked in optimal ex situ conditions

3 replicates of
n = 50 seeds

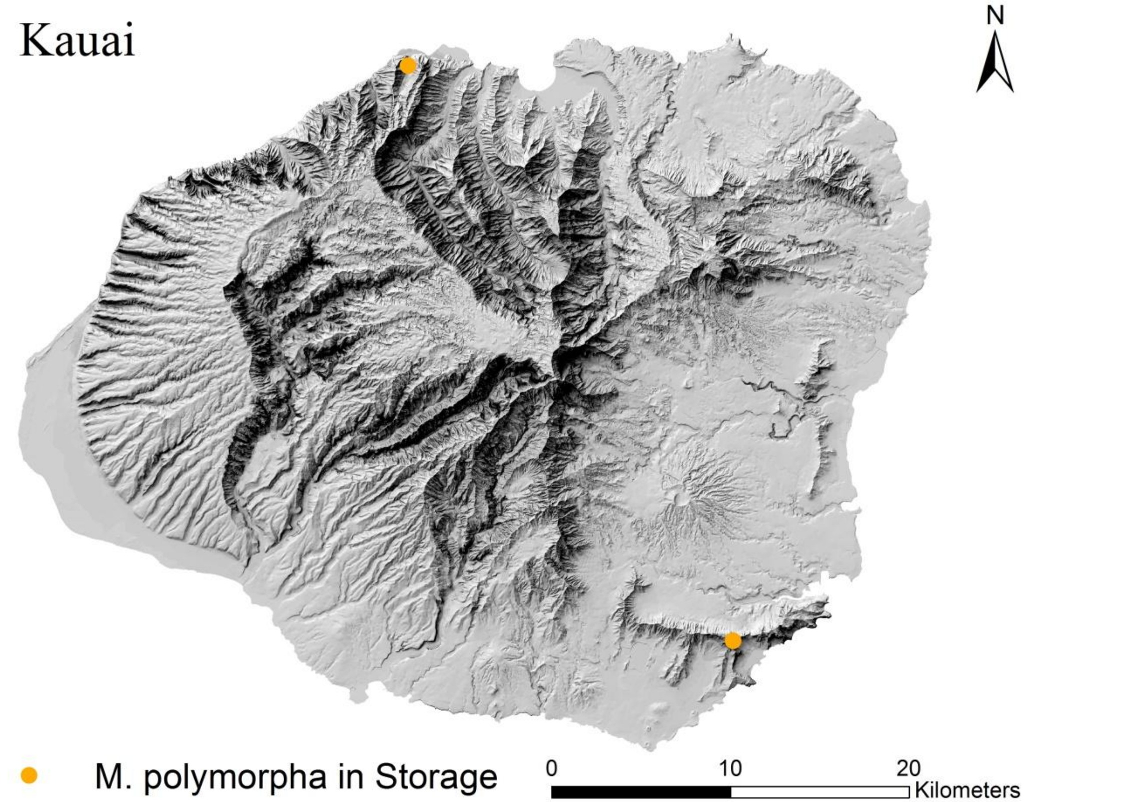
Accession ID	Island	Seed Storage Temp	Collection Year
100009	Kauai	-18C	2010
120614	Kauai	-18C	2012

METHODS

Long term seed viability banked in optimal ex situ conditions

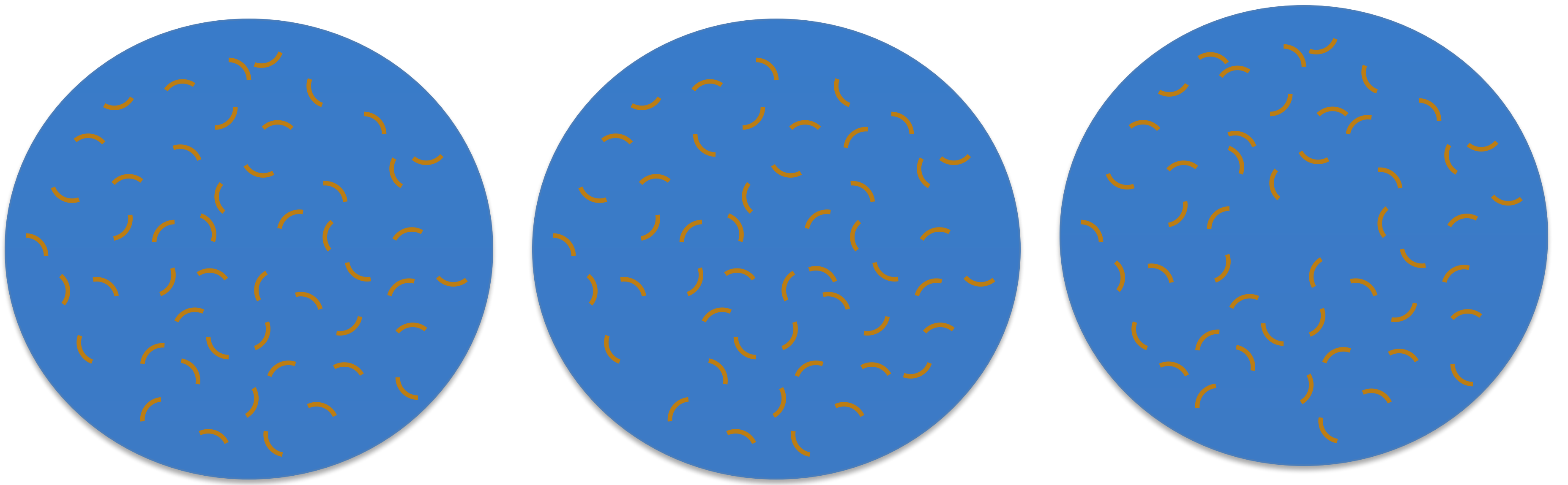
3 replicates of
n = 50 seeds

Accession ID	Island	Seed Storage Temp	Collection Year
100009	Kauai	-18C	2010
120614	Kauai	-18C	2012



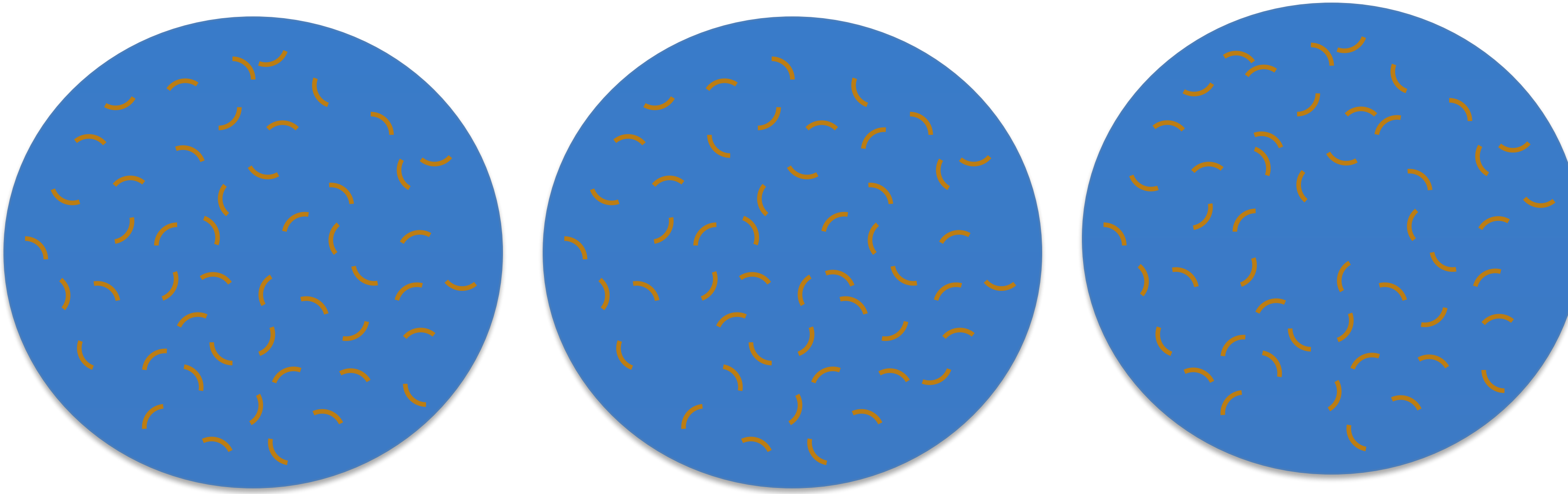
METHODS

Germination



METHODS

Germination

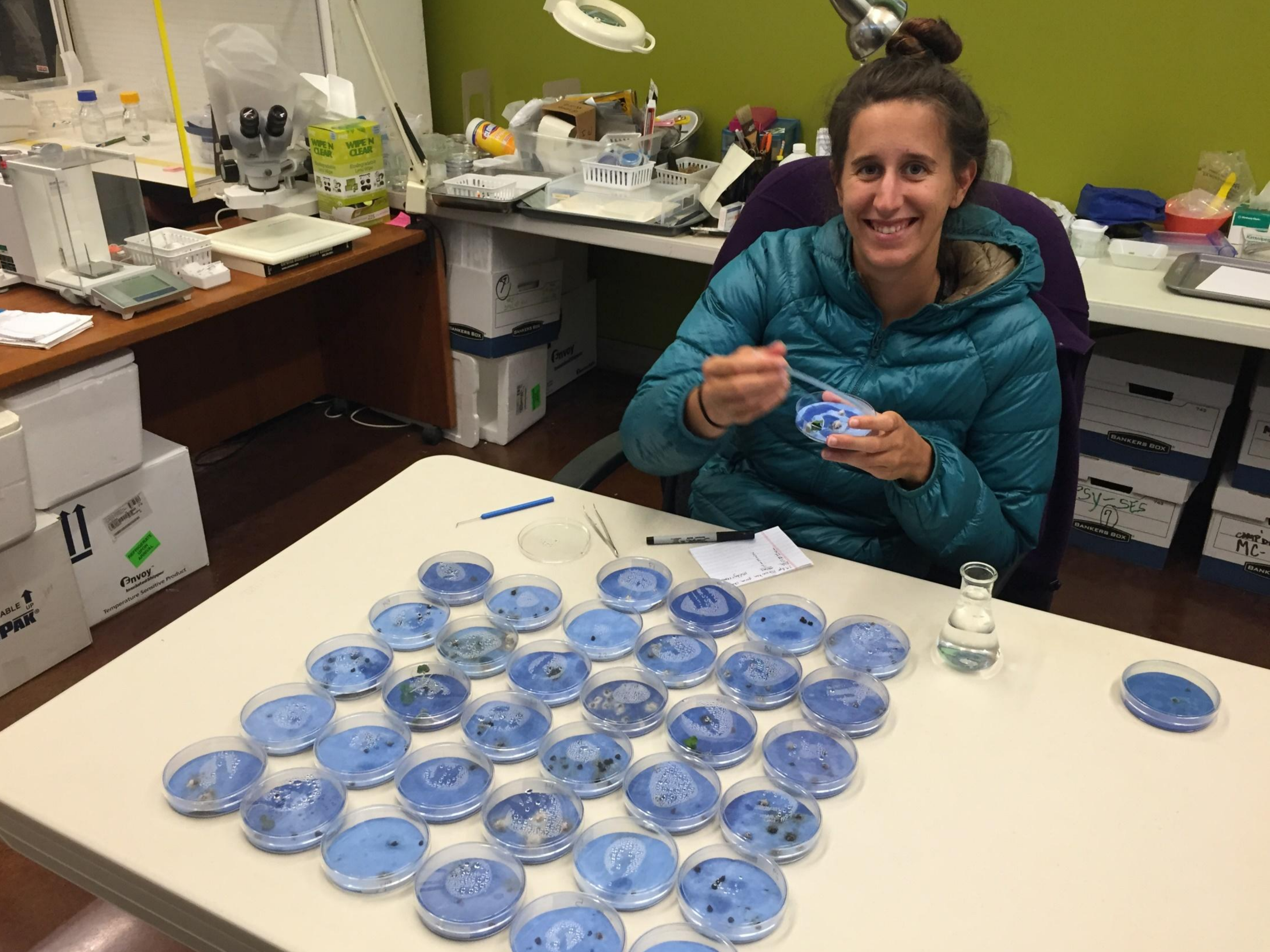


12 hours
30°C

12 hours
20°C

Drake DR (1993) Germination requirements of metrosideros polymorpha, the dominant tree of hawaiian lava flows and rain forests. Biotropica 25:461–467.

Lilleeng-Rosenberger, KE (2005) Growing hawaii's native plants. Mutual Publishing LLC, Honolulu HI.


WIPE 'N CLEAR
BANKERS BOX
Temperature Sensitive Product

1. How do seeds of ʻōhiʻa respond after entry into herbarium (PTBG) using current curation protocols?

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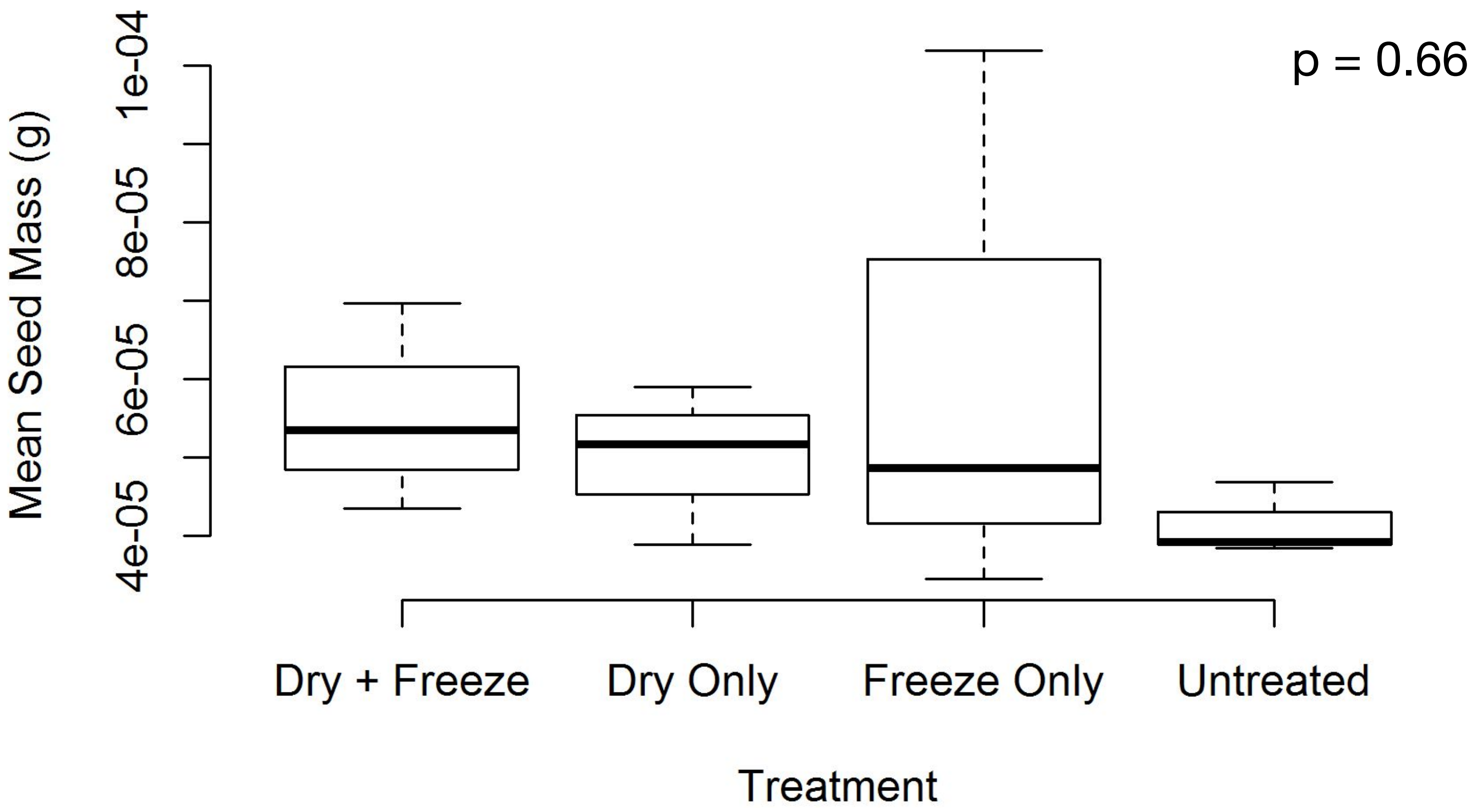
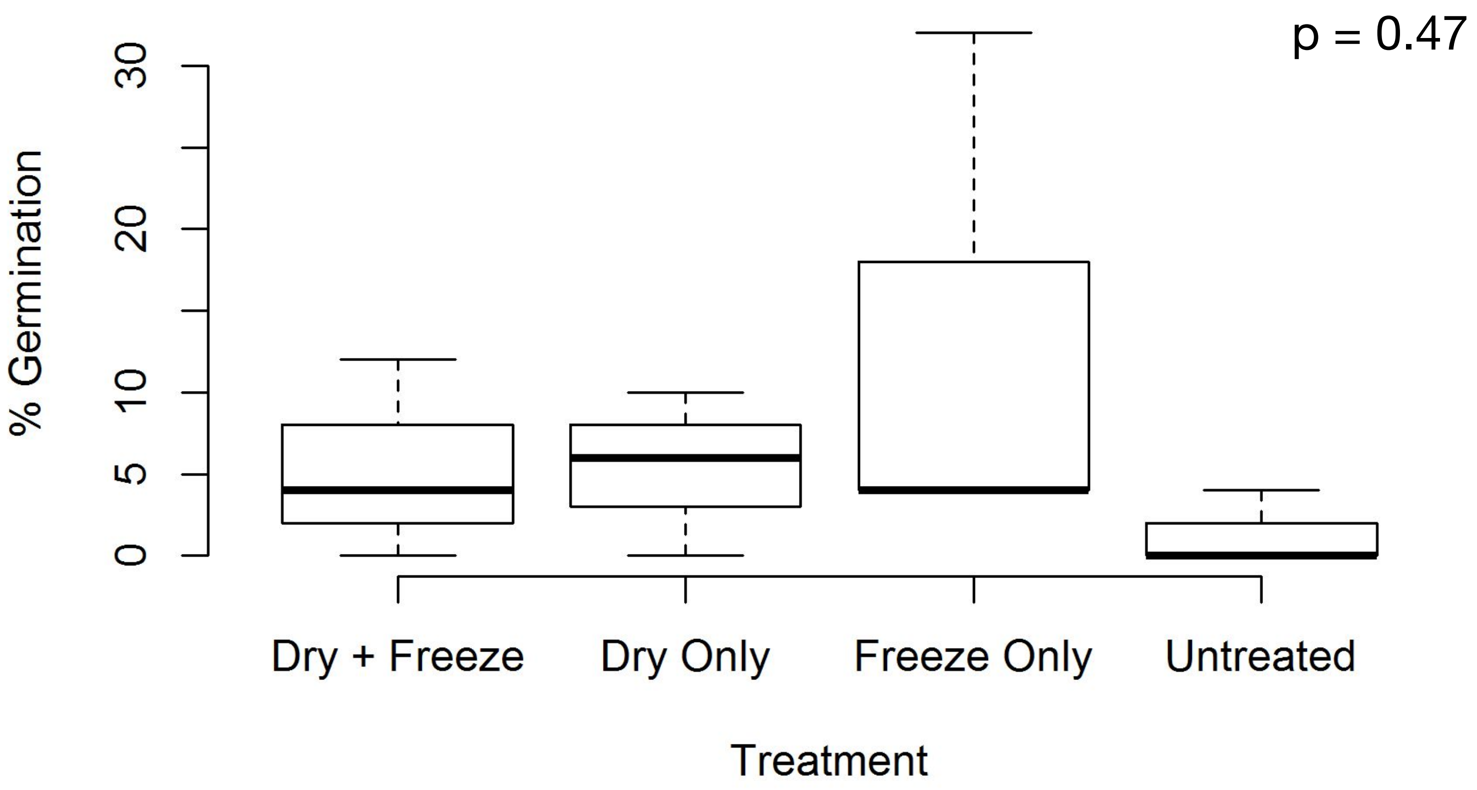
Initial Seed Survival



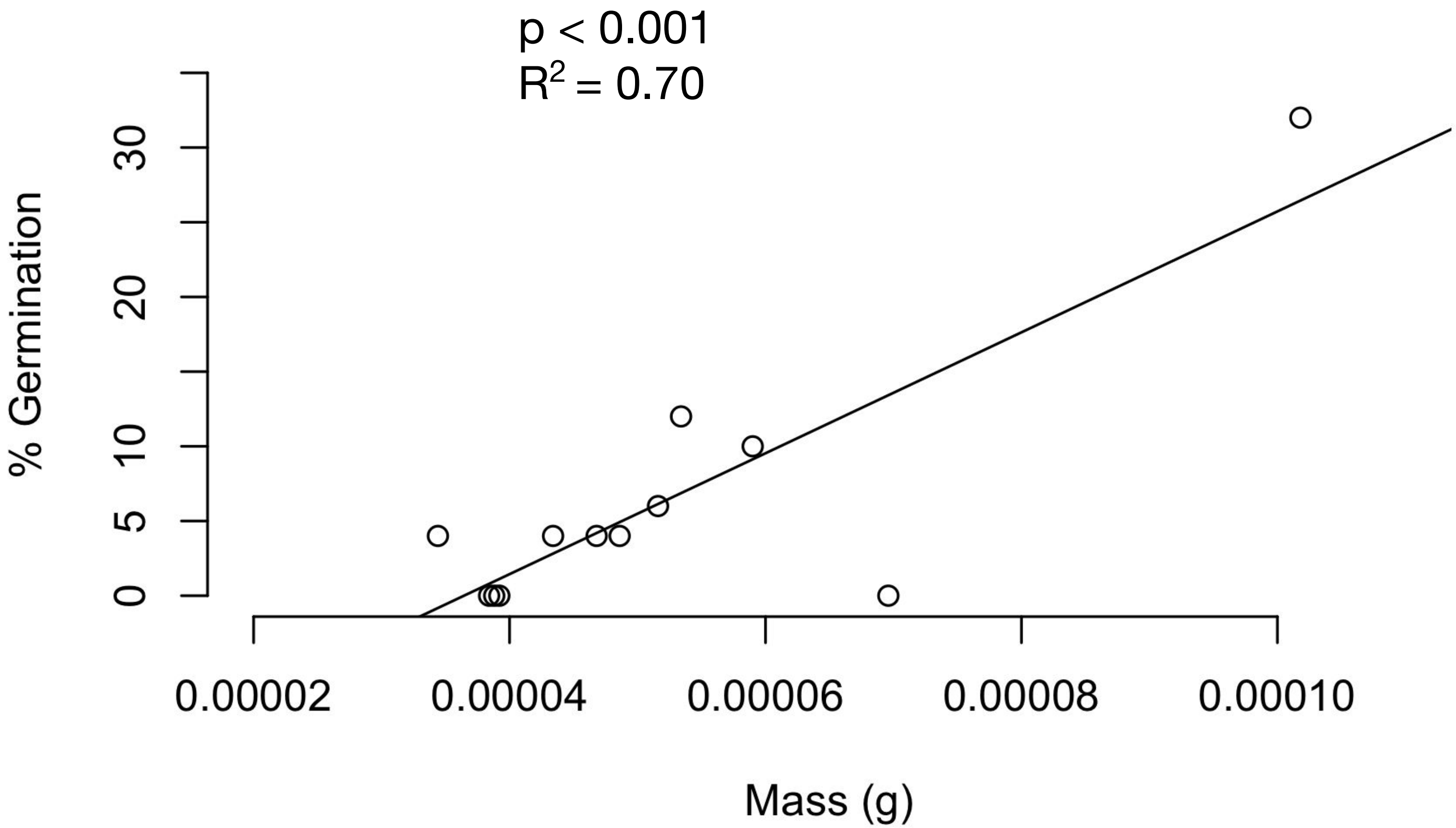
RESULTS & DISCUSSION

Initial Seed Survival



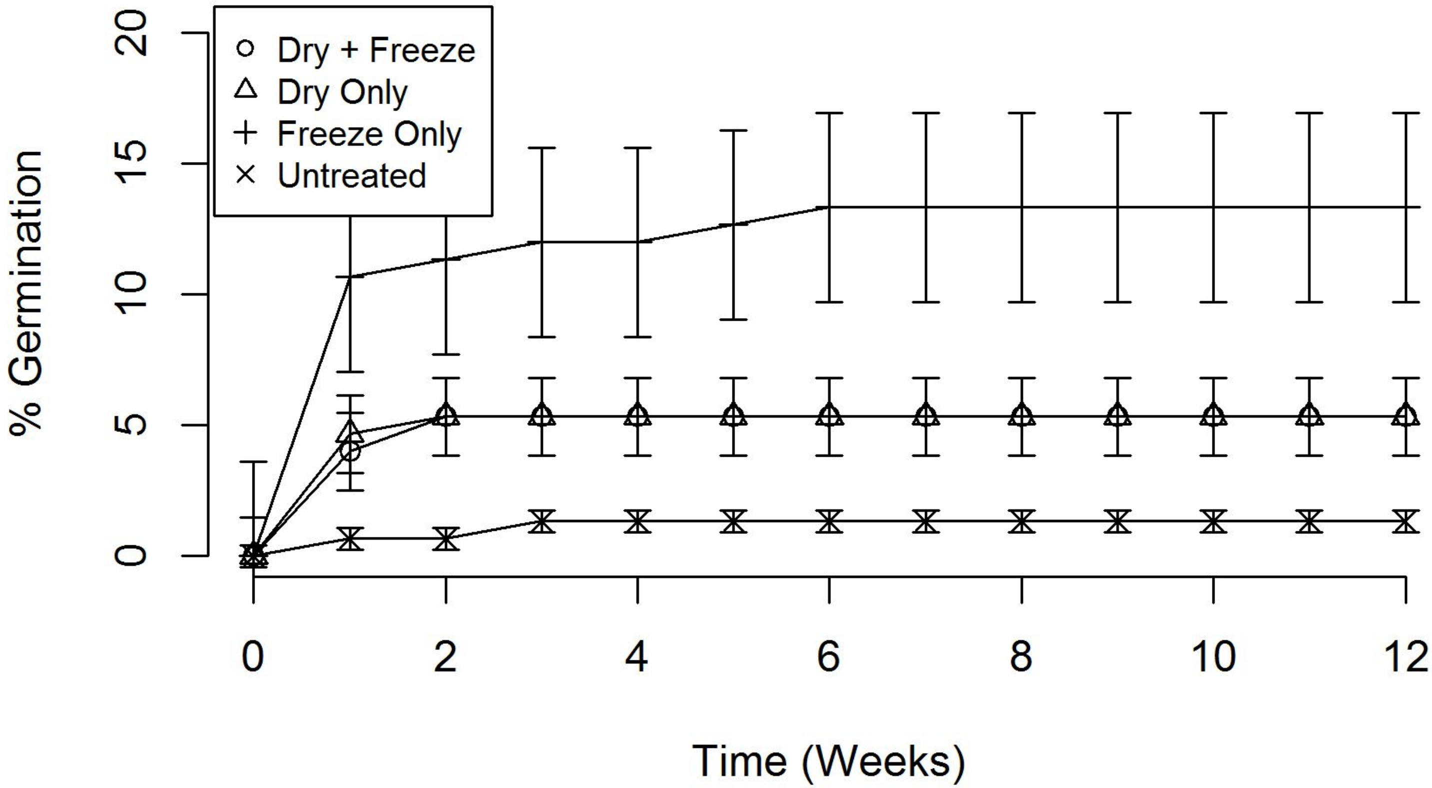
RESULTS & DISCUSSION

Initial Seed Survival



RESULTS & DISCUSSION

Initial Seed Survival



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RESULTS & DISCUSSION

Viability from herbarium specimens

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Viability from herbarium specimens

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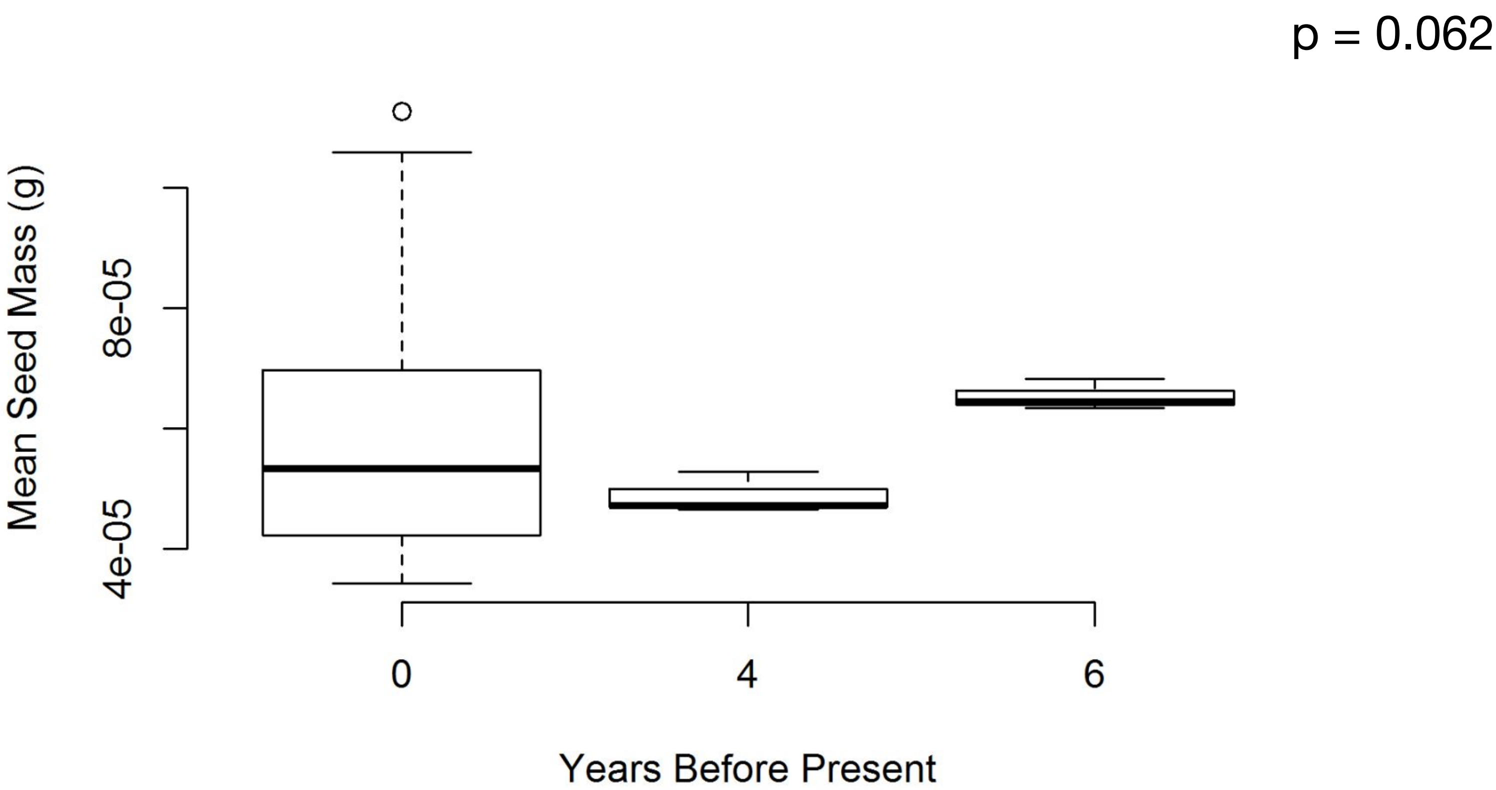
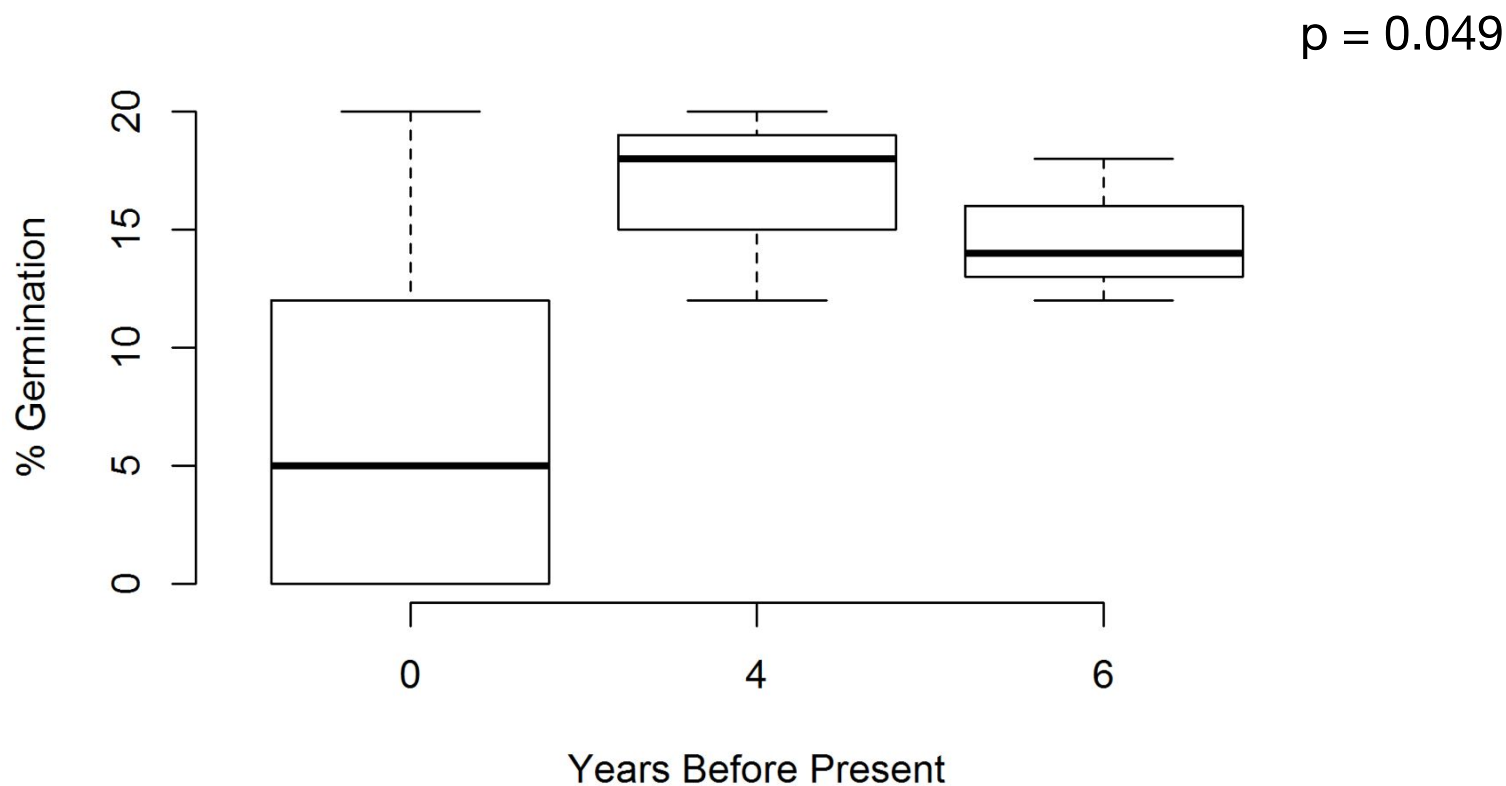
RESULTS & DISCUSSION

Viability from ex situ seed bank



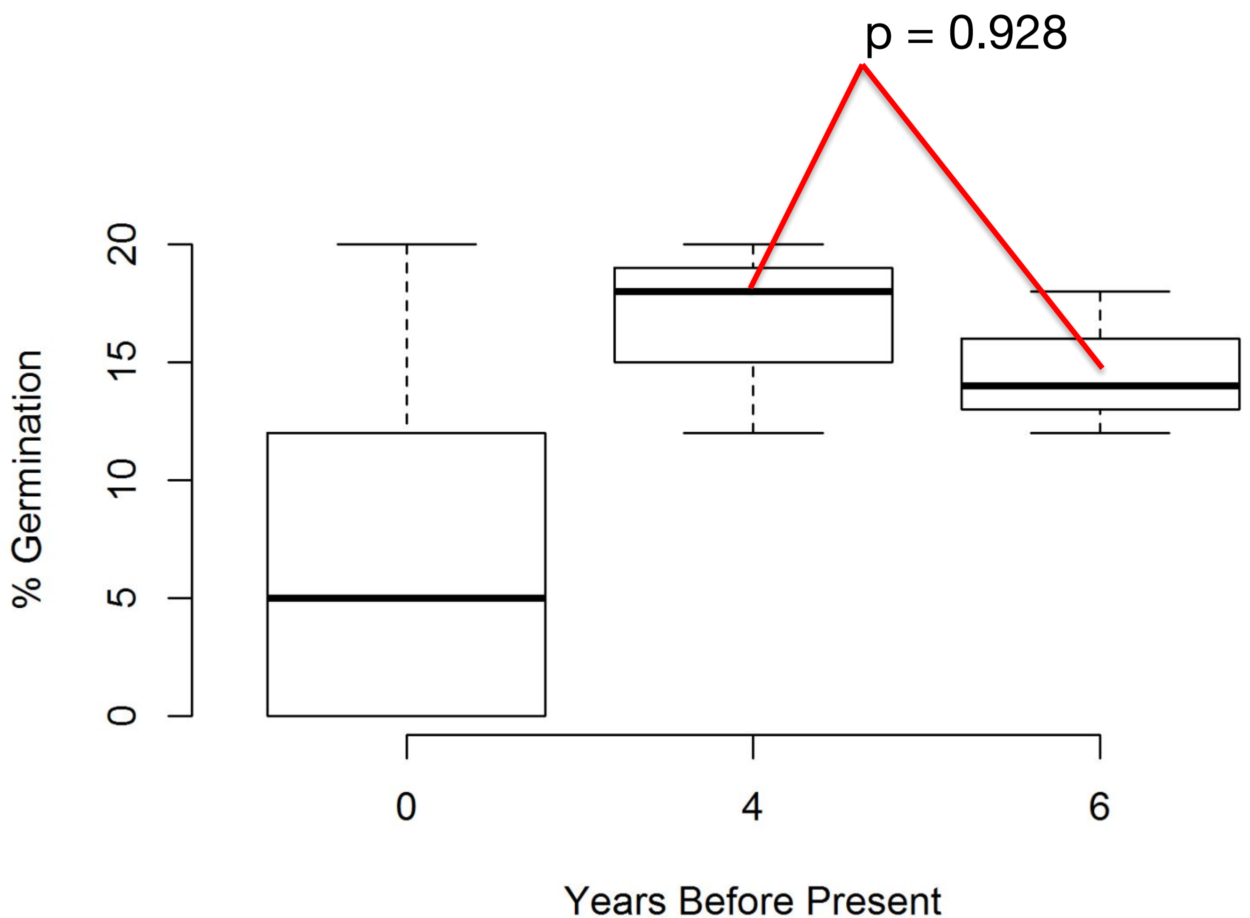
RESULTS & DISCUSSION

Viability from ex situ seed bank



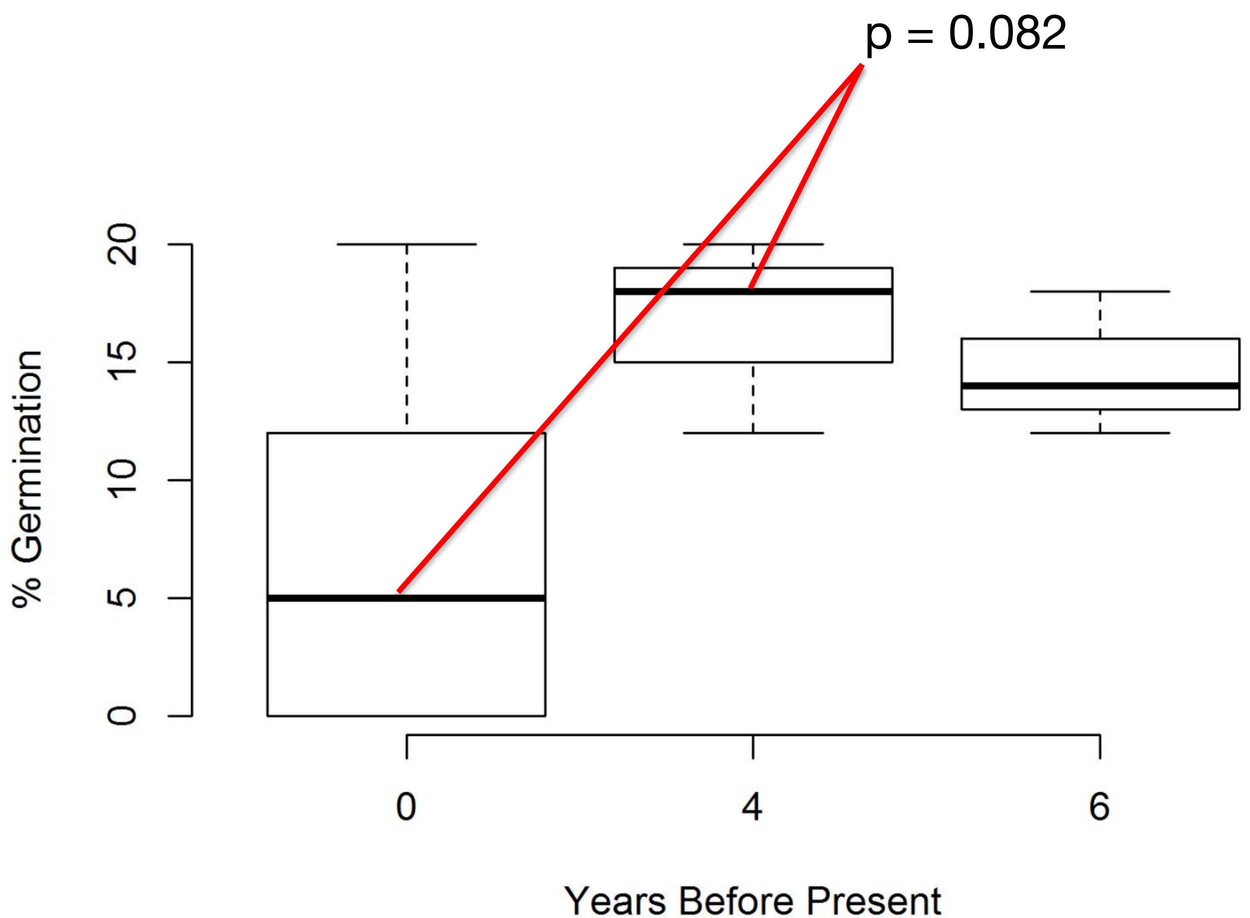
RESULTS & DISCUSSION

Viability from ex situ seed bank



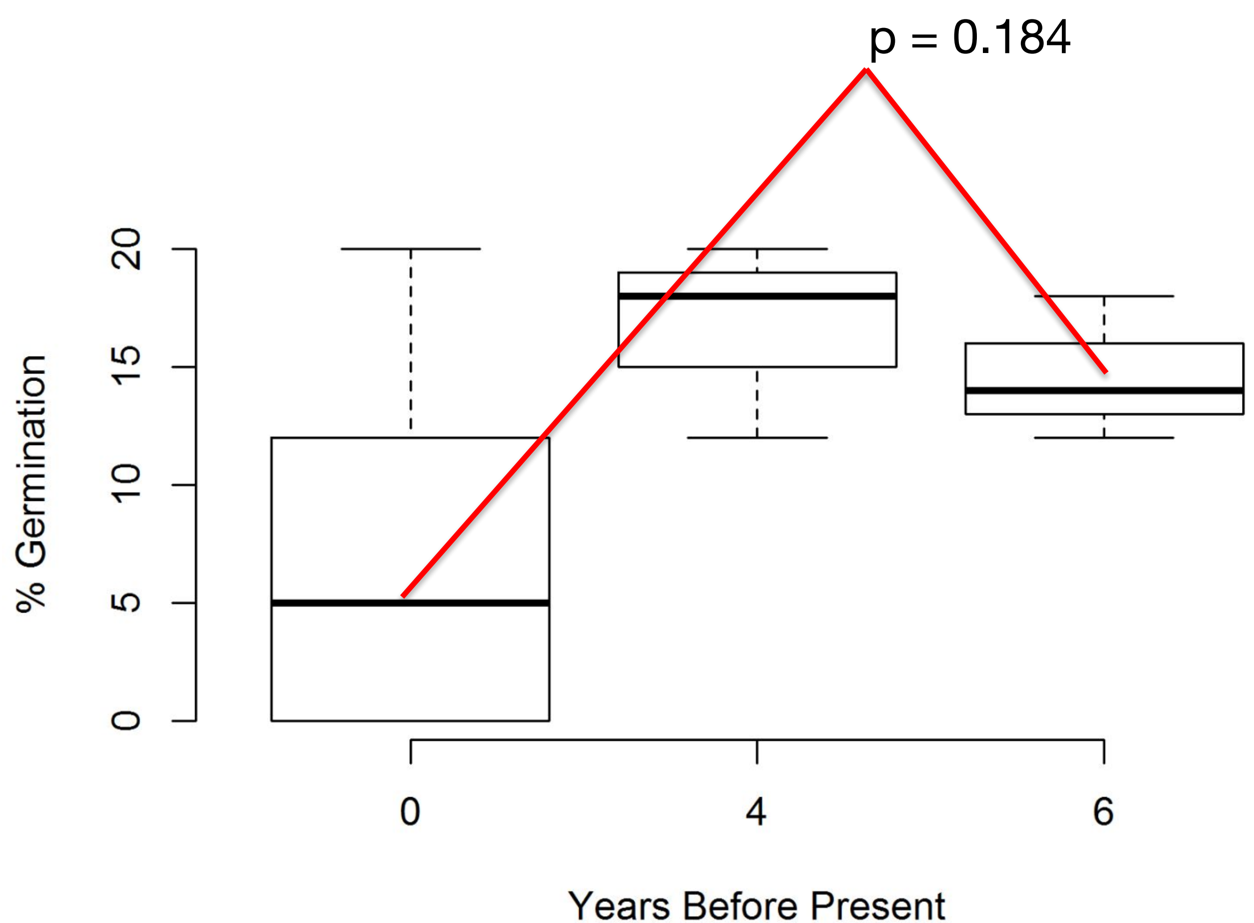
RESULTS & DISCUSSION

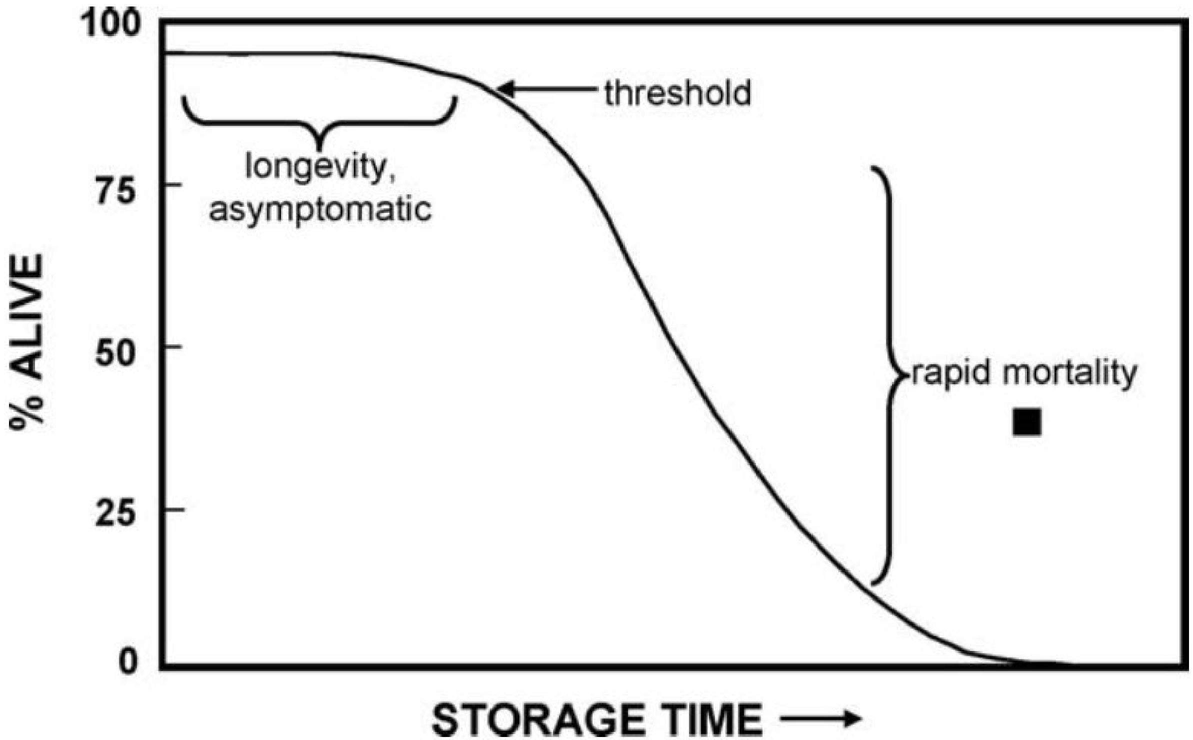
Viability from ex situ seed bank



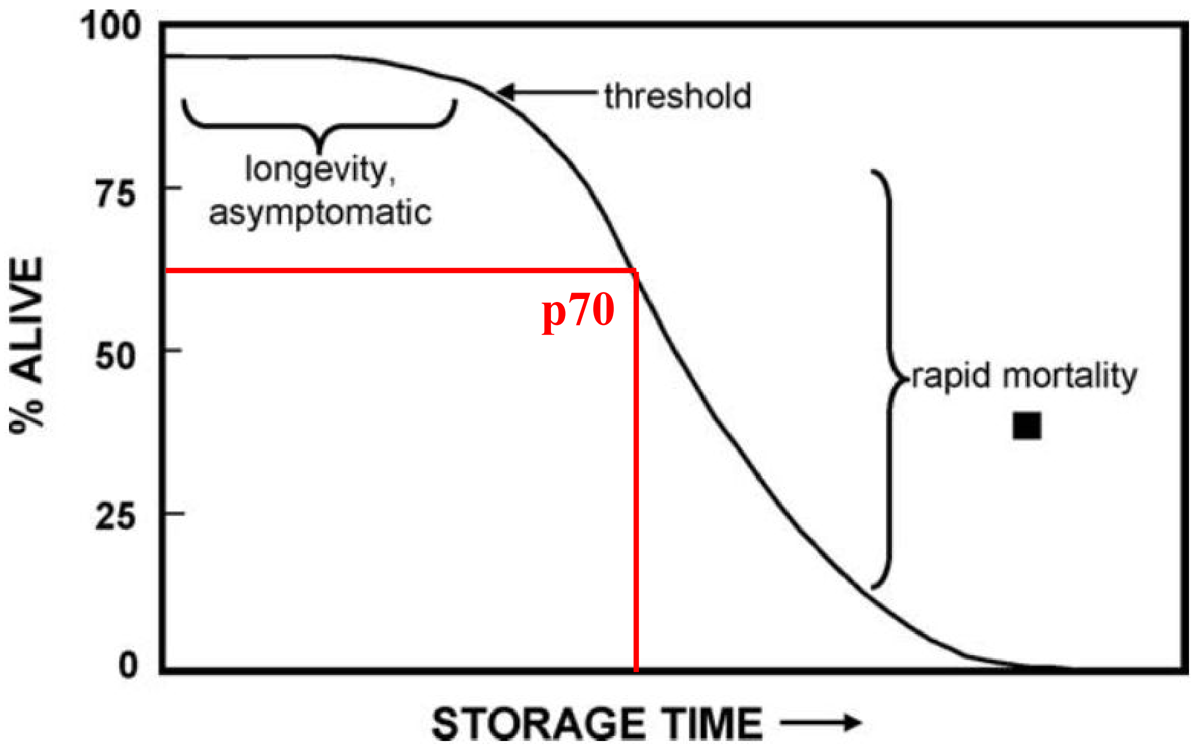
RESULTS & DISCUSSION

Viability from ex situ seed bank



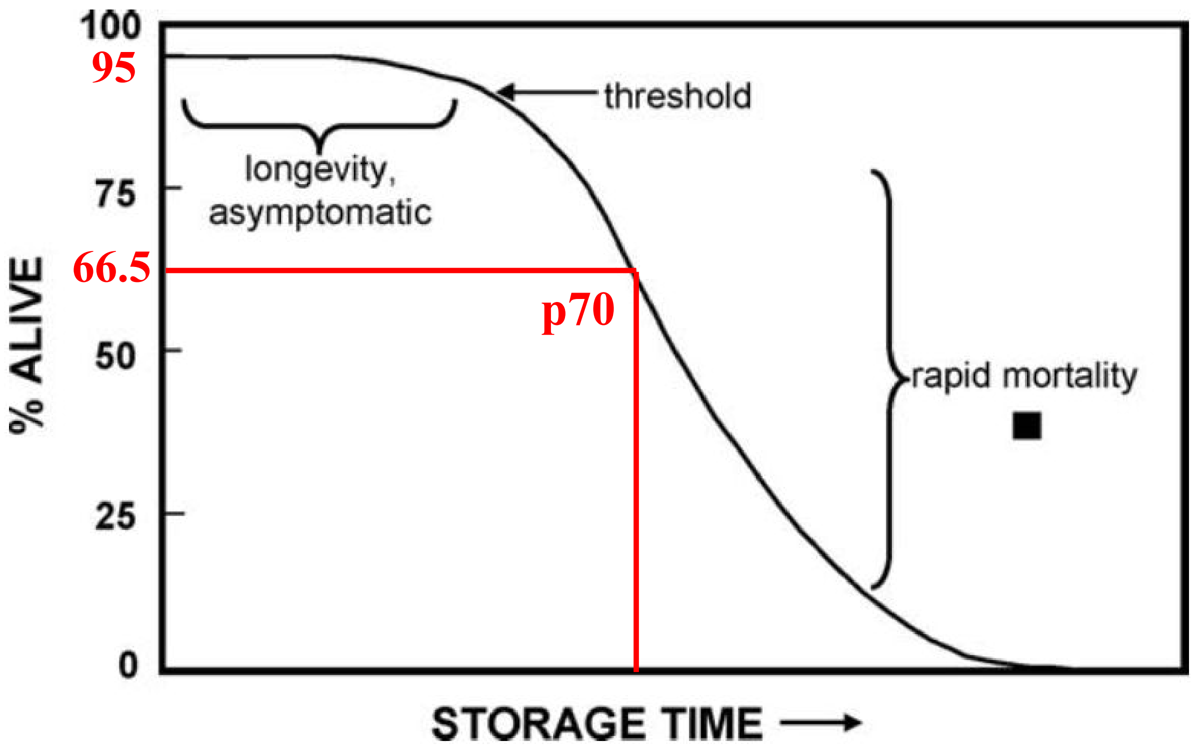


Walters C, Ballesteros D, Vertucci VA (2010) Structural mechanics of seed deterioration: Standing the test of time. Plant Science 179:565–573. doi: 10.1016/j.plantsci.2010.06.016



Walters C, Ballesteros D, Vertucci VA (2010) Structural mechanics of seed deterioration: Standing the test of time. Plant Science 179:565–573. doi: 10.1016/j.plantsci.2010.06.016

FUTURE DIRECTIONS



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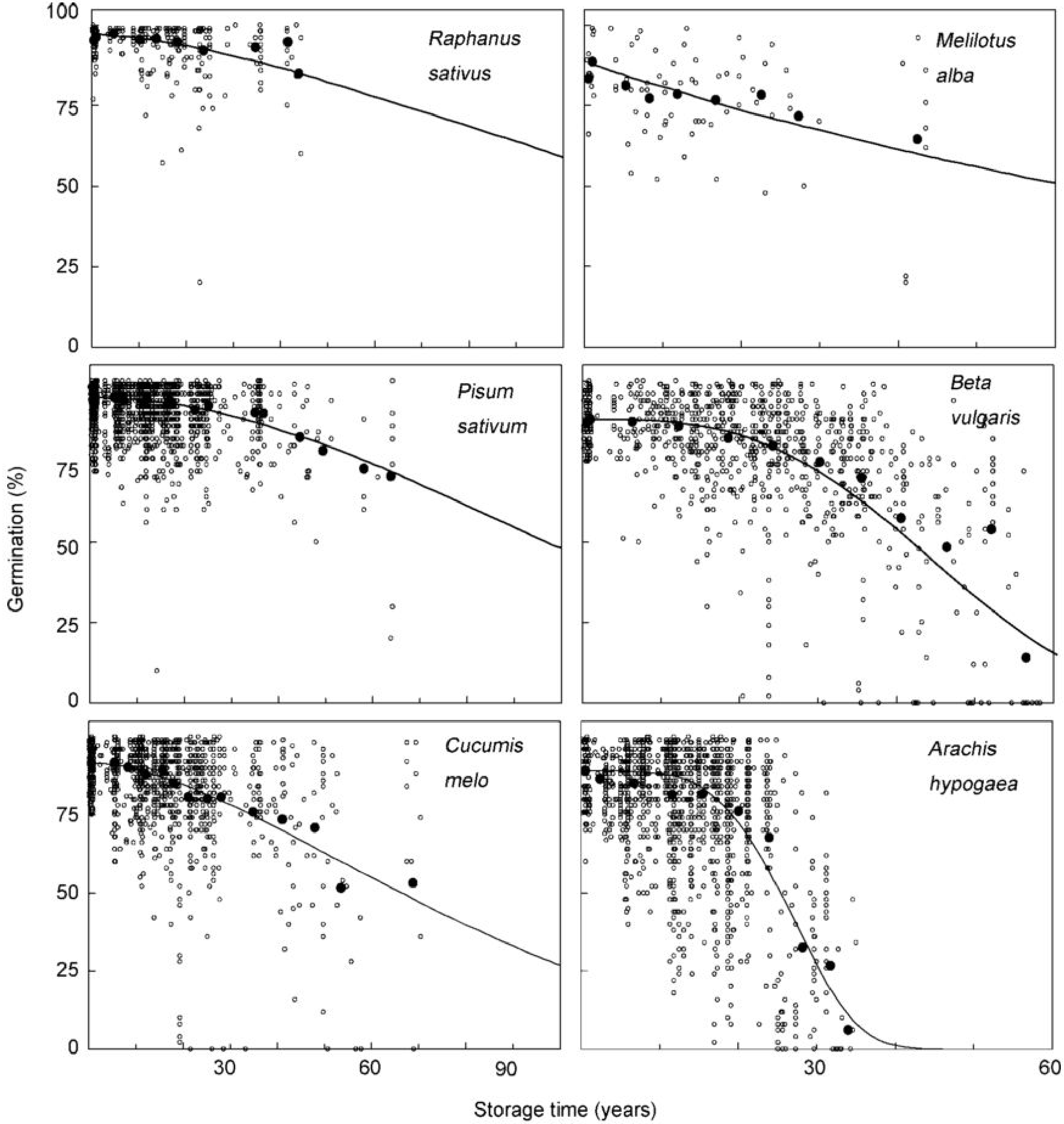
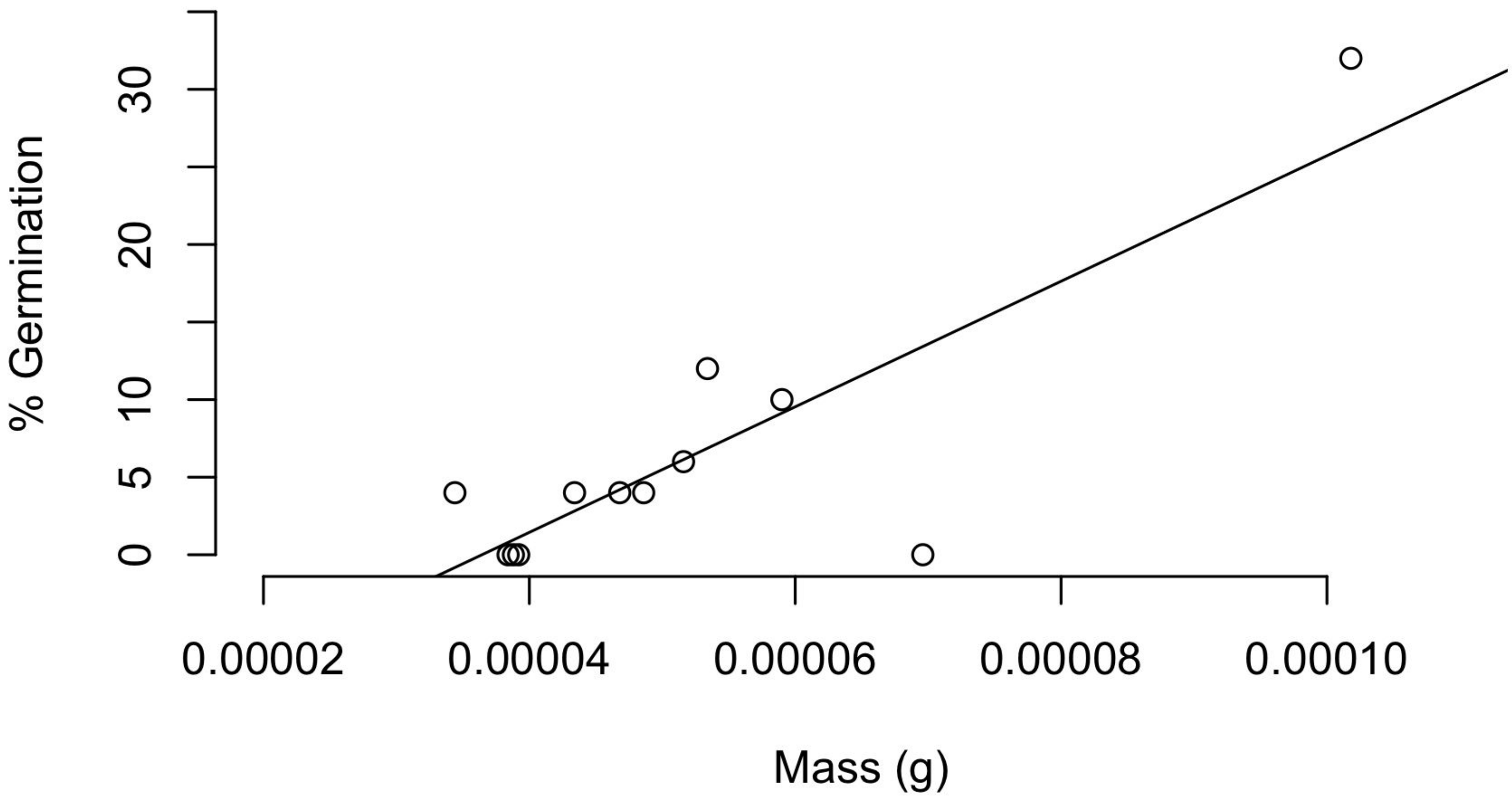
FUTURE DIRECTIONS



Walters, C., Wheeler, L. M., & Grotenhuis, J. M. (2005). Longevity of seeds stored in a genebank: species characteristics. Seed Science Research, 15(1), 1–20. https://doi.org/10.1079/SSR2004195

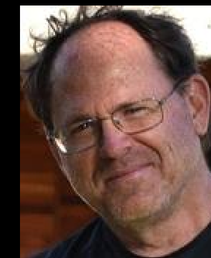
FUTURE DIRECTIONS



ACKNOWLEDGEMENTS

Seana Walsh for field support.

Margaret Clark for helping to check germination.



Tim Flynn for herbarium support

SCIENCE & CONSERVATION

NATIONAL TROPICAL BOTANICAL GARDEN

MAHALO!

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This and additional presentations available at http://nativeseed.info

NATIVE SEED NETWORK

Institute for Applied Ecology