

## Final Report: APGA Grant for *Alectryon macrococcus* var. *macrococcus*

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### Introduction:

A total of seven trips were made to locate, monitor and collect propagules of the rare and endangered Hawaiian Māhoe or *Alectryon macrococcus* var. *macrococcus*. *A. macrococcus*, is known as Māhoe or “twin” in Hawaiian, for its double fruits. Three trips were made in the Fall of 2016 - - two trips into Waimea Canyon and one to the remote Kalalau Valley on the inaccessible North Coast of Kauai. The Kalalau Valley population was found to be extirpated. The project then solely focused on the two populations left in Waimea Canyon (Koaie and Kawaiiki Valley). In the Spring of 2017, four follow-up trips were conducted into Waimea Canyon. All trees of this species are being hit by the black twig borer (*Xylosandrus compactus*) which kills its stems and reduces the vigor of established trees. Most of the trees in the Kawaiiki Valley and in the vicinity of Lonomea area of Waimea Canyon were found to be in feeble condition. The trees higher up in Koaie Canyon area of the Waimea Canyon where it is wetter were found to be more vigorous; this area was also where groups of previously unknown trees were discovered. Originally the plan was to bag and collect seed. However, only four trees were ever observed during the project period to have immature fruit. Weakened trunks due to the twig borer meant trees could not sustain climbing and bagging. Larger immature seed were collected and sent to Lyon Arboretum (Lyon) Tissue Culture Lab and San Diego Zoo’s (SDZ) Micropropagation Laboratory.

### Methodology:

Used known locations of plants in NTBG’s Rare Plant Database, which includes over 30 years of and collections and observations, plus herbarium vouchers to make a map of all the known Kauai *A. macrococcus* locations. Maps were added onto smartphones utilizing Collector ArcGIS and used to navigate and relocate individuals in the wild. New individuals were mapped and vouchered, if healthy enough, for future research and reference in PTBG and BISH herbarium.

Once trees were located, hiking trips were made to monitor the Waimea Canyon sub-populations and to survey for new individuals and monitor for phenology. Helicopters were utilized to get into steep and difficult to access rough terrain in Waimea Canyon. A 20 ft. zodiac was utilized to reach the Napali Coast Wilderness Preserve and to drop the field team off at the beach.

Our initial strategy was to relocate known and survey for new trees, then to bag and collect seed. Polyurethane bags (Rice Bag type material) were sewn by NTBG volunteers with Velcro closures. These bags deter and delay rodent predation and can be placed with one hand. However, few immature fruit were observed and bagging was not feasible due to the fragility of borer damaged trunks. Some Immature fruit was collected and sent intact and split equally between Lyon and SDZ Micropropagation Labs.

Herbarium vouchers were taken for newly discovered individuals. However the majority of trees were sterile and feeble and one voucher was made to represent the group. DNA samples were made for all vouchers by tearing up young green leaves and placing them in silica gel. All samples are stored at NTBG’s herbarium and Botanical Research Center.

If trees were healthy enough airlayers were set. In the fall on 2016, airlayers were set with regular Sphagnum moss and both Hormex powered hormone and CloneX rooting gel. In spring of 2017 we used Premium New Zealand Sphagnum moss (a commercial grade *S Cristatum*) and just the CloneX rooting gel. Upright young woody stems were selected first whenever possible. We removed the bark at twice the diameter of the stem, and applied hormone. A handful of dampened sphagnum moss was molded around the cut and treated area. The moss was then wrapped in clear plastic, zip ties were used to secure both ends. Lastly the clear plastic was wrapped in aluminum foil.



Photo 1: Showing Kupu Intern Randy Umetsu wrapping scored young branch in plastic and Sphagnum.

For removal, airlayers were cut 1-2 inches below root-ball when the roots were visible and plentiful. Root-balls of removed airlayers were placed in hard plastic cups and hand carried or helicoptered out.

Once in the nursery airlayers were potted in coarse media and placed in the mist house with 30 seconds of mist every 15 minutes and under 50% shade cloth. About three-fourths of the foliage was removed from the air-layer. Once new leaves and growth appeared, plants were moved to the nursery and placed in 80 percent shade. A light foliar fertilizer is applied once a week.



Photo 2: Showing rooted airlayers being flown out by helicopter to NTBG's nursery

## **Summary of Field Trips**

### **I. August 8-12, 2016**

Location: Kauai, Waimea Canyon

Participants: Natalia Tangalin (Field Botanist), Kamalani Chock (KUPU Intern), Emroy Griffin-Noyes (Limahuli Garden Preserve Manger)

Summary: Five day camping trip into Waimea Canyon to locate and collect fruit.

On August 10, flew in by helicopter. Spent August 10-11 tracking known existing *Alectryon macrococcus* var. *macrococcus* collection and observation points, collecting and setting air-layers since most trees did not have fruit. On August 12, the team spent 10 hours hiking out of the Canyon.

It has been a wet summer following 2015's dry winter, these unusual weather patterns may be contributing to the phenology we were observing. About two-thirds of the *Alectryon* trees were non-fertile, aside from the two trees with immature fruit; the rest had very young early inflorescences developing. Based on herbarium vouchers and past field botanist observations, were hoping to find and bag immature fruit, instead we set air-layers on trees that are healthy enough to sustain them. Set 1-3 per tree depending on health and suitability of branches.

Challenges: Attempted to drive in on August 8, but 4WD access road was impassible due to heavy rains.

Highlights: Over three days we visited 8 of the 10 known points for *Alectryon macrococcus* var. *macrococcus*. We set 28 air-layers and collected immature fruit from 2 trees. We opted to collect the fruit because bagging wasn't feasible without climbing the trees themselves and potentially causing damage to the trunks weakened by the black twig borer.



Photo 3: Helicopter landing for airlayer pick-up

## II. August 24-26, 2016

Location: Kalalau Valley

Participants: Natalia Tangalin (Field Botanist), Emory Griffin-Noyes (Limahuli Preserve Rare Plant Manager), Mike DeMotta (NTBG Curator of Living Collections)

Summary: Kalalau Valley is a secluded Valley on the Napali Coast within Napali Coast State Wilderness Park. It is only accessible by a 12 mile hiking trail, 3 hour boat ride, or helicopter. On August 24th a three person team boated in to Kalalau State Park, we were dropped offshore and swam in with our gear. The next day we spent 6 hours hiking up Kalalau Valley to the back wall and the native forest patch where the *Alectryon macrococcus* trees are known to occur. The skeletal trunk of one dead *Alectryon macrococcus* was observed to be still standing. One skeletal dead tree was found. This population of approximately 6 trees was last observed by

NTBG research biologist in 2013 (K. Wood, personal communication, July 22, 2016). Trees were said to be in poor condition.

Challenges: Last 6 known trees were dead. Not enough time for exploratory survey to discover new trees.

### III. Sept 23, 2017

Location: Waimea Canyon

Participants: Natalia Tangalin (Field Botanist), Kamalani Chock (KUPU Intern)

Summary: Monitoring day trip.

Challenges: Long hike in. The trail is called the "Root Trail" because of the steepness and necessity grabbing onto tree roots to get down. Once in Koaie Canyon, the first group of Alectryon with airdlayers is about an hour downstream.

Highlights: A new individual was located on the way to check airdlayers placed on the trees in the Bonamia area. It was sterile with 5 main trunks and lots of new shoots. Four air-layers were set. On tree NT4653 two additional airdlayers were set and 5 immature fruit were collected and sent to Lyon's Tissue Culture Lab. Additionally two *Bonamia menziesii* fruit were collected.

## Road to Koaie and Root Trail Closed November 3, 2016 – March 24, 2017

### IV. March 28, 2017

Location: Day hiking and monitoring of airdlayers

Participants: Natalia Tangalin (Field Botanist), Randy Umetsu (KUPU Intern)

Summary: Monitoring day trip. Check airdlayers and for fruit in the know upper three *A. macrococcus* trees.

Highlights: Found a new group of ten *A. macrococcus* were discovered. This group is the healthiest we have observed with individuals having trunks up to 30 ft. tall.

Collected a robust airdlayer from NT4831. Also monitored a second root ball that was less robust so it was left for the next trip.



Photo 4. Showing root ball of airtlayer on NT4831

#### **V. April 4-5, 2017**

Location: Waimea Canyon

Participants: Natalia Tangalin (Field Botanist), Ben Nyberg (GIS Specialist)

Purpose: To check and retrieve the airtlayers in Kawaiiki Valley and to check along the river above Lonomea where NTBG database shows old points.

Challenges: Ben was swarmed by honey bees and stung about 6 times on the head and neck, he was also stung on the hand and arm. This is uncharacteristic since they were not disturbed. *A. macrococcus* trees in this lower drier zone of the Canyon are mostly feeble and hit hard by black twig borer.



Photo 5 a-c GIS Specialist 3 days after encountering angry bees and bad roads.

Highlights: Collected two airdropers of the three set on this group. Located a new tree but it was too feeble to take any airdropers. Checked a subgulch where 2-3 *Alectryon* trees were observed by NTBG botanist. Found signs of landslides in the area and extremely degraded relic forest with numerous landslides and no *A. macrococcus* trees.

#### **VI. April 17-18, 2017**

Location: Waimea Canyon

Participants: Natalia Tangalin, Field Botanist, Chiaime Neagle (NTBG Conservation Technician), Kama Chock (KUPU Intern)

Summary: Flew in via helicopter to check corridor for *A. macrococcus*

Highlights: Discovery of a second individual of *Mezoneurom kavaiense*

Challenges: Previously known trees in this were not relocated and are believed to be gone. Area is heavily degraded by goats, pigs, weeds, and erosion.

### **VII. April 18-19**

Participants: Natalia Tangalin, Field Botanist, Mike DeMotta (Curator), Randy Umetsu (KUPU Intern)

Summary: Set airdlayers on a group of newly discovered *A. macrococcus*.

Highlights: Discovered a group of 17 healthy trees on the way to the newly discovered group of ten trees. Did 39 airdlayers on 18 individual trees. Collected 9 airdlayers that had been placed in the Fall of 2016. Observed three trees with immature fruit.

Challenges: It took so much time setting airdlayers on the 17 newly discovered trees that we did not make it to the other new group of 10 trees near the base of trail.

### **VIII. April 21, 2017**

Location: Koaie Canyon

Participants: Natalia Tangalin (Field Botanist), Maggie Sporck-Koehler (DLNR Hawaii Botanist), Randy Umetsu (KUPU Intern)

Purpose: Set airdlayers on the new group of 10 *A. macrococcus* found March 28, 2017.

Challenges: Steady heavy drizzle all day slowed our work setting the airdlayers and applying the rooting gel was more difficult. Because the drive and hike take ten hours, we only had about two hours of working time. On the drive out the Kawaikoi River, had risen a few feet and was not safe to cross so we had to walk 10 miles along the dirt road to a payphone.

Accomplishments: Set eighteen airdlayers on 10 healthy *A. macrococcus* trees. Got out of Canyon safe and retrieved truck next day with no problems.



## Incidental Discoveries of Importance

### I. Rediscovery of *Bonamia menziesii* in Waimea Canyon

While looking for *A. macrococcus* we discovered a new population of about 15-30 *Bonamia menziesii* and collected 8 seed. This is currently only the second population known of *Bonamia menziesii* known on Kauai; after this same team discovered a population on the East side of Kauai earlier this year. It is a U.S. listed Endangered Species in the Convolvulaceae family. On subsequent trips both seed and cuttings were collected and are growing in NTBG's nursery.



Photo 6. *Bonamia menziesii* in Waimea Canyon

## II. Discovery of *Melanthera micrantha subsp. micrantha*

*Melanthera micrantha subsp. micrantha* is a Kauai endemic, Plant Extinction Prevention (PEP) program species and U.S. Federally listed endangered species. There is one other known patch in the Waimea Canyon, which is being monitored by the PEP program. This is now the second known location consisting of three small patches comprising 5-15 plants. Juveniles were observed in August but gone and likely eaten by goats in April of 2017. Seed and cuttings were collected to be stored and grown at NTBG.



Photo 7. *Melanthera micrantha subsp. micrantha*



Photo 8. *Melanthera micrantha subsp. micrantha*

- III. Discovery of a second individual of *Mezoneurom kavaense* (H.Mann) Hillebr. Also known as *Caesalpinia kavaensis*, a Hawaiian endemic mesic to dry-forest tree with a hardwood that was valued for toolmaking by the early Hawaiians. On the island of Kauai, it was thought to be extinct in the wild. The last documented plant of this species on Kauai occurred in an enclosure in the Waimea Canyon and died after Hurricane *Iniki* in 1992. In 2011 NTBG relocated a single individual in a hanging valley of Koaie Canyon. A second previously unknown individual was located in that same valley while surveying for *Alectryon*. Seed was collected and is being grown by NTBG.



Photo 9 : *Mezoneurom kavaense* (H.Mann) Hillebr.



Photo 10: Close-up of *Mezoneurom* flower

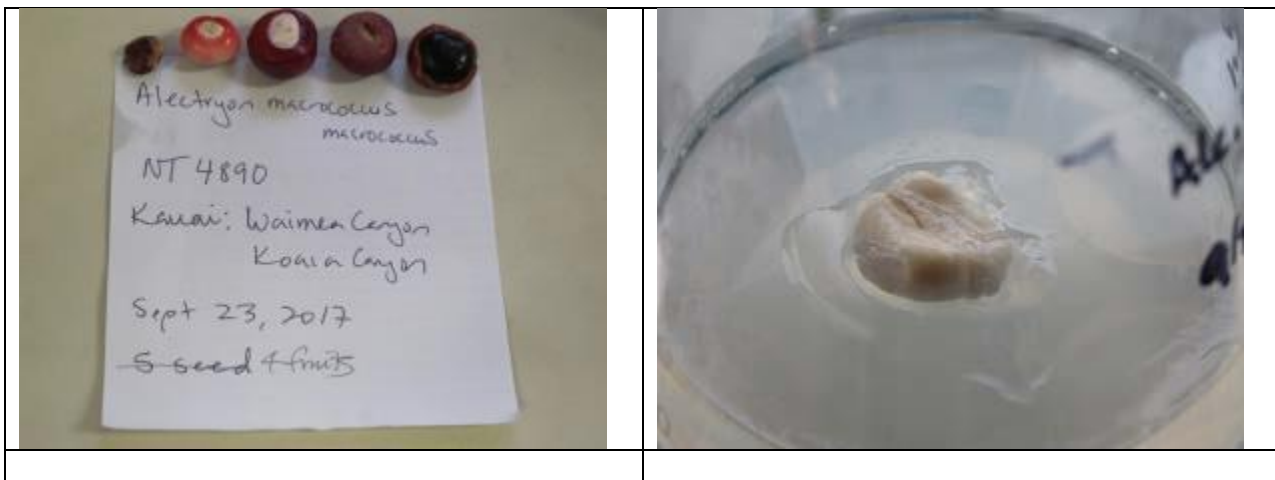
Project Accomplishments:

Total of 56 living *A. macrococcus*

## Condition of trees

- 64 % Normal or basically healthy condition
- 37.5 % Feeble Condition (> 50% of canopy dead)
- 11 % Dead
- 95 airlayers set on 56 different *A. macrococcus* trees
- Fruit collected
  - 13 fruit total were collected.
    - 6 fruit from 2 accessions / trees to Lyon Arboretum's Micropropagation Lab.

“We just processed the 4 fruits you sent but of the 5 seeds that were inside, one was partially eaten and rotted and another was too immature to have anything to process after cleaning (2 seeds on the left in first picture). So we were able to sow the 3 remaining seeds that were the larger ones on the right. The picture on the right is what the seed looks like after we process it--the seed in the flask is about the size of a quarter. Will let you know if we get any germination.” (C. Yamamoto, personal communication, September 26, 2016)



- San Diego Zoo's Micropropagation Lab.
  - 7 fruit sent to San Diego Zoo Micropropagation Lab from two accessions



Photo 11. *Alectryon macrococcus* fruit

- 11 airlayers in NTBG's nursery



Photo 12. Air-layer on *Alectryon macrococcus*



Photo 13. KUPU intern Kama places Air-layer on *Alectryon*

Table 1. Airlayer setting in the different subpopulations

Location	Date	# of living Trees found	Airlayers set	Airlayers coll.	Air-layers remainig
	3/28/17	10	0	0	0
	4/21/17	+0	18	0	18
Sub-Totals		10	18	0	18
	8/4/16	1	4	0	4
	3/23/17	+0	0	1	3
	4/18/17	+2	5	1	4
Sub-Totals		3	9	2	4
	3/23/17	18	39	0	39
Sub-Totals					
	8/4/16	2	5	0	5
	9/23/16	+0	3	0	0
	3/23/17	+0	0	0	0
Sub-Totals		2	8	0	1
	4/19/2017	4	4	0	4
Sub-Totals		4	4	0	4
	4/19/17	8	12	0	12
	8/10/16	1	0	1	0
		9	12	3	4
	8/10/16	5	7	0	7
	4/18/17	1	0	4	0
Sub-Totals					
	8/11/16	6	3	0	3
	4/4/2017	1	0	2	0
		# of living Trees found	Airlayers set	Airlayers collected	Air-layers reaming
<b>Totals</b>		<b>56</b>	<b>95</b>	<b>11</b>	<b>68</b>

Table 2. Airlayers set for over 3 months

	Total set > 3 months	Dead	Healed	Missing	Rooted	Remains
	4	0	0	0	2	2
	6	2	2	3	0	1
	12	4 (1 root & dead)	0	1	3 (1 root & dead)	4

	7	1	0	1	5	0
	3	1	0	0	2	0
Total	32	8	2	5	12	7
%		31	6	16	41	22

#### Summary of Airlayer Success:

Rooting rate is about 41%. Based on the limited observation of four airlayers, where the rooting took place in ColneX treated stems, when the project resumed in the Spring, we chose to use the CloneX gel exclusively. Our sample size of eleven trees showed slightly better results with the CloneX gel, but the sample size is too small to make more than observational correlations. CloneX gel is much easier to work with in field conditions. The purple color also makes it easier to see if the coverage is complete on the white cambium. We did observe one airlayer that rooted and died. Twig borers damage was observed in most of the dead airlayers including the one that had rooted. Due to the road closure, we collected airlayers starting a five months versus the proposed three months. It is likely that at three months, the root ball would have been unable to support the propagule. The later than expected harvest did not significantly impact the outcome.



Photo 14: Showing Randy Umetsu and Natalia Tangalin with collected air-layers

#### How this project impacts this species:

- This project is redirecting search efforts higher in the Canyon. Previously this species was believed to predominately occur in lower and in drier forest; botanist however discovered the two of the largest and healthiest up higher in the wetter part of the Canyon where they were thought to be less common. Wetter conditions likely allows for sap production and resistance to the twig borer.

- 56 living trees were located, the current population of 300, it is likely high given the condition of the known trees and the forest in Waimea Canyon. Rodents are getting all the mature seed and the vigor of every.

#### Future Planned Work:

- Return in July 2017 to check the remaining 68 airlayers
- Survey higher in Koaie Canyon for additional sub-populations.
- Collect and care for Alectryon air-layers.
- Establish viable Ex-Situ populations as they become ready for outplanting.
- Search for new individual in the extirpated Kalalau population
- Work with DOFAW to implement permanent metal tagging to replace temporary plastic tagging for Population Reference Codes for future tracking of founders.

Table 1. Summary of Waimea Canyon work (all NT#'s include a sterile leaf voucher and DNA collection per NTBG protocol).

			0	11-Aug-16	0		0		dead
			0	11-Aug-16	0		0		feeble
			0	11-Aug-16	0		0		feeble
			1	11-Aug-16	1		1	NT4671	feeble
			0	4-Apr-17	1		0	NT4836	
			1	11-Aug-16	1		1	NT4672	feeble
			0	4-Apr-17	1		0	NT4837	
			0	11-Aug-16	0		0	/	feeble
			0	11-Aug-16				NT4673	feeble
			0	4-Apr-17			0	observation	feeble
			1	21-Mar-17	0		1	NT4828	normal
			10	21-Mar-17	0		10	NT4829	normal
			1	21-Mar-17	0		1	/	normal
			2	21-Mar-17	0		2		normal
			1	21-Mar-17	0		1	NT4830	normal
			3	21-Mar-17	0		3	/	normal
			0	21-Mar-17	0		0	/	normal
			0	21-Mar-17	0		0	/	normal
			0	21-Mar-17	0		0	/	normal
			1	21-Mar-17	0		1	NT4831, NT4849	normal
			1	19-Apr-17	0		1		normal
			0	19-Apr-17	0		0		normal
			0	19-Apr-17	0		0		normal
			3	19-Apr-17	0		3		normal
			1	18-Apr-17	0		1		feeble
			2	18-Apr-17	0		2		feeble
			2	18-Apr-17	1		4	NT4849	normal
			0	28-Mar-17	1		3	NT4831	
			4	23-Sep-16	0		4	NT4889	observation



[REDACTED]	[REDACTED]	[REDACTED]	4	18-Apr-17	0		4	NT4846	normal
[REDACTED]	[REDACTED]	[REDACTED]	3	18-Apr-17	0		3	NT4847	normal
[REDACTED]	[REDACTED]	[REDACTED]	3	18-Apr-17	0		3		normal
[REDACTED]	[REDACTED]	[REDACTED]	3	18-Apr-17	0		3		normal
[REDACTED]	[REDACTED]	[REDACTED]	3	18-Apr-17	0		3		normal
[REDACTED]	[REDACTED]	[REDACTED]	4	18-Apr-17	0		4		normal
[REDACTED]	[REDACTED]	[REDACTED]	3	18-Apr-17	0		3		normal
[REDACTED]	[REDACTED]	[REDACTED]	4	18-Apr-17	0		4		normal
[REDACTED]	[REDACTED]	[REDACTED]	2	18-Apr-17	0		2		normal
[REDACTED]	[REDACTED]	[REDACTED]	2	18-Apr-17	0		2		normal
[REDACTED]	[REDACTED]	[REDACTED]	4	18-Apr-17	0		4		normal