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# Annual Report on: Recovery Initiative (RCI) September 2015—July 2016

(Cooperative Agreement No. F140C00847)

ENVIROSURVEY\_SIERRA\_BERMEJA\_REPORT\_2





# **Cooperative Agreement No: F14OC00847:**

Implementation of priority recovery actions for Puerto Rico's southwest endangered plant species that lead to the down listing of *Eugenia woodburyana*, delisting of *Sthalia monosperma* and simultaneously, in the same type of habitat, prevent the extinction of four listed plants of Puerto Rico.

#### Overview of nursery, seed material collection and propagation:

On September, 2014 the Cooperative Recovery Initiative (CRI) began with an assessment of the greenhouse to evaluate the infrastructure and space availability for the propagation of tree material. We also started the seeds material collection, and visits to the Maria Luisa Farm and El Conuco Farm. These two properties are managed for conservation by the Puerto Rico Conservation Trust "Para La Naturaleza", and harbor natural populations of the target endangered species (Aristida chaseae, A. portoricensis, Eugenia woodburyana and Vernonia proctorii). We also visited Los Gemelos Farm and Lozada's Farm, both private lands adjacent to the Cartagena Lagoon NWR "Tinaja Track" (Figure 1). The visits were made to have an overview of the habitat and to get familiar with the Eugenia woodburyana for the future assessment of the natural populations. Eugenia woodburyana seeds material were collected from natural populations (F<sub>1</sub>) in Sierra Bermeja, specifically from El Conuco Farm, La Tinaja Track, and Cerro Mariquita. The latter two areas are within the Cartagena Lagoon National Wildlife Refuge. Also, we collected seed material in a planted population (F<sub>2</sub>) in the vicinity of Sierra Bermeja (Figure 2). Since reintroduction and population enhancements efforts will be conducted within Sierra Bermeja, all seed material was collected from this geographical area, and we avoid collecting material from other areas with different environmental conditions (i.e., Guánica and Susúa Commonwealth Forest). The aim of this was to maintain the genetic integrity of the natural populations of E. woodburyana that evolve and are adapted to the environmental conditions at Sierra Bermeja.

We also visited the natural populations of *Stahlia monosperma* to have an idea of the different habitats (i.e., Sierra Bermeja, Los Pozos, Punta Melones, Punta Guaniquilla, Boquerón Country Club, and Boquerón Wildlife Refuge; Figure 3) where the species naturally occurs, and then return to those areas to conduct an assessment of those natural populations. The *Stahlia* 

monosperma seeds were collected from different areas in the municipality of Cabo Rojo, including both natural (F<sub>1</sub>) and planted (F<sub>2</sub>) populations (Figure 4). Remnants of natural populations were targeted to secure the species genetic diversity contained in populations that are not usually propagated. However, due to the absence of seed material we further collected seeds from planted individuals.

In addition, the ongoing project includes a habitat enhancement by planting native trees and shrub material. The native trees seeds material were collected in different areas in the municipality of Cabo Rojo (i.e., Punta Melones, Cabo Rojo National Wildlife Refuge, Los Pozos, Finca El Conuco, Joyuda, and Sierra Bermeja) and Lajas (i.e., Laguna Cartagena National Wildlife Refuge and La Tinaja Track in Sierra Bermeja) (Figure 5).

The seeds of all species were germinated and propagated between October 2014 and June 2016. As of to date, we have propagated about 393 *Eugenia woodburyana*, about 405 *Stahlia monosperma*, and about 886 native trees of 15 different species.

# Endangered, threatened and native trees propagation:

#### Eugenia woodburyana:

The seeds were collected in different areas of Sierra Bermeja: El Conuco (Para La Naturaleza) and La Tinaja Tract (Cartagena Lagoon NWR) (both natural populations), and a private land in the vicinity of Sierra Bermeja (F<sub>2</sub> source from the natural populations at Cerro Mariquita, Sierra Bermeja) (Figure 3). Field observations suggest that *Eugenia woodburyana* trees responds to rain events and moisture availability producing new growth (i.e., branches, leaves, flowers, and fruits). If the rain events are followed by a drought period, the flowers and immature fruits are aborted, reducing the amount of fruits (seeds) available. Some factors that may affect the seeds production in these areas may be rainfall and moisture availability, severe drought stress and insect pests. As a general rule we avoid over collecting seed material from natural populations, and we sample the entire population to capture the genetic diversity.

Fresh material collected from Sierra Bermeja was cleaned by removing the outer fleshy cover (exocarp) to minimize fungi contamination during the germination. These seeds were allowed to dry for 3-4 days at room temperature until the seed coat cracks and the cotyledons get exposed.

The cotyledons are visibly light green, and this color is a good indicator of the viability of the seed. After initial trials it was determined that this process accelerates and maximizes the germination. Seeds were set to germinate on a 1:1 Pro-mix "high porosity" and perlite, and watered every two days. Seedlings were transplanted to 1 gallon plastic pots after they already develop about 3 pairs of leaves.

From the approximately 905 seeds of *E. woodburyana* collected, only 393 germinated. This is about 43% survival, from germination to transplanting in pots. Due to the rarity and the infrequent flowering and low fruit set of the species we had to collect seeds from the ground. Thus, we consider the germination percentage as high since the majority of the collected seeds did not present healthy cotyledons and embryo (e.g., larvae insect feeding on the cotyledons or cotyledons showing signs of drought stress) at the time of placing on the germination bench. We identified one of the insect affecting *E. woodburyana* as a weevil (family: *Curculionidae*) (Figure 6). The germination process for *Eugenia woodburyana* takes place between 10 to 20 days as long as the embryo is exposed. If the embryo is not exposed, the germination process takes about 15 to 60 days.

# Stahlia monosperma:

The availability of *Stahlia monosperma* seeds depends on environmental conditions as flowering and fruit set vary depending rainfall and moisture availability. The lack of connectivity and distance between flowering trees is another factor that may influence fruit production. Fruit availability is also affected by over collection by staff from other conservation agencies (e.g., PR Department of Natural and Environmental Resources [PRDNER]). The long periods of drought, which resulted in a low fruit set and development during 2015, and the over collection of fruits, precluded us from producing the amount of *Stahlia monosperma* seedlings projected for 2015. However, between May and June, 2016 we collected and planted about 999 seeds from different sources. A total of 405 of these seeds germinated and were transplant in pots for eventual planting in the field. As of July 2016, there is about 41% survival of the seedlings produced.

We collected *Stahlia monosperma* seeds from planted populations at PRDNER Boquerón Forest Office, Saman Road in Cabo Rojo, and the Boquerón Post Office. Also, we collected seeds from the natural populations in Cabo Rojo Country Club and Punta Guaniquilla. The germination

process was well documented as we conducted various germination treatments to determine which resulted in the best germination of the seeds. The treatments consisted in (1) opening and removing the fleshy exocarp of the fruit and expose the cotyledons (seed); then the seed was placed in shady area with good air flow for about two days and then placed in the propagator above the soil surface; (2) drying the whole fruit for two days in a shady place with good air flow and then put it in the propagator above and below the soil surface; and (3) the whole fruit was placed in the propagator above and below the soil surface without any other treatment. We used the Pro-mix "high porosity" and perlite (1:1) as germination media. The seed germination process occurred between 4 to 15 days on vigorous seeds before placed in the propagator no matter treatment they (Figure 7). *Stahlia monosperma* seeds continued sprouting for about 30 to 40 days on all treatments.

Table 1. Native Trees Propagated as of summer 2016.

Native tree species	Number of trees produced	Germination (%)
Bourreria succulenta	12	11
Capparis baducca	27	28
Capparis flexuosa	32	23
Capparis indica	18	12
Cocoloba microsthachya	50	25
Crescentia linearifolia	44	24
Erythroxylon brevipes	11	14
Eugenia ligustrina	96	61
Eugenia monticola	2	12
Eugenia sessiloflora	23	77
Eugenia spp.	134	81
Ouratea litoralis	103	31
Reynosia urcinata	26	31
Sabal causiarum	250	87
Tabebuia heterophylla	58	39
Total	886	N/A

Table 2 Enadangered Trees Propagated as of summer 2016.

Endangered species	Number of trees produced	Germination (%)		
Eugenia woodburyana	393	43		
Stahlia monosperma	405	41		

# **Reintroduction/Enhancement Efforts and Populations Assessments:**

#### Site selection and reintroduction:

The reintroduction site for both listed and native trees was selected based on the proximity to other natural populations of E. woodburyana, close proximity to an old livestock water trough that was converted in a water cistern and is now used for irrigation, and also because the site is a secondary forest with natural recruitment a native trees that are conspecific with E. woodburyana. Thus, the area provides suitable habitat conditions for the establishment of this endangered species, and for the future natural recruitment once planted material reach reproductive size. The exact location is at La Tinaja Land Tract, just south of Laguna Cartagena (Figure 8), and is within the Cartagena Lagoon National Wildlife Refuge (La Tinaja Track), and thus managed for conservation. The area shows an open understory with canopy gaps and is very close to an intermittent creek. The selected area was favored due to its gentle slope, which minimizes the risk of landslides that could affect the planted individuals in the future (Figure 9). Another advantage of this site is that there are firebreaks along the property, minimizing the threats of fire. The quality of the habitat was evidence by the presence of Ottoschulzia rhodoxylon (palo de rosa), an endemic and endangered tree, native trees like Bucida buceras (ucar), Capparis flexuosa, Capparis indica, Eugenia spp., Guaiacum officinale (guayacan), Pisonia albida (corcho bobo). However, the overall area shows several exotics species that may require management such as Megathyrsus maximus (yerba de guinea) and Leucaena leucocephala. However, the overall quality of the habitat outweighs the threat by exotic plants and native vegetation is evidently recovering.

As mentioned above, close to the planting site we establish an irrigation system (Figure 10) starting with a galvalume roof structure over an old livestock water trough to collect rain water. Then we connected a PVC water line to the water trough and extended it along the main trail to a 530 gallons water tank reserve. The water lines continue along the edge of the main trail to different spots along the selected reintroduction site. Trees are irrigated manually with a hose connected to a tap. An electric water pump is used to move the water from the water trough to the reserve tank. This is a watering system that provides for the long term maintenance of the planted material, and provides for future planting efforts. The proximity of the site to a dirt road pose a logistic advantage as the planted trees can be regularly monitored, including the initial follow up of the individuals immediately after planting (i.e., initial survival), and the long term record of the status of the individuals (e.g., growth rate, flowering and fruit production).

# Planting events:

- The first planting event of forty (40) Eugenia woodburyana and fifteen (15) native trees (Coccoloba microstachya, Cordia rickseckeri, Eugenia ligustrina, and Gymnanthes lucida) was conducted on November 7, 2014. Trees were planted following the contour of the terrain and using the available canopy gaps (Figure 9). Each Eugenia woodburyana tree was marked with a flagging and with a permanent aluminum numbered tag (series 1 40). The planted area was fenced to prevent access of feral horses that access the refuge, which represent a real threat by trampling of the planted trees. The planted material was watered once or twice a week depending on the availability of water due to rains seasonality. This planting was a trial to verify the suitability of the site, and to determine the maintenance requirements and any logistic needs that should be addressed before implementing future reforestation efforts.
- The second planting event of fifty (50) Eugenia woodburyana and fifty (50) native trees (Coccoloba microstachya, Crescentia linearifolia, Erithroxylon brevipes, Eugenia ligustrina, Eugenia spp., Ouratea litoralis and Reynosia urcinata) occurred on October 27, 2015.

- The third planting event of thirty five (35) Eugenia woodburyana and fifty (50) native trees (Capparis baduca, Capparis indica, Coccoloba microstachya, Erithroxylon brevipes, Eugenia ligustrina, Eugenia spp., Ouratea litoralis, Reynosia urcinata) was conducted on December 29, 2015.
- The fourth planting event of forty (40) Eugenia woodburyana and fifteen (15) native trees (Capparis indica, Crescentia linearifolia, Erithroxylon brevipes, Eugenia ligustrina, Eugenia sessiliflora, Eugenia spp., Ouratea litoralis and Tabebuia heterophylla) was conducted on March 10, 2016.
- The fifth planting event of thirty (30) Eugenia woodburyana and twenty (20) native trees (Capparis indica, Crescentia linearifolia, Erythroxylon brevipes, Eugenia ligustrina, Ouratea litoralis and Tabebuia heterophylla) occurred on May 20, 2016.

We followed the same procedure conducted on the first planting effort during all five planting events. The total number of trees planted in La Tinaja Tract is one hundred and ninety five (195) *Eugenia woodburyana* (Figure 8), and one hundred and fifty (150) native trees. Basal diameter (mm) and height (cm) was recorded for each individual of *Eugenia woodburyana* as well as its general condition (e.g., broken branches and other signs) as baseline information for the long term monitoring of this recovery effort. The overall mortality of planted individuals of *E. woodburyana* was related to exposure to sun. Survival of planted material was higher at shaded areas dominated by native shrubs. Only twenty four trees of 195 planted trees died. The general survival for the planted population of *E.woodburyana* is 88%. Sixty four of the total of 195 were planted exposed to sunlight and 15 of them died, these is about 77% of survival; 131 of the total of 195 were planted under shade and 5 of them died, these is about 96% of survival; and four of the total of 195died by horses, these is about 2% of death. Initial planting effort was negatively affected by trampling of seedlings by feral horses, thus any further planting effort was within a fenced area to exclude horses (Figure 11 & Figure 11.1).

# **Assessment of Natural Populations:**

This ongoing task started on March 17, 2015 with a site visit to Maria Luisa Farm as in the past Service biologists Omar Monsegur and Ivan Llerandi identified individuals of Eugenia woodburyana in this property. This property is under a conservation easement managed by Para La Naturaleza (Puerto Rico Conservation Trust). The property lies within Sierra Bermeja and its northwest boundary is adjacent to La Tinaja Track (Cartagena Lagoon NWR) (Figure 12). Thus the monitored individuals are an extension of the natural populations within the Cartagena Lagoon NWR. We have collected data on tree height (m), DBH (cm), canopy cover (%), phenology, pests, evidence of cuttings or damages to the trees, epiphytes, tagged the individuals, and took GPS coordinates (Figure 13). From March 2015 to May 2016 we have counted a total of 301 adults, 161 seedlings, 46 saplings, and 441 immature adults, for a grand total of 949 individuals of Eugenia woodburyana within the Maria Luisa Farm. This farm and other adjacent properties have plenty of suitable habitats to assess, so we will continue with the population evaluation in 2016. Also, there are other federally listed species within the Maria Luisa Farm: Aristida portoricensis, Aristida chaseae, Ottoschulzia rhodoxylon and Vernonia proctorii. We recorded GPS coordinates for all observed clusters of these endangered species. During the assessments s we have observed different grazing animals (i.e., cows, goats, and horses). These animals have impacted the habitat by making trails, stepping on seedlings and eating the vegetation. Goats have been observed grazing on the endangered Aristida chaseae. Thus, we infer that these animals are at least suppressing the natural recruitment of these species and the expansion of the natural populations. Therefore, developing a management plan for this property (María Luisa) is critical for the conservation of these endangered plants.

The north section of Maria Luisa Farm occupies about 254.04 acres in the Subtropical Dry Forest Life Zone (Holdridge 1967; Ewel and Whitmore 1973) of southwestern Puerto Rico. The highest point on the property (220m) lies a few meters below the Cerro Mariquita (277m), which is the highest peak in Sierra Bermeja. The vegetative cover in the target area at Maria Luisa Farm is not uniform. Among the trees species that we documented are the *Bucida buceras* (úcar), *Bursera simaruba* (almacigo), *Capparis flexuosa*, *Capparis indica*, *Coccoloba microstachya* (uverillo), *Croton humilis* (crotón), *Eugenia ligustrina* (palo de murta), *Eugenia* 

sessiliflora, Eugenia spp., Guaiacum officinale (guayacán), Guapira fragrans (corcho), Leucaena leucocephala, Myrciaria floribunda, Pisonia albida (corcho bobo), Trichilia hirta, and other shrub species, grasses and fast growing herbaceous plants (no grasses). In general, mid- to high-elevation slopes on the property show forest cover (secondary forest). The southern facing slopes of the property are highly degraded, and the vegetation is predominantly composed of exotics, and the overall area is used for grazing.

#### **Fire Breaks Implementation:**

It was agreed that fire breaks would be contracted to an external source by Envirosurvey, Inc. Therefore, we asked Refuges for the specifications of the fire breaks in order to provide the contractor with specific detailed instructions. On February 2015, we had a meeting with the former refuge manager, Oscar Díaz, and other Service staff from Refuges and Ecological Services. The need of a section 7 consultation for this activity was discussed during the meeting. As we understand, the section 7 consultation was completed. However, as of the date of this report we have not received instructions and specifications from Refuges for the fire breaks. To begin the process we also need a map with the specific areas were the fire breaks will be constructed.

#### **Private Land Agreements:**

We are working on an agreement with the Escabi family, owners of the Maria Luisa Farm. The agreement proposes the construction of a fence to exclude grazing animals from the area where the core of the populations of endangered species are found (Figure 14). As discussed with Refuges and Ecological Services, we are proposing to protect approximately forty five acres of land by building a fence. This piece of land is adjacent to La Tinaja Tract, and therefore, once the fence is set, the forty five acres will be an extension of the Refuge. Moreover, the proposed fence will secure viable populations of *A. chaseae*, *A. portoricensis*, *E. woodburyana*, *O. rhodoxylon* and *V. proctorii*. At this moment we have several landowners with the possibility of reaching agreements to introduce listed tree populations on the surrounding of Sierra Bermeja.

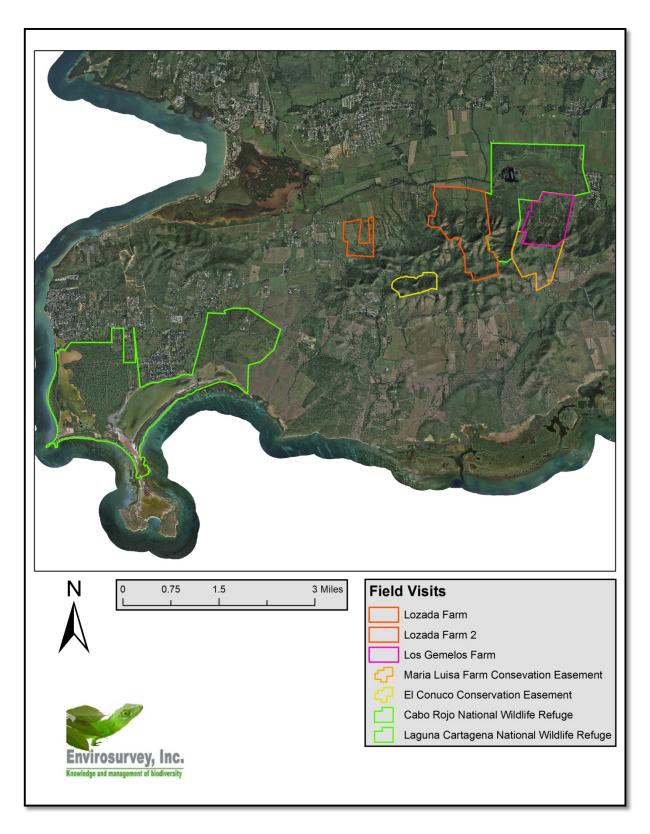
#### **Future Actions:**

- Continue *Eugenia woodburyana* natural population assessment in the Lozada Farm and Conuco Farm. Lozada property is also an extension of the Cartagena Lagoon NWR (Cerro Mariquita), and harbors populations of *A. chaseae*, *A. portoricensis*, *E. woodburyana*, *Lyonia truncata* var. *protorii* and *V. proctorii*, and Conuco Farm also harbors natural populations of *A. chaseae*, *E. woodburyana* and *V. proctorii*.
- Continue *S. monosperma* natural population assessment in the Punta Guaniquilla, Boquerón Country Club and Sierra Bermeja. The assessment will also include material of *S. monosperma* planted decades ago within Cartagena Lagoon.
- Continue with the population enhancement of *E. woodburyana* and the reintroduction of *S. monosperma* in La Tinaja Tract.
- Continue conversations with the land owners of Maria Luisa Farm (Escabí Family) for the proposed agreement to build a fence to exclude grazing animals (Figure 14) and protect the natural populations of *A. chaseae*, *A. portoricensis*, *E. woodburyana*, *O. rhodoxylon* and *V. proctorii*.
- Once the population assessments at Lozada Farm are ongoing, evaluate the feasibility of developing an agreement with the land owner to protect *A. chaseae*, *A. portoricensis*, *E. woodburyana*, *Lyonia truncata* var. *protorii* and *V. proctorii*. If feasible evaluate the possibility of planting further material of *E. woodburyana* and *S. monosperma*.
- Continue the scouting of properties with suitable habitat within Sierra Bermeja and Peñones de Melones for possible agreements.
- Continue seed collecting and propagation of both native tree species, and endangered material (*E. wodburyana* and *S. monosperma*).

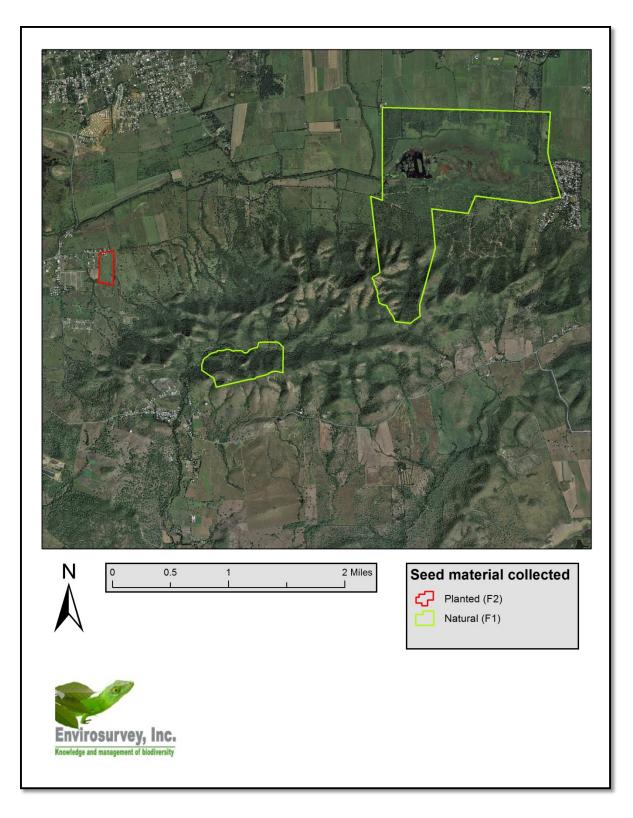
# **Budget Status:**

Concept	September 2014 - September 2015	October 2015 – August 2016	Total
Personnel	39,832	30,045	69,877
Supplies/Equipment*	6,281	45	6,326
Contractual**	3,507	40	3,547
Indirect charges	0	1,711	1,711
<b>Total (\$):</b>	49,620	31,841	81,461

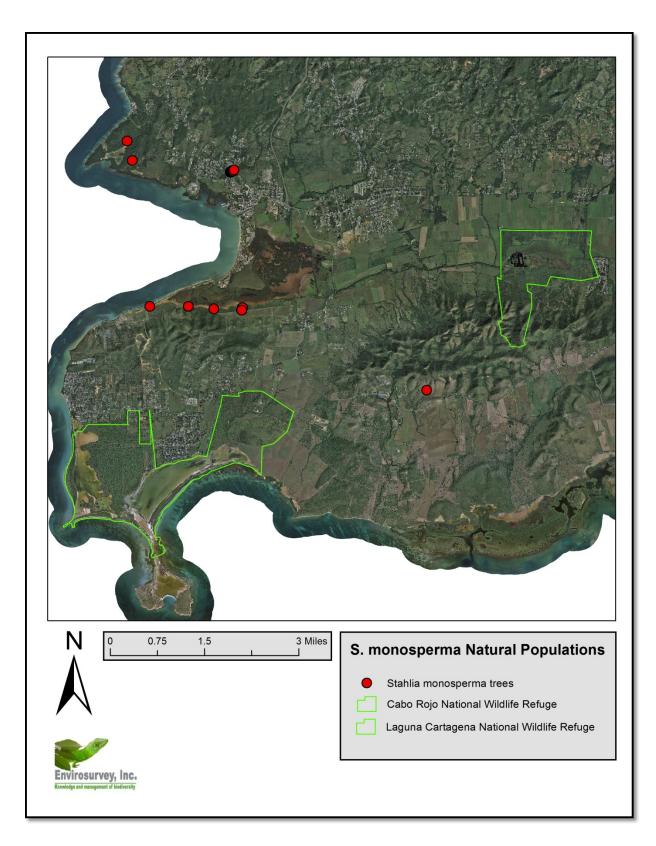
<sup>\*</sup>Includes: materials required for the installation of the fence and the irrigation system, and field equipment.
\*\* Includes issuing subcontracts for the fence and irrigation system, and for the development of GIS maps.



**Figure 1.** Cabo Rojo NWR and Cartagena Lagoon NWR (green), and target properties for the development of conservation agreements.



**Figure 2.** Source of seed material from planted individuals of *Eugenia woodburyana*.



**Figure 3.** Location of the known natural populations of *Stahlia monosperma* within the municipality of Cabo Rojo.

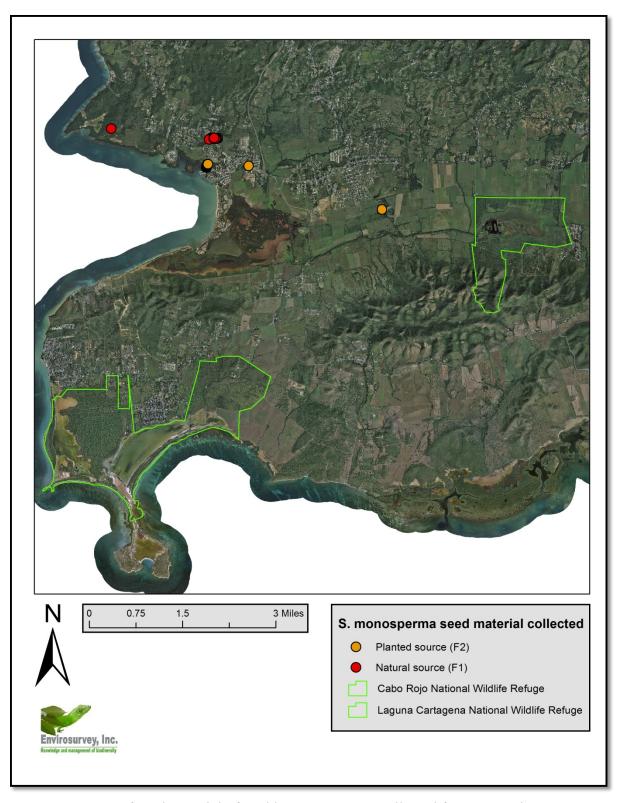


Figure 4. Source of seed material of *Stahlia monosperma* collected for propagation.

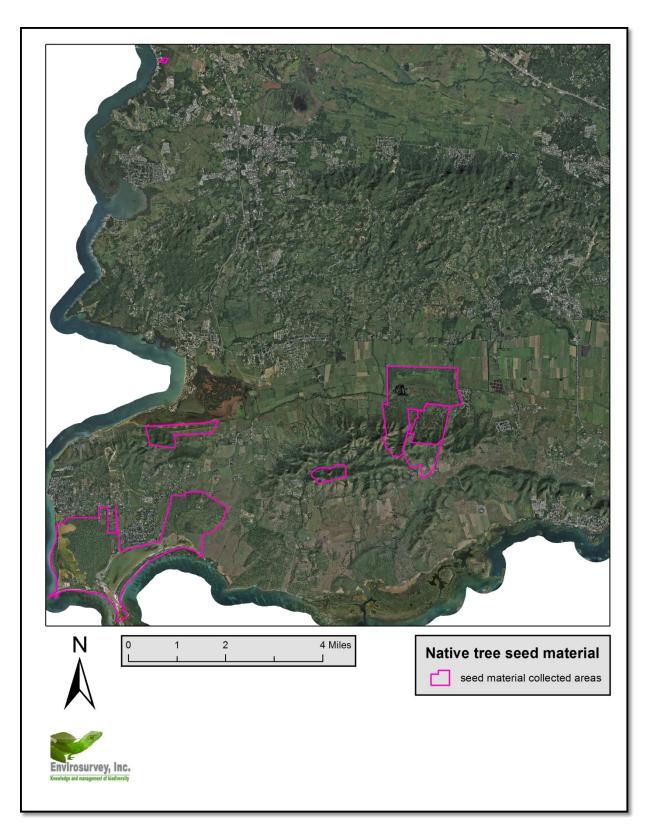


Figure 5. Seed collection areas of native tree species.



Eugenia woodburyana fruit



Eugenia woodburyana exposed embryo and seedling



Grain weevil in the larvae stage



Grain weevil in the pupal stage

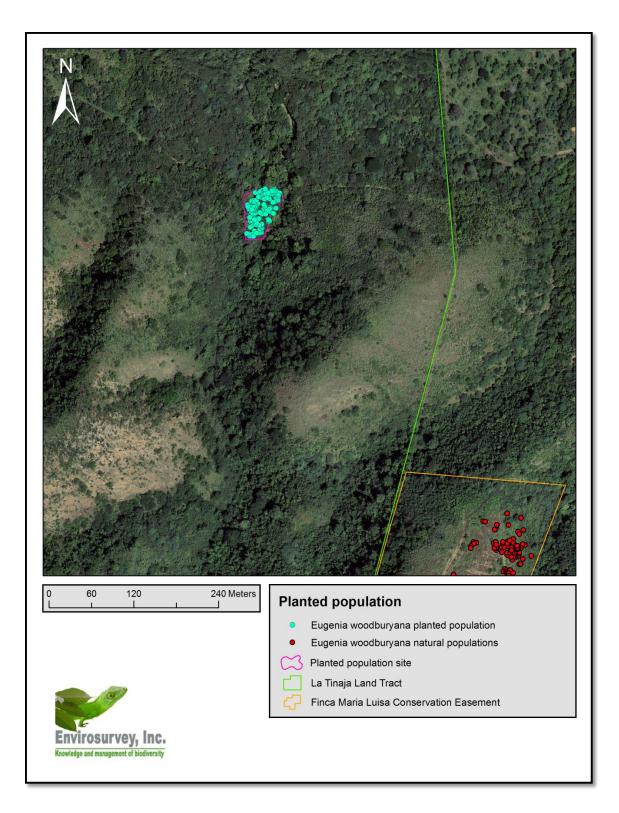


Sitophilus spp., Grain weevil adult

Figure 6. E. woodburyana seed and seedling, and stages of insect found affecting seed material.



**Figure 7.** Characteristic small seedling of *S. monosperma* few days after germination.



**Figure 8.** Reintroduction site of *E. woodburyana* at La Tinaja. The site is just at the base of the north facing slopes and adjacent to natural population of the species.



Figure 9. Planting areas before and after (from left to right).



Figure 10. Irrigation system on La Tinaja Tract planting site.



Feral horses on La Tinaja Tract, Lajas, Sierra Bermeja.



Figure 11. Fence construction on La Tinaja Tract in the first planting site.



Figure 11.1. Planted Eugenia woodburyana and evidence (fecal) of the feral horses.

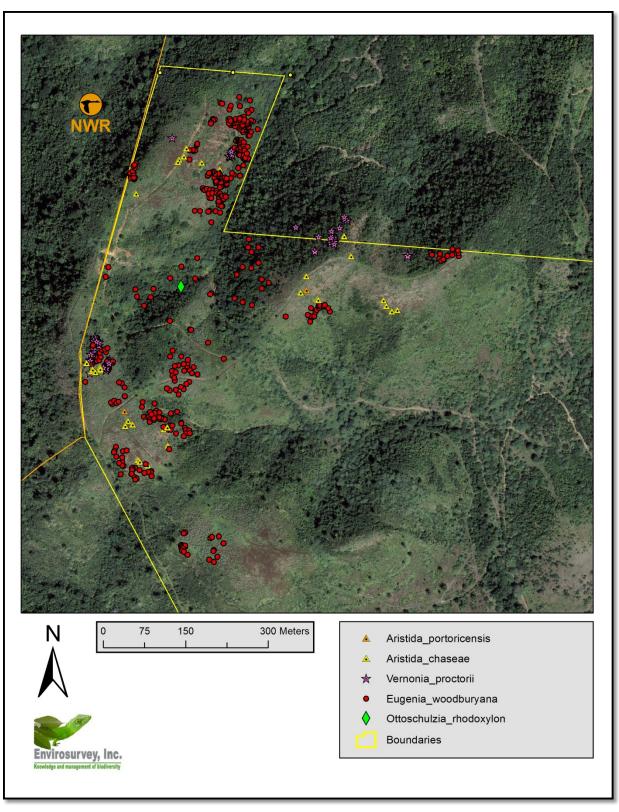
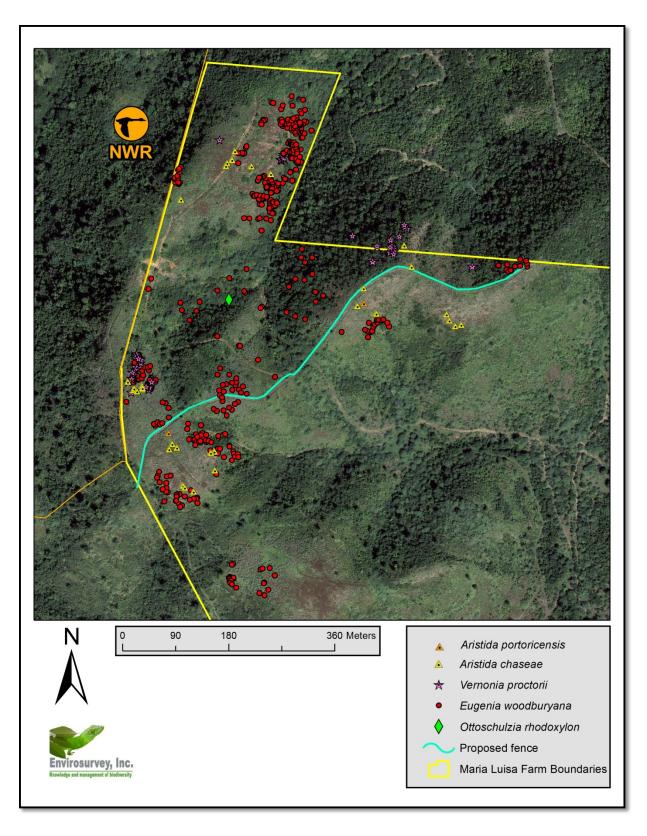


Figure 12. Natural populations of federally listed species at Maria Luisa Conservation Easement.



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roject Na	me	Sierra Ber	meja (CRI)										
ide Name		Finca Mar	ia Luisa o Fir	nca Escabi									
ocation		Carr. 303 I	Km 6.8 Lajas,	, Puerto Rico									
Tags #	Height	DBH		Phenology	Pests	Cut	Epiphytes	GPS/WP	Comments	LAT	LONG	#POWP	
	(m)	(cm)	cover (%)			evidence	evidence						
1	3.5	3.8	70	N/A	No	No	Yes	EW1	multiple stems 12, (lichens - tillandsias)	17.998034	-67.097878		
II .	-	5	-	-	ı	-	-	II .					
"	-	4	-	-	-	-	-	"					
"	-	3	-	-	ı	ı	-	ıı .					
II .	-	4.1	-	-	ı	-	-	II .					
"		2.5	-	-	-	-	-	"					
"		4.6	-	-	-	-	-	"					
"	-	3.8	-	-	-	1	-	"					
"		2.5	-	-	-	-	-	"					
"	_	2.5	-	-	-	-	-	"					
II .	-	3.4	-	-	-	-	-	ıı .					
II .	-	/	-	-	-	-	-	ıı .	dead				
2	4	5.7	70	dried fruits	termite	No	Yes	EW1	multiple stems 4 (1 stems $\downarrow$ 2.5cm DBH), vines	17.998034	-67.097878		
II .	-	3.9	-	-	-	-	-	ıı .					
II .	-	3.2	-	-	-	-	-	ıı .					
3	3	5.1	75	N/A	termite	No	Yes	EW1	multiple stems 3, (vines - tillandsias)	17.998034	-67.097878	3	
II.	-	3.6	-	-	-	•	-	"					
ıı.	-	/	-	-	-	-	-	"	dead				
4	4	5	60	N/A	termite	No	Yes	EW2	vines - tillandsias	17.998024	-67.097921		
"	-	2.5	-	-	-	-	-	"		·			
5	3	4.2	20	N/A	No	No	Yes	EW2	vines	17.998024	-67.097921	3	
6	2.5	3.7	80	N/A	No	No	Yes	EW3	multiple stems 5 (3 stems ↓2.5cm DBH), lichens - tillandsias	17.998041	-67.097885		
II	-	2.7	-	-	-	-	-	ıı ı					
7	2.6	3.7	25	N/A	termite	No	Yes	EW3	vines - tillandsias	17.998041	-67.097885	2	
"	-	3.8	-	-	-	-	-	"					
												8	Tota
ame of b	iologist o	collecting	data: José G.	Martinez, R	icardo A. A	lbarracín ai	nd Grace Ve	ega					
		17, 2015						_					

**Figure 13.** *E. woodburyana* with flag and identification number and example of data sheet for the assessment of natural populations.



**Figure 14.** Proposed fence to protect the core populations of the federally listed species at Maria Luisa Conservation Easement.







Eugenia woodburyana



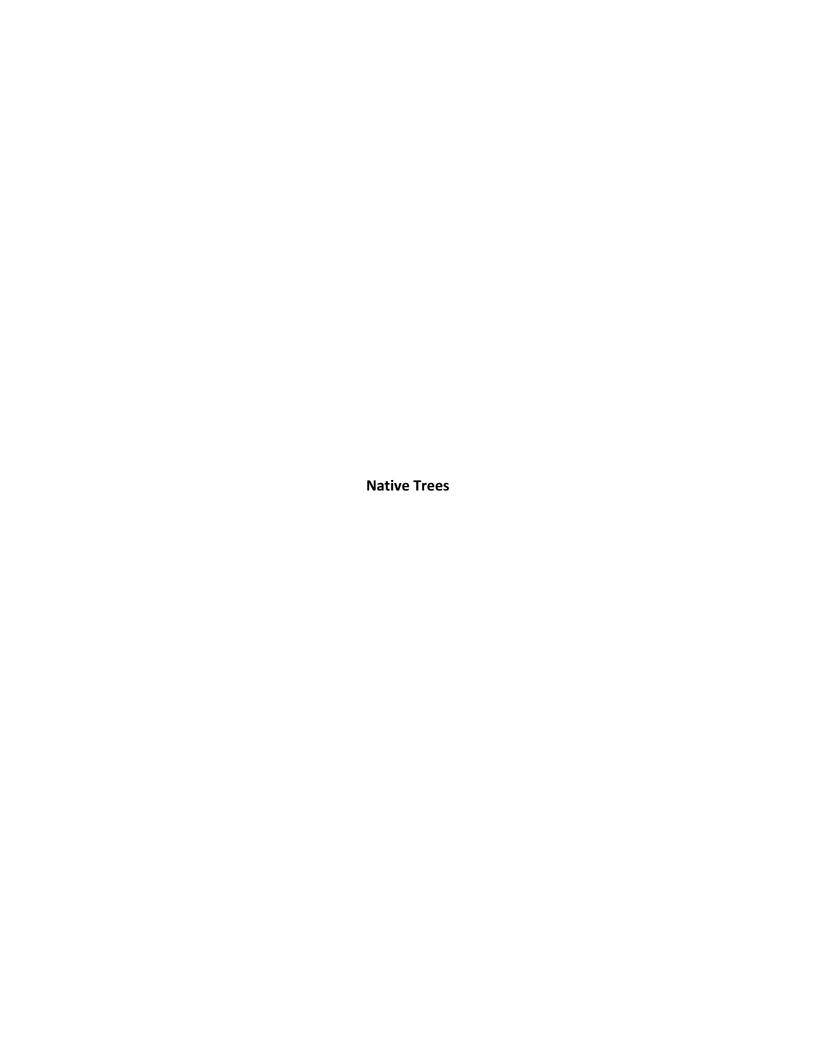
Eugenia woodburyana



Stahlia monosperma



Stahlia monosperma





Capparis baduca



Capparis flexuosa



Capparis indica



Coccoloba microstachya





Crescentia linerifolia



Erithroxylum brevipes



Ouratea litoralis



Eugenia ligustrina



Eugenia monticola





Eugenia sessiliflora



Eugenia spp.



Eugenia spp.



Reynosia uncinata



Reynosia uncinata



Sabal causiarum



Tabebuia heterophylla