Project number: 152511623
Target species: *Acacia belairioides*

Final Report
(Main results)

Introduction

Acacia belairioides Urb. (Fabaceae: Mimosoideae) is one of the strict endemics of the serpentine of Holguín, Cuba (Borhidi, 1996) at greater risk of extinction (Carmenate, 2013), where it grows as an emerging tree in xeromorphic thorny scrub. Currently holds the category of Critically Endangered species, based on its very restricted distribution (Gonzalez-Torres *et al*., 2016). Up to now the status of their populations was unknown, although Bisse (1988) considered it a very scarce species that needed conservation actions.

Among the main threats to the habitat of *Acacia belairioides* are inadequate forest management, direct cutting of adult individuals, fires and invasion by exotic species such as marabú (*Dricrostachys cinerea* (L.) Wrighr et Arn.) (Gómez *et al*., 2015). The recent delimitation of two protected areas (*Reserva Florística Manejada Ceja de Melone*s and *Reserva Natural Cerro Galano*), which include a large part of the range of distribution of *A. belairioides* is an important strength for the conservation of this species, so it is necessary to go deeper into The knowledge of the key features of their life history.

In this sense, the present project contributes to the conservation of this threatened tree by characterizing the habitat structure, determining the pattern of spatial arrangement of individuals, estimating population size and structure, the characterization of the seminal traits, the evaluation of the germination and determination of the effect of the seed predators on germination, as well as the characterization of seedling establishment microsites. This information was used as a starting point for the design and implementation of a species recovery plan in the protected areas where it grows, which included the training of technical personnel, the establishment of a nursery and the population reinforcement in one of the worked localities.

Size and population structure

The population structure and density of *Acacia belairioides* were determined in the localities of San Andrés, La Cejita-Los Ranchos, Ceja de Melones and Cerro Galano, belonging to Holguin's ultramafic nucleus. The area of patch occupancy was calculated in each locality, which once the density of individuals within the patches was known, served as a basis for estimating the population size. The individuals were grouped into seven height classes: <1 m (seedlings and juveniles); 1.1-2m (immature individuals); 2.1-3 m; 3.1-4 m; 4.1-5 m; 5.1-6 m and > 6 m (adult individuals).

Acacia belairioides is sensitive to the degradation of its habitat, because rarely it develops in the areas affected by the forestry activity or the fires. In the prospected

localities the population showed a displaced structure towards the seedling and juvenile classes. The proportion of seedlings (25.1%) was lower than that of juveniles (45.6%), which do not correspond strictly to the classical inverted *J* population structure. Although the population structure of Acacia belairioides was indicative of an expanding population, the contribution of the seedling and juvenile stages to the maintenance of the population size was low if one takes into account the abrupt decrease in the proportion of individuals of the following class of state (immature individuals). This class showed a decrease in abundance of 37.3%, suggesting a high mortality in the early stages of the species life cycle. The classes corresponding to adult individuals had similar proportions, ranging from 3.0 to 6.0%.

The individuals of *Acacia belairiodes* showed a pattern of aggregate spatial arrangement, forming patches of occupation with few adults and a great abundance of seedlings and juveniles around them. The average density of the patches was 0.160 individuals/m^2.

The Cerro Galano subpopulation reached the highest density values with 0.27 individuals/m^2. It occupied an area of 59 ha in a very preserved site. So far, 66 adults have been registered in this locality, although this number must be much higher given the high level of conservation of the site and the difficulties of access. The sub-population of La Cejita-Los Ranchos occupied an area of 5.6 ha and 53 adult individuals were inventoried. The density of this subpopulation was 0.056 individuals/m^2. The largest subpopulation was that of Melones, which occupied an area of 168 ha, with a density of 0.167 individuals/m^2. In this subpopulation 364 adults have been registered so far. For its part, San Andrés was the smallest subpopulation affected by anthropization, with an area of 0.05 ha, a density of 0.192 individuals/m^2 and only 7 adults.

Total population estimates that *Acacia belairioides* in Holguin's serpentine has an area of 19,370 presence has (193.7 km^2). The area of occupation of the subpopulations studied was 232.65 ha (2.32 km^2), with an effective size of 490 adult individuals, for a density of 2.1 adults/ha. Of the estimated area of occupancy, 99.5% is within the boundaries of protected areas *Reserva Natural Cerro Galano* (25.3%) and *Reserva Florística Manejada Ceja de Melones* (74.6%).

Regeneration Microsites

The regeneration sites of *Acacia belairioides* were characterized in 92 points at intervals of 1 m, along 8 transects centered on the same number of adult individuals of the species and oriented in the direction of the slope. It was considered as a busy microsite when a seedling or juvenile of *A. belairioides* was found within a radius of 20 cm from the selected point. At each point, the following variables were estimated: distance to the nearest adult, distance to nearest co-specific seedling, distance to nearest grass, distance to nearest shrub, shrub cover, herbaceous cover, slope direction, inclination and pH soil. When no seedlings were found at the point, it was considered as an unoccupied microsite and the same variables were measured. A multiple regression was used to determine whether the

variables evaluated were associated with the presence or absence of *A. belairioides* seedlings.

From the 46% of the evaluated microsites were occupied by seedlings of *Acacia belairioides*. Recruitment to the breeding adults was very high and the abundance of seedlings decreased as the distance to the parental tree increased. Sixty-four percent of the seedlings were located less than three meters from the nearest adult and no more than 7 m of the parents were observed. The average distance of a seedling of *A. belairioides* to its nearest coespecific was 34.47 cm, to the nearest grass, 10.02 cm and to the nearest shrub 50.2 cm. In all cases, the values obtained were very variable (CV> 80%). There were no significant differences in shrub cover ($t = -1.19$, $p = 0.23$), herbaceous cover ($t = 1.11$, $p = 0.90$) and soil rockiness ($t = 1.05$, $p = 0.29$) among occupied and unoccupied microsites. The distance to the nearest adult was significantly lower in the occupied microsites ($t = -2.82$, $p = 0.005$) than in the unoccupied ones. The slope slope ($t = 1.99$, $p = 0.04$) was higher and the soil pH was slightly more basic ($t = 3.51$, $p = 0.0007$) in the occupied and unoccupied microsites. The presence/absence of *A. belairioides* seedlings was significantly associated with the interaction between the distance to the nearest coexistent tree, slope inclination, rock cover and soil pH ($R^2 = 0.23$, $F = 7.91$, $p = 0.00$).

From these results it is inferred that microsites close to adult individuals, with high rockiness, soft slopes (5-132º) and an average pH of 5.9, favor the in situ regeneration of *Acacia belairioides*. Regeneration of the species is likely to be strongly limited by seed dispersal, which would explain why no seedlings were found more than 7 m from potential parents. Although no differences were found in the shrub and herbaceous cover between occupied and unoccupied microsites, it is possible that the progeny survival is higher in vegetation covered sites, given the extreme conditions of the clearings in the Holguin serpentine thickets (Gómez e*t al*., 2013).

Seed predation by bruchids

Two species of bruchids (*Stator bottimeri* and *Sennius fallax*) were identified as predators of the seeds of *Acacia belairioides*. To determine the possible influence of these beetles on the regeneration of *Acacia*, the effect of bruchid attack on physical dormancy and germination was evaluated. The infestation was 32.5% of the seeds in the soil, with two types of damage: penetration hole of the larva and adult emergency operculum. The size of the seed was not related to the number of infested seeds, nor to the initial moisture content or the distribution of biomass to the seed coat. Seeds damaged with a pit in the seminal covering (adult emergency operculum) did not germinate. In contrast, seeds with a penetration hole of the larva achieved a final germination of 19.3%, which was superior to the control treatment or intact seeds (8.4%), but less than 93.9% obtained for intact seeds subjected to acid scarification (Sanchez *et al*., 2017).

These results suggest that under natural conditions, bruchids infestation could accelerate the germination of *Acacia belairioides* seeds, to break the physical

dormancy. This could be beneficial as long as the level of damage does not compromise the viability of the seed, the infestation occur in a period where there is high soil moisture and the seed has been dispersed in a suitable microsites. Seed collection in the field for *in situ* and *ex situ* conservation actions should be performed taking into account signs of bruchids infestation. In order to guarantee successful results, the collection of infested seeds should be avoided, since their germination was low with respect to intact seeds subjected to artificial scarification.

Dormancy and germination requirements

The seeds of Acacia belairioides presented physical dormancy. This seed trait was tested by imbibition tests, and a pregermination treatment with concentrated sulfuric acid, after which 95% final germination was obtained, which was well above 8% germination of the intact seeds. Germination occurred both in light and in the dark, so the seeds were considered indifferent photoblastism, therefore, they can germinate on and below the soil surface. The optimal range of germination varied in fluctuating temperatures from 25/30°C to 25/35 °C, and at 25 ° C as constant temperature. On the other hand, the alternating temperature range of 25/40°C was sublethal for germination and the range of 25/45 ° C was lethal. These last ranges of temperatures could be scenarios that find the seeds once deforestation actions take place, or induced by the Global Climatic Change (IPCC, 2014).

Conservation Actions

The results of the population study did not modify the threat category of Critically Endangered *Acacia belairioides* (González-Torres *et al*., 2016), but the criteria for its allocation. From the information collected, the criteria would be as follows: CR; B2ab (ii, iii, iv). According to the present study, the presence extension (Criterion B1) and the population size (Criterion C) used by González-Torres *et al*. (2016) were higher than those established by the IUCN methodology (2001) for the Critically Endangered category. However, the reduced area of occupation of the population is an element of high vulnerability to the occurrence of fortuitous events that justify keeping the species in the category of maximum threat.

According to the results of the research on population structure, germination, seed predation and regeneration microsites, a species recovery plan was designed and implemented at sites most affected by anthropization within protected areas. Although San Andrés is the smallest subpopulation and affected by habitat degradation, no reinforcement actions were carried out in this subpopulation because it is outside of protected areas and any action taken would not be sustainable over time. The location of La Cejita-Los Ranchos was selected because it is part of the *Reserva Florística Maneja Cejas de Melones* and because in this subpopulation limitations were detected in natural regeneration.

The conservation strategy designed included *ex situ* reproduction in nursery conditions and population reinforcement. From the results of research on germination and dormancy, a protocol of propagation was developed for the multiplication of *Acacia belairioides* under nursery conditions, directed to the technical personnel of the protected areas where the species grows and the

National Botanical Gardens Network, which will contribute to *in situ* and *ex situ* conservation respectively. Other complementary actions of the bread included the training of the technical personnel of the protected areas and the environmental education with the communities that coexist with the species, for which a foldable (printing 200) and an informative poster (two) were designed. The long-term objective of the recovery plan is to ensure that the proposed actions are part of the management plan for protected areas where the species grows, to ensure the sustainability of actions over time.

The main conservation action of the plan was the population reinforcement carried out in the town of La Cejita-Los Ranchos. For this, about 1000 seeds of the species were collected in June 2016. Healthy seeds were selected, without signs of infestation by bruchids, which were subjected to an acid scarification process to eliminate physical dormancy and germinated at the optimal temperature of 25/30 °C. The final germination percentage of the lot was 93.9%. Transplanting of 250 seedlings to polyethylene bags with substrate of the locality where the plantation was carried out was carried out.

The nursery was established in the Botanical Garden of Holguín, to 18 km to the W of the locality La Cejita-Los Ranchos. The bags were placed in semi-shade and under an irrigation regime on alternate days, which was progressively reduced to a weekly frequency to achieve the acclimatization of the seedlings to the water conditions of their natural habitats. The new individuals were kept in nursery conditions for a year, until the next rainy season. The survival, height and number of branches of each individual were monitored. At one year of establishing the nursery, the survival of the cohort was 60% and the average height of the individuals was 26.8 cm. All individuals experienced a change in the state of the seedling phase to juvenile, evident by the appearance of horizontal branches, with an average of 4 branches per individual.

The population reinforcement was carried out in June of 2017 (locality of La Cejita-Los Ranchos), when 150 new individuals were planted. Planting sites were chosen according to the results obtained in the research on the regeneration microsites of the species. Also seedlings or juveniles of *Acacia* were given to the National Botanical Garden of Cuba (Havana) and to the Flora and Fuana Conservation Center "Quinta de los Molinos" (Havana). At the moment, financing is being managed to continue the monitoring of the plantation for at least one year and to develop new actions of population reinforcement.

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