

Guaiacum unijugum

Propagation Manual

Threatened flora of Baja California Sur, México



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Introduction

The genus *Guaiaacum* consists of 4-8 species and is commonly termed “lignum vitae” (Dertien & Duvall, 2014). The genus is well recognized throughout the Caribbean and Central America due to its beauty, medicinal properties, and unique trait as one of the world’s densest hardwoods. Two species in the genus, *G. sanctum* and *G. officinale* have been historically utilized for their hardwood (which is resistant to rot and sinks in water) as well as for medicinal purposes such as treating rheumatism and syphilis (Francis, 1993).

Although other members of the genus are more widely distributed and occur within several protected areas in Mexico, *G. unijugum* has a very limited distribution that does not overlap any protected sites (Lopez-Toledo et al. 2011; McCauley et al. 2010). *Guaiaacum unijugum* is thought to have been historically uncommon since it was first collected and misidentified as *G. sanctum* (Brandeggee, 1891). Later, Brandeggee identified it as a separate species and described it as rare and endemic to Baja California Sur, Mexico (Brandeggee, 1915). Last assessed by the IUCN Red List in 2018, *G. unijugum* is Critically Endangered under criteria C2a(i); D. The genus *Guaiaacum* is internationally protected by CITES Appendix II; however, there are no established species-specific protection policies for *G. unijugum*.

During the course of the study, a species survey was conducted, over 2,000 seeds were collected, a successful germination trial was developed, and almost 600 seedlings of *G. unijugum* were propagated. An additional population was also documented outside of the protected area in Cabo Pulmo National Marine Park, over 6 km north of the previously northernmost population in Bahia los Frailes. Although further research is needed to understand the gaps in successful conservation of *G. unijugum* (i.e. restoration and long term survival studies), it is important that the genetic material of the known remaining individuals is safeguarded from extinction. The objective of this protocol is to provide readers with recommendations in order to successfully propagate this Critically Endangered species.

Physiology and Phenology

The flowering period of *G. unijugum* is expected around May - June, and the flowers are a light blue-purple (Figure 1). The fruits are small green capsules when they are not ready for collection. When the fruits mature, they are yellow (Figure 2). They open and expose 1-3 (rarely up to 5) seeds covered by red arils (Figures 3 and 4). You can expect the fruits to ripen between August and September.



Figure 1: *Guaiacum unijugum* flower. Photo by Diego Alejandro Gomez Hoyos.

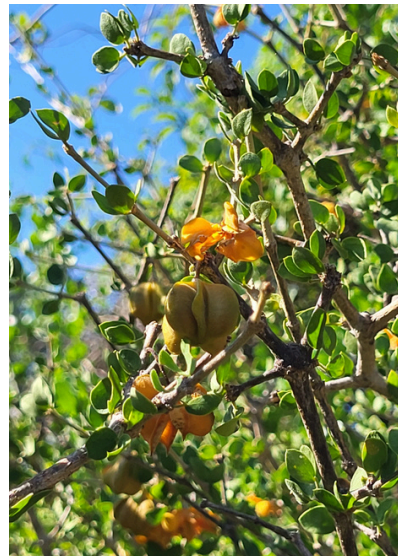


Figure 2: Mature fruit of *Guaiacum unijugum*.



Figure 3: *Guaiacum unijugum* seeds.



Figure 4: *Guaiacum unijugum* seeds.

Seed Collection

Materials

- Wire
- Wire clippers
- Tags
- GPS
- Paper or cloth bags
- Markers or pens for labeling

Prior to collecting any fruits or seeds, every individual should be tagged and geolocated in order to track maternal lineage. The wood of *G. unijugum* is dense, and nails could not permeate the wood, so individuals were tagged using wire (Figure 5). Before placing a new tag on any individual, verify that it has not been previously tagged. Label the paper or cloth bags with the date of collection, location, and the mother tree tag ID to match the corresponding individual you will collect from (Figure 6). Avoid non-permeable containers such as plastic as they maintain a high humidity which may cause fungus to spread. This may negatively affect seed viability.



Figure 5: Tagged mother tree.

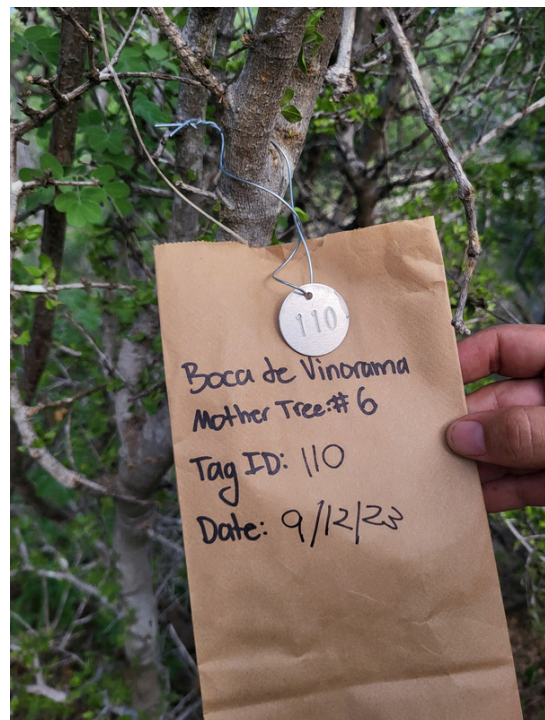


Figure 6: Labeled seed collection bag.

According to the Center for Plant Conservation Best Plant Conservation Practices (2019), it is advised not to collect “more than 10% of an individual or population seed production in one season and no more than 10 out of 90 years.” This is especially important because natural regeneration of *G. unijugum* has been observed in the wild, and the survival of wild individuals has not been studied. When collecting fruits and seeds, be careful not to step on the seedlings.

Pick the whole fruits off of the branches when they are yellow and have opened up to expose the seeds covered in their red arils. You may collect fruits when they are yellow but have not yet fully opened. Verify that the arils have already turned red. If the fruits are still closed, you can check if the arils are red by splitting one of the fruits open. If the arils are not red, wait until they have ripened prior to harvesting. While in the field, set the paper or cloth bags in the shade and avoid exposing them to direct sunlight. Exposure to direct sunlight will dry them faster and may affect their viability.

It is recommended to wait until the capsules are found open and the red arils of the mature seeds are exposed, as seen in Figure 3. When the fruits are mature, the seeds hang and can be gently plucked off. However, if it is not possible to wait, it is acceptable to collect fruits if they look like those in Figure 2. The fruits in Figure 2 can be harvested because they are yellow and when they are cut open, the arils are red. To test for maturity, collect a couple fruits and cut them open in the field. If the arils inside are not yet bright red, wait until they are ready. If they are confirmed to be red, you may proceed with collection. Collecting fruits when they are slightly premature will result in a more tedious seed cleaning process because it is more difficult to cut open the fruits when they are still closed.

Seed Cleaning

After collecting the fruits, separate them from the seeds (Figures 7a and 7b). This is best done within two days of collection when the fruits are still fresh and soft because the seeds easily come off of the capsules. If the fruits are allowed to dry, they become tough and difficult to separate. Once the fruits have been separated, clean the seeds and prepare them for storage. Clean the seeds by removing the red fleshy aril from the dark brown seed (Figure 7c). The aril usually slips away from the seed. We processed all of the seeds by removing the red fleshy aril as previous studies (Alexander, 1966) and personal observations (Schultz, pers. comm. 2023) of germination in *G. sanctum* have resulted in significantly higher germination rates when the aril is removed. Once the seeds are clean, they can be stored in paper bags at ambient room temperature for up to four months as long as they are kept dry. More studies are needed in order to better understand ideal seed storage conditions and seed viability.

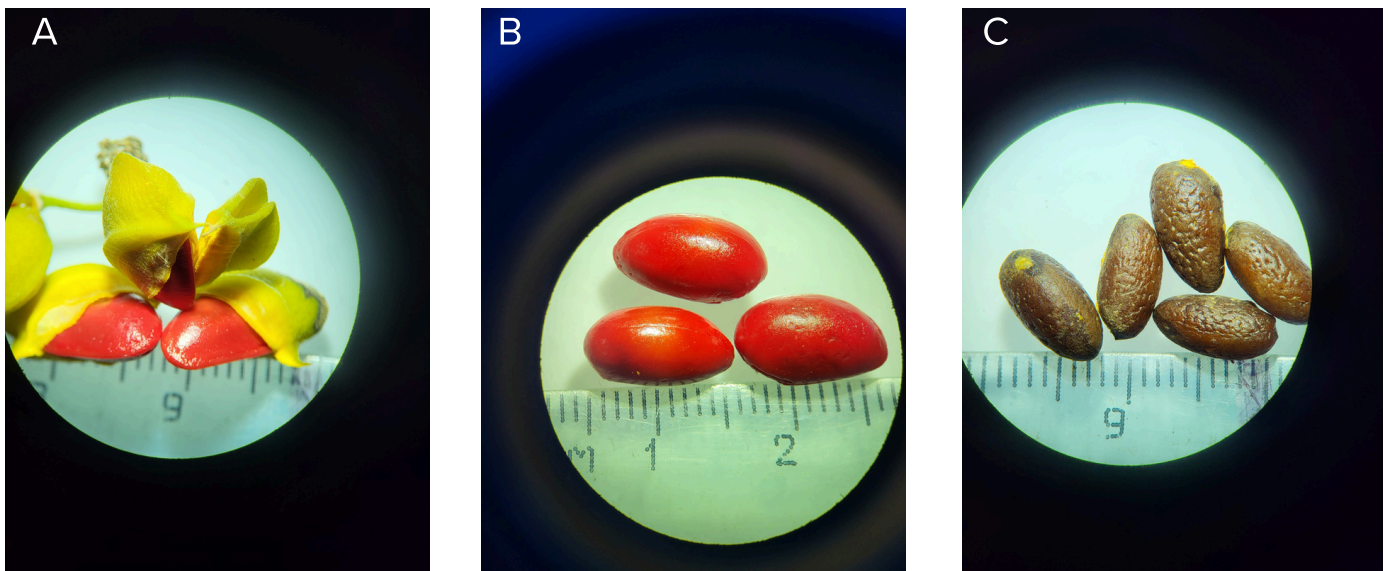


Figure 7: *Guaiacum unijugum* seeds under a microscope demonstrating a) ripe fruit exposing the seeds covered by red arils, b) seeds after they have been separated from the fruit with intact aril, and c) seeds after they have been cleaned and are ready for storage or germination.

Germination

Materials

- Markers or pens for labeling
- Paper towels
- Containers with lids or resealable plastic bags
- Paper envelopes
- Gibberellic Acid
 - To produce a 200 ppm solution, we mixed 1.0 g of BioGIB (Figure 8) into 500 mL of water. In case you access a different product, follow this rule: 1 ppm = 1 mg pure solute/ 1000 mL water



Figure 8: BioGIB
Gibberellic Acid

Seed treatments (recommended):

Germination trials conducted by V. Garcia Balderas indicate that seeds of *G. unijugum* achieve the highest germination rates when either exposed to 200 ppm Gibberellic Acid (GA) for 24 hours or stored for 30 days prior to sowing, with no significant difference in germination between the two treatments. Combining both treatments does not improve germination beyond what either produces alone. Therefore, we recommend applying only one treatment based on scheduling needs: storing seeds for 30 days when time allows, or treating with 200 ppm GA for 24 hours when seeds must be sown sooner. Both treatments are described below.

Storage (30 days)

Label a paper envelope and a resealable plastic container with the date of collection, location, and mother tree ID. Store cleaned seeds in labeled paper envelope for 30 days at ambient room temperature (Figure 9). After 30 days, soak the seeds for 24 hours by placing them in paper towels that have been moistened with pure water and placing the seeds and paper towels in the labeled plastic container. Replace the paper towels after 24 hours to prevent growth of fungi. After an additional 24 hours (48 hours total), the seeds are ready to be sown.

No storage, 200 ppm GA

After seed cleaning (no storage), label each resealable plastic container with the collection date, location, and mother tree ID. Soak the seeds for 24 hours by placing them in paper towels that have been moistened with pure water. After 24 hours, replace the paper towels with new paper towels that have been soaked in **200 ppm GA** (Figure 10). After an additional 24 hours (48 hours total), the seeds are ready to be sown.

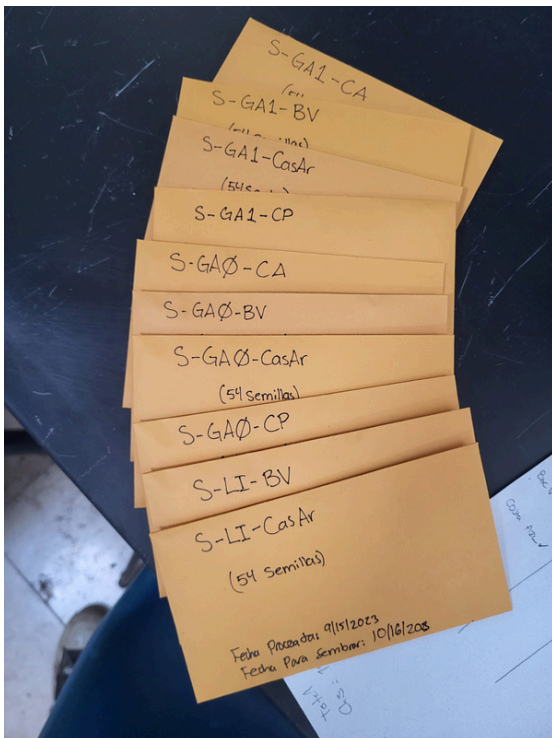


Figure 9: Labeled paper envelope for 30 day seed storage.

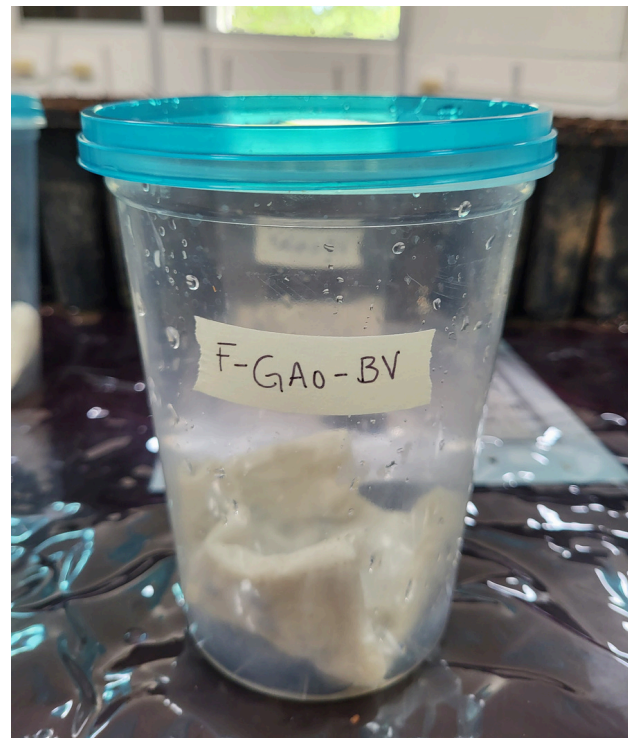


Figure 10: Labeled plastic container with seeds soaked in 200 ppm gibberellic acid.

Sowing the seeds and preparing the nursery

Materials

- 54 cell seedling tray (64 cm L x 30 cm W x 14 cm H)
 - Each cell is 5 cm L x 5 cm W x 14 cm H
- Substrate mixture
 - ProMix FLX and Vigoro Professional Soil Mix (1:1 ratio). This soil mix is generic for germinating seeds and is not specific to a habitat.
 - Media contents for ProMix FLX:
 - Perlite: 25-35%
 - Peat Moss: 65-75%
 - Media contents for Vigoro Professional Soil Mix:
 - Peat Moss
 - Coco coir
 - Pine bark compost
 - Zeolite
 - Perlite
 - Fertilizer: 350 g of NPK 25-5-15 per cubic meter
- Shovel (1-2)
- Plastic tarp (optional)
- Shade house

Seedlings should be started in trays that accommodate a long taproot. *Guaiacum unijugum* seedlings develop deep roots, which may be an adaptation to the limited water availability in their natural habitat.

While the seeds soak, prepare the substrate mixture. If preparing a large volume of substrate mixture, place a plastic tarp on the ground and then mix the substrate evenly by using a shovel. Once the substrate is evenly mixed, fill the pots 90% full without compacting the soil and plant one seed per cell at about 1 cm deep (Figure 11a). Cover loosely with substrate (Figure 11b). For the first five weeks, seedling trays were kept indoors where they had access to filtered sunlight and kept the soil consistently moist during the entire experiment by watering every couple of days. The seeds are quite small, so it is important to maintain a moist soil mixture as allowing the substrate to dry may decrease the viability of the seeds after they have been sown.



Figure 11: Prepared seedling trays before (a) and after (b) covering with substrate.

Nursery conditions

Seed trays were kept indoors in bright indirect light at ambient room temperature, and watered every 2-4 days. After five weeks, the seedling trays were moved to a nursery outside where they had access to filtered sunlight and part shade from a nearby tree. In order to expose seedlings to homogenous conditions, the trays were rotated and moved randomly in the nursery every week.

Seedlings germinate approximately one month after sowing, and grow their first set of true leaves soon thereafter (Figure 12).



Figure 12: Seedlings after one month of sowing. Shortly after they grow their first set of true leaves, they are taken outside to the nursery.

Seedling Care and Transplanting

Seedlings were transplanted into larger individual pots (25 cm X 12 cm X 12 cm) between three and four months after germinating. Although the seedlings had only grown about 1-2 cm in height, root growth had been extensive. After transplanting, seedlings were watered every 3-4 days on average, keeping the soil moist but not saturated. The watering schedule was adjusted based on how quickly the the soil was drying up.

Two different soil mixtures were prepared based on the availability of resources at the nursery:

- **Soil mixture 1**

- Soil mix of ProMix FLX, Vigoro Professional Soil Mix, Perlite, and Vermiculite (1:1:1:1 ratio). This substrate mix is generic for germinating seeds and is not specific to a habitat.

- **Soil Mixture 2**

- Soil mix of ProMix FLX, Vigoro Professional Soil Mix, Perlite, Vermiculite, and soil from Sierra La Laguna (locally sourced by the nursery; 1:1:1:1:1 ratio). This soil mix is generic for germinating seeds and is not specific to a habitat, but this soil mixture has a higher silt content as it includes soil from the region. We did not measure the effect of soil substrate on seedling growth.

Once the substrate mixture was prepared, pots were filled about two thirds of the way with the substrate. It is important to place seedlings at the exact soil level at which they had been growing prior to transplanting. Burying seedlings too deep may cause the stems to rot over time.

Seedlings were transplanted in the shade of trees to prevent the direct heat of the sun from stressing the young roots (Figure 13). Transplanted seedlings were kept in the shade and watered deeply to ensure all of the roots were soaked. By evening, they were moved to the nursery under shade cloths.



Figure 13: Transplanting and verifying that each *G. unijugum* individual is properly tagged.

Seedling Care

Seedlings were watered early in the morning or late in the evening when they were not exposed to the sun so that their leaves did not burn. In order to keep the substrate moist, a deep watering schedule was followed every 2-3 days depending on the weather and nursery conditions, allowing the top 3 cm of the soil to dry out slightly between waterings so that the seedlings were not waterlogged.

Guaiaacum unijugum seedlings are very slow growing (Figure 14). Seedlings reach approximately 35 cm in height after three years, and are ready to be transplanted into the ground.



Figure 14: *Guaiaacum unijugum* seedlings five months after transplanting. By this time, many of the seedlings have developed a second and third set of true leaves.

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