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Controlled Breeding of *Coccoloba cowellii* Britton (Polygonaceae), a Threatened Endemic Species

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Abstract

Context: *Coccoloba cowellii* Britton is an endemic plant to Camagüey province, Cuba, which has been reported in critical danger of extinction, though phytochemical studies have revealed the presence of promising compounds with antioxidant activity. Hence, the need for standardizing the potential economic exploitation of the species through preservation has encouraged scholars to study the features of the seeds and germination.
Aim: To assess *C. cowelli* seed dormancy, and evaluate their germination under controlled conditions.
Methods: Anatomical and physiological evaluation of *C. cowellii* seed dormancy. A standard factorial experiment was designed to evaluate the germination capacity under controlled conditions, consisting of three factors, one with two levels, and the other with three levels. All the possible combinations were evaluated, totaling 18 experimental units.
Results: The presence of non-dehydration sensitive *C. cowellii* seeds was checked, along with the absence of factors that can delay their physiological maturity. There were differences in terms of germinative efficiency resulting from scarification as pre-germinative treatments, rather than by inhibition. A significant decrease was observed 180 days following the harvest, while the best results were achieved with the utilization of substrate collected from the natural habitat.
Conclusions: *C. cowellii* seeds can be dehydrated and stored at room temperature to preserve embryo viability of at least 50% of them. The feasibility of germination under controlled conditions favored the implementation of *ex-situ* controlled steps, the generation of plant material for further research, and the existence of a potential for sustainable production.

Keywords: *Cuban flora, threatened species, ex-situ handling, conservation, germination.*

Introduction

One of the Goals for sustainable development of the 2030 Agenda is, Adopting urgent and significant measures to reduce natural habitat degradation, stop the loss of biodiversity, protect threatened species, and prevent their extinction (the United Nations, 2018). These priorities are included in the regulatory documents issued by the Ministry of Science, Technology, and the Environment, in Cuba (CITMA, 2021).

This study offers the possibility of dealing with the species *Coccoloba cowellii* Britton (Polygonaceae), which has exclusive endemism to the province of Camagüey, Cuba, on ultramafic soil ecosystems (Méndez et al., 1988; Méndez et al., 2005; Martínez & Reyes, 2015).

C. cowellii is a 2-3 m high shrub with few branches, with racemiform, multi-flower, conspicuous red inflorescences. The flowers are anatomically perfect, but unisexually in dioic plants. The fruit is achene, ovoid, glabrous (6 x 3 mm), with an acute apex. The pericarp is covered by a thickened hypanthium, with tepals on the top, which turns from red to violet-black when ripened. The seeds have a ruminate perisperm and endosperm (Castañeda, 2014).

C. cowellii is threatened with extinction, and it has been reported under the Critical Danger category (CD), with a population spreading under 10 Km^2, and severely fragmented due to anthropic constructions, such as settlements, road grids in different directions, and power lines. Consequently, the number of specimens, locations, and subpopulations has been reduced continuously (González et al., 2016).

Phytochemically, this is a promising species. The ethanolic extract from the leaves has demonstrated a high antioxidant activity (Méndez, 2019) due to the high phenolic compound contents, especially condensed tannins, and flavonoids (Méndez et al. 2019).

The need for standardizing the potential economic exploitation of the species through preservation has encouraged scholars to study the plant breeding features. Accordingly, this paper pursues the following: To assess *C. cowelli* seed dormancy and evaluate their germination under controlled conditions.

Materials and Methods

A research field was established to collect the propagation material. It was located northeast of the community of Albaisa, on the following coordinates: 21.434000. -77.834695. It was selected for the high conservation level of the original vegetation, proper *C. cowellii* structure, the high presence of adult individuals, the proximity to research facilities, and the access to the area.

The mother plants with sexually female behavior were marked for collection. The fruit (achene) was harvested when the color of hypanthium and tepals on the top turned violet-black. To prevent previous falls, the inflorescence was covered with a Marquisette fabric bag.

The anatomical and physiological evidence was studied to evaluate seed dormancy, according to Baskin & Baskin (2014). At first, germination was stimulated directly in the fruit, with no previous processing, starting on the day of harvest. The embryo viability was studied as well when the propagation material collected in the field was dehydrated and stored.

The fruits were placed on absorbent paper for drying, at room temperature indoors. For preservation and storage, the propagation material was kept in a closed room, at room temperature, as described by Vargas-Simón & Pire (2010), using *Coccoloba uvifera* L.

The germination of the propagation material was tested after drying, at room temperature in the shade, using two previous treatments: imbibition and scarification.

All the propagation material was dampened in Petri dishes, containing 10 ml distilled water, in an RTOP 310 chamber, at 28 ºC, constant temperature. The method described by Díaz & Molinelli (2018) was used to determine the amount of water that seeds can absorb, and it was calculated based on the weight throughout the process with an analytical balance (Sartorius, BSA124S). The weight readings were performed at 0.5, 1, 2, 4, 8, 12, 18, and 24 hours following the start of imbibition. The seeds were drained and dried on absorbent paper for 10 minutes, before each weighing. An inhibition curve was constructed with the data collected.

Concerning the second pre-germinative treatment, three groups were set up. In one group, the seeds were totally scarified; in another, mid-scarification was made; and in the other, the seeds were not scarified.

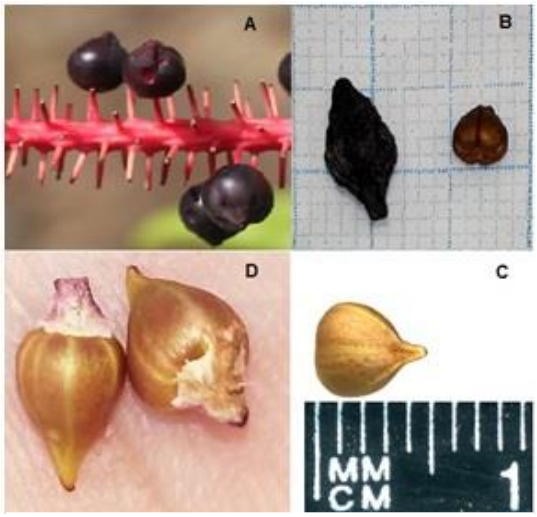

Fig. 1. Different scarification levels: A) the Fruit with thickened hypanthium, and the fleshy tepals of the mother plant. B) Right: the fruit with thickened hypanthium and flechy tepals after six months of storage. Left: totally scarified seed. C) The fruit without the thickened hypanthium and fleshy tepals (mid scarification), measured in mm. D) The fruit without the thickened hypanthium and fleshy tepals (mid scarification).

Scarification (Figure 1) was done manually, using a scalpel carefully not to damage the embryo. The three levels established contrasted as follows:

-Without scarification: All the structures of the propagation material were conserved.

-Mid-scarification: The thickened hypanthium was removed (including the apex-fixed tepals), but the fruit (achene) was left intact with the seed.

-Total scarification: The thickened hypanthium was removed (including the apex-fixed tepals) and the pericarp (achene) to expose the seed.

The tests included the fruit (regardless of the scarification level), recently harvested (less than 30 days of collection), and dehydrated and stored in paper bags kept in a room, at room temperature, for six months.

The germination study was done as described by Soledad (2018) and Alonso (2018). A standard factorial experiment was designed, consisting of three factors, one with two levels, and the other with three. All the possible combinations were evaluated, totaling 18 experimental units.

The factors were,

1). Fruit storing periods (dry in the shade, kept in paper bags at room temperature); two levels: fresh fruit (less than 30 days of harvesting), and stored fruit (for six months).

2). Propagation material scarification levels; three levels: no scarification, mid-scarification, and total scarification.

3). Types of substrate used for germination; three levels: river sand, soil from the natural habitat, and wormcast.

The propagation material of substrates was not sterilized.

Overall, 450 seeds were set to germinate, at a rate of 25 per experimental unit. All the tests were made in an RTOP 310 germination chamber. Two 12h photoperiods (lighting and dark) were used, while the relative humidity was constant (95%). With the lights on, the temperature was 30 ºC, whereas, in the dark, it was 25 ºC. The germination indicator was the emergence of the plumula.

The statistical analysis of the germination percent relied on an analysis of variance, multiple rank-sum test, mean comparison test, mean discrimination by LSD (Fischer), and regression coefficient. The statistical data processing was performed using SPSS 23.

Results and Discussion

General considerations on *C. cowellii* seeds

A well-developed embryo was corroborated through anatomical evidence shown by the *C. cowellii* seeds, along with the imbibition capacity right after harvesting and emergence, both in the radicle and the whole plant, after a few days. Massive germination was completed directly in the fruit, with no previous processing, starting the day of harvest, which permitted verifying the absence of factors that delay physiological maturity.

In harsh natural conditions, the species has easily adjusted because of its quick germination, faster than other plants. Similar reports have been studied (Chávez, 2017). However, it differs from other results that have proven the presence of physiological dormancy in other species of this genus, such as *C. diversifolia* Jacq. and *C. uvifera* (Sánchez, et al., 2019).

Nevertheless, the presence of primary dormancy; that is, one caused by the existence of a hard protecting surface (achenes) that hinders water and oxygen entry, and activates the embryo, creating favorable conditions for handling the seeds.

However, the typical behavior of recalcitrant seeds cannot be established. On the contrary, the humidity was reduced to lower than previous levels when harvesting (without compromising the embryo's viability), store the seeds at room temperature for six months, and keep their germination capacity of, at least, 50%.

The results demonstrated that the seeds can be preserved at room temperature, to delay and adjust sowing to the *ex-situ* handling needs. Nonetheless, at six months of storage under the conditions described, the germinative potency decreased by 50%. Hence, further studies will have to evaluate the response of the propagation material to controlled desiccation and storage at low temperatures, more accurately, to raise the levels of conservation efficiency.

Seed imbibition

Imbibition was used as a previous treatment for germination (regardless) of the scarification level, both in the fresh fruit and the stored fruit (six months). The results are shown in Figure 2.

Most water was absorbed by the hypanthium and the tepals on top of the propagation material, almost over 60% of the amount of water retained by the achene, and 80% by the one retained in the seed. However, in the first two cases, not all that humidity benefited the embryo directly.

After 18 hours of imbibition, the mass increased continuously, depending on the level of scarification. It underwent a 0.22 g increase without scarification; the ones with a mid-scarification had a 0.08 g increase, and the total scarification showed a 0.05 g increase compared to the initial values.

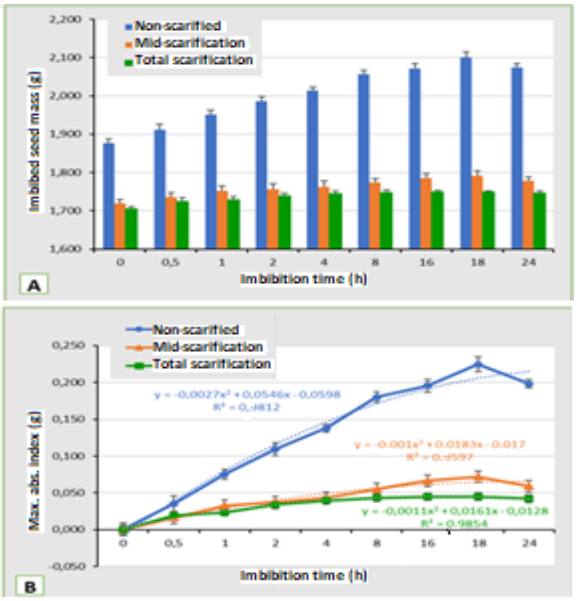

Fig. 2. Effect of scarification type on the water-absorption dynamic on *C. cowellii* seeds overtime. A) Fresh mass of imbibed seed, B) Maximum water absorption index. The error bars were estimated for every treatment and evaluation time. Mid-scarification consisted of the removal of the hypanthium from the fruit, whereas total scarification required the removal of the achene testa.

The propagation material in the scarification level absorbed water in the first eighteen hours past wetting. The stage with the fastest water absorption increase (Suárez & Melgarejo, 2010) only lasted an hour, when this substance went through the tissue, while nutrient stabilization and mobilization needed 18 hours approximately.

This study demonstrated that imbibition does not have a negative effect on *C. cowellii* germination. It behaved differently from other dry fruit species, such as *Jatropha curcas* L., in which prolonged water exposure caused a linear reduction of germination and longer time needed so that this process takes place (Lozano-Isla et al., 2017).

Germination

The previously imbibed seeds began to germinate five days after, regardless of the storage time. Germination reached 100% when scarification was performed, and the soil from the original plant habitat was added. These results showed that, generally, there are conditions to ensure the breeding of this species sexually, and through traditional methods. However, the experiments helped determine the most effective alternatives.

Based on the statistical analysis, significant differences were only found in relation to the number of days to the beginning of germination, between the seeds with some level of scarification and the ones with no scarification. The latter required about seven days to complete plumula emergence, whereas the former completed it in about thirteen days (Table 1A).

Table 1. Effect of scarification type on *C. cowellii* seed germination from functionally-female flowers A) Days needed for germination; B) Germination percentage

Treatments	Mean	Mean typical error	Variation coefficient (%)
	A) Days to germination (days)		
NS*	12.6 b**	1.20	47.3
MS	6.6 a	0.48	36.1
TS	7.5 a	0.81	53.8

Treatments	B) Germination percentage (%)		
NS	84.0 b	0.07	0.45
MS	100.0 a	0.00	0.0
TS	100.0 a	0.00	0.0

*NS: non-scarified; MS: mid-scarification; TS: total scarification. **Means with equal scripts did not differ statistically, according to ANOVA and Tukey HSD, $p \geq 0.05$ and n=25

It matched the findings in *Triplaris brasiliana* (*Polygonaceae*), where the concentrated H_2SO_4-scarified seeds at 30 ºC, for 10 minutes, on Kraft paper in tightly-closed glass tubes, reached 92% germination, against 29% of the non-scarified plants (Correia de Araujo et al., 2018).

Table 1B shows the statistical analysis of the results in terms of germination, depending on the scarification level, with significant differences. The seed responded to the top release, so the time to embryo emergence could be cut down, increasing germination efficiency.

Table 2 shows the statistical analysis of the results in terms of the effect of the substrate type on *C. cowellii* seed germination, at different storage times. The seeds stored at room temperature were observed to significantly reduce the germinative power compared to the fresh seeds, from the sixth month on, thus demonstrating that the seeds can be stored for a long time, up to 180 days.

There were significant differences as to the days needed for germination in the different substrate types and the storage time. In the seeds stored for a month, the substrate that required the fewest days (approximately 6) for plumula emergence was the soil from the original habitat. That behavior differed in relation to the seeds stored for the longest time,

with the best results observed in river sand. Concerning the germination percentage, no significant differences were observed between factors stored for a month (the best) and stored for six months.

C. cowellii behaves differently from other Polygonaceae species, such as *Rumex turcomanicus* Czerep, whose fruit was stored for four months, and showed the greatest germination percentage (95.51%) (Alirezaie & Azizi, 2015). In *Muehlenbeckia astonii* Petrie, also part of the same family, a better conservation response was observed, as the seeds planted six months after the collection showed 85.2% germination (Wotton, 2018).

The best results were observed in the soil collected in the original habitat (Figure 3). However, large-scale production would affect the natural vegetation, so further research studies should focus on mixing different substrates in order to reduce the volumes of soil removed from the ecosystems.

Table 2. Effect of the substrate type on *C. cowellii* seed germination, at different storage times A) Days needed for germination; B) Germination percentage

Table 2. Effect of the substrate type on *C. cowellii* seed germination, at different storage times A) Days needed for germination; B) Germination percentage

Storing time	Substrate type	Mean	Mean typical error	Variation coefficient (%)
		A) Days to germination (days)		
1 month	RS*	2.8 a**	0.50	17.5
	HS + Hu	4.1 a	0.46	11.2
	HS	8.1 bc	0.84	10.4
	RS + Hu	4.2 a	0.42	10.0
	Hu	9.5 cd	0.57	6.0
6 months	RS	10.2 d	0.48	4.7
	RS + Hu	10.7 d	0.75	7.0
	HS	6.3 b	0.35	5.5
	RS + Hu	9.5 cd	0.60	6.4
	Hu	10.4 d	0.75	7.2
Storing time	Substrate type	B) Germination percentage (%)		
1 month	RS	85.0 ab	10.00	11.8
	RS + Hu	76.7 ab	10.93	14.3
	HS	98.3 a	1.67	1.7
	RS + Hu	58.3 bc	24.55	42.1
	Hu	66.7 ab	9.28	13.9
6 months	RS	20.0 cd	11.55	57.7
	RS + Hu	5.0 d	2.89	57.7
	HS	40.0 bcd	5.00	12.5
	RS + Hu	8.3 d	4.41	52.9
	Hu	18.3 cd	7.26	39.6

*RS: River sand, HS + Hu Habitat soil + Humus, HS: Habitat soil, RS + Hu: River sand + Humus, Hu: Worm casting
**Means with equal scripts did not differ statistically, according to ANOVA and Tukey HSD, p≥0.05 and n=25

The germination of the species begins five days after sowing.

Imbibition and scarification were used as pre-germinative treatments suitable for more efficient *C. cowellii* germination, depending on the human and material resources available.

The seeds scarified could germinate in fewer days (seven) than the non-scarified seeds. Germination reached 100% when scarification was performed, and the soil from the original plant habitat was added, to both the fresh and stored seeds.

The propagation material can be preserved at room temperature to extend and adjust sowing to the *ex-situ* handling needs. However, the highest germination efficiency was observed when sowing took place 30 days after harvest.

Fig. 3. *Germinated C. cowellii* plantlets in soil from the natural habitat, with 100% success.

The seeds stored for six months showed a lower germinative power, and the best germination results were in the soil collected from the natural habitat. This behavior was observed likewise in the fresh seeds, which could germinate totally in the two scarification levels.

In situ, C. cowellii breeding can be handled for better plant conservation, ensuring the plant material for further research, and even, introducing new individuals in natural populations, if deemed necessary and advisable.

Conclusions

Although *C. cowellii* seeds can be classified, in general terms, as non-dormant, the presence of at least primary dormancy creates favorable handling conditions (desiccation, storage, etc.).

Author contribution statement

Andrys Martínez Proenza: Research conception and implementation, analysis of the results, redaction of the report.

Isidro Méndez Santos: Research conception, analysis of the results, redaction of the report.

Oscar Concepción Laffitte: Research conception, analysis of the results, statistical analysis.

Conflict of interest statement

Not declared.

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References

Alirezaie, M., & Azizi, M. (2015). Seed Harvesting Time Affects Seedling Emergence, Vigour and Growth: Case Study of *Rumex turcomanicus* Czerep. (Polygonaceae). *Notulae Scientia Biologicae*, *5*(2), 244-248. https://www.academia.edu/46996737/Seed_Harvesting_Time_Affects_Seedling_Emergence_Vigour_and_Growth_Case_Study_of_Rumex_turcomanicus_Czerep_Polygonaceae_

Alonso, W. (2018). *Evaluación de tres tratamientos pregerminativos y tres sustratos para la producción de semilleros de guayacán (Guaiacum sanctum l.), bajo condiciones de invernadero y análisis histológico e histoquímico de su semilla*. (Tesis en opción al título académico de Master. Universidad de San Carlos de Guatemala). Guatemala. https://acreditacion.ccqqfar.usac.edu.gt/qf/Anexo11.pdf

Baskin, C., & Baskin, J. (2014). *Seeds Ecology, Biogeography, and Evolution of Dorman-cy and Germination. Second Edition*. Academic Press is an imprint of Elsevier, San Diego, USA, 12-200. https://books.google.com.cu/books?hl=es&lr=&id=vXfNCgAAQBAJ&oi=fnd&pg=PP1&dq=Baskin,+C.+%26+Baskin,+J.+(2014).+Seeds+Ecology,+Biogeography,+and+Evolution+of+Dormancy+and+Germination.&ots=-ridWgGVye&sig=y7tw1p4ydW_qGN5zs51Xfkgs9kU&redir_esc=y#v=onepage&q&f=false

Castañeda, I. (2014). *Taxonomía y filogenia del género Coccoloba (Polygonaceae) en Cuba*. (Tesis en opción al grado científico de Doctor en Ciencias Biológicas. Universidad de La Habana), Cuba.

Chávez, M. (2017). Síndromes de domesticación en plantas. *Desde el Herbario (CICY)*, *9*, 79–83. http://www.cicy.mx/sitios/desde_herbario/

CITMA (Ministerio de Ciencia Tecnología y Medio Ambiente de la República de Cuba). (2021). *Estrategia Ambiental Nacional 2021/2025*. https://www.citma.gob.cu/estrategia-ambiental-nacional/

Correia de Araujo, J., Duarte, J., Inajal, M., Ferreira, L., & Marques, V. (2018). Morphometric characterization, germination and conservation of *Triplaris brasiliana* cham. seeds (Polygonaceae). *Ciência Florestal, Santa Maria*, *28*(3), 949-959. http://dx.doi.org/10.5902/1980509833363

Díaz, M. & Molinelli, M. (2018). Caracterización del fruto y la semilla de Ruprechtia apetala (polygonaceae) en relación con la entrada de agua y la germinación. *Bonplandia*, *27*(1), 5-22. https://www.researchgate.net/publication/326003263

González, L., Palmarola, A., González-Oliva, L., Bécquer, R., Testé, E., & Barrios, D. (Eds.). (2016). Lista roja de la flora de Cuba; *Bissea 10* (número especial 1), 1-352. https://www.researchgate.net/publication/309313148_Lista_Roja_de_la_Flora_de_Cuba_-_2016

Lozano-Isla, F., Miranda, P., & Pompelli, M. (2017). Germination behavior of *Jatropha curcas* L. after different imbibition times. *Peruvian Journal of Agronomy*, *1*(1), 32-38. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwjoiJexjvr0AhW1VTABHVOGC_oQFnoECAUQAQ&url=http%3A%2F%2Frevistas.lamolina.edu.pe%2Findex.php%2Fjpagronomy%2Farticle%2Fdownload%2F1065%2Fpdf_4&usg=AOvVaw2rteIxueKMsqypaLdtZcBx

Martínez, E., & Reyes, O. (2015). Caracterización de la vegetación de la meseta de San Felipe en Camagüey, Cuba, con propósitos de conservación. *Revista del Jardín Botánico Nacional*, *36*, 19-30.

http://www.rjbn.uh.cu/index.php/RJBN/article/view/185/0

Méndez, D. 2019. *Actividad antioxidante y bacteriostática de Coccoloba cowellii Britton*. (Tesis presentada en opción al Título Académico de Máster en Biotecnología. Universidad de Oriente). Santiago de Cuba. Cuba.

Méndez, D., Molina Pérez, E., Spengler, I., Escalona-Arranz, J., & Cos, P. (2019). Chemical composition and antioxidant activity of *Coccoloba cowellii* Britton. *Revista Cubana de Química*, *31*(2), 185-198. http://scielo.sld.cu/scielo.php?script=sci_abstract&pid=S2224-42120190002001 85&lng=en&nrm=iso&tlng=en

Méndez, I., Castillo, M., Trujillo, R., & Martínez, V. (1988). Algunas consideraciones acerca de las plantas serpentinícolas presentes en la flora de Sierra de Cubitas. *Revista del Jardín Botánico Nacional*, Universidad de La Habana, *9*(2), 65-70. https://www.jstor.org/stable/42596854

Méndez, I., Risco, R., & Reyes, M. (2005). Flora and vegetation of the ultramafic outcrop at Camagüey, Cuba. En R. Boyd, A. Baker and J. Proctor, (Eds.), Memorias de la IV Conferencia Internacional sobre Ecología de Serpentina: 21-26 abril 2003, en *Science Review*s, Reino Unido, 91 – 96.

Méndez, J. (2016). *Osmoacondicionamiento de semillas de Beaucarnea gracilis Lem. como tratamiento pregerminativo.* (Tesis presentada en opción al Título Académico de Máster en Biotecnología. Universidad Autónoma Metropolitana Iztapalapa). México D.F. México. https://repository.unad.edu.co/bitstream/handle/10596/11217/1114453102.pdf?sequence=1&isAllowed=y

Naciones Unidas. (2018). *La Agenda 2030 y los Objetivos de Desarrollo Sostenible: una oportunidad para América Latina y el Caribe*. LC/G.2681-P/Rev.3. Santiago. https://www.cepal.org/es/temas/agenda-2030-desarrollo-sostenible

Sánchez, J., Pernús, M., Torres-Arias, Y., Barrios, D., & Dupuig, Y. (2019). Dormancia y germinación en semillas de árboles y arbustos de Cuba: implicaciones para la restauración ecológica. *Acta Botánica Cubana*, *218* (2), 77-118. http://revistasgeotech.com/index.php/abc/article/view/290

Soledad, M. (2018). *Calidad morfo-fisiológica y sanitaria de semillas de Ruprechtia apetala WEDD. (Polygonaceae) para su domesticación*. (Tesis Para optar al Grado Académico de Magister en Ciencias Agropecuarias. Universidad Nacional de Córdoba). Córdova, Argentina. https://rdu.unc.edu.ar/handle/11086/6505

Suárez, D., & Melgarejo, L. (2010). *Biología y germinación de semillas. Laboratorio de fisiología y bioquímica vegetal.* Departamento de biología. Universidad Nacional de Colombia. https://www.researchgate.net/publication/258627099

Vargas-Simón, G., & Pire, R. (2010). Efecto de dos condiciones de almacenamiento en la germinación de semillas de uva de playa (Coccoloba uvifera (L.) Jacq.). *Revista. Facultad Agronomía* (LUZ). *27*, 559-573. https://www.researchgate.net/publication/265597554_Efecto_de_dos_condiciones_de_almacenamiento_en_la_germinacion_de_semillas_de_uva_de_playa_Coccoloba_uvifera_L_Jacq

Wotton, D. (2018). Seed germination, dormancy and longevity in the endangered shrub *Muehlenbeckia astonii* (Polygonaceae). *New Zealand Journal of Botany*, *56* (3), 331-341. http://dx.doi.org/10.1080/0028825X.2018.1491862