Emergence of *Passiflora caerulea* seeds simulating possible natural destinies

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Abstract — **Introduction**. The emergence of *P. caerulea* L. seeds was studied for the first time, in order to obtain useful information for cultivation of this plant with ornamental, edible and medicinal values. **Materials and methods**. Different treatments (aril removal, mechanical and chemical scarification and combinations of them) were performed on fresh seeds before sowing. The treatments simulated different paths that the seeds may follow in nature in this ornithochorus species, those which small growers could reproduce before cultivation. Seed emergence was recorded periodically for 16 months in a greenhouse. Seed emergence initial time, percentage and speed were calculated. **Results and discussion**. Seeds immersed in hydrochloric acid (chemical scarification) did not germinate, but the rest of the treatments were successful. Emergence percentages were low in most of the treatments and speed was slow in all of them. The minimum seed emergence initial time was 25 days. The curve of the accumulated number of seeds emerged followed an oscillating sigmoid pattern. **Conclusion**. It is suspected that dormancy exists, which will be dealt with in another paper.

Argentina / *Passiflora caerulea* / seeds / seed dispersal / germination / dormancy breaking

Émergence des graines de *Passiflora caerulea* en simulant leurs possibles destinées naturelles.

Résumé — **Introduction**. L'émergence de la graine de *P. caerulea* a été étudiée pour la première fois, afin d'obtenir des informations pour la culture de cette espèce qui a des applications ornementales, comestibles et médicinales. **Matériel et méthodes**. Différents traitements (élimination de l'arille, scarification mécanique et chimique et combinaisons des deux traitements) ont été appliqués à des graines fraîches avant leur semis. Les traitements ont simulé les différentes destinées que peuvent suivre, dans la nature, les graines de cette espèce disséminée par ornithochorie ; ils ont été tels qu'ils puissent être reproduits avant culture par les petits cultivateurs. L'émergence des graines a été enregistrée périodiquement pendant 16 mois sous serre chaude. Le temps, le pourcentage et la vitesse d'émergence ont été enregistrés. **Résultats et discussion**. Les graines immergées dans l'acide chlorhydrique (scarification chimique) n'ont pas germé, mais tous les autres traitements ont été efficaces. Les pourcentages d'émergence ont été bas dans la plupart des traitements et la vitesse a été lente dans tous les cas. Le temps minimum du début de l'émergence a été de 25 jours. La courbe du nombre cumulé de graines ayant montré une émergence a suivi un modèle d'oscillation sigmoïde. **Conclusion**. L'existence d'une dormance des graines de *P. caerulea* est suspectée. Cette étude fera l'objet d'un prochain travail.

Argentine / *Passiflora caerulea* / graine / dissémination des graines / germination / levée de dormance

1. Introduction

The family Passifloraceae has 18 genera and nearly 630 species distributed in tropical and subtropical regions all over the world [1]. Five genera grow in the New World, four of which are exclusively from America. In this continent, most of the species from the genus *Passiflora* are found in Central and South America [1]. *P. caerulea* occurs in Brazil, Bolivia, Chile, Paraguay and Uruguay, and extends to the province of Buenos Aires in Argentina. It is the most widely distributed *Passiflora* species in this last country, comprising 18 provinces.

P. caerulea renders successfully in xerophytic areas and wet forests, specially at the forest's edges. It grows on modified, sandy, clayey or rocky soils. It can be found from sea level up to 1400 m above sea level. This climber spreads over other plants and over wire fences at path edges and railways, both in rural and urban areas [1].

P. caerulea is also the Argentine *Passiflora* species most well known for its many uses: medicinal [2], edible [3] or ornamental [4]. The fruit is employed in health care [5] and as food [6, 7]. It can be eaten fresh as an ingredient of marmalades, syrups, beverages, juices [8], stews [9] or ice creams [10]. Although the entire mature fruit can be ingested, the most important part to be consumed is the pulp, which is sweet according to Ragonese and Martínez Crovetto [9], acid according to Luna Ercilla [11] and insipid according to Vanderplank [12] and the present authors. It is consumed by different American ethnic groups: the Toba from eastern Chaco [13] and the Maka [14], who eat the mature fruits raw and boil or bake the immature ones, discarding the seeds and pericarp. The fruits can yield as much as 3000 $kg \cdot ha^{-1}$ [11]. The vines last at least 4 years, before the stems are attacked by insects, so a plantation could be productive at least for that time, similar to *P. edulis* [15]. The advantages of *P. caerulea* over other *Passiflora* species whose cultures have already been set up are that it is a good grafting trunk for its rusticity [16] and that it is resistant to illnesses to which the other species are susceptible [11]. Despite the several uses of the fruit, *P. caerulea* is grown solely for its flowers in the USA [17] and the UK [12].

In Argentina, *P. caerulea* is propagated by seeds and cuttings [18]. Despite its wide distribution, its abundance is diminishing in some regions, at least in Buenos Aires' surroundings. This decrease is due in part to the increasing urbanisation (as it was a frequent species in lots and houses' wire fences) and in part to its collection and sale in herbal shops. As no commercial crops have been propagated, harvesting without replanting threatens the natural populations [8].

The studies on reproductive biology of this species involved the self-compatibility system, the floral ecology, the reproductive success and seed production [19–22]. Its later life cycle stages have not been studied.

The fruit is a berry whose seeds, (6.41 ± 0.02) mm long, (3.90 ± 0.02) mm wide and (2.64 ± 0.02) mm high (means for n = 20), are covered by a structure related to zoophilus dispersion (aril). The orange epicarp, the red aril and the fruit remaining on the plant although mature and the light aroma suggest ornithochory [23]. Although it has been reported that the fruit is highly sought after by birds [7], there are almost no observations on the manipulation details of the seeds by them. Deginani [24] reported that some birds eat only the aril and discard the seed, which would constitute synzoochory [25], but she also observed that they swallow the seed without processing it (Deginani, pers. commun.).

With respect to seed emergence, species belonging to genera of *Passifloraceae,* such as *Paropsia vareciformis* and *Tetrameles nudiflora*, did not show dormancy [26] but some *Passiflora* species did [27]. Dormancy seems to be due to physical constraints [27] in the species tested. Nevertheless, there are few studies on seed emergence in this genus [27], involving other species [28–30]. The treatments undertaken on the seeds include aril removal, desiccation, soaking, mechanical and chemical scarification [31, 32], fermentation [33] and stimulation with gibberellic acid [34].

Considering the economic importance and the conservation problems of *P. caerulea* noted above, our purpose was to

Table I.
Treatments applied to *Passiflora caerulea* seeds simulating natural outcomes, tested to induce the seedling emergence (Argentina).

Treatment	Simulation
With aril	Fallen seed
Without aril	Aril removed by a bird
With aril + soaking in water 24 h at room temperature	Fallen seed wet by rain
Without aril + soaking in water 24 h at room temperature	Aril removed by a bird + seed wet by rain
Without aril + mechanic scarification	Aril removed by a bird + seed coat damage
Without aril + chemical scarification 10 min	Aril removed by a bird + passage through digestive tract (considering time)
Without aril + chemical scarification 10 min + hot water (36 °C)	Aril removed by a bird + passage through digestive tract (considering time and temperature)
Without aril + mechanic scarification + chemical scarification 10 min + hot water (36 °C)	Aril removed by a bird + seed coat damage + passage through digestive tract (considering time and temperature)
Without aril + chemical scarification 10 min + hot water (36 °C) + washing 5 min with water at room temperature	Aril removed by a bird + passage through digestive tract (considering time and temperature) + washing by rain

examine the seed emergence pattern of this promising crop from the standpoint of either *in situ* or *ex situ* plant preservation and propagation.

2. Materials and methods

Mature fruits were collected from the natural population that grows on the campus of Ciudad Universitaria, Capital Federal, Argentina. The experiment took place between January 2003 and June 2004. Before sowing, the seeds received nine different treatments aiming to simulate possible natural outcomes (*table I*).

Mechanical scarification was performed with sand paper and pure hydrochloric acid was used for chemical scarification. Water was heated to 36 °C.

Seeds (324 seeds, 36 seeds per treatment) were sown in plastic divided trays filled with soil: perlite 3:1 with one seed per cell. The treatment distribution was randomly assigned. The trays were placed inside a greenhouse, under a natural photoperiod, where ambient temperature and relative humidity were recorded with a digital thermohygrometer, which varied from 12–42 °C and 43–99%, respectively. The soil was watered with tap water every day. Seedling emergence was registered daily. The following parameters were obtained for each treatment:

– Emergence initial time (T_0): days from sowing to emergence of the first seedling [35];

– Germinability (P): emergence percentage;

– Vigour (V): estimated by the emergence speed, which is defined by $V = [(a/1 + b/2 \ldots x/n) \times 100] / S$, where a, b... x represent the quantity of seeds that germinated after 1, 2, … n days after imbibition and S is the total seeds sown.

The resultant values of P and V were assigned to the categories that appear in López *et al*. [36].

Table II.
Emergence percentage, initial time and speed for seeds of *Passiflora caerulea* submitted to different treatments before sowing (n = 324 seeds, 36 seeds per treatment).

Treatment	Germinability (emergence %)	Emergence initial time (t_0)	Vigour[1]
With aril	22 b	165	0.1324
Without aril	8 a	162	0.0507
With aril + soaking in water 24 h at room temperature	19 b	80	0.1059
Without aril + soaking in water 24 h at room temperature	33 b	25	0.2569
Without aril + mechanic scarification	22 b	183	0.0575
Without aril + chemical scarification 10 min	0 a	0	0
Without aril + chemical scarification 10 min + hot water (36 °C)	0 a	0	0
Without aril + mechanic scarification + chemical scarification 10 min + hot water (36 °C)	0 a	0	0
Without aril + chemical scarification 10 min + hot water (36 °C) + washing 5 min with water at room temperature	0 a	0	0

[1] Vigour (or emergence speed) is defined by $V = [(a/1 + b/2 ... x/n) \times 100] / S$, where $a, b ... x$ = quantity of seeds that germinated after 1, 2, … n days after imbibition and S is the total of seeds sown.

a, b: values with different letters differ significantly ($X^2 = 28$; $p < 0.01$).

A frequency analysis with contingency tables was used on the germinability data. *Posteriori* contrasts were performed as needed, using the partition method by the degrees of freedom in tables of r×2 [37]. Percentages are shown in the text, for comparative purposes.

3. Results

Chemical scarification with hydrochloric acid prevented emergence, whether it was accompanied or not by other types of scarification (*table II*). The emergence percentages of the seeds submitted to the other treatments were low, except the 24-h soaking without aril. Seeds with that treatment resulted in the shortest emergence initial time. The emergence seed percentage was low in all the cases where emergence took place (*table II*).

By comparing the seeds from which the aril was removed, the emergence percentage and vigour were greater when some treatment was combined with the aril removal.

The cumulative emergence curves (*figure 1*) showed an imbibition phase of (2 to 5) months, depending on the seed treatment before sowing; that phase is followed by an increase for (1 to 3) months until reaching a steady speed (*figure 1*). A new increment for at least (1 to 2) months was observed in the treatments with soaking and mechanic scarification approximately 12 months later, until they came back to stabilisation (*figure 1*).

4. Discussion

The treatments that simulate what happens in nature were not the more successful ones, because in natural conditions it may be more profitable for the species that a certain quantity of seeds remain in the soil bank during a period of time. Nevertheless, it was considered convenient to face the first approximation to emergence in *P. caerulea* simulating natural destinies. Even in some endozoochorus species, seed emergence is possible only when all the factors imitating the digestive tract passage of the dispersing agents have been simulated [25]. Moreover, differences in seed emergence have been encountered between digestive tracts of different dispersing agents [25].

The possible outcomes simulated in this study do not exclude one another. For example, in spite of birds ingesting seeds with arils, some of the seeds fall to the ground during manipulation of the fruit, so the seeds that follow each destiny have the probability (although different) of germinating. During the development of our research, Passeriformes were observed feeding from hanging fruits: great kiskadees (*Pitangus sulphuratus*, Thyranidae), chalk-browned mockingbirds (*Mimus saturninus*, Mimidae) and rufous-bellied thrushes (*Turdus rufiventris*, Turdidae). They ingested seeds covered with the aril. Likewise, seeds with arils were found below *P. caerulea* plants, where leaf-cutting ants tried to transport them, a fact that also seems to have been observed by Deginani [1]. Some of those seeds displayed scars on the aril, which could have been done by the bill of a bird or by the ants.

In our work, the maximum seed emergence percentage and the minimum seed emergence initial time occurred in the seeds without arils and soaked for 24 h at room temperature (25 °C). In *P. edulis* (both forms), seed emergence accelerated slightly when the aril was removed [38]. Soaking the seeds devoid of an aril seems to favour imbibition and, consequently, emergence. Perhaps the aril prevents permeability to water.

Although chemical scarification with sulphuric acid is more common, for *Passiflora edulis* [32], *Vicia* spp. [39], *Trifolium* spp. [40] or *Medicago* spp. [41], for example, in

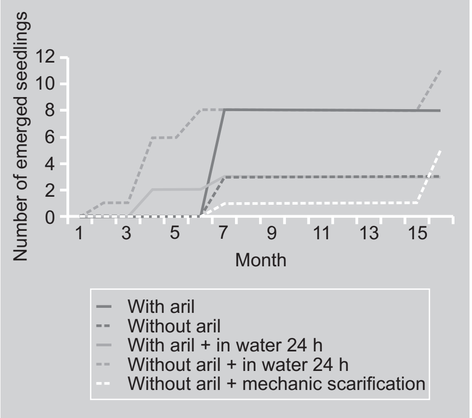

Figure 1.
Curves of the cumulated number of *Passiflora caerulea* seeds which showed an emergence according to different treatments applied. Four treatments implicating chemical scarification gave no emerged seedlings.

the present paper, chemical scarification was performed with hydrochloric acid in order to more closely simulate the digestive tract of an avian frugivore. Although an average immersion time close to the retention time observed in birds that eat berries [42] was used, the results were negative. Schoniger [31] obtained emergence of seeds from *Passiflora mollissima* with hydrochloric acid, but this author immersed the seeds for a longer period (2–4 h) in a more diluted solution and combined this treatment with desiccation, both with and without aril, and with and without mechanic scarification. The likely difference in the success might be due to the degree of acidity employed, that of the present work being harmful, or because the seeds sown by Schoniger [31] underwent a desiccation period.

The seed emergence initial time is also variable when different species and experiments are compared. According to Caicedo Ramírez and González [43], soaked seeds sown in soil could emerge 10 weeks later. Salinero Corral and Fernández Lorenzo [44] noted seed emergence 14 days after planting, but the main emergence flux occurred between the third and fourth weeks. There are also differences with respect to the extent of emergence. According to Williams and Buxton [28], emergence was staggered over 8 months or even longer. This data may have been underestimated, because they did not follow the experiment for more time. The present work detected seed emergence over 16 months.

The low levels of emergence obtained in the majority of the treatments, and the increase observed when the aril was removed and the seeds were soaked, make one suspect that physical dormancy exists in *P. caerulea,* which is currently being studied (Mendiondo and Amela García, in prep.). Besides, further tests with alternating temperatures and light regimes are also needed.

All the cumulative emergence curves for the different treatments showed an oscillating sigmoid pattern, one of the non-linear kinetic patterns commented on by López *et al.* [36], due to the absence of a factor acting continuously, which is usual in normal emergence processes. Williams and Buxton [28] found a similar pattern for *P. mollissima*: emergence began 2 weeks after sowing, increased for 10 weeks and remained stable for 5 months. This would indicate that a group of seeds remaining in the soil are capable of germinating, which would be interesting to investigate from an ecological viewpoint, as well as to take into account for culture multiplication. In order to be able to establish which type of periodicity [45] *P. caerulea* exhibits, it is necessary to continue the studies for several years.

5. Conclusion

Passiflora caerulea presented, in spite of the majority of the treatments applied, low levels of seed emergence. This species seems to have physical dormancy. It displayed an oscillating sigmoid pattern in the cumulative emergence curves during the 16-month duration of the experiment.

This work is the first of a series of studies concerning the methods to overcome the seed dormancy in *P. caerulea*.

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Emergencia de semillas de *Passiflora caerulea* simulando destinos naturales posibles.

Resumen — **Introducción**. La emergencia de las semillas de *P. caerulea* L. fue estudiada por primera vez, con el fin de obtener información útil para el cultivo de esta planta con valor ornamental, alimenticio y medicinal. **Material y métodos**. Diferentes tratamientos previos a la siembra (remoción del arilo, escarificación mecánica y química y combinaciones de los anteriores) se aplicaron a semillas frescas. Los tratamientos simularon distintos destinos que las semillas de esta especie ornitocora pueden seguir en la naturaleza, aquéllos que pequeños productores podrían reproducir antes del cultivo. La emergencia de las semillas fue registrada periódicamente durante 16 meses en un invernáculo. Se calcularon el tiempo inicial, porcentaje y velocidad de emergencia. **Resultados y discusión**. Las semillas sumergidas en ácido clorhídrico (escarificación química) no germinaron, pero el resto de los tratamientos fue exitoso. Los porcentajes de emergencia fueron bajos en la mayoría de los tratamientos y la velocidad fue baja en todos ellos. El menor tiempo inicial de emergencia fueron 25 días. La curva de la cantidad de semillas emergidas acumulada siguió un patrón sigmoide oscilante. **Conclusión**. Se sospecha que existe dormición en las semillas de *P. caerulea*, cuyo estudió será tratado en un trabajo futuro.

Argentina / *Passiflora caerulea* / semilla / diseminación de semillas / germinación / salida de la dormición