

INVITED ARTICLE

A new approach towards the so-called recalcitrant seeds¹

Claudio José Barbedo^{2*}

ABSTRACT - Water is essential, irreplaceable, and indispensable for any kind of carbon-based-life metabolic activity. Water-dependent living beings are the expected pattern in nature. However, some organisms can survive for some time at a minimum water content, such as seeds of some species (orthodox seeds). Nevertheless, the expected standard life behavior is found in seeds of another group of species, the so-called recalcitrant seeds, which are sensitive to desiccation. A huge range of different behaviors can be found between these two groups, leading authors to consider that orthodoxy and recalcitrance is not an all-or-nothing situation. Notwithstanding, we are still too far from understanding the differences and similarities between all these kinds of seeds and this has been a serious barrier to the development of plant conservation technologies. A new approach to understanding the differences between these seeds is presented here based on seed maturation, environmental influences, and evolution. From this point of view, all kinds of seed behavior are contemplated and, consequently, some new perspectives are considered for the recalcitrant seed conservation technology, the most intensely desired technology nowadays in this area.

Index terms: seed conservation, seed maturation, seed physiology.

Nova abordagem para as assim chamadas sementes recalcitrantes

RESUMO - A água é essencial, insubstituível e indispensável para qualquer tipo de atividade metabólica da vida baseada no carbono. A dependência dos seres vivos por água é o padrão esperado na natureza. No entanto, alguns organismos podem sobreviver por algum tempo com um teor mínimo de água, como sementes de algumas espécies (sementes ortodoxas). No entanto, o comportamento padrão de vida esperado é encontrado em sementes de outro grupo de espécies, as chamadas sementes recalcitrantes, que são sensíveis à dessecação. Entre esses dois grupos há uma enorme variedade de comportamentos diferentes que levaram autores a considerar que a ortodoxia e a recalcitrância não são uma situação de tudo ou nada. Não obstante, ainda estamos muito longe do entendimento das diferenças e semelhanças entre todos esses tipos de sementes e isso tem sido um sério entrave para o desenvolvimento de tecnologias de conservação de plantas. Aqui, uma nova abordagem para entender as diferenças entre essas sementes é apresentada tendo como base a maturação de sementes, as influências ambientais e a evolução. Sob este ponto de vista, todo o tipo de comportamento das sementes é contemplado e, conseqüentemente, são consideradas algumas novas perspectivas para a tecnologia de conservação de sementes recalcitrantes, que é a mais intensamente desejada hoje em dia nesta área.

Termos para indexação: conservação de sementes, maturação de sementes, fisiologia de sementes.

Introduction

The development of humankind up to the level of present-day civilizations may have begun with the change

from nomadic to sedentary habit, allowing the formation of groups established at fixed places and thus the construction of clans, towns, villages, and cities (Barbedo et al., 2013). The identification of nomadic or sedentary habits can sometimes

¹Submitted on 07/09/2018. Accepted for publication on 08/03/2018.

²Instituto de Botânica, Núcleo de Pesquisa em Sementes, 04301-012 – São Paulo, SP, Brasil.

*Corresponding author <claudio.barbedo@pesquisador.cnpq.br>

be modified by archaeological research, such as that which changed the knowledge about the peoples of the Inner Asia in the first millennium BC, formerly regarded as nomads but who, in fact, already had signs of animal domestication and cultivation of wheat and barley (Chang, 2018). However, it is very likely that the establishment of human in certain places depended on the development of the first agricultural techniques, allowing the production of food when, where, and whenever it was necessary, removing the need for the search for animals, fruits, and other products.

The transition from collecting and hunting to agriculture and grazing radically reshaped human societies, leading to global changes that culminated in changes in biodiversity and even in the earth's atmosphere. Given the significant results of this transition, it is not surprising that the origin and spread of domestic products and the emergence of agriculture remain topics of lasting interest to both the academic community and the general public. In the eastern Mediterranean, for instance, in the south of the Levant, archaeologists came to the consensus that domestication of animals (starting with goats and then with sheep) occurred about a thousand years after the domestication of cultivated plants (Zeder, 2008 and references therein).

In fact, historically the development of civilization and the development of agriculture coincide. As agricultural production became increasingly efficient, less time was needed to obtain food and shelter, leaving more time for other activities, such as contemplation, art, and poetry (Shoemaker, 2010). In order for the first agricultural techniques to be applied, it was essential that the plants used had seeds able of being stored and therefore tolerant to desiccation (Barbedo et al., 2013). Curiously, the earliest known civilizations are from regions with a markedly cold or dry season and therefore with a great prospect of having plants producing desiccation tolerant seeds so that they could pass through these seasons in a quiescent state, germinating when environmental conditions were favorable. These seeds are generically called orthodox.

It is always important to remember that the dry, quiescent, sometimes even dormant condition characterizes an exception in the world of living beings, not just on our planet. Although the definition of what life is, to this day, finds no global consensus (there are as many definitions of life as there are life-defining authors), carbon-based life finds the most common elements in the universe, and for that life, water is essential for the solvent-compound binomial in the water-carbon relation (Leprince and Buitink, 2015; Westall and Brack, 2018). Therefore, the desiccation-sensitive behavior is the most expected for living beings and is found in seeds of another group of species, the generally and globally called recalcitrant. However, these two extremes are not the only known behaviors, and several others

are identified among them.

Although several groups around the world have studied, for decades (and some continue to study), these different behaviors, few are the groups that remain to study recalcitrant seeds.

The research with recalcitrant seeds

Formation and persistence of research groups on unorthodox seeds is a very difficult task. Working with recalcitrant seeds requires, at the same time, speed and precision, characteristics that do not always go together. Firstly, because of the difficulty in obtaining seeds, which are often produced by tree species and in short periods of the year. Production often coincides with rainy seasons, making it difficult to access plants and transport seeds to laboratories. The whole processing, including pulping, selection, and experiment setup, must be very fast because seeds lose water easily (they are always with high levels of water in the harvest) and can initiate germination or deterioration at always very high rates (Barbedo et al., 2013). Therefore, the results do not rarely present huge deviations, limiting the analyses and conclusions by the researcher. It takes several cycles for the information to gain reliability, reducing the scientific production of the groups that study such seeds. This often becomes a major disincentive to project continuity and researchers (and even research groups) often migrate to studies with orthodox seeds.

Among the groups that persist in the area, the panel that was demonstrated up to two decades ago (Barbedo and Bilia, 1998) did not change much afterward (Figure 1). The majority of the studies are basically focused on desiccation tolerance (Mello et al., 2010; Corsato et al., 2012; Chapell et al., 2015; Amoedo and Ferraz, 2017; Gasparin et al., 2017; Subbiah et al., 2017; Chandra and Keshavkant, 2018; Plitta-Michalak et al., 2018), identification of storage behavior (Comin et al., 2014; Joshi et al., 2015; Vicente et al., 2016; Joshi et al.,

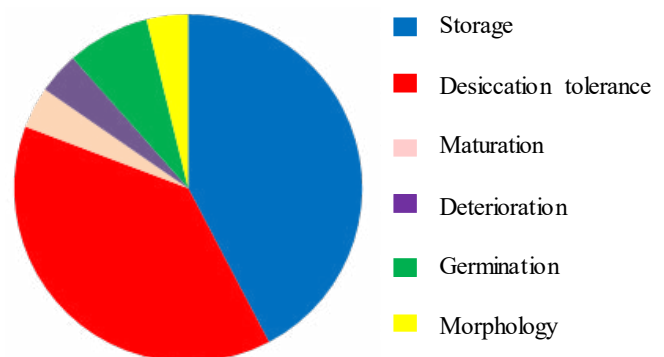


Figure 1. Distribution of the researches on recalcitrant seeds in Brazil, by area, in the period from 2010 to 2015.

2017; Plitta-Michalak et al., 2018), analysis of deterioration and evaluation of techniques to extend the storage period (Barbedo and Cicero, 2000; Andréo et al., 2006; Bonjovani and Barbedo, 2008; Ballesteros et al., 2014; Lan et al., 2014; Parkhey et al., 2014; Garcia et al., 2015; Ming-Yue et al., 2015; Umarani et al., 2015; Araldi et al., 2016; Hossel et al., 2016; Félix et al., 2017), and rare studying other aspects such as seed maturation (Avila et al., 2009; Lamarca et al., 2013b; Mata et al., 2013; Schultz et al., 2014; Araldi and Coelho, 2015; Shibata and Coelho, 2016; Souza et al., 2018a, b), whose importance will be discussed later, and ecological (Ramlall et al., 2015; Vaz et al., 2016; Porto et al., 2018) or germinative aspects (Moothoo-Padayachie et al., 2016). An analysis, albeit superficial, of studies developed in Brazil and in the world shows small differences. Studies directly related to storage, such as tolerance to desiccation and cold, cryopreservation, and storage itself, reach about 70% in Brazil, compared to 80% in the world (Table 1). Works on ecological studies also resemble (5%). The highest differences are in studies on maturation and conditions for germination: 11% in Brazil versus 4% in the world. These results may be due to the immediate technological demands of the species studied in Brazil, which require some decision on the procedures of collection and formation of seedlings.

Studies that seek to increase the storage period, despite important advances, still does not achieve substantial gains, obtaining an extension for a few months or, exceptionally, years. Some advances have been obtained, for instance, with fungus control (Figure 2A) during storage, one of the main problems since seeds are stored moist and often at temperatures that do not totally inhibit the development of microorganisms (Oliveira et al., 2011; Parisi et al., 2013; Françoso and Barbedo, 2014; Hennipman et al., 2017). Studies

Table 1. Distribution of the researches carried out with recalcitrant seeds in the Brazilian and world literature based on a survey of the last 20 years of articles that contain the expression “recalcitrant seeds”. Source: Google/Scholar, July 2, 2018.

Central subject of the publication	Distribution according to the origin of the publication (%)	
	Brazil	World
Desiccation tolerance	30	44
Storage	35	23
Cryopreservation	3	14
Ecology	5	5
Maturation	11	4
Germination	11	4
Other	4	6

on desiccation tolerance, although essential, are not exclusive to seeds, but include ferns (Fallard et al., 2018), mosses (Xiao et al., 2018), fungi (Jackson et al., 2006), algae (Cardon et al., 2018), and even animals such as insects (Kellermann et al., 2018) and amphibians (Eads et al., 2012), evidently each within certain limits of interest in the specific areas. However, even extreme limits of desiccation tolerance are not exclusive to seeds, being studied, for instance, in microorganisms (Mascarin et al., 2018), resurrection plants (Sun et al., 2018), rotifers (Nowell et al., 2018), and larvae of insects (Sogame and Kikawada, 2017) and crustaceans (Janis et al., 2017). However, it is still a long way from understanding enough to develop a technology to store recalcitrant seeds for periods as long as those of classical orthodox seeds. This is the biggest challenge today for seed technologists working with recalcitrant seeds.

In this review, we tried to offer a new approach to understand the differences between these seeds, with special focus on the differences in maturation processes of different seeds and environmental influences on 1) natural selection processes for the evolution of species, producing the large differences between species and 2) phenotypic variations within each species, between regions or production seasons. This approach, far from intending to be the only one, allows the inclusion of all sorts of seed behavior found.

Strategies to ensure the perpetuation of the species with recalcitrant seeds

Seeds are popularly known as structures that need to acquire water to start germination. Therefore, the idea that they are dry structures is settled. This is probably because seeds often used by the human being are, for the most part, orthodox, especially those of agricultural species. Because they are dry, the idea that they can be stored for long periods is also well established.

In contrast, the sensitivity to desiccation of recalcitrant seeds entails the almost impossibility of storage for long periods. However, it is important to remember that there are also orthodox seeds of low natural longevity (Figure 2B), even when they are almost totally dehydrated (Barbedo et al., 2002; Martini-Neto and Barbedo, 2015). In both cases, species were forced to develop other strategies to ensure the availability of propagules able of colonizing the environment when suitable conditions are present (Barbedo and Bilia, 1998).

Among these strategies, one can mention the almost constant supply of propagules throughout the year (Figure 2C), as with several palm trees (Garcia and Barbedo, 2016). For instance, in three *Arecaceae* species (*Bactris gasipaes*, *Euterpe edulis*, and *Syagrus romanzoffiana*), it is possible to

observe that in only a very short period of the year (one to three months depending on the year) there is no production of seeds with the capacity to germinate, from both ripe and immature fruits. Another interesting strategy is the elongation of the maturation cycle and the anticipation, in this cycle, of the maximum germination capacity so that there are also seeds able of being dispersed and germinating even still very immature (Figure 2D), as occurs with *Poincianella pluviosa* (Silva et al., 2015). The process of formation and maturation of these seeds happens in about a year, but from the second third of the cycle, these seeds have the capacity to germinate and form seedlings. The capacity of seeds to survive for long periods in submerged conditions, avoiding water loss and death by desiccation, is also an interesting strategy adopted by some species (Figure 2E), such as *Inga vera* (Okamoto and Joly, 2000) and *Eugenia stipitata* (Calvi et al., 2017).

Nevertheless, certainly one of the most interesting strategies is that developed by *Eugenia* species of the Myrtaceae family

(Figure 2F). We have been studying these interesting seeds for over 15 years, and each time they are even more fascinating. These seeds are intolerant to desiccation, losing viability when their water content is reduced to below 15 to 45%, depending on the species (Delgado and Barbedo, 2007).

The first important characteristic of seeds of these species of *Eugenia*, apparently rare in nature, is the ability to germinate and produce seedlings even when more than half of their reserve tissue is removed (Silva et al., 2003; 2005; Delgado et al., 2010; Prativiera et al., 2015). This ability may be related to a co-evolution with predatory insects since seeds with more than half of the cotyledons consumed by insect larvae are frequently found (Côrtes et al., 2009; Silva and Pinheiro, 2009; Teixeira and Barbedo, 2012; Gaggeti et al., 2016). Therefore, reserves are produced in quantities much higher than those required for producing new plantlets.

Another important characteristic is that, in addition to the single embryonic axis, which is microscopic (Justo et al., 2007),

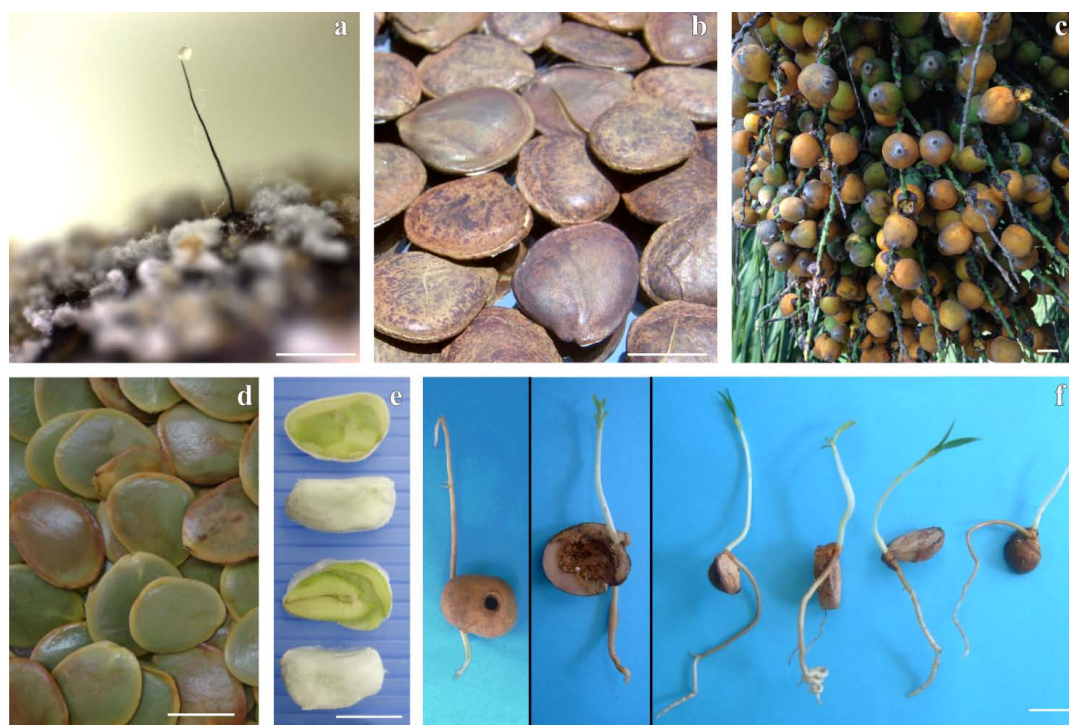


Figure 2. Seeds of species of the Brazilian flora. Surface of recalcitrant seed of *Eugenia pyriformis* covered by fungi of the genus *Fusarium*, *Penicillium*, and *Ceratocystis* (a); seeds of *Caesalpinia echinata* (brazilwood), an example of seeds highly tolerant to desiccation, but which remain viable for only a few months (b); raceme with fruits of *Syagrus romanzoffiana* at different maturation stages (c); seeds of *Poincianella pluviosa*, which has a long maturation period, but from a very early stage can disperse seeds with the capacity to germinate (d); seeds of *Inga vera*, which tolerate hypoxia conditions for several days (e); seeds of *Eugenia cerasiflora*, which have the capacity to produce roots and seedlings from cotyledon tissues: from left to right, seed drilled by insect larvae, damage caused internally by this insect, and seed fractioned into four parts, each of them producing a new seedling (f). Photos: C.F. Franoso, M.R. Bonjovani, D.M. Molizane, L.F. Delgado, V.A. Garcia, C.J. Barbedo.

these seeds are able of producing new roots and even entire seedlings from cotyledon cells (Anjos and Ferraz, 1999; Calvi et al., 2016), a characteristic rarely found in other species (Malik et al., 2005; Joshi et al., 2006; Asomaning et al., 2011). However, it is rare the formation of more than one root or seedling from a single seed at the same time. Apparently, there is a well-established control to prevent reserves of being depleted in simultaneous germination, and a second root formation only begins when the first root is lost (Amador and Barbedo, 2011; Delgado and Barbedo, 2011; Amador and Barbedo, 2015). This allows the same seed to experience adverse conditions without necessarily losing the ability to propagate the species. However, at each new root production, there is a need for increasing periods for the differentiation of these tissues (Alonso, 2018). Considering the successive possible germinations and the fact that more than four germinations have already been computed, these seeds can remain in the field able to form new plantlets until a new crop of seeds is produced.

The recalcitrance from the perspective of maturation

Regardless of dividing the seed into three groups (or slightly more than three) or into a gradient from the most orthodox to the most recalcitrant, little is known about the evolutionary aspects of this difference among them. Are recalcitrant seeds derived from orthodox or vice versa? Or were there alternations, sometimes in the direction of the orthodox and sometimes in those recalcitrant? It is even more difficult to know the factors involved in this evolution since not even the relation among seeds of different behaviors is known.

Grouping always finds exceptions for any classification system that wants to encompass all known behaviors. When we think about desiccation tolerance, we can group them (although systems of experimentation are not always totally unquestionable); when considering storage potential, grouping is also possible. This is true for several other characteristics, such as tolerance to low temperatures and freezing, vivipary, and differences in size, even with very interesting formulas for determining the degree of recalcitrance by the volume to tegument thickness ratio (Daws et al., 2006). However, when all variables related to recalcitrance/orthodoxy are considered, there are always exceptions. For instance, when comparing seeds of *Caesalpinia echinata* with those of *Eugenia brasiliensis* and *E. involucrata*, the former are highly tolerant to desiccation, surviving to water contents as low as 5% (Martini-Neto and Barbedo, 2015), which would classify them as orthodox but, under a refrigeration temperature, they remain viable for only a few months (Barbedo et al., 2002). On the other hand, seeds of *E. brasiliensis* and *E. involucrata*,

highly susceptible to desiccation (they do not tolerate water contents below 45%, Delgado and Barbedo, 2007; 2012) and therefore recalcitrant, can be stored under refrigeration for over six months (Barbedo et al., 1998; Kohama et al., 2006).

Recently, a model was proposed based on the degree of maturation in which the seeds are found when disconnected from the mother plant (Barbedo et al., 2013). In this model, the classical orthodox behavior would be considered the maximum development and maturation to be reached by seeds with all properties (dry matter content, desiccation tolerance, germinative capacity, vigor, etc.), reaching their maximum before seed dispersion. As this detachment is anticipated, some properties would not reach their maximum, and the seed would drift away from the classical orthodox behavior, approaching the recalcitrant behavior. The further from maturity it went, the more recalcitrant it would be, and all the behaviors would be contemplated in that model. Some conceptual advances in this model could be considered and will be discussed below.

Starting from the traditional behavior of changes in classical orthodox seeds during their maturation process, changes in water content can be used (Figure 3A) as a physiological reference of the chronological evolution, from fertilization and zygote formation to seed dispersion. Taking as an example the dry matter content and the ability of seeds to germinate, after cell divisions the dry matter of seeds will increase until it reaches a maximum (Figure 3B), which for many authors would characterize the point of physiological maturity. Similarly, germinability does not arise instantaneously for all seeds and, as maturation progresses, more and more seeds are acquiring this capacity until reaching a maximum (Figure 3B).

With the evolution of species, these curves could be differentiated from each other (Barbedo et al., 2018), each of them adapting to the most favorable conditions of that environment, sometimes anticipating this maximum, sometimes delaying it (Figures 3C, F). For instance, an early dispersion could be more favorable to the species in a given environment, making it more adapted the individuals that accumulated faster dry matter in their seeds, even though sacrificing the maximum accumulated (Figure 3C). This change would occur at a genotypic level, increasing the frequency of alleles related to this adaptation and fixing the new characteristic in the new species (Mayr, 2009). Therefore, this behavior would become the standard for that new species. According to environmental conditions, seed dispersion could occur closer to the end of the standard maturation process, or earlier (Barbedo et al., 2018). The different moments of releasing seeds from the mother plant would produce the different degrees of "recalcitrance" (Barbedo et al., 2013).

However, there are many variations in behavior, not only explained by these different moments of release. This behavior could undergo changes as a function of environmental conditions at each new seed formation. These would derive from geographical or temporal differences, for example.

In each different region, or in different years, soil and climate conditions, for instance, could anticipate the accumulation of reserves, the germinability, and even the dispersion itself (Figures 3D, E, G, H). The maximum values to be reached could also vary, for example, by sacrificing a higher dry matter accumulation or a higher percentage of germination in favor of an early release of seeds in the environment (Figure 4), characterizing a variation in a phenotypic level, therefore always dependent on environmental conditions. This variation

was observed in some species, such as *Aesculus hippocastanum* (Daws et al., 2004), *Eugenia pyriformis* (Lamarca et al., 2016), and *Inga vera* (Lamarca and Barbedo, 2015). Note that the observer of that species, in that year and location, would not be able to perceive this anticipation since it would only be able to obtain the data as presented in Figures 3E and 4. This observer would consider that dispersion as being the moment of maximum possible development for that species.

The same could be said about the germinability. If a species needs to disperse its seeds early, it would need to anticipate the maximum germination, as simulated in Figure 3F. This evolutionary investment could be at the expense, for example, of the investment in the capacity to store for long periods or even in the desiccation tolerance (Figure 5).

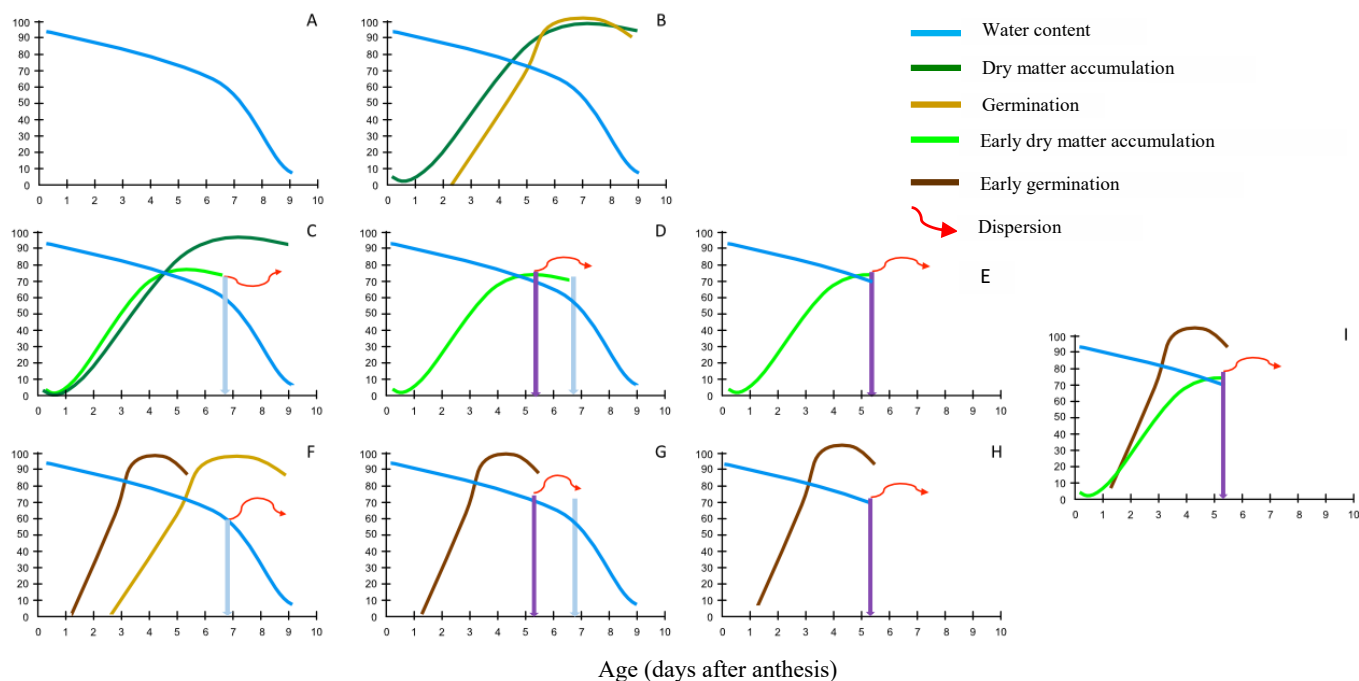


Figure 3. Variations in water content (A) and dry matter content and germination capacity (B) in seeds over maturation. Seed dispersion would occur at the end of the maturation drying, i.e., at the lowest value of water content. By mutation and natural selection processes, the species could change the traditional curve to one with an early dry matter accumulation, which may be due to an early dispersion, as indicated by the arrow (C). Standardized curves with generic behavior for species with classical orthodox seeds (B) could, therefore, be altered to adapt to the conditions of the environment in which each species is selected. The early maximum dry matter accumulation for an early dispersion (C) could require less investment in this accumulation, sacrificing part of the total amount to be accumulated. Thus, there would be a new pattern of dry matter accumulation for this species. This pattern could also undergo environmental action at each production cycle depending on the year and location. This action could further anticipate the moment of dispersion (D). However, the observer at this location and year would have access only to the information until the moment of dispersion (E) and could consider this the pattern of the species. Likewise, there could be variations in the acquisition of the germinative capacity (F). An even greater early dispersion would not affect the germinative capacity since its maximum was also much early (G), leading the observer to conclude that this is the maximum possible development for the species (H). This observation could be performed for all seed characteristics of that species, in that year and location (I).

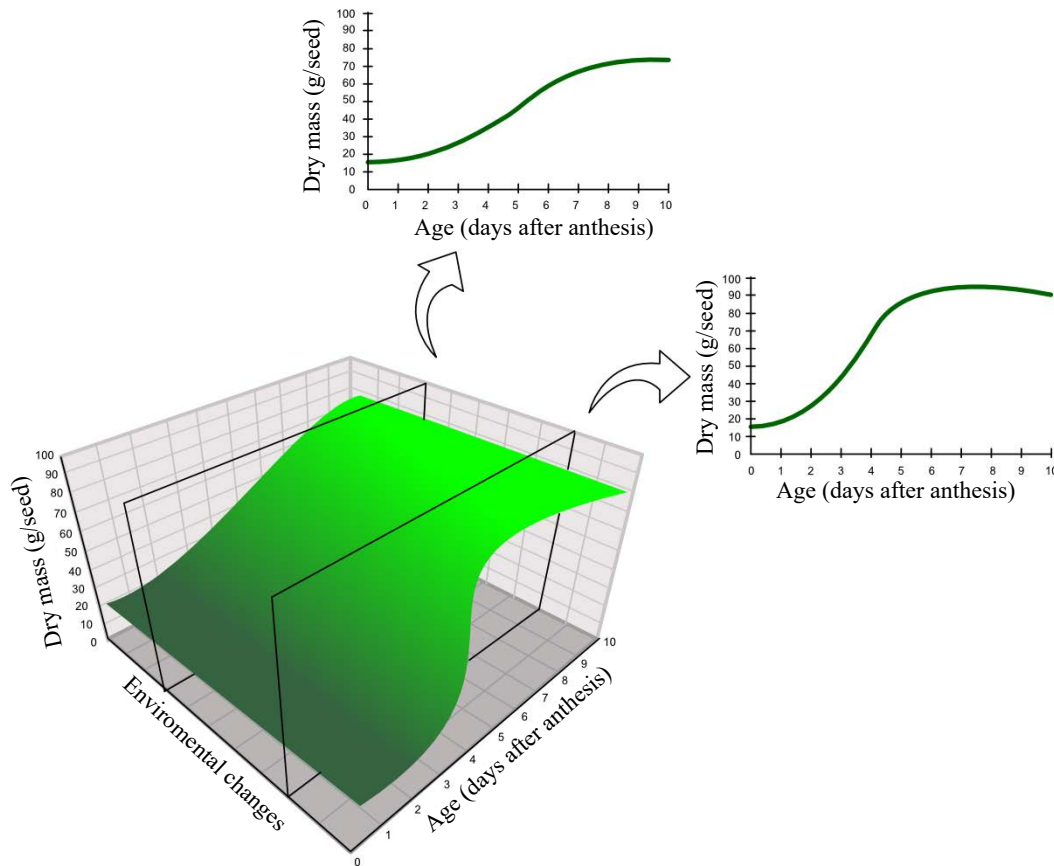


Figure 4. Hypothetical diagram of the environmental action on the reserve accumulation during seed maturation. The frontal observation by the age axis (X-axis) with the dry matter axis in the vertical (Y-axis) would produce a two-dimensional graph, characterizing the expected pattern for the species. The environmental variables (Z-axis) would alter this pattern, producing a response surface. The dry matter accumulation curve would be the result of traversing the standard curve on the Z-axis. Going through the back or front, we would have the oscillations in the dry matter accumulation of the same species in different years, or in different regions, i.e., the phenotypic variation. A cut-off plane at a given point, characterizing the conditions of the environment in a particular year and location, would show the resulting curve as a two-dimensional graph: if it is more in front, it will show an early accumulation in relation to a cut more in back, as illustrated in the two-dimensional graphs resulting from two cut-off planes of the response surface.

However, it would guarantee that an early dispersion could still be performed with the maximum of seeds in conditions to germinate and colonize the environment (Figure 3G). Again, the observer of this species, in this location and year, could interpret the maximum in germination as the maximum possible development of these seeds (Figure 3H). Thus, the observer of that species, in that location and year, would interpret changes in dry matter accumulation and germination capacity before its dispersion as normal (Figure 3I). This would be true for all other characteristics, for instance, desiccation tolerance and ability to maintain the viability for long storage periods (Figures 5 and 6), ability to germinate under suboptimal conditions, development of antioxidant

systems, and various aspects considered in seed vigor. This observer could interpret that sensitivity to desiccation and short longevity, the main separators of recalcitrant, orthodox, and intermediate groups, could be a standard for “mature” seeds of species, and not that seeds actually shortened their whole process of maturation, anticipating the dispersion.

Thus, for each seed characteristic, changes in values and moments of peaks could represent advantages over the environment, allowing the species with such characteristics to be selected. The anticipation of the maximum in germination may have demanded to sacrifice part of the lot, and the maximum in germination would be below the maximum possible. However, if a seed had environmental advantages in

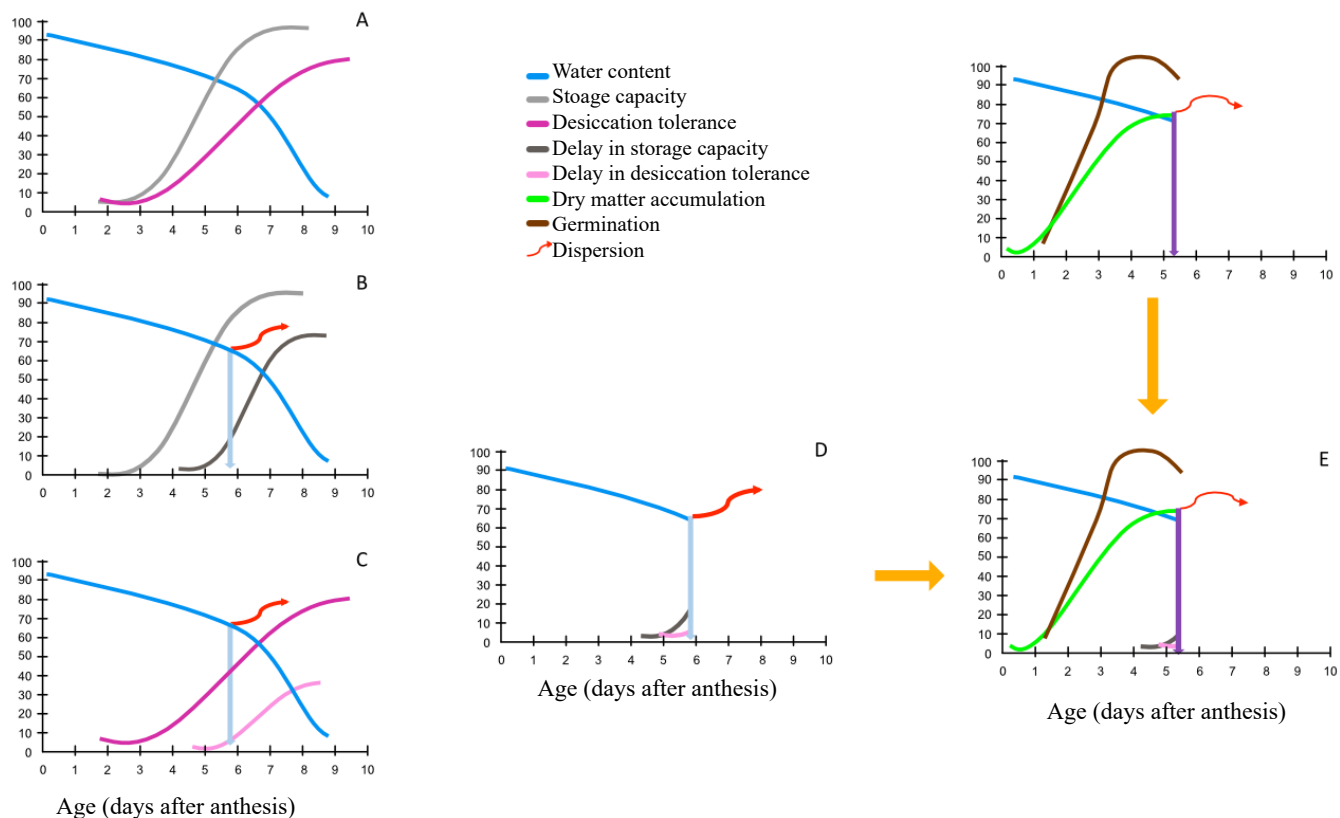


Figure 5. Variations in water content, desiccation tolerance, and storage capacity (A) in seeds during their maturation. By mutation and natural selection processes, the species could dispense with the investment in prolonged storage (B) and desiccation tolerance (C), delaying the onset of these processes. The observer would only have access to the information until the moment of dispersion (D) and could consider this the maximum maturation potential of seeds of the species. The species could adopt this strategy to guarantee high germination rates already at the beginning of seed maturation (E, derived from Figure 3), perhaps due to an early dispersion in an environment favorable to colonization and highly competitive. Similar to the dry matter and germination curves, a new pattern of desiccation tolerance and longevity would be obtained and the observer of this species would consider the set of characteristics of the seeds dispersed as the pattern of a mature seed (E).

being dispersed and germinating immediately after shedding to colonize the environment at that time, it would not require much from the vigor or even from the desiccation tolerance. For such seeds, it would be highly desirable to delay the onset of desiccation tolerance development. If the environment did not become favorable in that year and location, it could continue its development, completing the maturation and all other favorable characteristics. The evolutionary cost of maintaining all this potential, however, could not allow the establishment of the species, being necessary to prioritize this or that characteristic.

It would be interesting, now, to imagine the dynamics of all these lines of maturation moving back and forth, up and down, producing all manner of behavior in seeds of different species. This model allows including a great

diversity of behaviors among seeds of different species. For instance, seeds of *Caesalpinia echinata* are highly tolerant to desiccation (Martini-Neto and Barbedo, 2015), but they cannot be stored under hot or cold conditions for more than a few months (Barbedo et al., 2002), being necessary their freezing (Mello et al., 2013). Lamarca and Barbedo (2012) demonstrated that a fast deterioration of these seeds occurs through oxidative processes. Therefore, it is likely that, in the evolution process, the species has anticipated the beginning of the development of desiccation tolerance, sacrificing the development of antioxidant systems. On the other hand, it may also have sacrificed the development of a strong glassy state, which would guarantee a higher capacity to withstand a prolonged storage (Walters, 2015).

Seeds of some species of *Eugenia*, such as *E. brasiliensis*,

unlike those of *C. echinata*, may have delayed the beginning of the development of desiccation tolerance, being highly sensitive (Delgado and Barbedo, 2007), but invested a little more in storability (Kohama et al., 2006) or even in alternative ways of staying in the environment for long periods with the capacity to produce new plants (Teixeira and Barbedo, 2012; Alonso, 2018). The regenerative capacity of *Eugenia* seeds, together with their potential to germinate and produce plantlets with less than half of the original reserve tissue (Silva et al., 2003; 2005; Teixeira and Barbedo, 2012), could

be interpreted as an evolutionary investment that would bring dry matter accumulation to very close to the Y-axis. Thus, for instance, changes in the dry matter of seeds over maturation cannot be observed (Lamarca et al., 2013b).

Once established a new pattern of maturation of seeds of the species, we would still have to consider the influence of the environment on each cycle of production of these seeds. Therefore, we would have two important sources of variation determining seed behavior. The first source of variation would be genotypic, establishing the behavior pattern for the

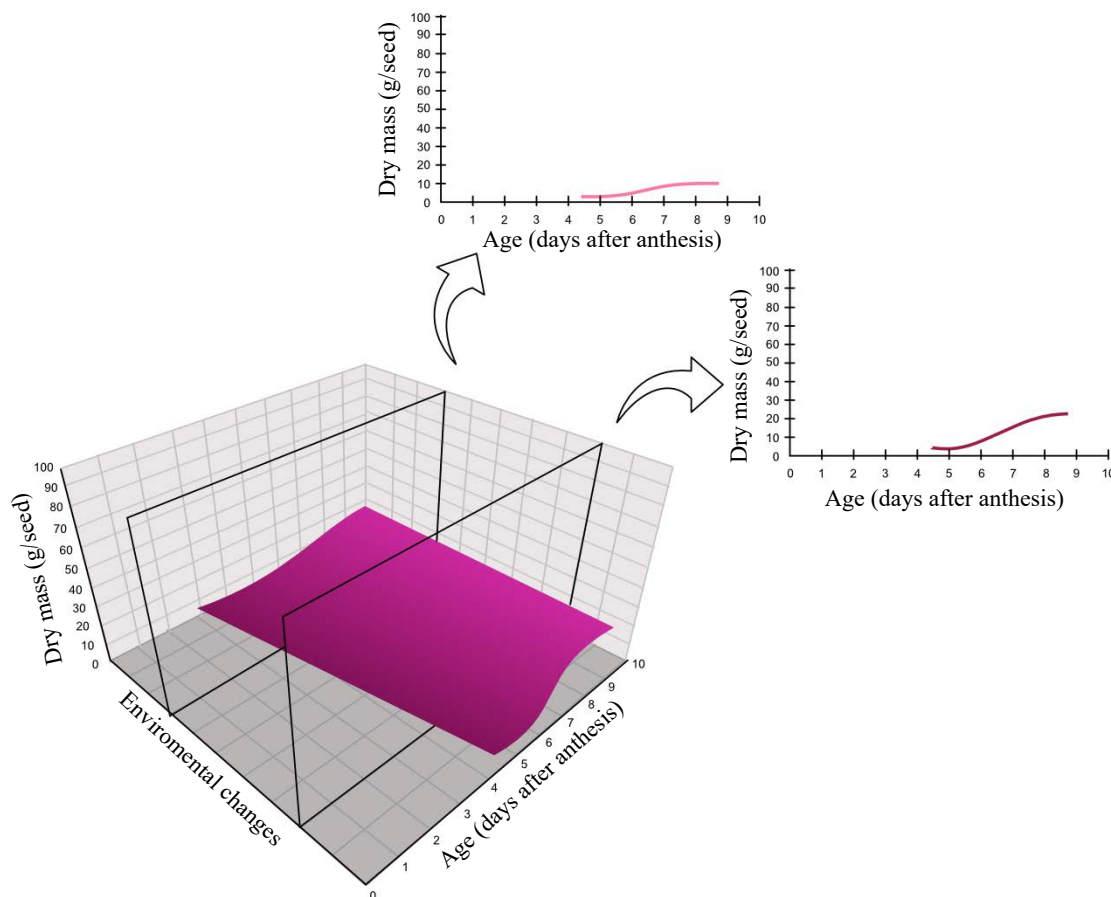


Figure 6. Hypothetical diagram of the environmental action on the acquisition of desiccation tolerance during seed maturation. The frontal observation by the age axis (X-axis) with the desiccation tolerance axis in the vertical (Y-axis) would produce a two-dimensional graph, characterizing the expected pattern for the species. The environmental variables (Z-axis) would alter this pattern, producing a response surface. The desiccation tolerance curve would be the result of traversing the standard curve on the Z-axis. Going through the back or front, we would have the oscillations in the desiccation tolerance of the same species in different years, or in different regions, i.e., the phenotypic variation. These oscillations could also be obtained by applying special techniques of induction of desiccation tolerance such as osmoconditioning and application of reduced glutathione. A cut-off plane at a given point, characterizing the conditions of the environment in a particular year and location, would show the resulting curve as a two-dimensional graph: if it is more in front, it will show a high desiccation tolerance in relation to a cut more in back, as illustrated in the two-dimensional graphs resulting from two cut-off planes of the response surface. However, note that the oscillations did not allow reaching the high levels of the classical orthodox seeds.

species in relation to its evolution and could be interpreted by variations in the curves of different characteristics, sometimes anticipating the process, sometimes delaying it; sometimes reaching higher peaks, sometimes reducing the maximum values to be reached. These variations would be responsible for the great differences between orthodox, recalcitrant, and intermediate seeds, creating gradients from the most orthodox to the most recalcitrant, with all the variations of behavior observed until today. This is graphically represented in Figures 3 and 5, with examples of changes in dry matter content, germinability, storability and desiccation tolerance.

The second important source of variation would be the phenotypic, i.e., the environment changing the standard behavior of the species. These variations would be responsible for small differences between seeds within the same species, leading to the different results obtained in different works with the same species, such as in *Caesalpinia echinata* (Borges et al., 2005; Mello et al., 2011; Leduc et al., 2012), *Aesculus hippocastanum* (Daws et al., 2004), *Eugenia pyriformis* (Lamarca et al., 2011; Scalon et al., 2012; Lamarca et al., 2016), and *Inga vera* (Lamarca et al., 2013a; Bilia et al., 1999; Barbedo and Cicero, 2000; Lamarca and Barbedo, 2015). It is important to note that the further away from the extreme of orthodoxy the species is, the more the environment may influence it at each cycle.

For a visualization of these variations, we could imagine that the curves of Figures 3 and 5 are actually profiles of response surfaces of Figures 4 and 6. These surfaces would have, on the axis of depth, the variations of the environment changing the standard behavior of the species. Thus, imagining the X-axis as the time during maturation and the Y-axis as the characteristic quantification (for instance, germination percentage, g of dry matter, desiccation tolerance, or storage time), the Z-axis would correspond to the environmental variations that could alter each line (Figures 4 and 6). Thus, to see the dry matter accumulation of seeds of a species in a particular region in a given year, it would be necessary to “slice” the 3D graph at some point. It could be, for instance, in the cut-off planes of the axis of environmental variations, as illustrated in Figures 4 and 6. Cutting at those points would change the response to dry matter accumulation for the graphs derived from the cuts.

Because evolution does not cease, this same response surface, which today represents small environmental variations within the species, on an evolutionary scale could also be interpreted as the accumulation of variations that resulted, or will result, in the formation of a new seed behavior of a new species. In addition, the Z-axis, i.e., the axis of the influence of environmental variations, could also be seen as the result of the application of artificial induction techniques, such as tolerance to desiccation or cold, already demonstrated

for some species (Bonjovani and Barbedo, 2008; Leduc et al., 2012; Kalemba and Ratajczak, 2018). Even dormancy could be seen as one more of the lines, or response surfaces, with possibilities of changes depending on the environment, regardless of the separation into orthodox and recalcitrant. Thus, dormancy must be separated from specific stages of maturation, as proposed by Barbedo et al. (2013), and may even be induced (Molizane et al., 2018). Therefore, unlike the previous models, which considered dispersed recalcitrant seeds as effectively mature, and that always found several exceptions, all the behaviors are contemplated in this model. Of course, there is still insufficient information in the literature to allow technology to be used to prolong the maturation period of the most recalcitrant seeds so that they can survive the drying and be stored for long periods. However, for a better understanding of this process, it is possible to study in a greater depth the immature phases of classical orthodox seeds, comparing them with the so-called “mature” recalcitrant ones. The best understanding of factors responsible for their early dispersion could bring great advances in terms of germplasm conservation and substantially assist in the preservation of plant biodiversity, especially of endangered species.

Concluding remarks

About a century ago the first seeds susceptible to desiccation, i.e., with behavior different from that expected for seeds in general, began to be studied (Kidd, 1914). Half a century later, to ensure the validity of storage predictability equations, these seeds were isolated from the classic ones and named recalcitrant seeds (Roberts, 1973). Since then, many new species have been, and continue to be, discovered, as well as new seed behaviors (Barbedo and Bilia, 1998). Except for practical purposes, by defining which species have seeds that can be stored or not, the orthodox-recalcitrant dichotomy limits a better understanding of the broad spectrum of physiological responses presented by seeds, which should be understood as a gradient of responses (Walters, 2015). One way of interpreting this gradient could be by the degree of maturation in which seeds are dispersed, i.e., the more immature, the more recalcitrant (Barbedo et al., 2013).

In this review, we tried to advance this model, presenting two sources of variation. In the first, with a more stable level (genotypic) that fixes certain characteristics for seeds of a species, it is understood that the evolution adapted the classic modifications of seed characteristics during its formation, aiming at anticipating its dispersion in the face of environments that demanded the fastest establishment of the species. In the second, with a more unstable level

(phenotypic), it is considered that those characteristics can still be influenced by the specific environmental conditions of that year and location. Here, examples from the literature were shown demonstrating these different responses for the same species when the research is carried out with seeds from different years or regions. These modifications have very restricted limits since larger modifications would depend on longer binding time to the mother plant. These small modifications correspond, for instance, to the displacement of desiccation tolerance and storage potential curves described by Barbedo et al. (2013).

The effect of the environment on each production cycle, which was considered above as causing phenotypic variations, could also be seen as caused by applications of artificial techniques, which would simulate some condition of seed maturation (Barbedo et al., 2013). It should be noted, however, that they would also be limited effects, evidently not comparable to the effective complementation of seed maturation in the mother plant. An example would be the inductions of tolerance to desiccation or cold when harvested seeds are submitted to some type of stress (Leduc et al., 2012; Bonjovani and Barbedo, 2014).

Evidently, this is a subject where there are still more doubts than certainties and new approaches must always be considered. However, it is important to note that, unlike previous models, in this, all known seed behaviors are contemplated. If, in fact, the degree of seed recalcitrance is due to evolutionary adaptations that modified the curves of dry matter accumulation, desiccation tolerance, development of germinability, storability, vigor, among others, few advances are expected to increase the viability conservation of these seeds during the classic storage, even under refrigerated conditions. Studies would be needed to extend the maturation period of seeds in the mother plant, preventing their “early” dispersion and approaching them to maturation cycles of orthodox seeds. However, it is not yet possible to know whether the genes linked to the development of desiccation tolerance, for example, were lost in the species with recalcitrant seeds, or if they were only switched off. Or even if both conditions are found, depending on the species. Some recent studies have clues on the relationship between certain genes and the evolution of seed characteristics during maturation (Delahaie et al., 2013; Zinsmeister et al., 2016). Apparently, there is some lack of process inducers such as the acquisition of desiccation tolerance and longevity enhancement and not necessarily the conditions for the processes to occur. It is also possible that in recalcitrant seeds there is a silencing of those inducers. However, much remains to be investigated in this area.

Currently, it is very far from a possible control over

the maturation cycle of seeds, in the sense of enlarging it, since little is known about the maturation itself. Important information would be obtained from studies with the immature (or recalcitrant) stages of orthodox seeds, but such studies are also scarce. In a more encouraging way, it is important to recognize the progress that has been made, with an increasing number of research groups interested in these seeds and with an increasing number of studied species. Studies performed with the same species under different environmental conditions, varying the location or time of seed production, become essential to clarify which factors are more or less influenced by the environment. In this sense, the exchange of information between groups of different regions is essential. Studies focusing on specificities, such as those anatomical and biochemical, or those related to desiccation tolerance, are very important, but it must be clear that they are not the only ones, and other areas, such as the ecological and technological, are equally important in an information feedback system.

Acknowledgments

To the Editors of the Journal of Seed Science for the invitation to produce this review. To Dr. Debora Manzano Molizane for the elaboration of the Figures of this article. To Dr. Adelianna Saes Coelho Barbedo and Dr. João Paulo Naldi Silva for the critical reading of the manuscript. To Dr. R.C.L. Figueiredo-Ribeiro and Dr. D.C. Centeno, with whom I have developed most of these ideas. To all my students, from the past and present, without whom many of the essential works of this review would not exist.

References

- ALONSO, C.R. Germinação múltipla e sequencial de sementes como estratégia de propagação em espécies de *Eugenia* (Myrtaceae). Dissertação de Mestrado. São Paulo, Instituto de Botânica, 2018.
- AMADOR, T.S.; BARBEDO, C.J. Potencial de inibição da regeneração de raízes e plântulas em sementes germinantes de *Eugenia pyriformis*. *Pesquisa Agropecuária Brasileira*, v.46, p.814-821, 2011. <http://www.scielo.br/pdf/pab/v46n8/05.pdf>
- AMADOR, T.S.; BARBEDO, C.J. Germination inhibits the growth of new roots and seedlings in *Eugenia uniflora* and *Eugenia brasiliensis*. *Journal of Seed Science*, v.37, p.241-247, 2015. http://www.scielo.br/pdf/jss/v37n3/2317-1537-jss-37-03-2317_1545v37n3150595.pdf
- AMOEDO, S.C.; FERRAZ, I.D.K. Seed quality evaluation by tetrazolium staining during a desiccation study of the recalcitrant seeds of *Carapa guianensis* Aubl. and *Carapa surinamensis* Miq. - Meliaceae. *African Journal of Agricultural Research*, v.12, p.1005-1013, 2017. <https://doi.org/10.5897/AJAR2016.11854>

- ANDRÉO, Y.; NAKAGAWA, J.; BARBEDO, C.J. Mobilização de água e conservação da viabilidade de sementes recalcitrantes de ingá (*Inga vera* Willd. subsp. *affinis* (DC.) T.D. Pennington). *Revista Brasileira de Botânica*, v.29, p.309-318, 2006. <http://www.scielo.br/pdf/00D/rbb/v29n2/a12v29n2.pdf>
- ANJOS, A.M.G.; FERRAZ, I.D.K. Morfologia, germinação e teor de água das sementes de araçá-boi (*Eugenia stipitata* ssp. *sororia*). *Acta Amazonica*, v.29, p.337-348, 1999. <http://www.scielo.br/pdf/aa/v29n3/1809-4392-aa-29-3-0337.pdf>
- ARALDI, C.G.; COELHO, C.M.M. Establishment of post-harvest early-developmental categories for viability maintenance of *Araucaria angustifolia* seeds. *Acta Botanica Brasílica*, v.29, p.524-531, 2015. <http://www.scielo.br/pdf/abb/v29n4/0102-3306-abb-29-04-00524.pdf>
- ARALDI, C.G.; COELHO, C.M.M.; MARASCHIN, M. Metabolic profile of Brazilian pine embryos and megagametophyte of stored seeds. *African Journal of Agricultural Research*, v.11, p.760-768, 2016. <https://doi.org/10.5897/AJAR2015.10054>
- ASOMANING, J.M.; OLYMPIO, N.S.; SACANDE, M. Desiccation sensitivity and germination of recalcitrant *Garcinia kola* Heckel seeds. *Research Journal of Seed Science*, v.4, p.15-27, 2011. <http://docsdrive.com/pdfs/academicjournals/rjss/2011/15-27.pdf>
- AVILA, A.L.; ARGENTA, M.S.; MUNIZ, M.F.B.; POLETO, I.; BLUME, E. Maturação fisiológica e coleta de sementes de *Eugenia uniflora* L. (pitanga), Santa Maria, RS. *Ciência Florestal*, v.19, p.61-68, 2009. <https://periodicos.ufsc.br/index.php/biotemas/article/viewFile/2175-7925.2012v25n3p11/22796>
- BALLESTEROS, D.; SERSHEN; VARGHESE, B.; BERJAK, P.; PAMMENTER, N.W. Uneven drying of zygotic embryos and embryonic axes of recalcitrant seeds: challenges and considerations for cryopreservation. *Cryobiology*, v.69, p.100-109, 2014. <https://doi.org/10.1016/j.cryobiol.2014.05.010>
- BARBEDO, C.J.; BILIA, D.A.C. Evolution of research on recalcitrant seeds. *Scientia Agricola*, v.55 (especial), p.121-125, 1998. <http://www.scielo.br/pdf/sa/v55nspe/3161.pdf>
- BARBEDO, C.J.; CICERO, S.M. Effects of initial quality, low temperature and ABA on the storage of seeds of *Inga uruguensis*, a tropical species with recalcitrant seeds. *Seed Science and Technology*, v.28, p.793-808, 2000.
- BARBEDO, C.J.; KOHAMA, S.; MALUF, A.M.; BILIA, D.A.C. Germinação e armazenamento de diásporos de cerejeira (*Eugenia involucrata* DC. - Myrtaceae) em função do teor de água. *Revista Brasileira de Sementes*, v.20, p.184-188, 1998. <http://dx.doi.org/10.17801/0101-3122/rbs.v20n1p184-188>
- BARBEDO, C.J.; BILIA, D.A.C.; FIGUEIREDO-RIBEIRO, R.C.L. Tolerância à dessecação e armazenamento de sementes de *Caesalpinia echinata* Lam. (pau-brasil), espécie da Mata Atlântica. *Revista Brasileira de Botânica*, v.25, p.431-439, 2002. <http://www.scielo.br/pdf/rbb/v25n4/a07v25n4.pdf>
- BARBEDO, C.J.; CENTENO, D.C.; FIGUEIREDO-RIBEIRO, R.C.L. Do recalcitrant seeds really exist? *Hoehnea*, v.40, p.583-593, 2013. <http://www.scielo.br/pdf/hoehnea/v40n4/01.pdf>
- BARBEDO, C.J.; SILVA, J.P.N.; FRANÇOSO, C.F.; PARISI, J.J.D. Armazenamento de sementes. In: BARBEDO, C.J.; SANTOS JR., N.A. (Eds.). *Sementes do Brasil*. São Paulo: Instituto de Botânica, 2018. p.79-106.
- BILIA, D.A.C.; NOVENBRE, A.D.L.C.; MARCOS-FILHO, J. Desiccation tolerance and seed storability of *Inga uruguensis* (Hook. et Arn.). *Seed Science and Technology*, v.27, p.77-89, 1999.
- BONJOVANI, M.R.; BARBEDO, C.J. Sementes recalcitrantes: intolerantes a baixas temperaturas? Embriões recalcitrantes de *Inga vera* Willd. subsp. *affinis* (DC.) T.D. Penn. toleram temperatura sub-zero. *Revista Brasileira de Botânica*, v.31, p.345-356, 2008. <http://www.scielo.br/pdf/rbb/v31n2/v31n2a17.pdf>
- BONJOVANI, M.R.; BARBEDO, C.J. Induction of tolerance to desiccation and to subzero temperatures in embryos of recalcitrant seeds of inga. *Journal of Seed Science*, v.36, p.419-426, 2014. <http://www.scielo.br/pdf/jss/v36n4/a06v36n4.pdf>
- BORGES, I.F.; DEL GIUDICE NETO, J.; BILIA, D.A.C.; FIGUEIREDO-RIBEIRO, R.C.L.; BARBEDO, C.J. Maturation of seeds of *Caesalpinia echinata* Lam. (brazilwood), an endangered leguminous tree from the Brazilian Atlantic Forest. *Brazilian Archives of Biology and Technology*, v.48, p.851-861, 2005. <http://www.scielo.br/pdf/babt/v48n6/27430.pdf>
- CALVI, G.P.; ANJOS, A.M.G.; KRANER, I.; PRITCHARD, H.W.; FERRAZ, I.D.K. Exceptional flooding tolerance in the totipotent recalcitrant seeds of *Eugenia stipitata*. *Seed Science Research*, v.27, p.121-130, 2017. <https://doi.org/10.1017/S0960258517000125>
- CALVI, G.P.; AUD, F.F.; FERRAZ, I.D.K.; PRITCHARD, H.W.; KRANER, I. Analyses of several seed viability markers in individual recalcitrant seeds of *Eugenia stipitata* McVaugh with totipotent germination. *Plant Biology*, v.19, p.6-13, 2016. <https://onlinelibrary.wiley.com/doi/epdf/10.1111/plb.12466>
- CARDON, Z.G.; PEREDO, E.L.; DOHNALKOVA, A.C.; GERSHON, H.L.; BEZANILLA, M. A model suite of green algae within the Scenedesmaceae for investigating contrasting desiccation tolerance and morphology. *Journal of Cell Science*, v.131, doi: 10.1242/jcs.212233, 2018. <http://jcs.biologists.org/content/131/7/jcs212233>
- CHANDRA, J.; KESHAVKANT, S. Desiccation-induced ROS accumulation and lipid catabolism in recalcitrant *Madhuca latifolia* seeds. *Physiology and Molecular Biology of Plants*, v.24, p.75-87, 2018. <https://doi.org/10.1007/s12298-017-0487-y>
- CHANG, C. *Rethinking Prehistoric Central Asia*. London: Routledge, 2018. 144p.
- CHAPELL, J.H.; WANG, Y.; COHN, M.A. Recalcitrant and orthodox *Spartina* seeds or isolated embryos exhibit similar leachate patterns immediately following desiccation. *Seed Science Research*, v.25, p.247-254, 2015. <https://www.cambridge.org/core/journals/seed-science-research/article/recalcitrant-and-orthodox-spartina-seeds-or-isolated-embryos-exhibit-similar-leachate-patterns-immediately-following-desiccation/016E4D4C613A2A7E9016815D3B162CF1>
- COMIN, A.; PEREIRA, L.D.; MACIEL, C.G.; CHIES, J.; MUNIZ, M.F.B. Secagem e armazenamento de sementes de *Eugenia uniflora* L. *Revista Brasileira de Ciências Agrárias*, v.9, p.84-90, 2014. <https://doi.org/10.5039/agraria.v9i1a2786>

- CORSATO, J.M.; FERREIRA, G.; BARBEDO, C.J. Desiccation tolerance in seeds of *Annona emarginata* (Schldtl.) H. Rainer and action of plant growth regulators on germination. *Brazilian Journal of Plant Physiology*, v.24, p.253-260, 2012. <http://www.scielo.br/pdf/bjpp/v24n4/a04v24n4.pdf>
- CÔRTEZ, M.C.; CAZETTA, E.; STAGGEMEIER, V.G.; GALETTI, M. Linking frugivore activity to early recruitment of a bird dispersed tree, *Eugenia umbelliflora* (Myrtaceae) in the Atlantic rainforest. *Australian Ecology*, v.34, p.249-258, 2009. <https://doi.org/10.1111/j.1442-9993.2009.01926.x>
- DAWS, M.I.; LYDALL, E.; CHMIELARZ, P.; LEPRINCE, O.; MATTHEWS, S.; THANOS, C.A.; PRITCHARD, H.W. Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe. *New Phytologist*, v.162, p.157-166, 2004. <https://doi.org/10.1111/j.1469-8137.2004.01012.x>
- DAWS, M.I.; GARWOOD, N.C.; PRITCHARD, H.W. Prediction of desiccation sensitivity in seeds of woody species: a probabilistic model based on two seed traits and 104 species. *Annals of Botany*, v.97, p.667-674, 2006. <https://doi.org/10.1093/aob/mcl022>
- DELAHAIE, J.; HUNDERTMARK, M.; BOVE, J.; LEPRINCE, O.; ROGNIAUX, H.; BUITINK, J. LEA polypeptide profiling of recalcitrant and orthodox legume seeds reveals ABI3-regulated LEA protein abundance linked to desiccation tolerance. *Journal of Experimental Botany*, v.64, p.4559-4573, 2013. <https://doi.org/10.1093/jxb/ert274>
- DELGADO, L.F.; BARBEDO, C.J. Tolerância à dessecação de sementes de espécies de *Eugenia*. *Pesquisa Agropecuária Brasileira*, v.42, p.265-272, 2007. <http://www.scielo.br/pdf/pab/v42n2/16.pdf>
- DELGADO, L.F.; BARBEDO, C.J. Atividade inibidora da germinação em extratos de sementes de *Eugenia uniflora* L. *Revista Brasileira de Sementes*, v.33, p.463-471, 2011. <http://www.scielo.br/pdf/rbs/v33n3/09.pdf>
- DELGADO, L.F.; BARBEDO, C.J. Water potential and viability of seeds of *Eugenia* (Myrtaceae), a tropical tree species, based upon different levels of drying. *Brazilian Archives of Biology and Technology*, v.55, p.583-590, 2012. <http://www.scielo.br/pdf/babt/v55n4/a14v55n4.pdf>
- DELGADO, L.F.; MELLO, J.I.O.; BARBEDO, C.J. Potential for regeneration and propagation from cut seeds of *Eugenia* (Myrtaceae) tropical tree species. *Seed Science and Technology*, v.38, p.624-634, 2010. <https://doi.org/10.15258/sst.2010.38.3.10>
- EADS, A.R.; MITCHELL, N.J.; EVANS, J.P. Patterns of genetic variation in desiccation tolerance in embryos of the terrestrial-breeding frog, *Pseudophryne guentheri*. *Evolution*, v.66, p.2865-2877, 2012. <https://doi.org/10.1111/j.1558-5646.2012.01616.x>
- FALLARD, A.; RABERT, C.; REYES-DÍAZ, M.; ALBERDI, M.; BRAVO, L.A. Compatible solutes and metabolites accumulation does not explain partial desiccation tolerance in *Hymenoglossum cruentum* and *Hymenophyllum dentatum* (Hymenophyllaceae) two filmy ferns with contrasting vertical distribution. *Environmental and Experimental Botany*, v.15, p.272-279, 2018. <https://doi.org/10.1016/j.envexpbot.2018.02.002>
- FÉLIX, F.C.; ARAÚJO, F.S.; FERRARI, C.S.; PACHECO, M.V. Dessecação e armazenamento de sementes de *Adonidia merrillii* (Becc.) Becc. *Revista Brasileira de Ciências Agrárias*, v.12, p.86-91, 2017. <http://www.redalyc.org/articulo.oa?id=119050448014>
- FRANÇOSO, C.F.; BARBEDO, C.J. Tratamentos osmóticos e térmicos para controle de fungos em sementes de grumixameira (*Eugenia brasiliensis* Lam.) e pitangueira (*Eugenia uniflora* L.). *Hoehnea*, v.41, p.541-552, 2014. <http://www.scielo.br/pdf/hoehnea/v41n4/0073-2877-hoehnea-41-04-0541.pdf>
- GAGGETI, B.L.; PIRATELLI, A.J.; PIÑA-RODRIGUES, F.C.M. Fruit color preference by birds and applications to ecological restoration. *Brazilian Journal of Biology*, v.76, p.955-966, 2016. <http://www.scielo.br/pdf/bjb/v76n4/1519-6984-bjb-1519-698405115.pdf>
- GARCIA, V.A.; BARBEDO, C.J. Estudo fenológico de *Bactris gasipes* Kunth, *Euterpe edulis* Mart. e *Syagrus romanzoffiana* (Cham.) Glassman no Vale do Ribeira, SP, Brasil. *Hoehnea*, v.43, p.135-149, 2016. <http://www.scielo.br/pdf/hoehnea/v43n1/2236-8906-hoehnea-43-01-0135.pdf>
- GARCIA, C.; COELHO, C.M.M.; MARASCHIN, M.; SOARES, F.L.F.; GUERRA, M.P.; WILHELM-FILHO, D. Biochemical changes in *Araucaria angustifolia* (Araucariaceae) zygotic embryos during the storage. *Revista de Biologia Tropical*, v.63, p.1185-1196, 2015. <https://doi.org/10.15517/rbt.v63i4.16415>
- GASPARIN, E.; FARIA, J.M.R.; JOSÉ, A.C.; HILHORST, H.W.M. Physiological and ultrastructural responses during drying of recalcitrant seeds of *Araucaria angustifolia*. *Seed Science and Technology*, v.45, p.112-129, 2017. <https://doi.org/10.15258/sst.2017.45.1.01>
- HENNIPMAN, H.S.; SANTOS, A.F.; VIEIRA, E.S.N.; AUER, C.G. Qualidade sanitária e fisiológica de sementes de *Araucaria* durante armazenamento. *Ciência Florestal*, v.27, p.643-654, 2017. <http://ainfo.cnptia.embrapa.br/digital/bitstream/item/161478/1/2017-Alvaro-CF-Qualidade.pdf>
- HOSSEL, C.; HOSSEL, J.S.A.O.; ALEGRETTI, A.L.; WAGNER JUNIOR, A.; DALLAGO, A. Hidrocondicionamento e atmosfera modificada ou controlada para a conservação da viabilidade de sementes de cerejeira-da-mata. *Brazilian Journal of Applied Technology for Agricultural Science*, v.9, p.99-106, 2016. <https://doi.org/10.5935/PAeT.V9.N2.11>
- JACKSON, M.A.; ERHAN, S.; POPRAWSKI, T.J. Influence of formulation additives on the desiccation tolerance and storage stability of blastospores of the entomopathogenic fungus *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes). *Biocontrol Science and Technology*, v.16, p.61-75, 2006. <https://doi.org/10.1080/09583150500188197>
- JANIS, B.; UVERSKY, V.N.; MENZE, M.A. Potential functions of LEA proteins from the brine shrimp *Artemia franciscana* - anhydropobiosis meets bioinformatics. *Journal of Biomolecular Structure and Dynamics*, published on line, 2017. <https://doi.org/10.1080/07391102.2017.1387177>
- JOSHI, G.; ARUNKUMAR, A.N.; GOWDA, B.; SRINIVASA, Y.B. Production of supernumerary plants from seed fragments in *Garcinia gummi-gutta*: evolutionary implications of mammalian frugivory. *Current Science*, v.91, p.372-376, 2006. www.iisc.ernet.in/currensci/aug102006/372.pdf

- JOSHI, G.; PHARTYAL, S.S.; KHAN, S.S.; ARUNKUMAR, A.N. Recalcitrant morphological traits and intermediate storage behaviour in seeds of *Mesua ferrea*, a tropical evergreen species. *Seed Science and Technology*, v.43, p.121-126, 2015. <https://doi.org/10.15258/sst.2015.43.1.13>
- JOSHI, G.; PHARTYAL, S.S.; ARUNKUMAR, A.N. Non-deep physiological dormancy, desiccation and low-temperature sensitivity in seeds of *Garcinia gummi-gutta* (Clusiaceae): a tropical evergreen recalcitrant species. *Tropical Ecology*, v.58, p.241-250, 2017. http://www.tropecol.com/pdf/open/PDF_58_2/3.%20Joshi%20et%20al.pdf
- JUSTO, C.F.; ALVARENGA, A.A.; ALVES, E.; GUIMARÃES, R.M.; STRASSBURG, R.C. Efeito da secagem, do armazenamento e da germinação sobre a micromorfologia de sementes de *Eugenia pyriformis* Camb. *Acta Botanica Brasílica*, v.21, p.539-551, 2007. <http://www.scielo.br/pdf/abb/v21n3/a04v21n3.pdf>
- KALEMBA, E.M.; RATAJCZAK, E. The effect of a doubled glutathione level on parameters affecting the germinability of recalcitrant *Acer saccharinum* seeds during drying. *Journal of Plant Physiology*, v.223, p.72-83, 2018. <http://doi.org/10.1016/j.jplph.2018.02.010>
- KELLERMANN, V.; HOFFMANN, A.A.; OVERGAARD, J.; LOESCHKE, V.; SGRÓ, C.M. Plasticity for desiccation tolerance across *Drosophila* species is affected by phylogeny and climate in complex ways. *Proceedings of the Royal Society of London B*, v.285, 20180048, 2018. <http://dx.doi.org/10.1098/rspb.2018.0048>
- KIDD, B.A.F. The controlling influence of carbon dioxide in the maturation, dormancy and germination of seeds. Part II. *Proceedings of the Royal Society B*, v.87, p.609-625, 1914. <http://rspb.royalsocietypublishing.org/content/royprsb/87/599/609.full.pdf>
- KOHAMA, S.; MALUF, A.M.; BILIA, D.A.C.; BARBEDO, C.J. Secagem e armazenamento de sementes de *Eugenia brasiliensis* Lam. (grumixameira). *Revista Brasileira de Sementes*, v.28, p.72-78, 2006. <http://www.scielo.br/pdf/rbs/v28n1/a10v28n1.pdf>
- LAMARCA, E.V.; SILVA, C.V.; BARBEDO, C.J. Limites térmicos para a germinação em função da origem de sementes de espécies de *Eugenia* (Myrtaceae) nativas do Brasil. *Acta Botanica Brasílica*, v.25, p.293-300, 2011. <http://www.scielo.br/pdf/abb/v25n2/a05v25n2.pdf>
- LAMARCA, E.V.; BARBEDO, C.J. Short storability of *Caesalpinia echinata* Lam. seeds as a consequence of oxidative processes. *Hoehnea*, v.39, p.577-586, 2012. <http://www.scielo.br/pdf/hoehnea/v39n4/06.pdf>
- LAMARCA, E.V.; BARBEDO, C.J. Sensibilidade à dessecação de embriões de *Inga vera* Willd. formados sob diferentes condições ambientais. *Revista Árvore*, v.39, p.1083-1092, 2015. <http://www.scielo.br/pdf/rarv/v39n6/0100-6762-rarv-39-06-1083.pdf>
- LAMARCA, E.V.; BONJOVANI, M.R.; FARIA, J.M.R.; BARBEDO, C.J. Germinação em temperatura sub-ótima de embriões de *Inga vera* subsp. *affinis* obtidos sob diferentes condições ambientais. *Rodriguésia*, v.64, p.877-885, 2013a. <http://www.scielo.br/pdf/rod/v64n4/v64n4a15.pdf>
- LAMARCA, E.V.; PRATAVIERA, J.S.; BORGES, I.F.; DELGADO, L.F.; TEIXEIRA, C.C.; CAMARGO, M.B.P.; FARIA, J.M.R.; BARBEDO, C.J. Maturation of *Eugenia pyriformis* seeds under different hydric and thermal conditions. *Anais da Academia Brasileira de Ciências*, v.85, p.223-233, 2013b. <http://www.scielo.br/pdf/aabc/v85n1/0001-3765-aabc-85-01-223.pdf>
- LAMARCA, E.V.; CAMARGO, M.B.P.; TEIXEIRA, S.P.; SILVA, E.A.A.; FARIA, J.M.R.; BARBEDO, C.J. Variations in desiccation tolerance in seeds of *Eugenia pyriformis*: dipsersal at different stages of maturation. *Revista Ciência Agronômica*, v.47, p.118-126, 2016. <http://www.scielo.br/pdf/rca/v47n1/0045-6888-rca-47-01-0118.pdf>
- LEDUC, S.N.M.; SILVA, J.P.N.; GASPAR, M.; BARBEDO, C.J.; FIGUEIREDO-RIBEIRO, R.C.L. Non-structural carbohydrates in immature seeds of *Caesalpinia echinata* (Leguminosae) are involved in the induction of desiccation tolerance. *Australian Journal of Botany*, v.60, p.42-48, 2012. <https://doi.org/10.1071/BT11236>
- LAN, Q.Y.; LUO, Y.L.; QIU, Y.P.; JIN, B.Q.; HE, H.Y.; YIN, S.H. Storage of recalcitrant seeds of *Antiaris toxicaria* (Moraceae). *Seed Science and Technology*, v.42, p.97-100, 2014. <https://doi.org/10.15258/sst.2014.42.1.11>
- LEPRINCE, O.; BUITINK, J. Special issue on desiccation biology. *Planta*, v.242, p.367, 2015. <https://doi.org/10.1007/s00425-015-2356-7>
- MALIK, S.K.; CHAUDHURY, R.; KALIA, R.K. Rapid in vitro multiplication and conservation of *Garcinia indica*: A tropical medicinal tree species. *Scientia Horticulturae*, v.106, p.539-553, 2005. <https://doi.org/10.1016/j.scienta.2005.05.002>
- MARTINI-NETO, N.; BARBEDO, C.J. Viability of brazilwood seeds (*Caesalpinia echinata* Lam.) stored at room temperature in controlled atmospheres. *Journal of Seed Science*, v.37, p.93-101, 2015. <http://www.scielo.br/pdf/jss/v37n2/2317-1537-jss-2317-1545v37n2142340.pdf>
- MASCARIN, G.M.; KOBORI, N.N.; JACKSON, M.A.; DUNLAP, C.A.; DELALIBERA JR, I. Nitrogen sources affect productivity, desiccation tolerance and storage stability of *Beauveria bassiana* blastospores. *Journal of Applied Microbiology*, v.124, p.810-820, 2018. <https://doi.org/10.1111/jam.13694>
- MATA, M.F.; SILVA, K.B.; BRUNO, R.L.A.; FELIX, L.P.; MEDEIROS FILHO, S.; ALVES, E.U. Maturação fisiológica de sementes de ingazeiro (*Inga striata*) Benth. *Semina: Ciências Agrárias*, v.34, p.549-565, 2013. <https://doi.org/10.5433/1679-0359.2013v34n2p549>
- MAYR, E. *O que é a evolução*. Rio de Janeiro: Rocco, 2009. 342p.
- MELLO, J.I.O.; BARBEDO, C.J.; SALATINO, A.; FIGUEIREDO-RIBEIRO, R.C.L. Reserve carbohydrates and lipids from the seeds of four tropical tree species with different sensitivity to desiccation. *Brazilian Archives of Biology and Technology*, v.53, p.889-899, 2010. <http://www.scielo.br/pdf/babt/v53n4/v53n4a19.pdf>
- MELLO, J.I.O.; CENTENO, D.C.; BARBEDO, C.J.; FIGUEIREDO-RIBEIRO, R.C.L. Changes in carbohydrate composition in seeds of three tropical tree species submitted to drying and storage at freezing temperature. *Seed Science and Technology*, v.39, p.465-480, 2011. <https://doi.org/10.15258/sst.2011.39.2.18>

- MELLO, J.I.O.; FIGUEIREDO-RIBEIRO, R.C.L.; BARBEDO, C.J. Sub-zero temperature enables storage of seeds of *Caesalpinia echinata* Lam. *Journal of Seed Science*, v.35, p.519-523, 2013. <http://www.scielo.br/pdf/jss/v35n4/14.pdf>
- MING-YUE, J.I.; ZHANG, N.; YAN, Q.; WEN, B. Storage behaviour characteristics of recalcitrant *Podocarpus nagi* seeds. *Plant Diversity and Resources*, v.37, p.63-70, 2015.
- MOLIZANE, D.M.; JULIO, P.G.S.; CARMELLO-GUERREIRO, S.M.; BARBEDO, C.J. Physical, physiological and anatomical changes in *Erythrina speciosa* Andrews seeds from different seasons related to the dormancy degree. *Journal of Seed Science*, v.40, n.3 p.329-339, 2018.
- MOOTHOO-PADAYACHIE, A.; VARGHESE, B.; PAMMENTER, N.W.; GOVENDER, P.; SERSHEN. Germination associated ROS production and glutathione redox capacity in two recalcitrant-seeded species differing in seed longevity. *Botany*, v.94, p.1103-1114, 2016. <https://doi.org/10.1139/cjb-2016-0130>
- NOWELL, R.W.; ALMEIDA, P.; WILSON, C.G.; SMITH, T.P.; FONTANETO, D.; CRISP, A.; MICKLEM, G.; TUNNACLIFFE, A.; BOSCHETTI, C.; BARRACLOUGH, T.G. Comparative genomics of bdelloid rotifers: insights from desiccating and nondesiccating species. *PLoS Biology*, v.16, e2004830, 2018. <https://doi.org/10.1371/journal.pbio.2004830>
- OKAMOTO, J.M.; JOLY, C.A. Ecophysiology and respiratory metabolism during the germination of *Inga sessilis* (Vell.) Mart. (Mimosaceae) seeds subjected to hypoxia and anoxia. *Revista Brasileira de Botânica*, v.23, p.51-57, 2000. <http://www.scielo.br/pdf/rbb/v23n1/v23n1a06.pdf>
- OLIVEIRA, C.F.; OLIVEIRA, D.C.; PARISI, J.J.D.; BARBEDO, C.J. Deterioração de sementes de espécies brasileiras de *Eugenia* em função da incidência e do controle de fungos. *Revista Brasileira de Sementes*, v.33, p.520-532, 2011. <http://www.scielo.br/pdf/rbs/v33n3/15.pdf>
- PARISI, J.J.D.; BIAGI, J.D.; BARBEDO, C.J.; MEDINA, P.F. Viability of *Inga vera* Willd. subsp. *affinis* (DC.) T.D. Penn. embryos according to the maturation stage, fungal incidence, chemical treatment and storage. *Journal of Seed Science*, v.35, p.70-76, 2013. <http://www.scielo.br/pdf/jss/v35n1/10.pdf>
- PARKHEY, S.; NAITHANI, S.C.; KESHVKANT, S. Protein metabolism during natural ageing in desiccating recalcitrant seeds of *Shorea robusta*. *Acta Physiologia Plantarum*, v.36, p.1649-1659, 2014. <https://doi.org/10.1007/s11738-014-1540-x>
- PLITTA-MICHALAK, B.P.; NASKRĘT-BARCISZEWSKA, M.Z.; KOTLARSKI, S.; TOMASZEWSKI, D.; TYLKOWSKI, T.; BARCISZEWSKI, J.; CHMIELARZ, P.; MICHALAK, M. Changes in genomic 5-methylcytosine level mirror the response of orthodox (*Acer platanoides* L.) and recalcitrant (*Acer pseudoplatanus* L.) seeds to severe desiccation. *Tree Physiology*, v.38, p.617-629, 2018. <https://doi.org/10.1093/treephys/tpx134>
- PORTO, K.C.N.; NUNES, Y.R.F.; RIBEIRO, L.M. The dynamic of recalcitrant seed banks of *Mauritia flexuosa* (Arecaceae) reveal adaptations to marsh microenvironments. *Plant Ecology*, v.219, p.199-207, 2018. <https://doi.org/10.1007/s11258-017-0788-9>
- PRATAVIERA, J.S.; LAMARCA, E.V.; TEIXEIRA, C.C.; BARBEDO, C.J. The germination success of the cut seeds of *Eugenia pyriformis* depends on their size and origin. *Journal of Seed Science*, v.37, p.47-54, 2015. <http://www.scielo.br/pdf/jss/v37n1/2317-1537-jss-37-01-00047.pdf>
- RAMLALL, C.; VARGHESE, B.; RAMDHANI, S.; PAMMENTER, N.W.; BHATT, A.; BERJAK, P.; SERSHEN. Effects of simulated acid rain on germination, seedling growth and oxidative metabolism of recalcitrant-seeded *Trichilia dregeana* grown in its natural seed bank. *Physiologia Plantarum*, v.153, p.149-160, 2015. <https://doi.org/doi:10.1111/ppl.12230>
- ROBERTS, E.H. Predicting the storage life of seeds. *Seed Science and Technology*, v.1, p.499-514, 1973.
- SCALON, S.P.Q.; NEVES, E.M.S.; MASETO, T.E.; PEREIRA, Z.V. Sensibilidade à dessecação e ao armazenamento em sementes de *Eugenia pyriformis* Cambess. (uvaia). *Revista Brasileira de Fruticultura*, v.34, p.269-276, 2012. <http://www.scielo.br/pdf/rbf/v34n1/v34n1a36.pdf>
- SCHULTZ, D.G.; ORO, P.; VOLKWEIS, C.; MALAVASI, M.M.; MALAVASI, U.C. Maturidade fisiológica e morfometria de sementes de *Inga laurina* (Sw.) Willd. *Floresta e Ambiente*, v.21, p.45-51, 2014. http://www.scielo.br/pdf/floram/v21n1/aop_floram_054713.pdf
- SHIBATA, M.; COELHO, C.M.M. Early harvest increases post-harvest physiological quality of *Araucaria angustifolia* (Araucariaceae) seeds. *Revista Biologia Tropical*, v.64, p.885-896, 2016. <http://www.scielo.sa.cr/pdf/rbt/v64n2/0034-7744-rbt-64-02-00885.pdf>
- SHOEMAKER, C.A. Plants and human culture. *Journal of Home & Consumer Horticulture*, v.1, p.3-7, 2010. https://doi.org/10.1300/J280v01n02_02
- SILVA, A.L.G.; PINHEIRO, M.C.B. Reproductive success of four species of *Eugenia* L. (Myrtaceae). *Acta Botanica Brasilica*, v.23, p.526-534, 2009. <http://www.scielo.br/pdf/abb/v23n2/v23n2a24.pdf>
- SILVA, C.V.; BILIA, D.A.C.; BARBEDO, C.J. Fracionamento e germinação de sementes de uvaia (*Eugenia pyriformis* Cambess. - Myrtaceae). *Revista Brasileira de Botânica*, v.26, p.213-221, 2003. <http://www.scielo.br/pdf/rbb/v26n2/a09v26n2.pdf>
- SILVA, C.V.; BILIA, D.A.C.; BARBEDO, C.J. Fracionamento e germinação de sementes de *Eugenia*. *Revista Brasileira de Sementes*, v.27, p.86-92, 2005. <http://www.scielo.br/pdf/rbs/v27n1/25185.pdf>
- SILVA, J.P.N.; CENTENO, D.C.; FIGUEIREDO-RIBEIRO, R.C.L.; BARBEDO, C.J. Maturation of seeds of *Poincianella pluviosa* (Caesalpinioideae). *Journal of Seed Science*, v.37, p.131-138, 2015. <http://www.scielo.br/pdf/jss/v37n2/2317-1537-jss-37-02-00131.pdf>
- SOGAME, Y.; KIKAWADA, T. Current findings on the molecular mechanisms underlying anhydrobiosis in *Polypedilum vanderplanki*. *Current Opinion in Insect Science*, v.19, p.16-21, 2017. <https://doi.org/10.1016/j.cois.2016.10.008>
- SOUZA, G.A.; DIAS, D.C.F.S.; PIMENTA, T.M.; ALMEIDA, A.L.; PICOLI, E.A.T.; ALVARENGA, A.P.; SILVA, J.C.F. Sugar metabolism and developmental stages of rubber tree (*Hevea brasiliensis* L.) seeds. *Physiologia Plantarum*, v.162, p.495-505, 2018a. <https://doi.org/doi:10.1111/ppl.12650>

- SOUZA, G.A.; DIAS, D.C.F.S.; PIMENTA, T.M.; CARDOSO, A.A.; PIRES, R.M.O.; ALVARENGA, A.P.; PICOLI, E.A.T.; Morpho-anatomical, physiological and biochemical changes in rubber tree seeds. *Anais da Academia Brasileira de Ciências*, v.90, p.1625-1641, 2018b. <http://dx.doi.org/10.1590/0001-3765201820170340>
- SUBBIAH, A.; PAMMENTER, N.W.; VARGHESE, B.; SERSHEN. Modelling desiccation sensitivity by integration of stress intensity and duration effects in flash-dried embryonic axes and zygotic embryos of recalcitrant seeds. *Seed Science and Technology*, v.45, p.650-664, 2017. <https://doi.org/10.15258/sst.2017.45.3.16>
- SUN, R.Z.; LIN, C.T.; ZHANG, X.F.; DUAN, L.X.; QI, X.Q.; GONG, Y.H.; DENG, X. Acclimation-induced metabolic reprogramming contributes to rapid desiccation tolerance acquisition in *Boea hygrometrica*. *Environmental and Experimental Botany*, v.148, p.70-84, 2018. <https://doi.org/10.1016/j.envexpbot.2018.01.008>
- TEIXEIRA, C.C.; BARBEDO, C.J. The development of seedlings from fragments of monoembryonic seeds as an important survival strategy for *Eugenia* (Myrtaceae) tree species. *Trees, Structure and Function*, v.26, p.1069-1077, 2012. <http://dx.doi.org/10.1007/s00468-011-0648-5>
- UMARANI, R.; AADHAVAN, E.K.; FAISAL, M.M. Understanding poor storage potential of recalcitrant seeds. *Current Science*, v.108, p.2023-2034, 2015. <https://doi.org/10.18520/cs%2Fv108%2Fi11%2F2023-2034>
- VAZ, T.A.A.; DAVIDE, A.C.; RODRIGUES-JUNIOR, A.G.; NAKAMURA, A.T.; TONETTI, O.A.O.; SILVA, E.A.A. *Swartzia langsdorffii* Raddi: morphophysiological traits of a recalcitrant seed dispersed during the dry season. *Seed Science Research*, v.26, p.47-56, 2016. <https://doi.org/10.1017/S0960258515000380>
- VICENTE, D.; OLIVEIRA, L.M.; TONETTI, O.A.O.; SILVA, A.A.; LIESCH, P.P.; ENGEL, M.L. Viabilidade de sementes de *Ocotea puberula* (Rich.) Ness ao longo do armazenamento. *Floresta e Ambiente*, v.23, p.418-426, 2016. <http://www.scielo.br/pdf/floram/v23n3/2179-8087-floram-2179-8087107414.pdf>
- WALTERS, C. Orthodoxy, recalcitrance and in-between: describing variation in seed storage characteristics using threshold responses to water loss. *Planta*, v.242, p.397-406, 2015. <https://link.springer.com/content/pdf/10.1007%2Fs00425-015-2312-6.pdf>
- WESTALL, F.; BRACK, A. The importance of water for life. *Space Science Review*, v.214 (50), p.1-23, 2018. <https://doi.org/10.1007/s11214-018-0476-7>
- XIAO, L.; YOBI, A.; KOSTER, K.L.; HE, Y.; OLIVER, M.J. Desiccation tolerance in *Physcomitrella patens*: rate of dehydration and the involvement of endogenous abscisic acid (ABA). *Plant, Cell and Environment*, v.41, p.275-284, 2018. <https://doi.org/10.1111/pce.13096>
- ZEDER, M.A. Domestication and early agriculture in the Mediterranean Basin: origins, diffusion, and impact. *Proceedings of the National Academy of Science*, v.105, p.11597-11604, 2008. <https://doi.org/10.1073/pnas.0801317105>
- ZINSMEISTER, J.; LALANNE, D.; TERRASON, E.; CHATELAIN, E.; VANDECASTEELE, C.; VU, B.L.; DUBOIS-LAURENT, C.; GEOFFRIAU, E.; LE SIGNOR, C.; DALMAIS, M.; GUTBROD, K.; DÖRMANN, P.; GALLARDO, K.; BENDAHDMANE, A.; BUITINK, J.; LEPRINCE, O. ABI5 is a regulator of seed maturation and longevity in legumes. *The Plant Cell*, v.28, p.2735-2754, 2016. <http://www.plantcell.org/content/28/11/2735>



This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.