Hoehnea 40(4): 583-593, 3 fig., 2013

Artigo de Revisão / Review Paper

Do recalcitrant seeds really exist?

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Received: 26.08.2013; accepted: 21.11.2013

ABSTRACT - (Do recalcitrant seeds really exist?). In the 70's, seeds were divided into two categories: recalcitrant and orthodox. In the 80's, it was necessary to create an intermediate category; from the 90's onwards, a gradient between orthodox and recalcitrant categories has been considered by several authors. Currently, the terms orthodox and recalcitrant are appropriate just for technological purposes, not for scientific studies. It seems that the differences between recalcitrant and orthodox seeds lie only on the maturity stage in which they are detached from the mother plant, the recalcitrant ones in a very immature stage. This implies that little progress should be expected to expand the storability of these recalcitrant seeds with the application of any treatment after harvesting. Efforts shall be focused on amplifying the maturation period of these seeds by keeping them linked to the mother plant until the maturation process has been completed.

Key words: conservation, desiccation tolerance, maturation, seed storage

RESUMO - (Sementes recalcitrantes realmente existem?). Na década de 1970, as sementes foram divididas em recalcitrantes e ortodoxas. Na década de 1980, foi necessário criar a categoria das intermediárias e, a partir de 1990, um gradiente entre as categorias ortodoxas e recalcitrantes tem sido considerado por vários autores. Atualmente, os termos ortodoxa e recalcitrante são apropriados apenas para fins tecnológicos, não para estudos científicos. As diferenças entre sementes recalcitrantes e ortodoxas parecem residir apenas no estádio de maturidade no qual se desprendem da planta mãe, as recalcitrantes em uma fase muito imatura. Isso implica que pouco progresso deve ser esperado para expandir a capacidade de armazenamento com a aplicação de qualquer tipo de tratamento após a colheita dessas sementes recalcitrantes. Esforços devem ser voltados para ampliar o período de maturidade das sementes, mantendo-as ligadas à planta-mãe até que o processo de maturação tenha sido concluído.

Palavras-chave: armazenamento, conservação, maturação, tolerância à dessecação

Introduction

Seeds are certainly included among the most important contributors to the establishment of villages, towns and countries. The development of agriculture was probably necessary at the beginning of civilizations, which allowed people to stay at the same place for long periods; it led mankind to produce its own food at different times of the seed production. Propagation of agricultural plants is mostly based on seeds. Therefore, the desiccation tolerance was essential by being one of the most important characteristics that allowed man to store seeds during times of prolonged scarcity of food. Hence, as agriculture was probably developed based on species with desiccation-tolerant seeds, the natural drying during the final maturation was usual and, during a long time, neither physiological nor biochemical studies on this subject had been performed.

Thereby the survival in an almost completely dry state was always the expected behaviour for seeds (Barbedo & Bilia 1998). However, and this is crucial to bear in mind, in nature this is a rare behaviour among living things.

In the 70's, Professor Eric H. Roberts showed that the seed viability period may be extended by lowering their temperature and moisture content throughout storage and, also, that this is predictable (Roberts 1973). However, he also described a group of species whose seeds showed different characteristics,

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because a decrease in their moisture content tended to decrease the viability period. Examples of these seeds were described long before the 70's, such as the ones of *Hevea* (Kidd 1914), *Acer* (Jones 1920), *Inga* (Castro & Krug 1951, Bacchi 1961), *Theobroma* (Zink & Rochelle 1964) and *Araucaria* (Prange 1964). Professor Roberts divided seeds into two groups: the predictable ones, which he called orthodox, and all the others, he called recalcitrant. Both terms are under current use, even after 40 years and this shows how important that work was. The predictable behaviour was based on the desiccation tolerance and, also, the ability to tolerate freezing temperatures. Both tolerances are essential for *ex situ* conservation in seed banks, and therefore the long-term storage of recalcitrant seeds has been a major challenge.

Since 1973 researchers have been studying the behaviour of seeds of different species and, despite the great amount of information accumulated over last 40 years, very few advances were actually obtained regarding the extension of the viability period of non-orthodox seeds. In terms of *ex situ* conservation, the most efficient current method to preserve species with recalcitrant seeds is the cryopreservation of embryonic axes (Sershen *et al.* 2012b, Hamilton *et al.* 2013, Walters *et al.* 2013) that, in spite of being far less expensive than maintaining growing plants, is considerably more costly than the storage of frozen dried seeds. Of course, this is not exactly a kind of seed storage. Thus, after nearly a century from the discovery of the first recalcitrant seed, one may find it difficult to understand this procedure as a real advance on the storage of recalcitrant seeds. We, seed researchers, have the responsibility to provide society with better results in terms of seed conservation.

It is about time to rethink all these concepts regarding recalcitrant seeds.

Tolerance and sensitivity to desiccation among seeds of different species

The ability to dry without dying is found in very few animals, plants and microbes, which allow them to survive during extended periods. For the desiccation-tolerant organisms, drying is often a strategy to avoid rapid deterioration of living tissues and this implies several advantages. In the dry state, orthodox seeds, for example, can be distributed widely (Alpert 2005), since they can be stored for long periods, whereas recalcitrant seeds developed mechanisms for a quick germination (Barbedo & Marcos Filho 1998, Obroucheva *et al.* 2012). In spite of not being immortal (Berjak 2006), seeds of some species can be stored for as long as thousands of years, such as *Nelumbo nucifera* Gaertn. (Shen-Miller *et al.* 1995) and *Phoenix dactylifera* L. (Sallon *et al.* 2008). Seed aging rates can also be slowed down by decreasing the environmental temperature (Reis *et al.* 2012, Bewley *et al.* 2013). Drying and low temperature are also important to control the fungal proliferation (Oliveira *et al.* 2011, Parisi *et al.* 2013). However, the seeds with relatively high lethal limits to desiccation (the so-called recalcitrant) must be maintained at high water content, and consequently at temperatures close to or above 0 °C (Smith & Berjak 1995, Wesley-Smith *et al.* 2004, Bonjovani & Barbedo 2008, Pasquini *et al.* 2012, Sershen *et al.* 2012a), and sometimes even the gaseous exchange should be avoided (Pasquini *et al.* 2012). Such conditions often allow the seeds to maintain a high metabolism (Sershen *et al.* 2012b). This is evident when compared to orthodox seeds. For instance, it was observed that on the orthodox seeds of *Arabidopsis thaliana* there is a switch in the metabolism during the desiccation phase (Fait *et al.* 2006), suggesting a decrease in respiratory rates as a preparation for a long period of storage. Conversely, the recalcitrant seeds of *Inga vera* Willd. show a different behaviour. They also have a shift in the metabolism, but this shift is aimed at increasing the efficiency of respiration and energy production until such seeds are detached from the mother plant (Caccere *et al.* 2013), that is, making it difficult their conservation.

Regardless the definitions of desiccation tolerance and the factors that are needed to develop or to guarantee it (Vertucci & Farrant 1995, Han *et al.* 1997, Barbedo & Marcos Filho 1998, Alpert & Oliver 2002, Berjak 2006, Pukacka *et al.* 2011), desiccation sensitivity is not an all-or-nothing situation. Instead, there are different levels amongst seeds of different species, and also among seeds within the same species, depending on the environmental conditions in which the seeds are developed (Berjak & Pammenter 1994, Walters 2000, Bovi *et al.* 2004, Daws *et al.* 2004, Lamarca *et al.* 2011, Delgado & Barbedo 2012, Pereira *et al.* 2012, Lamarca *et al.* 2013). Desiccation tolerance is also - and this may be the most important information - acquired progressively during the maturation of the same seed (Manfre *et al.* 2009, Hay *et al.* 2010, Ellis 2011). As a matter of fact, even a classical orthodox seed shows a recalcitrant behaviour for some time during its formation and maturation.

The physiological aspects of seed maturation

The most commonly variables analysed in seed maturation studies are seed dimensions, external characteristics (such as colour, texture, opacity and flexibility of the integument), water and dry matter contents, germinability, capacity to produce normal seedlings, and vigour (Borges *et al.* 2005, Marcos Filho 2005, Carvalho & Nakagawa 2012). Some of these studies can also include water potential, respiration rate and chemical composition (Borges *et al.* 2006, Ramya *et al.* 2012, Mata *et al.* 2013). Several works also include the fruit characteristics, mainly while establishing a visual diagnosis of the appropriate time to harvest or to identify the characteristics of the fruit that correspond to the best physiological quality of the seeds (Borges *et al.* 2005, Matheus *et al.* 2011). However, it is not surprisingly that both fruit and seed characteristics change according to the species and conditions in which the seeds are formed, making it difficult to standardize these characteristics in order to identify the physiological maturity of seeds. Nutrition and/or weather may change, for example, the final dry matter content of the seeds. Immature seeds produced over years under favourable environmental conditions can be heavier than the mature ones, from the same species or even in the same specimen, produced under unfavourable conditions (Borges *et al*. 2005, Lamarca *et al*. 2013). In many species, the size of the seed reaches its maximum early during maturation, remaining relatively unchanged (or with no evident changes due to the accuracy of the methods) up to the end of the process. The germination capacity often appears early during seed development (Hong & Ellis 1990, Borges *et al.* 2005, Lamarca *et al.* 2013) and can also reach different maximum values according to the conditions on which the seeds are formed. Therefore, although the general behaviour of maturation process can be predictable, each seed or fruit characteristic by itself is not reliable to define the real maturity stage of a seed, especially considering different species or, within the same species, from different regions or seasons. However, the water content is even more reliable, homogeneous and apparently more independent of the conditions under which seeds are formed. Even though the total amount of water in seeds can also be non-homogeneous among species, ages or regions of seed origin, unlike the other characteristics described above, water content is usually expressed in proportions (% or g g^{-1}). This can be expressed in relation to the fresh (wet basis - wb) or dry (dry basis - db) weight (Barbedo & Lamarca 2014), and could be used for comparisons among different cycles of seed maturation (Lamarca *et al.* 2013). In this way, it could be predictable. This is shown in figure 1, in which it is possible to notice that at the beginning of the seed formation the water content is very high, usually above 80% (wb), or 4.0 g g^{-1} (db, g water per g dry weight) for most of the angiosperms (Marcos Filho 2005). During seed maturation, water content decreases slowly and continuously until it reaches values near 50% (wb) or 1.0 g g^{-1} (db). From this point on, it is possible to obtain different results, and the largest differences can occur in the process depending on the species and the environmental conditions. However, it is crucial to emphasize that, whatever category seeds are classified (orthodox, recalcitrant or intermediate), the variation of the water content until this moment of the process is strictly the same. Considering this similarity, we hereby propose that the water content could be an indicator of how extended the maturation process was, and consequently, how recalcitrant or orthodox this seed can be, according to the degree of maturation achieved by such seed from this point on.

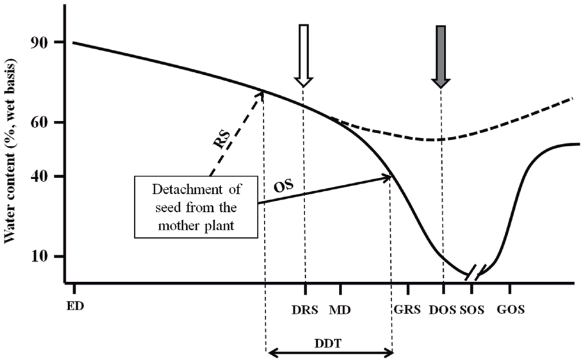

Figure 1. Schematic representation of the water content during maturation, dispersion (identified by the arrows), and germination of orthodox and recalcitrant seeds. ED: embryo starts development; RS: recalcitrant seeds; OS: orthodox seeds; DRS: dispersion of recalcitrant seeds; DOS: dispersion of orthodox seeds; DDT: development of desiccation tolerance; MD: beginning of maturation drying; GRS: germination of recalcitrant seeds; SOS: storage of orthodox seeds; GOS: germination of orthodox seeds. The dotted line represents the behaviour of recalcitrant seeds from the end of their maturation to the beginning of germination. SOS can vary from days to millennia (see text for more details). Considering OS as a reference point, the higher the period between RS and OS, the more the recalcitrance of the seed.

The anatomical and biochemical aspects of seed maturation

As already mentioned, desiccation tolerance is acquired progressively during seed maturation and several mechanisms have been presented as contributors to such tolerance. Among them are anatomical and biochemical modifications, which allow seed tissues to survive through a period of anabiosis. For example, some recent studies have characterized the cell wall folding and ultrastructure of the cell containing fewer organelles as well as low vacuolated cells in vegetative tissues, such as the so-called orthodox seeds (Moura *et al.* 2010) and also the resurrection plants (Moore *et al.* 2008, 2009). However, considering desiccation sensitive seeds, adaptations on the cell wall or subcellular structure are impaired. Caccere *et al.* (2013) showed that the proportion of arabinose-containing polysaccharides, which act as wall plasticizers, is substantially lower in *Inga vera* seeds (desiccation sensitive) than on desiccation tolerant seeds after shedding. It implies on mechanical damage of the seeds if submitted to desiccation. The lack of cell wall adjustment could indicate that those seeds are not yet ready for shedding, or in case of shedding, they would not be able to survive at low humidity in normal atmosphere. Slow drying of the sensitive seeds of *Trichilia emetica* Vahl also resulted in great seed damage (Kioko *et al.* 2006). In this case, slow drying until 0.5 g g^{-1} resulted in subcellular destruction, characterized by a complete detachment of the plasmalemma from the cell wall and coalescence of the cell. Similarly to *Inga vera* the cell wall was not ready yet to cope with water loss. Moreover, during maturation of *I. vera* seeds, Caccere *et al.* (2013) also observed an increase in vacuole number and size, in both cotyledons and embryonic axes, therefore indicating higher metabolic activity.

Not losing water and the presence of highly vacuolated seeds is consistent with a high metabolic activity in desiccation sensitive seeds, which is evident when compared to desiccation tolerant seeds after shedding. In *Arabidopsis thaliana* (L.) Heynh., for instance, there is a switch in the metabolism over the desiccation phase, suggesting a decrease in respiratory rates as a preparation for a long period of low water content (Fait *et al.* 2006). *Inga vera* seeds have a different behaviour as they also show a shift in the metabolism, but in this case, it is focused on increasing the efficiency of respiration and energy production until fruit shedding (Bonjovani 2011, Caccere *et al.* 2013). Interestingly, by comparing tolerant and sensitive seeds one can observe that in the first stages of development both types of seeds present high metabolism and high respiratory rates for energy supply during seed growth. Desiccation sensitive seeds of *Galanthus nivalis* L. and *Narcissus pseudonarcissus* L. are dispersed with high water content (> 50%) and develop the ability to germinate prior to desiccation tolerance acquisition. Considerable embryo development occurred in these seeds after shedding (Newton *et al.* 2013), when high metabolism is necessary to complete the growth. The authors suggested that seeds of those species are still immature at shedding, as already reported by Delgado & Barbedo (2012) for seeds of *Eugenia* species. This is in accordance with the results described by Huang *et al.* (2012), who performed a proteome profile of maize embryo. During the early and late stages of development, these embryos presented, as expected, different water content and could be a model for desiccation sensitivity and tolerance, with the same genetic background. In this study, the authors also found high metabolism and formation of reactive oxygen species (ROS), which could be eliminated in fully developed seeds by anti-oxidant enzymes synthesized at the later stages of development. The high metabolism found during the first stages of development is correlated with the presence of oxygen inside the seeds (Borisjuk & Rolletschek 2009) and is associated to its energy *status*, which can be indicated by the temporal and spatial ATP distribution (Rolletschek *et al.* 2004). This is essential for energy production and utilization throughout the carbon storage phase.

During the late stages of development, the metabolism of desiccation tolerant seeds might turn to the synthesis of protective molecules, such as some lipids, polyols or saccharides, which behaviour is not observed in sensitive seeds. Saccharides such as sucrose, for example, could be involved in the glass state formation of the cytoplasm (Crowe *et al.* 1998, Bryant *et al.* 2001) or might still interact with proteins for the same purpose, increasing viscosity as a way to reach lower metabolism. Moreover, it is suggested that changes in lipid quality also correlate with desiccation tolerance (Hoekstra 2005). By comparing seeds from four different species with a desiccation tolerance gradient, Mello *et al.* (2010) found an accumulation of fatty acids with higher degree of insaturation in the more tolerant seeds (*Erythrina speciosa* Andrews and *Caesalpinia echinata* Lam.) Such accumulation could contribute to the flexibility of cell membranes. Additionally, seeds of these species accumulated raffinose family oligosaccharides (RFOs), such as raffinose and stachyose (Mello *et al.* 2010), or polyols, such as pinitol and ciceritol (Borges *et al.* 2006), which could also contribute to glass state formation. Some studies suggest yet that the role of sucrose and RFOs as protective molecules do not depend only on their content, but rather depend on the sucrose/RFOs ratio (Steadman *et al.* 1996, Vandecasteele *et al.* 2011) and with sucrose/cyclitols ratio (Leduc *et al.* 2012).

Therefore, the biochemical and anatomical aspects studied previously suggest that during the first stages of development both sensitive and desiccation tolerant seeds behave similarly and have high metabolic activity. However, from a specific point during maturation on, tolerant seeds switch off the metabolism, whereas the sensitive seeds maintain an increased metabolic rate until detachment.

Seed detachment: the critical point

Considering the classic categorization between orthodox and recalcitrant seeds, some important points from seed maturation to germination are schematically shown in figure 1. Some of them are easily identifiable like shedding, radicle protrusion (germination), and maturation drying. However, the exact moment of the seed detachment from the mother plant is not easily identified because it would depend on the microscopic identification of the placental layer cell disruption. The maximum dry matter accumulation could be used to estimate this point, but in many cases it is difficult to establish exactly when the seed reaches such maximum, since the accuracy of the methods for this evaluation may not be sufficient to detect small increases in mass. In figure 1, this point was represented only conceptually. However, it would be decisive to determine it if one intends to define the degree of orthodoxy or recalcitrance of the seed, because this point establishes the end of ripening, and if this was completed or not. If one analyses some results with immature orthodox seeds, it would be easy to suppose they were obtained from recalcitrant seeds (Hong & Ellis 1990, Butler *et al.* 2009, Ramya *et al.* 2012, Newton *et al.* 2013). As a matter of fact, most of the results obtained with recalcitrant seeds would be almost perfectly adjusted to a specific period in the development of orthodox seeds (Delgado & Barbedo 2012, Perez *et al.* 2012), as represented in figure 2.

In addition to breaking the connection between the seed and parent plant, another natural event (i.e. not artificially induced) might interrupt the maturation process: the beginning of germination (figures 1 and 2) despite the seed is still connected to the mother plant. For instance, *Inga vera* can disperse the fruits with germinating seeds inside them, sometimes in an advanced process of germination (Bilia & Barbedo 1997, Bilia *et al*. 1999, Barbedo & Cicero 2000, Caccere *et al*. 2013, Parisi *et al*. 2013). Therefore, to understand the differences of seeds formation in distinct groups, it is essential to learn what blocks the germination in one type of seed, and does not in other seeds. However, there is not enough information in the current literature of the factors involved in this process.

Nevertheless, based on this concept (i.e. the complete maturation cycle of a recalcitrant seed fits a specific period of an orthodox seed maturation), some features would depend on how far the recalcitrant seed has developed and, consequently, a gradient of behaviour shall be found among seeds of different species. Therefore, desiccation or low temperature tolerance, viability period during storage and adaptability to unfavourable conditions for germination, among others, would depend on how far the seed developed. In fact, these characteristics also show a gradient for orthodox seeds depending on how far they are from their maximum physiological

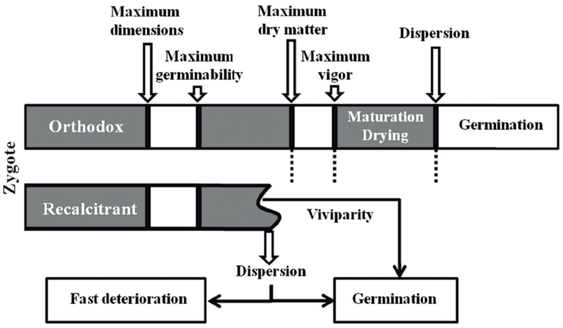

Figure 2. Schematic representation of the relationship of seed maturation between orthodox and recalcitrant seeds. For recalcitrant seeds, maximum vigor and maturation drying would never occur or would do so just partially, and maximum dry matter would be difficult to identify. At the end of the cycle, recalcitrant seeds would be detached from the mother plant and dispersed or would start to germinate into the fruit, thus dotted lines would not occur. After dispersion, since recalcitrant seeds are sensitive to desiccation (considering they are, in fact, immature orthodox seeds) they would die quickly if not germinate. For more details, see text.

quality. Such characteristics are usually considered for diagnosis of seed vigour and are related, among other factors, to maturity. This fact was widely reported for seeds of different species. The variation in such characteristics could also be found within the same species, among different populations, for both orthodox and recalcitrant seeds. Indeed, this does happen with the recalcitrant seeds of *Inga* (Bilia *et al.* 1999, Barbedo & Cicero 2000, Faria *et al.* 2004, Andréo *et al.* 2006, Parisi *et al.* 2013) as well as with the orthodox seeds of *Caesalpinia echinata* (Barbedo *et al.* 2002, Hellmann *et al.* 2006, Mello 2013). Moreover, the gradients should also occur on seeds of the same specimen, among different years, mainly if the seeds were developed under completely different environmental conditions. This was also reported for *Eugenia pyriformis* Cambess. (Lamarca *et al.* 2013) and *Inga striata* Benth. (Mata *et al.* 2013). Obviously, these gradients would not be identified if the conditions were not good enough to break the complete maturation process, for example, prolonging the shedding time. It is also obvious that there is the genetic component regulating the maximum potential for each individual, similar to what occurs with any other features.

The maximum seed longevity of a species is defined by the genetic information (Lgi). However, the potential longevity (Lpot), here defined as the expected time of maintenance of viability during storage, would be a result of the interaction among Lgi and: a) the reduction factors (Rf), such as the water content of the seed during storage, the ambient temperature, the presence of damage or microorganisms in seeds, and especially the degree of seed maturity at shedding, and b) improvement factors (If), such as dormancy and the addition of plant growth regulators, fungicides or insecticides. Therefore:

$$\mathrm{Lpot} = \mathrm{Lgi} - \mathrm{Rf} + \mathrm{If} \qquad (1)$$

It is possible that during natural selection of species in different ecosystems, the factors that promote the breaking of the maturation process and/or the early germination of the seeds have been established with more or less intensity in each species, and therefore, they could be, currently speaking, irreversible. Environmental conditions might have become highly advantageous to early germination seeds. This could be assured, for example, by eliminating inhibitors in those phases of the maturation process in which seeds have all the conditions to germinate but do not do it within their own fruit. It is important to bear in mind that the evolutionary process led the seeds to a high degree of specialization, by showing differences in chemical compounds, types of germination, degree of dormancy, development of structures and appendages to facilitate dispersion, among others. This specialization might also have occurred in the maturation process. However, if one compares data in the literature, it is at least curious the similarity among the processes occurring throughout the maturation between recalcitrant and orthodox seeds until the stages in which the seeds have water content close to 50 % (wb).

What is expected for the conservation of a recalcitrant seed?

All in all, the results obtained by different authors may allow us to infer that the so-called recalcitrant seeds are, in fact, orthodox seeds which have not completed their maturation. This could be due to the germination within the fruit or the dispersion in an immature stage. Therefore, the level of recalcitrance is related to how far the maturation was achieved as well as how far the germination advanced before seed shedding. Such seeds will probably not achieve the levels of desiccation tolerance and storability of the so-called orthodox seeds by artificial methods. Advances were obtained by the utilization of several techniques to increase desiccation tolerance and/or storability. These include physiological conditioning, induction of stresses, and application of plant growth regulators, among others. Therefore, extending the period the seed remains attached to the mother plant and avoiding germination before shedding could be a successful strategy for the conservation of the so-called recalcitrant seeds.

A theoretical model of the relationships discussed above could be described by the equations:

$$\mathrm{Y}_{\mathrm{dt}} = \mathrm{e}^{\mathrm{aw}} \qquad (2)$$

$$\mathrm{Y}_{\mathrm{Lpot}} = \mathrm{a}(10^{16})/\mathrm{w}^{9} \qquad (3)$$

where Y_{dt} is the expected desiccation tolerance, Y_{Lpot} is the expected potential longevity, a is a constant (here proposed as being about 0.06) and w is the water content (%, wb) at seed detachment. This is schematically shown in figure 3. It is important to notice that, according to this model, the shedding would not be the best indicator of the maturity degree achieved by the seeds, but rather the precise moment in which seeds are detached from the mother plant.

This is due to the fact that seeds of some species may remain inside the fruit for some time after the detachment. The water content in seeds at this point would identify the actual seed maturity degree, which would establish characteristics such as desiccation tolerance and storability, *i.e.*, the characteristics used to identify orthodox and recalcitrant seeds.

According to this model, artificial processes applied to detached seeds, like If in equation (1), could change values in equations (2) and (3) and move the lines in figure 3 slightly to the right, increasing the limit of desiccation tolerance and longevity. However, this effect would have narrow bounds. Better results would be obtained if the detachment could be delayed, *i.e.* changing value *w* in equation (3), and only then, the seed would be submitted to improving treatments. The study of immature orthodox seeds could be an interesting experimental issue to support this model. For example, immature seeds of *Caesalpinia echinata* L., which were still sensitive to desiccation at about 35 days after flowering (daa), did not increase desiccation tolerance by PEG treatments; however, by maintaining these seeds in the mother plant for 10 days more was enough to create desiccation tolerance by the same PEG treatment (Leduc *et al.* 2012). Another way to analyse this process would be the comparison of recalcitrant seeds from different populations of the same species, from different regions or years in case the cycle, the degree-days and/or the final water content of the seeds would be different. Conceptually, they would show different final characteristics in accordance with the degree of maturity, such as desiccation tolerance or the range of temperature to germinate.

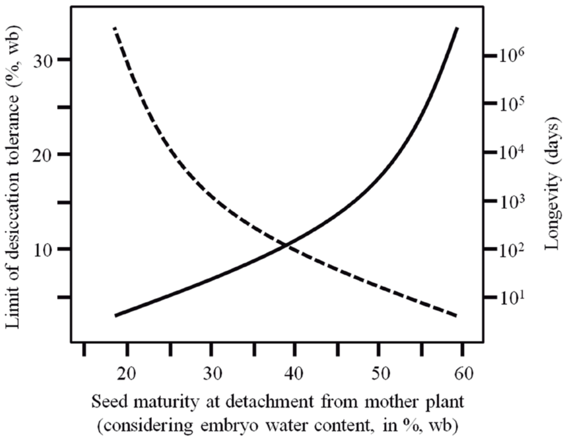

Figure 3. Schematic representation of the relationship among seed maturity, expected desiccation tolerance (solid line, $Y = e^{0.06x}$) and expected longevity (dotted line, $Y = [(6.0 \times 10^{16})\ x^{-9}]$). Since the seed is detached from the mother plant, water content should be determined, which is projected to the solid line to estimate the limit of desiccation tolerance expected, and to the dotted line to estimate the expected period of viability during storage.

In fact, there are some results in the literature that suggest that this does happen. For instance, Daws *et al.* (2004) showed that seeds of *Aesculus hippocastanum* L. from further North were shed less developed as a consequence of cooler temperatures during development, suggesting a quantitative explanation for intraspecies variability in recalcitrant seed traits. Moreover, Daws *et al.* (2006) observed that the level of desiccation-tolerance of seeds of *Acer pseudoplatanus* L. was dependent on the heat sums during maturation. The lower the heat sum until fruit dispersion the narrower the temperature range for germination, the higher the water content at shedding and the more the sensitivity of the seeds to desiccation. On the other hand, Lamarca *et al.* (2013) observed that hydric and thermal environmental variations during development influence the maturation of *Eugenia pyriformis* Cambess. seeds and determine the maturation cycle and the final seed quality. They also showed that the temperature range for germination was dependent on the growing degree-day.

Concluding remarks

Few advances have been obtained for the conservation of recalcitrant seeds and this might be the most confounding factor: what exactly is a recalcitrant seed? As already mentioned, Roberts proposed the term recalcitrant in 1973 and since then many reviews have been published around the world about this. Nonetheless, the classical distinction between orthodox and recalcitrant seeds is to be clarified, since the term recalcitrant could not be accurate for many species. The more species are studied, the more difficult it is to place their seeds into one of these categories (orthodox, recalcitrant or intermediate). Since the distinction of these terms was firstly a general one, taking into account only desiccation tolerance and lower longevity, seeds showing so many different characteristics needed to be placed in the same category. Some researchers proposed new classifications based on the existence of different degrees of recalcitrance. They included some features such as species habitat, longevity during storage, medium water content in which seed viability is 50%, chilling sensitivity, seed dormancy, immediate germination after shedding, among others. Actually, each classification system is quite useful for specific purposes. However, they are not really describing what exactly this or that seed is. As reported here, we do believe that the analysis of a seed according to its maturity level achieved while developing attached to the mother plant should be more accurate. Therefore, this could be useful to understand the behaviour and produce real advances for the so-called recalcitrant seeds, which may be simply immature dispersed seeds. Hence, efforts shall be focused on studies of complete maturation process of the so-called recalcitrant seeds as well as in the immature phase of the so-called orthodox seeds. This could allow the development of techniques to amplify the maturation period until it has been completed.

Acknowledgements

The authors wish to thank CNPq (Proc. 481484/2007-8, 477640/2009-5, 306537/2010-9) and FAPESP (2000/06422-4, 2002/12215-7, 2005/04139-7) for the financial support and fellowships. We also would like to thank Mr O. Vieira for the English review.

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