

Dormancy as exaptation to protect mimetic seeds against deterioration before dispersal

Pedro H. S. Brancalion*, Ana D. L. C. Novembre, Ricardo R. Rodrigues and Júlio Marcos Filho

Escola Superior de Agricultura 'Luiz de Queiroz', Universidade de São Paulo, Av. Pádua Dias 11, 13-418-900
Piracicaba, Brazil

*For correspondence. E-mail pedrohsb@yahoo.com.br

Received: 2 February 2010 Returned for revision: 11 February 2010 Accepted: 19 February 2010 Published electronically: 30 March 2010

• **Background and Aims** Mimetic seeds simulate the appearance of fleshy fruits and arilled seeds without producing nutritive tissues as a reward for seed dispersers. In this strategy of seed dispersal, seeds may remain attached to the mother plant for long periods after maturity, increasing their availability to naïve seed dispersers. The hypothesis that seed coat impermeability in many tropical Fabaceae with mimetic seeds serves as an exaptation to protect the seeds from deterioration and rotting while awaiting dispersal was investigated.

• **Methods** Seed coat impermeability was evaluated in five mimetic-seeded species of tropical Fabaceae in south-eastern Brazil (*Abarema langsdorffii*, *Abrus precatorius*, *Adenanthera pavonina*, *Erythrina velutina* and *Ormosia arborea*) and in *Erythrina speciosa*, a 'basal' species in its genus, which has monochromatic brown seeds and no mimetic displays. Seed hardness was evaluated as a defence against accelerated ageing (humid chamber at 41 °C for 144 h). Seed development and physiological potential of *O. arborea* was evaluated and the effect of holding mature seeds in pods on the mother plant in the field for a period of 1 year under humid tropical conditions was compared with seeds stored under controlled conditions (15 °C and 40 % relative air humidity).

• **Key Results** All five mimetic-seeded species, and *E. speciosa*, showed strong coat impermeability, which protected the seeds against deterioration in accelerated ageing. Most *O. arborea* seeds only became dormant 2 months after pod dehiscence. Germination of seeds after 1 year on the plant in a humid tropical climate was 56 %, compared with 80 % for seeds stored in controlled conditions (15 °C, 45 % relative humidity). Seedling shoot length after 1 year did not differ between seed sources.

• **Conclusions** Dormancy acts in mimetic-seeded species as an exaptation to reduce seed deterioration, allowing an increase in their effective dispersal period and mitigating the losses incurred by low removal rates by naïve avian frugivores.

Key words: *Ormosia arborea*, seed coat impermeability, seed hardness, seed development, seed adaptive traits, seed physiology, adaptation, frugivory.

INTRODUCTION

Many tropical tree species produce nutritive tissues in fleshy fruits or aril-bearing seeds to attract and reward animals for their dispersal services, establishing the well-known mutualistic interaction between this group of plants and frugivores (Howe and Smallwood, 1982; Seidler and Plotkin, 2006; Almeida-Neto *et al.*, 2008). However, some plant species have evolved a particular strategy of seed dispersal in which they take advantage of the seed dispersal services of frugivorous birds, by mimicking fleshy fruits or arilled seeds with so-called mimetic seeds that appear attractive but offer no rewards (Ridley, 1930; McKey, 1975; Van der Pijl, 1982; Galetti, 2002). Mimetic seeds are widespread in many plant families throughout the world occurring in at least 21 different genera, including herbs, vines, shrubs and predominantly trees. This strategy is remarkably common among Fabaceae, which have at least eight genera producing mimetic seeds (Ridley, 1930; McKey, 1975; van der Pijl, 1982).

In this asymmetrical relationship, plants with mimetic seeds take advantage of the appearance of fleshy fruits or arilled

seeds to attract dispersers without giving them a reward (Peres and von Roosmalen, 1996; Foster, 2008). This system is more efficient in attracting naïve (i.e. young or captive) avian frugivores (Barrows *et al.*, 1980; Galetti, 2002), that have not had sufficient experience to know that these particular mimetic seeds do not effectively offer nutritive tissue. As adult birds are less likely to consume this kind of seed, mimetic-seeded species normally have low seed removal rates, as compared with comparable fleshy fruited or aril-seeded species (Foster and Delay, 1998; Galetti, 2002; Andrieu and Debussche, 2007; Cazetta *et al.*, 2008; Foster, 2008).

Although the evolutionary ecology of mimetic seeds has been debated since the first monographs on seed dispersal (Ridley, 1930; Van der Pijl, 1982), their functional ecology, especially in relation to the role of seed coat impermeability, is still poorly understood (Peres and von Roosmalen, 1996; Foster and Delay, 1998; Galetti, 2002; Foster, 2008). Peres and van Roosmalen (1996) hypothesized that mimetic seeds ingested by terrestrial granivorous birds may be used as a grit to grind other seeds that are present in bird gizzards, and the attractive colours showed by mimetic seeds would be

important to make them easier to be found in leaf litter on the ground. This abrasive treatment could serve to overcome the dormancy of mimetic seeds and allow germination after dispersal (Foster and Delay, 1998). However, this hypothesis was not supported by the findings of Galetti (2002), who tested the passage of mimetic seeds through the digestive track of birds with and without muscular gizzards. He observed that seeds ingested by both kinds of birds showed lower germination than non-ingested seeds sown without prior treatment to overcome dormancy. In addition, Foster (2008) suggested that the possible abrasive scarification in granivorous birds' gizzards is not favourable for seedling establishment of mimetic-seeded species, as scarified seeds of *Ormosia macrocalyx* and *O. bopiensis* sown in a tropical forest gave rise to lower rates of seedling emergence than did intact seeds. This may have been the consequence of early germination of scarified seeds in a period of naturally lower water availability, which resulted in primary root desiccation following emergence.

An alternative explanation for the impermeable seed coats of mimetic seeds is that this type of dormancy is useful in avoiding deterioration while the seeds are 'waiting' for dispersers to come to take them away from the mother plant. To compensate for low seed removal rates by naïve avian frugivores, mimetic seeds can remain attached to the mother plant for some years after maturity, consequently increasing their effective dispersal period (Hammond, 1995; Galetti, 2002; Foster, 2008). To ensure the success of such a strategy, however, seeds must remain viable even if they experience environmental conditions that enhance physiological deterioration and insect predation, such as those commonly prevailing in moist tropical forests. Seed predation may be partially countered in these plant species by the accumulation of secondary compounds in mimetic seeds, especially alkaloids that deter most insect predators (Games *et al.*, 1974; Hargreaves *et al.*, 1974; Ramos, 1999; Galetti, 2002; Guimarães *et al.*, 2003; Cazetta *et al.*, 2008). However, little information is available on how mimetic seeds remain viable under environmental conditions normally conducive to deterioration and rotting. One important strategy to maintain viability is seed coat impermeability. This type of dormancy is caused by the structure or chemical composition of the seed coat, which blocks water uptake in seeds and consequently retards germination, as well as the metabolic reactions that normally culminate in seed deterioration (Baskin and Baskin, 1998).

It is well recognized that seed dormancy can optimize the timing of germination for plants in different vegetation types, and under varying environmental conditions (Baskin and Baskin, 1998). However, it may also reduce the extent of deterioration of mimetic seeds, thereby constituting an exaptation. An exaptation is the term applied to the secondary use or benefit of a trait already present in an organism for other adaptive reasons, as in traits fit for their current role but not primarily selected for it (Gould and Vrba, 1982). This alternative explanation for the adaptationist interpretation of seed dispersal patterns has already been confirmed for mutualisms occurring among several fleshy fruits and their respective animal dispersers (Jordano, 1995). It may also apply to the relationship between mimetic seeds and animal dispersers. Therefore the following hypothesis was investigated: that

seed coat impermeability protects mimetic seeds from deterioration and rotting under environmental conditions that are detrimental to seed survival, and that this putative protection could be considered as an exaptation to increase the effective dispersal period of mimetic seeds. It would not be a particular adaptation for mimetic seeds if basal, non-mimetic species from genus with mimetic seeds also show this protection.

MATERIALS AND METHODS

Studied species

Four mimetic-seeded Fabaceae tree species were studied, namely *Abarema langsdorffii* (Benth.) Barneby & J.W. Grimes, *Adenanthera pavonina* L., *Erythrina velutina* Wild. and *Ormosia arborea* Harms, as well as the widespread papilionaceous vine *Abrus precatorius* L. Mimicry of seed arils is produced in these species by the contrast of black and red in *A. precatorius* and *O. arborea*, and white and blue pigments in *A. langsdorffii*, or else by the presentation of completely red seed coats, in both *E. velutina* and *A. pavonina* (Fig. 1). Also studied were *Erythrina speciosa* Andrews, a putatively basal species of *Erythrina* with monochromatic, brown seed coats (Bruneau, 1996). Seeds from *A. langsdorffii*, *E. speciosa*, and *O. arborea* were harvested from five mother trees in moist tropical forests from São Paulo State, south-eastern Brazil, while seeds from *A. precatorius*, *A. pavonina* and *E. velutina* were harvested from three cultivated individuals for each species in Piracicaba-SP, Brazil.

Amongst the different Fabaceae genera with mimetic-seeded species, *Ormosia* has been the most studied as a model to investigate the ecology of mimetic seeds (Peres and van Roosmalen, 1996; Foster and Delay, 1998; Galetti, 2002; Foster, 2008). Seeds of *O. arborea*, the most widespread *Ormosia* species in south-eastern Brazil, maintain a morphological connection with the pod even after their complete dehiscence. This is in contrast to autochoric Fabaceae species in which pod dehiscence is followed by seed fall as a result of the presence of an abscission layer in the funiculum. The pods of *O. arborea* also remain attached to the mother plant, which, rather than performing the role of seed protection during development, simply acts as a support for the mimetic seeds even after seed development is completed.

Evaluation of seed coat impermeability

The five above-mentioned mimetic-seeded species were studied to evaluate if mimetic seeds have impermeable seed coats. *Erythrina speciosa* was also studied to evaluate if an impermeable seed coat is a plesiomorphic trait in *Erythrina* with mimetic seeds. For each species, hydration curves were evaluated in four samples of 25 seeds each, for both sandpaper-scarified seeds and non-scarified seeds. Seeds were kept moist between humidified sheets of paper in transparent plastic boxes kept at 25 °C. Before each weighing, seeds were dried on paper towel sheets to remove any free water on the seed coat surface.

Both scarified and non-scarified seed samples were weighed every 24 h during the hydration period, until primary root protrusion was detected in at least 25 % of seeds. This percentage

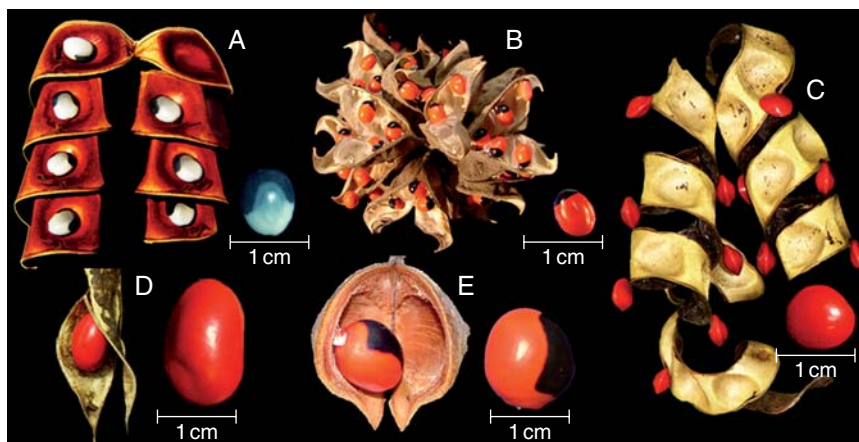


FIG. 1. Pods and mimetic seeds of *Abarema langsdorffii* (A), *Abrus precatorius* (B), *Adenantha pavonina* (C), *Erythrina velutina* (D) and *Ormosia arborea* (E).

was selected because, following primary root protrusion, an increase in water would likely be more related to seedling growth than to imbibition. A completely random design was used, using four replicates of 25 seeds for each species and treatment (scarified and non-scarified seeds).

Seed coat impermeability protection against physiological deterioration

In this experiment, whether impermeable coats protect both mimetic and non-mimetic seeds against physiological deterioration during accelerated ageing (AA) was investigated. Scarified and non-scarified seeds from all species except *A. langsdorffii*, for which there were not sufficient seeds, were used. There were five treatments in total: (I) non-scarified seeds, not submitted to AA (aiming to evaluate the germination of intact seeds); (II) scarified seeds, not submitted to AA (aiming to evaluate the maximum germination of seeds following dormancy breaking and without physiological stress); (III) non-scarified seeds, submitted to AA and without scarification after the treatment (aiming to evaluate if AA affects dormancy intensity); (IV) non-scarified seeds, submitted to AA followed by scarification (aiming to evaluate if impermeable coats protect seeds against AA and whether dormancy breaking was necessary after AA to assess the maximum germination); and (V) scarified seeds, submitted to AA (aiming to evaluate the germination of non-dormant aged seeds, which simulates mimetic seeds with permeable coats in conditions of physiological stress).

Seed scarification in concentrated (96%) sulfuric acid was carried out following recommendations from the scientific literature for each species, namely 10 min for *A. pavonina* (Kissmann *et al.*, 2008), and 40 min for both *O. arborea* (Lopes *et al.*, 2004) and *E. velutina* (Silva *et al.*, 2007). Seeds of *E. speciosa* and *A. precatorius* were also immersed in sulfuric acid for 40 min. Seeds were chemically scarified, rather than mechanically as described before, in order to remove the impermeability from the entire seed coat, assuring a better exposure of seeds to AA effects and also to better simulate the condition of naturally non-dormant seeds. This was not an attempt to simulate the seeds' passage through

a bird's stomach, but rather just a way to overcome seed hardness of the entire seed coat. After acid treatment, seeds were thoroughly rinsed in running water to remove all acid residues.

The normal conditions for the AA test (41 °C for 48 h) provide a test of seed quality (ISTA, 2001). However, the present aim was to test which, if any, of the dormant seeds of the species under study could survive especially harsh conditions. Hence, AA was carried out at 41 °C for 144 h, as determined by previous experimentation (data not shown). AA was performed using samples of 25 seeds spread in a single layer on a wire mesh screen suspended over 40 mL of water inside a plastic box (ISTA, 2001) and held in a 'water jacketed' incubator at 41 °C for 144 h. Seed moisture content after ageing was determined by the oven method (105 ± 3 °C for 24 h) (ISTA, 2001), before and after the ageing periods.

Germination tests were carried out by sowing seeds in 300 g of wet sand at 60% of water-holding capacity in plastic boxes 11 × 11 cm and 3 cm deep. The boxes were kept at 25 °C until no further seedling emergence was observed in scarified seed samples, when seed germination percentage was scored for all treatments. The viability of non-germinated scarified seeds was evaluated by the tetrazolium chloride test. Non-rotten excised seeds were immersed in a 0.075% tetrazolium chloride solution for 24 h at 30 °C, and stained embryos were classified as viable or non-viable according to the patterns determined by Moore (1972).

A completely random design was adopted, using eight replicates of 25 seeds per treatment. ANOVA tests were applied to germination percentage and to seed moisture content data, and subsequently conducted Tukey tests ($P \leq 0.05$) for multiple comparisons among means.

Seed development of Ormosia arborea

The acquisition of seed dormancy and seed coat colour in mimetic seeds was assessed during seed development of *O. arborea* in a mother tree (UTM 23k 0230003-7486288) in Piracicaba-SP, south-eastern Brazil, in a seasonally dry forest. According to Köppen's classification, this study site has a Cwa climate (high altitude tropical, with dry winters and rainy summers; 22°42'30"S latitude and 47°38'30"W

longitude; elevation 546 m a.s.l.; mean annual precipitation 1260 mm; mean annual temperature 21.5 °C). Seed harvesting started 45 d after anthesis (DAA) and continued at intervals of 30 d until 96% of the seeds became dormant (315 DAA). During this period, rainfall, mean relative air humidity and mean temperature were 1219 mm, 85.4% and 21.5 °C, respectively. Seed dry mass, seed moisture content, germination and dormancy percentages (evaluated as previously described) and seedling epicotyl length were evaluated for each seed's developmental stages. Seedling epicotyl length was measured with a ruler from the collar until primary leaf insertion.

A completely random design was used, using five replicates of ten seeds for each harvesting time. The tendency of each parameter under evaluation was graphically described using the mean \pm s.e. from each evaluation.

Physiological potential of O. arborea seeds kept attached to the pod on the mother plant after seeds became dormant

This experiment investigated whether mimetic seeds held on the mother plant after maturity in the field survive natural conditions that are favourable to their physiological deterioration. The origin of *O. arborea* seeds was the same as for those used in the previously mentioned experiment. When seeds became dormant (100% of impermeable coated seeds), a seed sample was collected and kept in the laboratory under controlled storage conditions (paper bags, 15 °C and 40% relative air humidity) whilst another sample remained on the mother plant exposed to natural environmental conditions. Only apparently healthy seeds were harvested for experiments and seeds with any signs of malformation were rejected.

Sub-samples from seeds stored in laboratory and from seeds attached to the mother tree were collected 3, 6, 9 and 12 months after seeds became dormant to evaluate germination percentage and seedling epicotyl length, as previously described. Each seed sub-sample included 120 seeds, of which 50 were used in germination tests (seeds scarified according to the same abrasive treatment described in seed hydration experiments), 50 seeds were used to evaluate dormancy percentage and 20 to evaluate seed moisture content. During this period, rainfall, mean relative air humidity and mean temperature were, respectively, 1339 mm, 87.3% and 21.1 °C.

A completely random design was adopted in a factorial scheme, with five replicates of ten seeds each and the factors 'source of seeds' (seeds kept attached to the pod on the mother plant and seeds kept under controlled storage conditions) and 'days after seeds became dormant' (0, 92, 184, 273 and 369 d). ANOVA tests (factorial scheme) and subsequently Tukey tests ($P \leq 0.05$) for multiple comparisons among means were applied.

RESULTS

Hydration curves of the scarified seed-samples showed deep seed coat impermeability for all species studied (Fig. 2). Scarified seeds showed regular water uptake, following the three-phased pattern proposed by Bewley and Black (1982).

The impermeable seed coat inhibited water uptake by most seeds during AA (Table 1; treatments III and IV), while

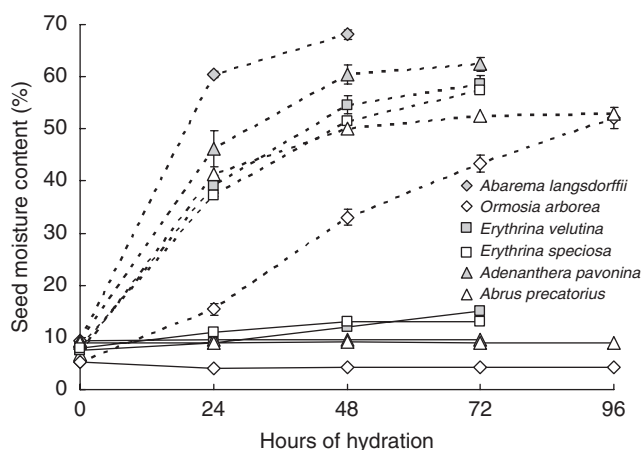


FIG. 2. Hydration curves of scarified (dashed lines) and non-scarified (continuous lines) seeds evaluated until the time of primary root protrusion (except for *O. arborea*). Data shown are the averages \pm s.e. from four replications of 25 seeds each.

scarified seeds submitted to AA (Table 1; treatment V) showed higher moisture content than seeds of the other treatments as a result of water absorption in the humidity chamber. The reduced germination of scarified seeds from all species after AA (Table 1; treatment V) provided clear evidence of physiological deterioration, while non-scarified seeds submitted to AA followed by scarification (Table 1; treatment IV) retained a similar high germination to that of scarified seeds not submitted to AA (Table 1; treatment II). ANOVA tests showed a statistically significant difference in germination percentage with an α -level of 0.01 among the treatments involved in the evaluation of seed hardness as protection to AA in *A. precatorius* ($F_{4,19} = 265.57$, $P < 0.0001$), *A. pavonina* ($F_{4,19} = 78.15$, $P < 0.0001$), *E. velutina* ($F_{4,19} = 82.05$, $P < 0.0001$), *E. speciosa* ($F_{4,19} = 69.74$, $P < 0.0001$) and *O. arborea* ($F_{4,19} = 129.92$, $P < 0.0001$). The impermeable seed coat protected all species against AA and there was little water absorption in the dormant seeds, with the exception of a portion of the *A. precatorius* seeds which imbibed water during AA (Table 1; treatments III and IV), and reached a higher seed moisture content (17%) than seeds not submitted to AA (Table 1; treatments I and II). This increase in moisture content during AA did not reduce dormancy, since seeds failed to germinate after AA when they were not scarified (treatment III). Furthermore, there was no evidence of deterioration since the germination of *A. precatorius* scarified after AA remained high (84%).

Ormosia arborea seed development followed the typical pattern shown by orthodox seeds, which is characterized by an intense seed drying phase at the end of the dry matter accumulation period. The drastic reduction in seed moisture content in the period between 225 and 255 DAA was a consequence of pod dehiscence, which brought seeds to a low moisture content status (Fig. 3A). Seed germination started at 195 DAA and reached its maximum value, together with seedling epicotyl length and seed dry mass values, at 255 DAA (Fig. 3B). This indicated that physiological maturity was reached around this same date. Morphological changes of *O. arborea* seeds in maturation are shown in Fig. 4.

TABLE 1. Seed moisture content (SMC) and germination (G) percentage of seeds submitted to the following treatments: (I) non-scarified seeds, not submitted to artificial ageing; (II) scarified seeds, not submitted to artificial ageing; (III) non-scarified seeds, submitted to artificial ageing and without scarification after the treatment; (IV) non-scarified seeds, submitted to artificial ageing and scarified after the treatment; and (V) scarified seeds, submitted to artificial ageing

| Treatment | <i>A. precatarius</i> | | <i>A. pavonina</i> | | <i>E. velutina</i> | | <i>E. speciosa</i> | | <i>O. arborea</i> | |
|-----------|-----------------------|-------|--------------------|-------|--------------------|-------|--------------------|-------|-------------------|-------|
| | SMC (%) | G (%) | SMC (%) | G (%) | SMC (%) | G (%) | SMC (%) | G (%) | SMC (%) | G (%) |
| I | 8.9 a | 0 a | 8.1 a | 4 a | 7.3 a | 39 b | 7.9 a | 8 a | 5.1 a | 0 a |
| II | 9.8 a | 78 b | 10.0 b | 63 c | 8.3 a | 89 c | 8.8 a | 92 b | 6.6 a | 80 c |
| III | 17.1 b | 0 a | 10.6 b | 10 a | 10.9 b | 38 b | 9.4 a | 14 a | 6.1 a | 0 a |
| IV | 17.8 b | 84 b | 10.9 b | 58 c | 11.4 b | 93 c | 9.2 a | 88 b | 6.3 a | 84 c |
| V | 33.1 c | 2.5 a | 28.9 c | 36 b | 30.7 c | 9 a | 34.7 b | 12 a | 26.8 b | 25 b |

Means followed by the same lower-case letters in the column are not different according to Tukey's test at $P < 0.05$.

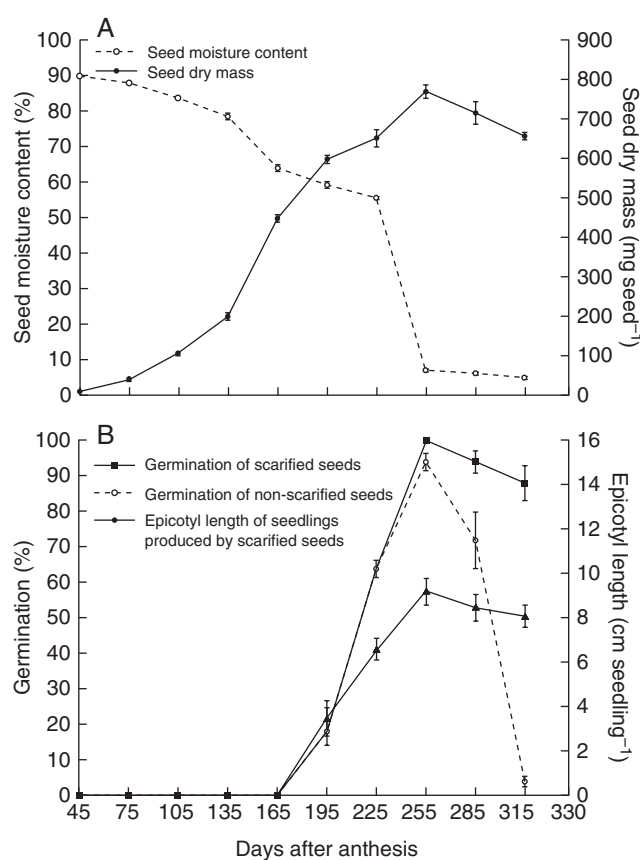


FIG. 3. Changes in seed dry mass and seed moisture content (A), and in seed germination, seed dormancy and seedling epicotyl length (B) during *Ormosia arborea* seed development. Data shown are the averages \pm s.e. from five replications of ten seeds each.

Significant morpho-physiological changes in seeds were observed after pod dehiscence. Seeds started to become dormant as seed moisture content was drastically reduced and the seed coat acquired its typical black–red colouring that creates mimicry, suggesting that this is a light-induced physiological phenomenon. Although maximum seed dry matter was attained at 255 DAA, the germination percentage of non-scarified seeds, which indirectly indicates the level of seed dormancy when compared with the germination of

scarified seeds, fell steadily until 315 DAA. This demonstrated the continuity of physiological changes in the seeds even after their maturity, when the physiological connection with the mother plant had ceased.

Seeds that remained attached to the pod in the mother plant for a year showed lower germination than seeds stored under controlled conditions ($F_{1,39} = 30.73$, $P = < 0.0001$; Table 2). Germination percentage fell in the first 3 months that seeds were attached to the pod on the mother plant ($F_{1,39} = 7.78$, $P = 0.0005$; Table 2), although no further significant decrease was observed in the following 9 months, allowing these seeds to keep a germination percentage around 55 % even after 1 year under field conditions. The high proportion of hard seeds (100 % of seeds were dormant in all evaluations), allowed seeds to maintain low moisture content levels after 3 (4.7 ± 0.3 %, $x \pm 1$ SD), 6 (4.1 ± 0.2 %), 9 (3.8 ± 0.1 %) and 12 (3.8 ± 0.1 %) months, even under the humid tropical climate characteristic of the study site.

Seedling epicotyl length did not differ between seeds stored under controlled conditions and seeds left attached to the pod on the mother plant (Table 2), but differed within each seed group according to the period that seeds remained on the mother plant or were harvested and stored ($F_{3,39} = 9.18$, $P = 0.0002$). An increase in seedling epicotyl length was observed in these two seed groups 3 months after all seeds became dormant, but no significant changes were observed during the following 9 months.

DISCUSSION

The hypothesis that dormancy protects mimetic seeds against deterioration before dispersal was supported by evidence of high seed-coat impermeability shown by all the species studied. Impermeable coats protected non-scarified seeds against AA because seeds did not imbibe water during the treatment. Thus the seeds did not undergo the physiological ageing that occurs when seeds are held at high temperature with high seed moisture content (McDonald, 1999). The increased seed moisture content seen in the aged seeds of *A. precatarius* may have been due to alterations in the seed coats resulting from temperature stress during AA. However, this did not either reduce the dormancy of the seeds or lead to their deterioration. The scarified seeds submitted to AA that failed to germinate were rotten or non-viable, and

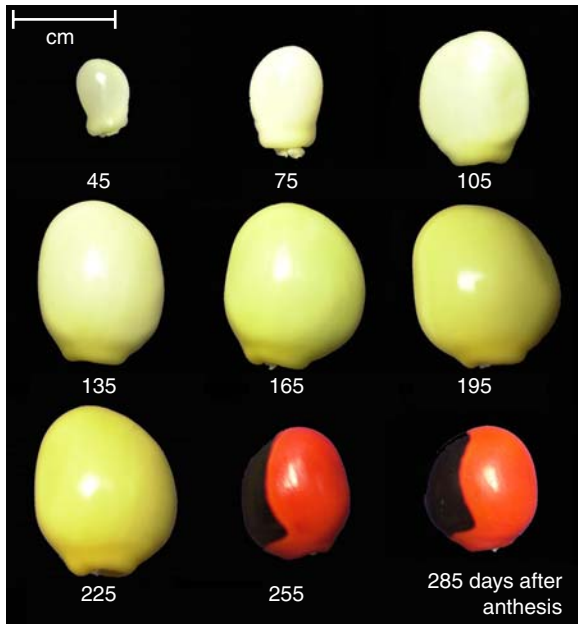


FIG. 4. *Ormosia arborea* seeds from different harvesting times during seed development.

therefore considered to be dead. These results support the hypothesis that seed hardness would be an effective protection against deterioration if seeds are exposed to a humid tropical environment while attached to the pod after maturity.

This hypothesis is reinforced by evaluating the physiological potential of *O. arborea* seeds kept on the mother plant after maturity. Even though these seeds did not show the same germination percentage as those of seeds stored under controlled conditions, they produced seedlings with the same vigour, which would effectively contribute to the perpetuation of the species. Since long rainy periods and high temperatures occur in the study area, germination around 55% and the presence of vigorous seedlings even after 1 year under field conditions following seed maturity suggest that coat impermeability can extend the seed dispersal period for the species under study without intense losses of viability, which can increase their chances of being consumed by naïve avian frugivores.

The decrease in germination percentage for seeds that remained on the mother plant was possibly related to the intense reduction in seed moisture content, ranging from

4.7% after seeds become dormant to 3.8% after 1 year in the field. This was a result of full seed exposure to sunlight in fully open pods. Impermeable coats avoid water absorption but allow water loss by seeds, therefore the decrease in seed moisture may have removed the water strongly associated to macromolecules by chemical absorption. The removal of this type of water enhances the deterioration rate in seed tissues by reducing their protection against free radical activity and protein denaturation (McDonald, 1999). In this extremely reduced water status, reactions mediated by enzymes do not occur and catabolic events prevail (Pammenter and Berjak, 2000). It is a probable collateral effect of keeping seeds exposed to field conditions for such a long period.

Although these results support the hypothesis that impermeable seed coats can reduce the physiological deterioration of mimetic seeds, but not avoid it, this dormancy mechanism may also be part of a mutualistic interaction between mimetic-seeded species and terrestrial granivorous birds (Peres and van Roosmalen, 1996; Foster and Delay, 1998). Indeed, if mutualism were the effective mode of interaction between mimetic-seeded species and terrestrial granivorous birds, one might expect the seeds to be liberated from the pod to the soil immediately after maturation, exposing them to terrestrial granivorous birds' foraging as soon as possible. In contrast, these seeds remain attached to the pod on the mother plant for long periods after dehiscence, probably 'waiting' for their ingestion by frugivorous birds in the canopy. As observed by Galetti (2002), *O. arborea* seeds can remain attached to the pod on the mother plant for up to 3 years. The maintenance of seeds in the tree canopy is contradictory to the idea that mimetic seeds are predominantly dispersed by terrestrial granivorous birds, as seeds are kept distant from that class of potential dispersers.

Another function frequently associated with seed dormancy is the spread of germination time, which increases chances of seedling survival (Fenner and Thompson, 2005). This function cannot, however, be the sole explanation for the presence of coat impermeability in mimetic seeds. As observed in seed development of *O. arborea*, only 4% of the seeds were dormant immediately after pod dehiscence. Onset of dormancy continued gradually after pod dehiscence; after 30 d, 22% of impermeable coated seeds were dormant, and 96% were dormant after 60 d. Thus, if seeds were readily ingested by frugivorous birds soon after pod dehiscence, most of the dispersed seeds would not be dormant.

TABLE 2. Comparison of germination percentage and epicotyl length of *Ormosia arborea* dormant seeds kept for 1 year under controlled storage conditions and attached to the pod on the mother plant

| Parameter | Seed source | Days after seeds become dormant | | | | |
|------------------------|--|---------------------------------|---------|---------|---------|---------|
| | | 0 | 92 | 184 | 273 | 369 |
| Germination percentage | Seeds kept attached to the pod on the mother plant | 92 Aa | 60 Bb | 56 Bb | 52 Bb | 56Bb |
| | Seeds kept under controlled storage conditions | 88 Aa | 84 Aa | 82 Aa | 80 Aa | 80Aa |
| Epicotyl length | Seeds kept attached to the pod on the mother plant | 8.8 Aa | 10.8 Ba | 10.7 Ba | 10.9 Ba | 10.8 Ba |
| | Seeds kept under controlled storage conditions | 8.5 Aa | 11.6 Ba | 11.1 Ba | 11.1 Ba | 11.8 Ba |

Before germination tests, dormancy was overcome by scarifying seeds in sandpaper.

Means followed by the same lower-case letters in the column or upper-case letters in the row, considering germination percentage separated from epicotyl length are not different according to Tukey's test at $P < 0.05$.

The retention of mimetic seeds on the mother plant after maturity could eventually bring disadvantages for species recruitment, as seed dispersal would not always occur in periods of optimal seed germination and seedling establishment. However, an impermeable seed coat could result in another advantage for mimetic seeds, in this situation, by optimizing germination timing and increasing the recruitment of seedlings over time (Foster, 2008).

Seed hardness has been considered a key trait for the functional ecology of mimetic seeds, independently of understanding the role of this trait in their dispersal strategy. However, the evolutionary pathway that resulted in coat impermeability in mimetic seeds is still unclear. The argument that an impermeable seed coat is an exaptation rather than an adaptation in mimetic seeds is reinforced by studying *Erythrina* phylogeny. Two main derived clades include all mimetic-seeded species, while species with brown or black seed coats (without mimicry) are present in more 'basal' clades (Bruneau, 1996). In addition to the present results obtained for *E. speciosa*, seed dormancy studies carried out with two species from this 'basal' clade, namely *E. crista-galli* (Silva et al., 2006) and *E. falcata* (Fowler and Bianchetti, 2000), indicated that these species have impermeable seed coats, suggesting this trait is a plesiomorphic, 'basal' character state in the genus *Erythrina*. *Ormosia* also has species with entirely black seeds (Rudd, 1965), but no phylogenetic-oriented studies have been made to date to investigate if the black coat is a plesiomorphic character state.

We speculate that mimetism is a seed trait that evolved in species whose common ancestor already had seeds with alkaloids and impermeable coats. The mutations that changed seed visual display probably increased the overall species fitness by deceiving avian frugivores and enhancing seed dispersal. Additionally, the presence of alkaloids and impermeable coats in these seeds created a favourable situation to fix mutations that help avoid the formation of an abscission layer in the funiculum, allowing an increase in seed dispersal period by keeping seeds attached to the pod long after seed maturity. It is well known that in many species of edible cereal grasses, reduced fruit dehiscence and seed shattering were artificially introduced by human selection, and expression of these traits have been shown to be controlled by monogenic heritage (Li et al., 2006, and references therein). Thus, it seems plausible that natural changes in this character could easily occur as an adaptive step in the seed dispersal strategy of tropical trees bearing mimetic seeds. Moreover, biochemical exaptations are recognized as playing a major role in the evolution of plant–insect relationships (Armbruster et al., 1997) and, in the case of mimetic-seeded species, the protection against insect seed predation resulting from the accumulation of secondary compounds may also be considered an exaptation.

In this light, we argue that mimetic seeds may have evolved as an apomorphic trait in plant groups wherein secondary compounds and impermeable seed coats were already present as plesiomorphic character states, so that an impermeable coat was secondarily 'useful' to the plant by increasing the dispersal period of mimetic seeds by protecting seeds against deterioration while they 'await' appropriate frugivores. Therefore, dormancy also could arguably be an exaptation in

mimetic-seeded species to reduce seed deterioration, allowing an increase in their effective dispersal period and minimizing the limitation imposed by low removal rates by naïve avian frugivores.

ACKNOWLEDGEMENTS

We thank Prof. Mauro Galetti and Dr James Aronson for useful comments on the manuscript. J. Marcos Filho and Ricardo Ribeiro Rodrigues thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support.

LITERATURE CITED

- Almeida-Neto M, Campassi F, Galetti M, Jordano P, Oliveira-Filho A. 2008. Vertebrate dispersal syndromes along the Atlantic forest: broad-scale patterns and macroecological correlates. *Global Ecology and Biogeography* 17: 503–513.
- Andrieu E, Debussche M. 2007. Diaspore removal and potential dispersers of the rare and protected *Paonia officinalis* L. (Paeoniaceae). *Botanical Journal of the Linnean Society* 157: 13–25.
- Armbruster WS. 1997. Exaptation link evolution of plant–herbivore and plant–pollinator interactions: a phylogenetic inquiry. *Ecology* 78: 1661–1672.
- Barrows EM, Acquavelha AP, Wesinsein PJS, Nosal RE. 1980. Response to novel food in captive, juvenile mockingbirds. *Wilson Bulletin* 92: 399–402.
- Baskin CC, Baskin JM. 1998. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. San Diego, CA: Academic Press.
- Bewley JD, Black M. 1982. *Physiology and biochemistry of seeds in relation to germination*. New York, NY: Springer-Verlag.
- Bruneau A. 1996. Phylogenetic and biogeographical patterns in *Erythrina* (Leguminosae: Phaseoleae) as inferred from morphological and chloroplast DNA characters. *Systematic Botany* 21: 587–605.
- Cazetta E, Zumstein LS, Melo-Júnior TA, Galetti M. 2008. Frugivory on *Margaritaria nobilis* L.f. (Euphorbiaceae): poor investment and mimetism. *Revista Brasileira de Botânica* 31: 303–308.
- Fenner M, Thompson K. 2005. *The ecology of seeds*. Cambridge: Cambridge University Press.
- Foster MS. 2008. Potential effects of arboreal and terrestrial avian dispersers on seed dormancy, seed germination and seedling establishment in *Ormosia* (Papilionoideae) species in Peru. *Journal of Tropical Ecology* 24: 619–627.
- Foster MS, Delay LS. 1998. Dispersal of mimetic seeds of three species of *Ormosia* (Leguminosae). *Journal of Tropical Ecology* 14: 389–411.
- Fowler JAP, Bianchetti A. 2000. *Dormência em sementes florestais*. Colombo: Embrapa Florestas.
- Galetti M. 2002. Seed dispersal of mimetic fruits: parasitism, mutualism, aposematism or exaptation? In: Levey DJ, Silva WR, Galetti M. eds. *Seed dispersal and frugivory: ecology, evolution and conservation*. Wallingford: CAB International, 177–191.
- Games DE, Jackson AH, Khan NA, Millington DS. 1974. Alkaloids of some African, Asian, Polynesian and Australian species of *Erythrina*. *Lloydia* 37: 581–588.
- Gould SJ, Vrba ES. 1982. Exaptation – a missing term in the science of form. *Paleobiology* 6: 4–15.
- Guimarães PR, José J, Galetti M, Trigo JR. 2003. Quinolizidine alkaloids in *Ormosia arborea* seeds inhibit predation but not hoarding by agoutis (*Dasyprocta leporina*). *Journal of Chemical Ecology* 29: 1065–1072.
- Hammond DS. 1995. Post-dispersal seed and seedling mortality of tropical dry forest trees after shifting agriculture, Chiapas, Mexico. *Journal of Tropical Ecology* 11: 295–313.
- Hargreaves RT, Johnson RD, Millington DS, et al. 1974. Alkaloids of American species of *Erythrina*. *Lloydia* 40: 454–470.
- Howe HF, Smallwood J. 1982. Ecology of seed dispersal. *Annual Review of Ecology and Systematics* 13: 201–228.
- ISTA. 2001. *International rules for seed testing*. Zurich: International Seed Testing Association.

- Jordano P. 1995.** Angiosperm fleshy fruits and seed dispersers: a comparative analysis of adaptation and constraints in plant–animal interactions. *American Naturalist* **145**: 163–191.
- Kissmann C, Scalon SPQ, Scalon Filho H, Ribeiro N. 2008.** Tratamentos para quebra de dormência, temperaturas e substratos na germinação de *Adenantha pavonina* L. *Ciência Agrotécnica* **32**: 668–674.
- Li C, Zhou A, Sang T. 2006.** Rice domestication by reducing shattering. *Science* **311**: 1936–1939.
- Lopes JC, Dias PC, Macedo CMP. 2004.** Tratamentos para superar dormência de sementes de *Ormosia arborea* (Vell.) Harms. *Brasil Florestal* **80**: 25–35.
- McDonald MB. 1999.** Seed deterioration: physiology, repair and assessment. *Seed Science and Technology* **27**: 177–237.
- McKey D. 1975.** The ecology of coevolved seed dispersal systems. In: Gilbert LE, Raven PH. eds. *Coevolution of animals and plants*. Austin, TX: University of Texas Press, 59–209.
- Mack AL. 2000.** Did fleshy fruit pulp evolve as a defence against seed loss rather than as a dispersal mechanism? *Journal of Bioscience* **25**: 93–97.
- Moore RP. 1972.** Interpretation of color differences in tetrazolium testing. *Seed Technologist News* **44**: 22–24.
- Pammenter NW, Berjak P. 2000.** Aspects of recalcitrant seed physiology. *Revista Brasileira de Fisiologia Vegetal* **12**: 56–69.
- Peres CA, von Roosmalen MGM. 1996.** Avian dispersal of mimetic seeds of *Ormosia lignivalvis* by terrestrial granivores: deception or mutualism? *Oikos* **75**: 249–258.
- Ramos MV, Teixeira CR, Bomfim LR, Madeira SVF, Moreira RA. 1999.** The carbohydrate-binding specificity of a highly toxic protein from *Abrus pulchellus* seeds. *Memórias do Instituto Oswaldo Cruz* **94**: 185–188.
- Ridley HN. 1930.** *The dispersal of plants throughout the world*. London: L. Reeve.
- Rudd VE. 1965.** The American species of *Ormosia*. *Contributions of the United States National Herbarium* **32**: 279–284.
- Seidler TG, Plotkin JB. 2006.** Seed dispersal and spatial pattern in tropical trees. *PLoS Biology* **4**: 1–6.
- Silva AJC, Carpanezzi AA, Lavoranti OJ. 2006.** Breaking of dormancy of *Erythrina crista-galli* seeds. *Boletim de Pesquisa Florestal* **53**: 65–78.
- Silva KB, Alves EU, Bruno RLA, Gonçalves EP, Braz MSS, Viana JS. 2007.** Quebra de dormência em sementes de *Erythrina velutina* Willd. *Revista Brasileira de Biociências* **5**: 180–182.
- Van der Pijl L. 1982.** *Principles of dispersal in higher plants*, 3rd edn. New York, NY: Springer-Verlag.