UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
Departamento de Botânica
Programa de Pós-Graduação em Biologia Vegetal
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AILTON GONÇALVES RODRIGUES JUNIOR

DORMÊNCIA FÍSICA EM SEMENTES: COMPREENDENDO O CICLO DE SENSIBILIDADE E OS COMPLEXOS *WATER GAP*

Physical dormancy in seeds: understanding the sensitivity cycling and water-gap complexes

Tese apresentada ao programa de Pós-Graduação em Biologia Vegetal do Departamento de Botânica do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutor em Biologia Vegetal.

Área de concentração: Fisiologia Vegetal e Ecologia

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Orientadora: Profa. Dra. Queila de Souza Garcia
Universidade Federal de Minas Gerais

Co-orientadora: Profa. Dra. Carol C. Baskin
University of Kentucky

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Dra. Queila de Souza Garcia (UFMG)

Dra. Andréa Rodrigues Marques Guimarães (CEFET)

Dr. Edvaldo Aparecido Amaral da Silva (FCA- UNESP)

Dra. Denise Maria Trombert de Oliveira (UFMG)

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SUMÁRIO

RESUMO GERAL

A dormência física (PY), caracterizada pela presença de uma camada impermeável no fruto ou semente, é um dos tipos de dormência mais recorrentes em sementes. No entanto, para uma melhor compreensão deste complexo mecanismo faz-se necessário uma investigação mais aprofundada sobre PY. Um modelo de duas etapas foi descrito para a superação da PY em sementes, em que a primeira etapa pode ser reversível entre dois estados ('insensível' ↔ 'sensível'), tornando-se permeável na segunda etapa. Esta característica faz com que as sementes com PY possam apresentar ciclo de sensibilidade, regulando assim o momento adequado para a germinação, o que tem sido relatado para espécies herbáceas de zonas temperadas. Outro fator importante sobre a PY é que o tamanho das sementes regula a dormência, entretanto, este mecanismo não é ainda compreendido. Estruturas como o hilo, a lente e a micrópila, atuam como canais de entrada de água (*water gaps*), sendo a lente a estrutura mais comumente relacionada a esta função. O pleurograma, uma estrutura cuja função é ainda desconhecida apresenta várias fissuras sinalizando para um possível papel na entrada de água. O gênero *Senna*, amplamente diverso e distribuído, cujas sementes apresentam PY, foi escolhido para investigar o ciclo de sensibilidade, o efeito do tamanho das sementes na dormência, a função e evolução do pleurograma e demais estruturas na PY. Para testar as hipóteses deste trabalho, as seguintes perguntas foram feitas: (1) Espécies de zona tropical e de hábito arbóreo podem apresentar ciclo de sensibilidade? (2) Se o tamanho das sementes regula a superação da dormência, como este mecanismo atua? (3) Qual é a função do pleurograma em sementes com PY? Este estudo confirmou que o ciclo de sensibilidade não é uma estratégia exclusiva de espécies herbáceas de zonas temperadas, ocorrendo na espécie arbórea tropical *Senna multijuga*. Nesta espécie, o tamanho da semente controla a superação da dormência, sendo este mecanismo regulado pelo conteúdo de água e a razão entre a espessura da camada paliçádica do *water gap* e a massa das sementes. Além disso, o pleurograma atua como um *water gap* em algumas sementes com dormência física. Resultados preliminares indicaram o caráter ancestral relacionado à funcionalidade do pleurograma, sendo uma estrutura presente em espécies primitivas dentro do gênero *Senna*, com a lente podendo ser uma estrutura funcional mais recente durante a evolução da PY.

Palavras-chave: Ciclo de sensibilidade, controle da dormência, dormência física, evolução da dormência, Fabaceae, pleurograma, *Senna*, sensibilidade mediada pelo tamanho da semente, tamanho da semente, *water gaps*.

ABSTRACT

Physical dormancy (PY), characterized by the presence of a water-impermeable layer in the fruit/seed coat, is one of the most recurrent kind of dormancy in seeds. However, an ample investigation about PY is still needed to understand better this complex mechanism. A two-stage model has been described to break PY in seeds, wherein the first step can be reversible between two stages ('insensitive' ↔ 'sensitive'), becoming permeable in the second step. This feature makes physically dormant seeds to present sensitivity cycling, which regulates the timing of germination, which has been reported for herbaceous species from temperate zones only. Another important aspect about PY is that the seed size controls the dormancy, but how this affects the dormancy is still unknown. Seed structures like hilum, micropyle and lens, act as water gaps, in which the lens is the most common kind of water gap. The pleurogram, a seed structure which function is still unknown, has several fissures which signalize for a possible role in water uptake. *Senna*, a highly diverse and widespread genus which seeds have PY only, was selected to test our hypothesis in relation to sensitivity cycling, the effect of seed size on dormancy, the function and evolution of the pleurogram and other structures on PY. To test our hypothesis, it was addressed the following questions: (1) Can species from tropical zones and tree habit have sensitivity cycling? (2) If the seed size mediates breaking PY, how does this mechanism work? (3) What is the function of pleurogram in seeds with PY? It was confirmed in this study that sensitivity cycling is not a trait of herbaceous species from temperate zones only. This also occurred for the tropical tree species *Senna multijuga*. In this species, seed size mediates dormancy break, which is regulated by the water content and the ratio between palisade layer thickness and seed mass. Further, the pleurogram was described as a water gap for some physically dormant seeds. Preliminary results indicate the ancestral character related to the functionality of the pleurogram, structure present in primitive *Senna* species. Also, the lens may be a most recent structure during the evolution of PY.

Keywords: Dormancy control, evolution of dormancy, Fabaceae, pleurogram, physical dormancy, seed size, seed size-mediated sensitivity, *Senna*, sensitivity cycling, water gaps.

INTRODUÇÃO GERAL

A dormência é caracterizada como um impedimento à germinação de sementes viáveis durante um período específico de tempo sob condições adequadas para que isto ocorra (Baskin e Baskin 2004, Finch-Savage e Leubner-Metzger 2006). A dormência física (PY) é um dos cinco tipos de dormência descritos por Baskin e Baskin (2004). Este tipo de dormência é caracterizado pela presença de uma camada impermeável no fruto ou na semente que impede a absorção de água (Baskin et al. 2000; Baskin 2003). Para as sementes se tornarem permeáveis, ou seja, superarem a dormência, diferentes estruturas atuam como canais de entrada de água (*water gaps*) (Baskin et al. 2000; Gama-Arachchige et al. 2013). Estes canais são formados em resposta a sinais ambientais específicos, ajustando a germinação ao período adequado ao estabelecimento da plântula (Baskin 2003).

Sementes com PY são encontradas praticamente em todas as regiões ao redor do mundo, sendo um dos tipos de dormência mais recorrentes (Baskin e Baskin 2014). No entanto, esta é a única *classe* de dormência que ainda não foi subdividida em *níveis* e *tipos* de acordo com a classificação de Baskin e Baskin (2004). A necessidade de um maior detalhamento desta classe de dormência para a compreensão das características deste tipo de dormência permitirá um maior entendimento deste mecanismo de controle da germinação e como ele afeta o *fitness* das espécies e assim poderá gerar subsídios para uma subdivisão desta *classe* de dormência.

Uma característica importante das sementes com PY é a presença de duas etapas para a superação da dormência. Este modelo de duas etapas foi proposto por Taylor (1981, 2005) e detalhado mais recentemente por Jayasuriya et al. (2008, 2009) e Gama-Arachchige et al. (2012). Na primeira etapa, as sementes precisam experimentar

condições específicas para tornarem-se ‘sensíveis’, mantendo-se ainda impermeáveis. Estas sementes ‘sensíveis’ são capazes de responder a condições ambientais específicas para superar a dormência. A segunda etapa deste processo é caracterizada pela superação da PY, ou seja, quando as sementes tornam-se permeáveis (Jayasuriya et al. 2008, 2009). Uma vez que a dormência é superada, ou seja, as sementes tornam-se permeáveis, elas não são mais capazes de retornar ao estado dormente. Sendo assim, esta última etapa, a perda da impermeabilidade, é irreversível (Jayasuriya et al. 2008). No entanto, a primeira etapa pode ser reversível e, neste caso, as sementes podem alternar entre estados ‘sensíveis’ e ‘insensíveis’. Esta característica foi denominada ‘ciclo de sensibilidade’ por Jayasuriya et al. (2008, 2009), sendo reportada para espécies herbáceas de ambientes temperados. No primeiro capítulo desta tese, foi investigado se esta característica é compartilhada com uma espécie arbórea tropical. Para isto, utilizamos a espécie *Senna multijuga*, que produz sementes com PY (Rodrigues-Junior et al. 2014).

Além do ciclo de sensibilidade, uma característica recorrente da PY é que o tamanho da semente afeta a superação da dormência (Russi et al. 1992, Schutte et al. 2014), em que sementes maiores germinam mais rapidamente do que sementes menores, evidenciando a complexidade do mecanismo de controle da dormência. Schutte et al. (2014) já havia sugerido um possível *trade-off* entre o tamanho das sementes e a persistência no solo em espécies com PY, e assim como Russi et al. (1992), estes autores descreveram que a espessura do tegumento estaria relacionada com o tamanho das sementes. Assim, no segundo capítulo, utilizando como modelo a espécie *S. multijuga*, foi investigado como o tamanho da semente afeta a superação da PY. O segundo capítulo desta tese é o primeiro trabalho a explicar como o mecanismo de

superação da dormência física é controlado pelo tamanho das sementes e como isto regula o momento adequado para que a germinação ocorra.

No capítulo subsequente, os estudos foram aprofundados com o intuito de se investigar a dormência física no gênero *Senna* e compreender se a grande variação morfológica das sementes deste gênero é indicativa de novos *water gaps* em sementes com PY. Para isto foram estudadas 11 espécies de *Senna* para caracterização dos canais de entrada de água (*water gaps*), sendo investigada a função das estruturas das sementes sobre a dormência física, com destaque para o papel do pleurograma. O pleurograma (quando presente na semente) é uma marca em ambas laterais no tegumento de espécies de Fabaceae (Caesalpinioideae e Mimosoideae) e Cucurbitaceae (Corner 1976, Werker 1997). Gunn (1981) sugeriu que o pleurograma pode atuar de modo semelhante ao hilo, funcionando como uma válvula higroscópica, entretanto, sua função ainda não havia sido descrita. No terceiro capítulo, a funcionalidade do pleurograma em sementes com PY foi investigada. Diferentes estruturas das sementes atuam como *water gaps*, variando entre as espécies. Assim, a possível atuação do pleurograma na dormência física como *water gap* evidenciaria a enorme variação de funcionalidade entre as estruturas das sementes.

Senna é um gênero altamente diverso e amplamente distribuído (Irwin and Barneby, 1982) em que todas as espécies descritas produzem sementes com PY. Este gênero surgiu no Eoceno precoce e a sua rápida diversificação é evidenciada pela ampla variação morfológica apresentada pelas sementes (Marazzi et al. 2006; Marazzi e Sanderson, 2010). Sendo um dos maiores gêneros da família Fabaceae (Marazzi e Sanderson, 2010) e produzindo unicamente sementes dormentes, *Senna* é um grupo adequado para a investigação da PY.

Desta forma, o gênero *Senna* foi utilizado para testar as hipóteses neste trabalho em relação ao ciclo de sensibilidade, efeito do tamanho das sementes, atuação e evolução do pleurograma e demais estruturas na PY em sementes. Assim, para testar estas hipóteses, as seguintes perguntas foram feitas: (1) Espécies de zona tropical e de hábito arbóreo podem apresentar ciclo de sensibilidade? (2) Se o tamanho das sementes modula a superação da dormência, como este mecanismo de controle da dormência atua? (3) Qual a função do pleurograma em sementes com dormência física?

REFERÊNCIAS BIBLIOGRÁFICAS

Baskin CC. 2003. Breaking physical dormancy in seeds – focusing on the lens. *New Phytologist* **158**: 229-232.

Baskin CC, Baskin JM. 2014. *Seeds: ecology, biogeography and evolution of dormancy and germination*, 2nd edn. San Diego: Elsevier/Academic Press.

Baskin JM, Baskin CC. 2004. A classification system for seed dormancy. *Seed Science Research* **14**: 1-16.

Baskin JM, Baskin CC, Li X. 2000. Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Species Biology* **15**: 139-152.

Corner EJH. 1976. *The seeds of dicotyledons*, vol. I. Cambridge: Cambridge University Press.

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. *New Phytologist* **171**: 501-523.

Gama-Arachchige NS, Baskin JM, Geneve RL, Baskin CC. 2012. The autumn effect: timing of physical dormancy break in seeds of two winter annual species of Geraniaceae by a stepwise process. *Annals of Botany* **110**, 637-651.

Gama-Arachchige NS, Baskin JM, Geneve RL, Baskin CC. 2013. Identification and characterization of ten new water gaps in seeds and fruits with physical dormancy and classification of water-gap complexes. *Annals of Botany* **112**, 69-84.

Gunn CR. 1981. Seeds of the Leguminosae. In: Polhill RM, Raven PH, eds. *Advances in Legume Systematics, Part. 2*. Kew: Crown Copyright, 913–926.

Irwin HS, Barneby RC. 1982. The American Cassiinae. *Memoirs of the New York Botanical Garden* **35**: 1-918.

Jayasuriya KMGG, Baskin JM, Baskin CC. 2008. Cycling of sensitivity to physical dormancy-break in seeds of *Ipomoea lacunosa* (Convolvulaceae) and ecological significance. *Annals of Botany* **101:** 341-352.

Jayasuriya KMGG, Baskin JM, Geneve RL, Baskin CC. 2009. A proposed mechanism for physical dormancy break in seeds of *Ipomoea lacunosa* (Convolvulaceae). *Annals of Botany* **103**: 433-445.

Marazzi B, Endress PK, de Queiroz LP, Conti E. 2006. Phylogenetic relationships within *Senna* (Leguminosae, Cassiinae) based on three chloroplast DNA regions: patterns in the evolution of floral symmetry and extrafloral nectaries. *American Journal of Botany* **93**: 288-303.

Marazzi B, Sanderson MJ. 2010. Large-scale patterns of diversification in the widespread legume genus *Senna* and the evolutionary role of the extrafloral nectaries. *Evolution* **64**: 3570-3592.

Marazzi B, Conti E, Sanderson MJ, McMahon MM, Bronstein JL. 2013. Diversity and evolution of a trait mediating ant-plant interactions: insights from extrafloral nectaries in *Senna* (Leguminosae). *Annals of Botany* **111**: 1263-1275.

Rodrigues-Junior AG, Faria JMR, Vaz TAA, Nakamura AT, José AC. 2014. Physical dormancy in *Senna multijuga* (Fabaceae: Caesalpinioideae) seeds: the role of seed structures in water uptake. *Seed Science Research* **24**: 147-157.

Russi, L., Cocks P. S., Roberts E. H. 1992. Coat thickness and hard-seededness in some *Medicago* and *Trifolium* species. *Seed Science Research* **2**: 243-249.

Schutte, B. J., Davis A. S., Peinado Jr. S. A., Ashigh J. 2014. Seed-coat thickness data clarify seed size–seed-bank persistence trade-offs in *Abutilon theophrasti* (Malvaceae). *Seed Science Research* **24**: 119-131.

Taylor, G. B. 1981. Effect of constant temperature treatments followed by fluctuating temperatures on the softening of hard seeds of *Trifolium subterraneum* L. *Australian Journal of Plant Physiology* **8**: 547-558.

Taylor, G. B. 2005. Hardseededness in Mediterranean annual pasture legumes in Australia: a review. *Australian Journal of Agricultural Research* **56**: 645-661.

Werker, E. 1997. *Seed anatomy*. Berlin: Borntraeger.

CAPÍTULO I

SENSITIVITY CYCLING IN PHYSICALLY DORMANT SEEDS OF THE NEOTROPICAL TREE *SENNA MULTIJUGA* (FABACEAE)

Manuscrito submetido à *Plant Biology*

Research paper

Sensitivity cycling in physically dormant seeds of the neotropical tree *Senna multijuga* (Fabaceae)

Ailton G. Rodrigues-Junior[1,*], Carol C. Baskin[2,3], Jerry M. Baskin[2] and Queila S. Garcia[1]

[1]Laboratório de Fisiologia Vegetal, Departamento de Botânica, ICB/Universidade Federal de Minas Gerais, Caixa Postal 486, 31270-901, Belo Horizonte, MG, Brazil.
[2]Department of Biology, University of Kentucky, Lexington, KY 40506, USA and
[3]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546, USA
**Correspondence. E-mail ailtongrodrigues@gmail.com/queila@icb.ufmg.br*

Short title: Sensitivity cycling in Senna seeds

Key words

Natural PY-break, neotropical tree species, physical dormancy, seed size-mediated sensitivity, survival strategies.

ABSTRACT

- Cycling of sensitivity to physical dormancy (PY) break has been documented in herbaceous species. However, it has not been reported in tree seeds, nor has the effect of seed size on sensitivity to PY-breaking been evaluated in any species. Thus, the aims of this study were to investigate how PY is broken in seeds of the tropical legume tree *Senna multijuga*, if seeds exhibit sensitivity cycling and if seed size affects induction into sensitivity.
- Dormancy and germination were evaluated in intact and scarified seeds from two collections of *S. multijuga*. The effects of temperature, moisture and seed size on induction of sensitivity to dormancy-breaking were assessed, and seasonal changes in germination and persistence of buried seeds were determined. Reversal of sensitivity was also investigated.
- Fresh seeds were insensitive to dormancy break at wet-high temperatures, and an increase in sensitivity occurred in buried seeds after they experienced low temperatures during winter (dry season). Temperatures ≤ 20 ºC increased sensitivity, whereas temperatures ≥ 30 ºC decreased it regardless of moisture conditions. Dormancy was broken in sensitive seeds by incubating them at 35 ºC. Sensitivity could be reversed, and large seeds were more sensitive than small seeds to sensitivity induction.
- Seeds of *S. multijuga* exhibit sensitivity cycling to PY-breaking. Seeds become sensitive during winter and can germinate with the onset of the spring-summer rainy season in Brazil. Small seeds are slower to become sensitive than large ones, and this may be a mechanism by which germination is spread over time.

Sensitive seeds that fail to germinate become insensitive during exposure to drought during summer. This is the first report of sensitivity cycling in a tree species.

INTRODUCTION

Seeds that do not imbibe water because of (a) water-impermeable cell layer(s) in the seed (or fruit) coat have physical dormancy (PY) (Baskin & Baskin 2004, 2014). For release of PY, a water-gap in the coat needs to be opened, thereby allowing water to enter the seed (Baskin *et al*. 2000; Baskin 2003; Turner *et al*. 2009; Gama-Arachchige *et al*. 2013). However, seeds may need to experience a sequence of specific environmental conditions for dormancy break to occur (Jayasuriya *et al*. 2008a,b; Gama-Arachchige *et al*. 2012). Upon opening of the gap, the seeds will germinate promptly over a wide range of conditions if they do not have physiological dormancy (PD) (i.e. in addition to PY).

Taylor (1981) proposed a two-stage temperature-dependent model for breaking PY, which explains the seasonal germination reported for water-impermeable seeds of herbaceous legume species. An environmental factor related to a specific season of the year is required for the occurrence of each of these two stages, and thus this model describes how the seasonality of germination in physically dormant seeds is controlled in the field (Taylor 1981). Taylor (1996) identified a temporal pattern of breaking PY for two annual species of *Medicago*. The first step occurred in the field in Western Australia during summer and resulted in seeds becoming "latent soft" but still water impermeable, i.e. they were sensitive to dormancy-breaking conditions. In the second step, the latent soft seeds became permeable after four diurnal cycles of 35/10 °C and incubation at 20 °C. That is, dormancy break did not occur unless seeds first entered the

'latent-soft' state. Reversibility of the first step in this dormancy-breaking process was described by Taylor & Revell (1999) as being temperature-modulated. Van Assche *et al*. (2003) also demonstrated that sensitivity to dormancy-breaking conditions could be reversed.

During the first step (induction of sensitivity), Taylor (1981) suggested that a weakening of the water gap (lens for legumes) was responsible for making the still dormant (or latent soft; Taylor 1996) seeds capable of responding to the second step of the dormancy breaking process. Taylor (2005) considered moisture to be a factor that accelerated the first step of seed softening. By preventing dehydration of the seeds, he showed that moisture accelerated seed softening; however, this process occurred even in dry conditions. Furthermore, in the second step, when latent soft seeds became permeable, temperature was an important factor (Taylor 1981, 2005).

Jayasuriya *et al*. (2008a,b, 2009a,b) interpreted these two steps for breaking PY as changes between insensitive and sensitive stages (sensitivity cycling) and reported the effect of moisture on this process. Sensitivity cycling has been reported only in seeds of Fabaceae and Convolvulaceae and only in herbaceous species of these two families (see Jayasuriya *et al*. 2009a; Baskin & Baskin 2014). However, although it seems that the two-step process has been reported in all species in which it has been sought (see table 2 in Gama-Arachchige *et al.* 2012), sensitivity cycling was not found in seeds of *Geranium carolinianum* or *G. dissectum* (Gama-Arachchige *et al*. 2012). Furthermore, the effect of seed size on induction of sensitivity to PY-breaking heretofore has not been evaluated.

To help fill these gaps in our of knowledge of PY-breaking, we studied the neotropical tree species *Senna multijuga* (Fabaceae, Caesalpinioideae), which occurs throughout many regions of Brazil but mainly in the Southeast (Carvalho 2004; Souza

& Bortoluzzi 2015). *Senna multijuga* produces desiccation-tolerant (Rodrigues-Junior *et al*. 2015) and physically dormant (Rodrigues-Junior *et al*. 2014) seeds. For dormancy to be broken naturally in *S. multijuga* seeds, an opening needs to be formed in the lens, which acts as a water gap (Rodrigues-Junior *et al*. 2014). The water-gap complex of this species is classified as Type-I simple (sensu Gama-Arachchige *et al*. 2013); however, the ecological aspects of dormancy break and germination of this species have not been determined in the field.

We hypothesized that sensitivity cycling is a feature of the physically dormant seeds of *S. multijuga* and that seed size affects the PY-breaking process. More specifically, the purposes of this study were to determine (1) how PY is broken in seeds of a tropical tree species in response to environmental conditions in the habitat; (2) if seeds exhibit sensitivity cycling in response to temperature and moisture; and (3) if seed size affects induction of sensitivity.

MATERIAL AND METHODS

Seed collection

Freshly matured dry fruits (pods) were collected in September 2014 from *S. multijuga* trees in two locations on the campus of Universidade Federal de Lavras, Lavras, Brazil [seed collection 1 (S1) (21° 13′ 39,34″ S, 44° 58′ 11,85″ W; seed collection 2 (S2) (21° 13′ 30,53″ S, 44° 58′ 27,12″ W)]. The region is a transition zone between the Atlantic Forest and Cerrado (Brazilian savanna). Seeds were removed manually from the pods and filled seeds separated from nonfilled seeds by flotation in water. Seeds without obvious insect damage that sank in water were blotted dry and placed in plastic trays in ambient room conditions [25±5 ºC, 40-60% relative humidity (RH)] for 24 h and then stored in sealed semipermeable plastic bags in the same conditions until the beginning of the experiments one week later. S1 seeds were heavier

than S2 seeds; mean seed mass 0.019±0.003 g for S1 and 0.009±0.002 g for S2 (P<0.01).

Seed dormancy and germination in controlled conditions

To evaluate the germination response of dormant and non-dormant seeds (both S1 and S2) to temperature, fresh scarified and non-scarified seeds from each seed collection were incubated in Petri dishes on germination paper moistened with distilled water in light/ dark (12/12 h; hereafter light) at constant (5 to 40 ºC at 5 °C intervals) and at alternating [30/10, 30/15, 30/20 and 35/20 ºC (12/12 h)] temperature regimes. The light source was cool white fluorescent tubes (40 µmol m^{-2} s^{-1}). Seeds were scarified by using sandpaper. Five replicates of 25 seeds were used in each treatment. Germination was monitored at 3-d intervals for 30 d; the criterion for germination was emergence of the radicle.

Seasonal changes in germination and persistence in soil

To assess changes in germination responses and persistence in soil, 45 nylon-mesh bags (0.5 mm mesh diameter) each of S1 and S2 seeds were buried in soil at a depth of 5 cm in November 2014 in an area where the species naturally occurs. Each bag contained 115 seeds. Five bags each of S1 and S2 seeds were exhumed at 2-4 mo intervals for 18 mo. Non-imbibed seeds were removed from the bags and tested for germination. Imbibition results in a clear change in seed colour and size, making imbibed seeds easy to detect. Five replicates of 25 exhumed, non-imbibed S1 and S2 seeds each were placed in Petri dishes on moist germination paper and incubated in light at 25, 35 and 30/20 ºC. Germinated seeds were counted at 3-d intervals for 30 d. After incubation, the remaining seeds were manually scarified with sandpaper and incubated at 25 °C to assess germination/viability. Soil temperature was monitored

throughout the experimental period by sensors (12-Bit Temperature Smart Sensor S-TMB-M006) buried 5 cm in the same place as the seeds and connected to a data-logger (HOBO® Micro Station Logger H21-002). Rainfall data for the region were obtained from the Instituto Nacional de Meteorologia (INMET).

Seed sensitivity after dry storage

To determine if storage in laboratory conditions affects seed sensitivity to dormancy breaking conditions, three samples containing four replicates of 25 S1 and S2 seeds each were stored dry in the laboratory (25±5 ºC) in semipermeable plastic bags for 0, 1 or 2 years. After each storage period, intact seeds were placed in Petri dishes on moist germination paper and incubated in light at 25, 35 and 30/20 ºC to evaluate germination. Germinated seeds were counted at 3-d intervals for 30 d. If sensitivity of seeds increases during dry storage at room temperatures, then germination at one or more of the test temperatures will increase significantly compared to that of fresh seeds.

Effects of temperature and moisture on sensitivity of stored seeds

The purpose of this experiment was to evaluate the effects of temperature and moisture on sensitivity to dormancy breaking conditions (based on Jayasuriya *et al*. 2008a). In this experiment, we used 1-year laboratory-stored seeds, which preliminary tests showed were still dormant. Seventy-two samples of seeds containing four replicates of 25 S1 and S2 seeds each were stored wet and dry at constant (15 - 30 ºC at 5 °C intervals) and at alternating (25/15 and 30/20 ºC) temperatures for 1, 3 and 6 mo (Fig. 1). After each storage period, germination was evaluated in light at 35 ºC for 30 d, conditions that previously had been shown to break dormancy of sensitive seeds. If sensitivity of seeds increases during wet or dry storage at different temperatures, then germination percentage will be significantly higher than that of 1-year-old laboratory-

stored seeds. Dormant seeds at the end of the germination test following 6 mo of storage in each temperature and moisture condition were scarified and incubated at 25 ºC to assess their viability via germination.

To determine if sensitivity could be reversed, after 6 mo of wet storage at all constant and alternating temperatures seeds were transferred to a dry condition at 30/20 ºC. After 3 mo of dry storage, four replicates of 25 S1 and S2 seeds each were placed into Petri dishes on moistened paper and tested for germination in light at 35 ºC for 30 d (Fig. 1). If sensitivity of seeds decreases during dry storage at 30/20 ºC, then germination percentage will be significantly lower than that of seeds after 6-mo of wet storage. Dormant seeds at the end of germination test following 3 mo of dry storage were scarified and incubated at 25 ºC to assess their viability via germination.

Effect of seed size on sensitivity induction

The effect of seed size on sensitivity to high temperature was evaluated for each seed collection. In this experiment, we used 1.5-year lab-stored seeds (seeds still dormant) and compared sensitivity after storage relative to seed size. S1 and S2 seeds were separated into two groups: large and small. Since large S1 seeds were twice the size of small S1 seeds, they could be easily separated visually. However, the validity of this separation was confirmed by weighing each seed. Furthermore, large S2 seeds were twice the size of small S2 seeds and this could be easily separated visually. However, the validity of the separation was confirmed by weighing each seed. Germination was evaluated at 35 ºC in light with four replicates of 25 S1 and S2 seeds for each size. Germinated seeds were counted at 3-d intervals for 30 d. If sensitivity of seeds differs in relation to seed size, then germination percentage will be significantly different between large and small seeds. At the end of the germination test, dormant S1 and S2 seeds of both sizes were scarified and incubated at 25 ºC to assess their viability via germination.

Statistical analyses

The experimental design was completely randomized. Seed mass data were tested for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett test) and then submitted to ANOVA at 5% probability; means were compared using the Tukey test at 5% probability. Germination data were analysed with a generalized linear model (GLM) (see table S1), and the means were compared using Fisher's Test (LSD). For the experiments with more than one factor (i.e. time, temperature, moisture and seedlot), the statistical model included the effects of the factors examined as well as their interactions. A Spearman's correlation analysis was performed among seed germination percentage, temperature, moisture condition and storage period to evaluate seed sensitivity (R Development Core Team 2011). SigmaPlot® software was used to design the graphs (Systat Software Inc., San José, California, USA).

RESULTS

Seed dormancy and germination in controlled conditions

Intact fresh S1 seeds germinated <20% and S2 <10% at all temperatures tested. Nevertheless, higher germination percentages ($P \leq 0.05$) were reached at 35 and 35/20 ºC than at the other temperatures in both seed collections. Highest percentage of imbibed seeds occurred at 40 ºC, but seeds failed to germinate at this temperature (Fig. 2A). Scarified seeds germinated to high percentages (≥60 %) at all temperatures tested (data not shown). However, scarified S1 seeds germinated to low percentages (23%) at 5 ºC, whereas no scarified S2 seeds germinated at this temperature. Scarified S1 and S2 seeds also failed to germinate at 40 ºC (Fig. 2B).

Seasonal changes in germination and persistence in soil

The period seeds experienced in the soil ($P<0.05$) and temperatures used for germination tests ($P<0.05$) affected the germination percentages, but there was no interaction between these two factors for S1 ($P>0.05$). Germination of fresh S1 seeds was ≤ 12% at all temperatures tested. After 2 mo of burial, germination reached 21.6% at 35 ºC, but there was little or no change at 25 and 30/20 ºC. Germination percentage increased throughout the period of burial, with highest values at 35 ºC. After 8 mo, 50% of the seeds germinated at 35 ºC; however, only 8 and 17.6% of the seeds germinated at 25 and 30/20 ºC, respectively. After 12 mo of burial, seeds germinated to 19, 46 and 20% at 25, 35 and 30/20°C, respectively (Fig. 3). For S2 seeds, there was an interaction between the length of burial period in soil and temperatures used for germination ($P>0.05$). S2 seeds were similar to S1 seeds in that they reached the highest germination percentages at 35°C. However, an increase in germination percentage of S2 seeds occurred only at 35 ºC, with little or no change at 25 and 30/20°C (Fig. 3). For both S1 and S2 seeds, germination percentages at 35°C increased after seeds were exposed to low temperatures (autumn/winter, dry season), and they remained high in subsequent evaluations (Fig. 3). Only a few seeds had imbibed or germinated inside the bags after burial for 6 mo. However, after 8 mo the presence of a fungal mass made it difficult to count seeds, and after 12 mo there was an insufficient number of seeds for further evaluation.

Seed sensitivity after dry storage

Germination percentage of S1 and S2 seeds at 35 ºC and 30/20 ºC increased with time of storage in laboratory conditions. A significant increase in germination at 35 and at 30/20 ºC occurred after 1 and 2 years of storage, with 35°C being optimal for germination. Germination at 25 ºC did not increase significantly with storage (Fig. 4).

Effects of temperature and moisture on sensitivity of stored seeds

At the beginning of this experiment, 50 and 39% of the S1 and S2 seeds, respectively, germinated at 35 ºC. Spearman's correlation for S1 seeds showed a moderately negative correlation between sensitivity and temperature (R=-0.40, P<0.001). Sensitivity also was correlated with moisture condition, but with a weak positive correlation (R=0.24, P<0.001).

There was no interaction between storage period and moisture condition at any of the temperatures tested for S1 seeds. Temperatures < 25 ºC increased sensitivity of S1 seeds (Fig. 5A, B). However, regardless of moisture condition, temperature < 20 ºC increased sensitivity of seeds throughout the storage period (P≤0.05), with highest sensitivity attained after 6 mo (Fig. 5A). At 20 ºC, wet-stored seeds were more sensitive than those dry-stored (P=0.015), and regardless of moisture condition seeds stored at this temperature for 6 mo had the highest sensitivity (P=0.017) (Fig. 5B). Moisture condition affected sensitivity of seeds stored at 25 ºC (P=0.046), with dry-stored seeds being less sensitive than wet-stored seeds (Fig. 5C). Storing seeds at 30 ºC tended to reduce sensitivity, unlike what occurred at 25/15ºC (Fig. 5D, E), and at 30/20 ºC sensitivity was reduced drastically regardless of moisture condition, with the lowest sensitivity after 1 and 3 mo of storage (P<0.001) (Fig. 5F).

There was a weak negative correlation for S2 seeds between sensitivity and time (R=-0.19, P=0.007) and sensitivity and temperature (R=-0.18, P=0.010). For S2 seeds, there was an interaction between storage period and moisture condition only at 30/20 ºC. However, storage at 15 ºC increased sensitivity, regardless of moisture condition (P=0.03), and at 20 ºC seeds stored under wet conditions had higher sensitivity than those stored under dry conditions (P<0.05) (Fig. 5G, H). As temperature increased (≥25 ºC), sensitivity of seeds tended to decrease, and at 30/20 ºC there was a strong reduction in sensitivity, with an interaction between storage period and moisture condition

(P=0.007) (Fig. 5I-L). At 30/20 ºC, there was a big reduction in sensitivity of wet-stored seeds after 1 mo, and after 3 and 6 mo sensitivity of both wet- and dry-stored seeds was low (P<0.001) (Fig. 5L). After 6 mo of storage in all temperature and moisture conditions, scarified S1 and S2 seeds germinated to ≥ 85%.

Seed sensitivity to dormancy break at 35 ºC could be reversed. There was a strong reduction in germination for S1 and S2 seeds after 3 mo of dry storage at 30/20 ºC regardless of the temperature during the previous period of wet storage (Fig. 6), confirming the reversal of sensitivity of these seeds. For example, after 6 mo of wet storage at 15 ºC, 73% of S1 seeds germinated at 35 ºC. However, if 6 mo of wet storage at 15°C was followed by 3 mo of dry storage at 30/20°C only 32% of the seeds germinated when subsequently tested at 35°C (Fig. 6). Scarified S1 and S2 seeds germinated to ≥ 90%.

Effect of seed size on sensitivity induction

Large and small S1 seeds had a mass of 0.02 and 0.01g, respectively, and large and small S2 seeds had a mass of 0.012 and 0.007 g, respectively. There was no interaction between seed collection and seed size on germination (P>0.05). However, seed size had an effect on germination (P≤0.05), with large seeds having higher sensitivity to high temperature (35 ºC) than small seeds (Fig. 7A, B). Furthermore, although large S1 seeds had twice the mass of large S2 seeds, the two collections had similar sensitivity to dormancy break at 35 ºC. Meanwhile, small S1 and S2 seeds germinated to only about 20% (Fig. 7A). After scarification, large and small L1 and L2 seeds germinated to approximately 80% at 25 ºC.

DISCUSSION

Most (75-98%) of the fresh intact seeds from both populations of *S. multijuga* incubated at 5, 10, 15, 20, 25, 30, 35, 35/20, 30/20, 30/15 and 30/10°C were water

impermeable and thus did not germinate. Seeds became permeable and germinated at 35 °C after they had been incubated wet or dry at temperatures ≤20 °C, had been buried in soil in the field during winter or had been dry-stored at room temperatures for 1 or 2 years. Thus, like the physically dormant seeds of the summer annual weed *Sida spinosa* (Malvaceae) (Baskin & Baskin 1984) if seeds of *S. multijuga* are incubated at one temperature and then moved to a higher temperature, the water gap opens and seeds imbibe water and germinate. That is, at a given temperature seeds of *S. multijuga* may become sensitive, but whether or not the lens opens depends on the seeds being exposed to a higher temperature than the one at which they became sensitive.

From the laboratory experiment, it appears that a temperature increase of 10-15 °C is needed to promote opening of the lens on sensitive seeds of *S. multijuga*. Since soil temperatures in the habitat of *S. multijuga* are lower (did not exceed 25 ºC) than air temperatures, sensitivity increased over time during burial. During the coldest month of the year (July), the maximum soil temperature did not exceed 20 ºC, thus contributing to the increase in sensitivity during winter. At ≤20 °C, sensitivity of seeds increased regardless of moisture condition, but with an increase in temperature seeds became more sensitive in wet than in dry conditions. The sensitivity of *S. multijuga* seeds to dormancy-breaking conditions could be reversed by high temperatures (≥30 °C), especially if seeds were dry. The alternating temperature regime of 30/20 ºC had the strongest effect on decreasing sensitivity. This temperature regime represents the mean maximum and minimum temperatures that occur during spring/summer in the region where *S. multijuga* grows, which suggests that sensitive seeds can respond accurately to natural conditions.

After burial in soil in the field, seeds of *S. multijuga* germinated to higher percentages at 35 than at 25 or 30/20 °C. In the tropical region where *S. multijuga*

grows, high temperatures are associated with an elevated amount of rainfall in spring/summer, and 35 °C is the maximum temperature that normally occurs in the study region. Thus, the high temperature requirement for the second step in the dormancy-breaking process allows the timing of germination of *S. multijuga* seeds in the field to coincide with the onset of the summer rainy season, when conditions for seedling establishment would be highly favourable.

A delay in rainfall or an unusually long dry period during the wet season could prevent dormancy breaking and reverse seed sensitivity. Several studies have reported the need for moisture for seeds to break PY (Martin *et al*. 1975; Van Klinken & Flack 2005; Jayasuriya *et al*. 2008a). In particular, the need for moisture to break PY was reported by Van Klinken *et al*. (2006) and Van Klinken & Goulier (2013) for four tropical legume species in Australia and by Fidelis *et al*. (2016) for some tropical legume species in Brazil, where dry heat (even at 150 ºC) did not break PY. Erickson et al. (2016) found that 70-100 °C wet heat was more effective in breaking PY in seeds of seven species of legumes in the Pilbara Region of northwestern Western Australia than dry heat.

Distinct strategies for timing of seed germination have been reported for other species with PY. For example, the physically dormant seeds of the summer annual vine *Ipomoea lacunosa* (Convolvulaceae) become sensitive during incubation on a moist substrate, with high temperatures being more effective than low temperatures (Jayasuriya *et al*. 2008a). Sensitive seeds become permeable when exposed to moist high temperature conditions, thereby promoting germination in summer. On the other hand, sensitive seeds become nonsensitive if they are stored dry at high (≥30 °C) or low (≤5 °C) temperatures. Thus, induction of *I. lacunosa* seeds into the nonsensitive state by drought conditions in mid- to late summer prevents germination in autumn and

consequently death of the cold-intolerant seedlings during winter. In contrast, the physically dormant seeds of the winter annual *Geranium carolinianum* (Geraniaceae) are made sensitive during incubation at high temperatures (≥20 °C) in either wet or dry conditions; during this time, the PD component (PY+PD) of the embryo is broken (Gama-Arachchige *et al*. 2012). The water gap on sensitive seeds of *G. carolinianum* opens when seeds are exposed to <20 °C; thus, seeds germinate in autumn. The cold-tolerant plants flower and produce seeds in spring, but freshly matured seeds cannot germinate in spring due to PD of the embryo and insensitivity of seeds to PY-break at spring temperatures.

The *S. multijuga* seed collection with the larger seeds overall (S1) had higher germination percentages than the one with smaller seeds overall (S2) in all experiments in this study. However, when S1 and S2 seeds were separated into two size classes, germination percentages were similar for large seeds of S1 and S2 and for small seeds of S1 and S2 (Fig. 7). Therefore, the distribution of seed size within each seed collection explains the differences found for these two seed collections, with S1 having a higher proportion of large seeds than S2.

In support of our hypothesis that seed size affects the two-step PY-breaking process, we found that after 1.5 years of dry storage at room temperatures more large than small seeds had become sensitive. Thus, small seeds potentially remain in the soil for a longer period of time than large seeds. Both large and small seeds of *S. multijuga* are dispersed in late winter/early spring and are insensitive to dormancy-break at high temperatures on a wet substrate (Fig. 8). Since seeds are not matured and dispersed until late winter/early spring, germination of most seeds would be delayed until at least the following year – after they had been exposed to winter temperatures and/or been buried. Non-dormant seeds and those that become sensitive in late winter or during burial

germinate in spring/summer, if there is adequate rainfall. Since large seeds become sensitive faster than small ones, it is expected that more large than small seeds would germinate in the first germination season. Further, any small (or large) sensitive seeds that fail to germinate will lose their sensitivity when summer drought periods occur. Nonsensitive seeds exposed to low temperatures the next winter will acquire sensitivity to PY-break, and dormancy potentially will be broken at the beginning of the next rainy season in spring/summer. Thus, in the second germination season potentially more small than large seeds would germinate. That is, one possible ecological consequence of differences in the induction of sensitivity in large and small seeds is that germination of a seed cohort with both sizes of seeds will be spread over more than 1 year.

Jayasuriya *et al*. (2008a) found maternal effects on sensitivity. In the present study, there were differences between seed collections in the requirements for induction of sensitivity. Lacerda *et al*. (2004) reported that maternal effects were responsible for variation in seed dormancy between two populations of *S. multijuga*. Furthermore, we showed that seed size plays a role in control of PY-break, wherein seeds have different degrees of sensitivity in relation to their size. The reason that seeds with PY germinate in fractions can be explained by a *continuum* of sensitivity (Jayasuriya *et al*. 2008a, 2009a), and seed size is a trait that can mediate these levels of sensitivity.

In conclusion, this is the first report of sensitivity cycling in seeds of a tree species with PY and that seed size can play a role in the induction of sensitivity. Since seeds of *S. multijuga* have a short-term soil seed bank (sensu Bakker *et al*. 1996), it is especially critical in terms of seedling survival that germination be timed to occur when the soil is sufficiently moist for good seedling growth. The rainy season is in spring/summer, thus high temperature (e.g. 35 °C) and high-moisture requirements for dormancy break of sensitive seeds ensure that seeds germinate during the warm wet

season. However, the presence of small seeds that are relatively slow to become sensitive would potentially spread germination of seeds in a particular cohort over at least two germination seasons.

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REFERENCES

Bakker J.P., Poschlod P., Strykstra R.J., Bekker R.M., Thompson K. (1996) Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandica*, **45**, 461-490.

Baskin C.C. (2003) Breaking physical dormancy in seeds – focusing on the lens. *New Phytologist*, **158**, 229-232.

Baskin C.C., Baskin J.M. (2014) *Seeds: ecology, biogeography and evolution of dormancy and germination*, 2nd edn. Elsevier/Academic Press, San Diego, US: 1587 pp.

Baskin J.M., Baskin C.C. (1984) Environmental conditions required for germination of prickly sida (*Sida spinosa*). *Weed Science*, **32**, 786-791.

Baskin J.M., Baskin C.C. (2004) A classification system for seed dormancy. *Seed Science Research*, **14**, 1-16.

Baskin J.M., Baskin C.C., Li X. (2000) Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Species Biology*, **15**, 139-152.

Carvalho P.E.R. (2004) Pau-cigarra – *Senna multijuga*. Embrapa Florestas, Colombo: 11 pp.

Erickson T.E., Merritt D.J., Turner S.R. (2016) Overcoming physical seed dormancy in priority native species for use in arid-zone restoration programs. *Australian Journal of Botany*, **64**, 401-416.

Fidelis A., Daibes L.F., Martins A.R. (2016). To resist or to germinate? The effect of fire on legume seeds in Brazilian subtropical grasslands. *Acta Botanica Brasilica*, **30**, 147-151.

Gama-Arachchige N.S., Baskin J.M., Geneve R.L., Baskin C.C. (2012) The autumn effect: timing of physical dormancy break in seeds of two winter annual species of Geraniaceae by a stepwise process. *Annals of Botany*, **110**, 637-651.

Gama-Arachchige N.S., Baskin J.M., Geneve R.L., Baskin C.C. (2013) Identification and characterization of ten new water gaps in seeds and fruits with physical dormancy and classification of water-gap complexes. *Annals of Botany*, **112**, 69-84.

Jayasuriya K.M.G.G., Baskin J.M., Baskin C.C. (2008a) Cycling of sensitivity to physical dormancy-break in seeds of *Ipomoea lacunosa* (Convolvulaceae) and ecological significance. *Annals of Botany*, **101**, 341-352.

Jayasuriya K.M.G.G., Baskin J.M., Baskin C.C. (2009a) Sensitivity cycling and its ecological role in seeds with physical dormancy. *Seed Science Research*, **19**, 3-13.

Jayasuriya K.M.G.G., Baskin J.M., Geneve R.L., Baskin C.C. (2009b) Sensitivity cycling and mechanism of physical dormancy break in seeds of *Ipomoea hederacea* (Convolvulaceae). *International Journal of Plant Sciences*, **170**, 429-443.

Jayasuriya K.M.G.G., Baskin J.M., Geneve R.L., Baskin C.C., Chien C.-T. (2008b) Physical dormancy in seeds of the holoparasitic angiosperm *Cuscuta australis* (Convolvulaceae, Cuscuteae): dormancy-breaking requirements, anatomy of the water gap and sensitivity cycling. *Annals of Botany*, **102**, 39-48.

Lacerda D.R., Lemos Filho J.P., Goulart M.F., Ribeiro R.A., Lovato M.B. (2004) Seed-dormancy variation in natural populations of two tropical leguminous tree species: *Senna multijuga* (Caesalpinioideae) and *Plathymenia reticulata* (Mimosoideae). *Seed Science Research*, **14**, 127-135.

Martin R.E., Miller R.L., Cushwa C.T. (1975) Germination response of legume seeds subjected to moist and dry heat. *Ecology*, **56**, 1441-1445.

R Development Core Team. (2011) *R: A language and environment for statistical computing*. Vienna, Austria, R Foundation for Statistical Computing. Available at http://www.R-project.org.

Rodrigues-Junior A.G., Faria J.M.R., Vaz T.A.A., José A.C. (2015) Loss of desiccation tolerance and storage behavior in germinating seeds of *Senna multijuga*: implications for seed germination and conservation. *New Forests*, **46**, 283-291.

Rodrigues-Junior A.G., Faria J.M.R., Vaz T.A.A., Nakamura A.T., José A.C. (2014) Physical dormancy in *Senna multijuga* (Fabaceae: Caesalpinioideae) seeds: the role of seed structures in water uptake. *Seed Science Research*, **24**, 147-157.

Souza V.C., Bortoluzzi R.L.C. (2015) *Senna* in lista de espécies da flora do Brasil. Jardim Botânico do Rio de Janeiro. <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB23157>.

Taylor G.B. (1981) Effect of constant temperature treatments followed by fluctuating temperatures on the softening of hard seeds of *Trifolium subterraneum* L. *Australian Journal of Plant Physiology*, **8**, 547-558.

Taylor G.B. (1996) Effect of the environment in which seeds are grown and softened on the incidence of autumn seed softening in two species of annual medics. *Australian Journal of Agricultural Research*, **47**, 141-159.

Taylor G.B. (2005) Hardseededness in Mediterranean annual pasture legumes in Australia: a review. *Australian Journal of Agricultural Research*, **56**, 645-661.

Taylor G.B., Revell C.K. (1999) Effect of pod burial, light, and temperature on seed softening in yellow serradella. *Australian Journal of Agricultural Research*, **50**, 1203-1209.

Turner S.R., Cook A., Baskin J.M., Baskin C.C., Tuckett R.E., Steadman K.J., Dixon K.W. (2009) Identification and characterization of the water gap in the physically dormant seeds of *Dodonaea petiolaris*: a first report for Sapindaceae. *Annals of Botany*, **104**, 833-844.

Van Assche J.A., Debucquoy K.L.A., Rommens W.A.F. (2003) Seasonal cycles in the germination capacity of buried seeds of some Leguminosae (Fabaceae). *New Phytologist*, **158**, 315-323.

Van Klinken R.D., Flack L. (2005) Wet heat as a mechanism for dormancy release and germination of seeds with physical dormancy. *Weed Science*, **53**, 663-669.

Van Klinken R.D., Flack L.K., Pettit W. (2006) Wet-season dormancy release in seed banks of a tropical leguminous shrub is determined by wet heat. *Annals of Botany*, **98**, 875-883.

Van Klinken R.D, Goulier J-P. (2013) Habitat-specific seed dormancy-release mechanisms in four legume species. *Seed Science Research*, **23,** 181-188.

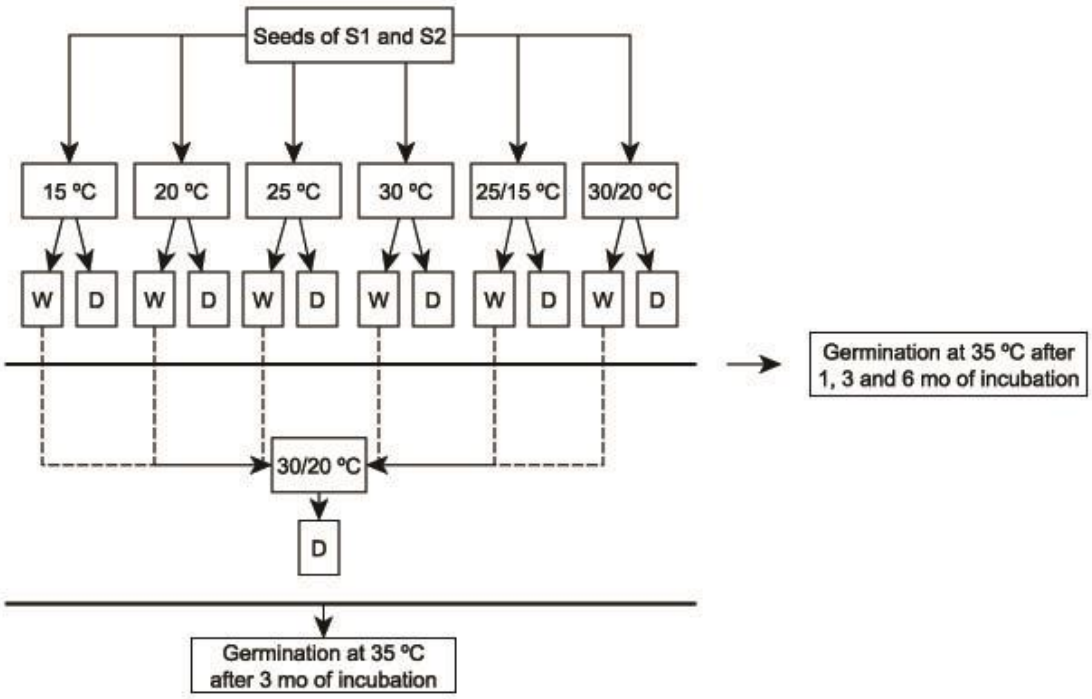

Fig. 1. Schematic diagram of the experiment to evaluate sensitivity of seeds to different temperatures in wet (W) and dry (D) conditions.

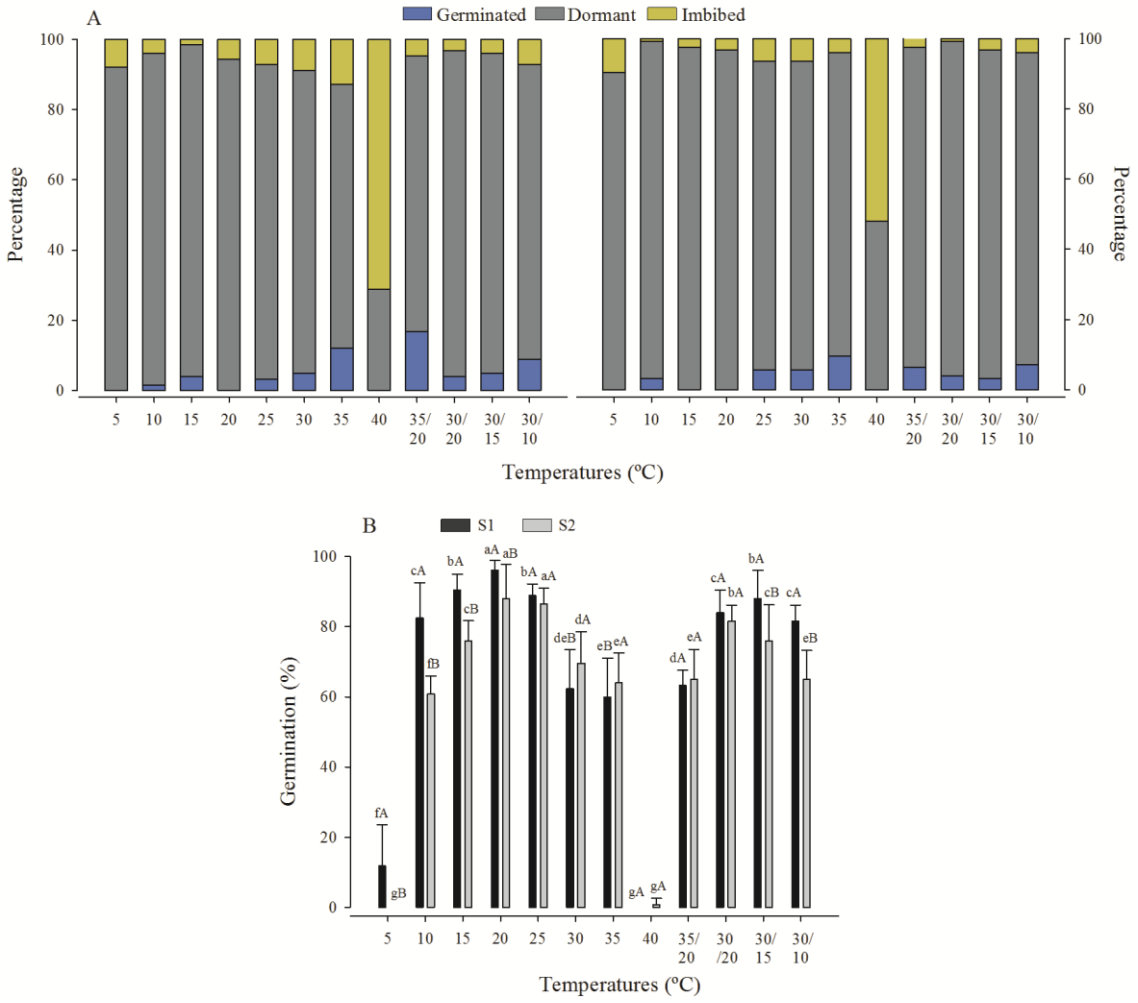

Fig. 2. Proportion of germinated, dormant and imbibed seeds after germination test at different constant and alternating temperatures for seed collections S1 (left) and S2 (right) (A). Germination (mean %± s.d.) of scarified S1 and S2 seeds at different constant and alternating temperatures (B).

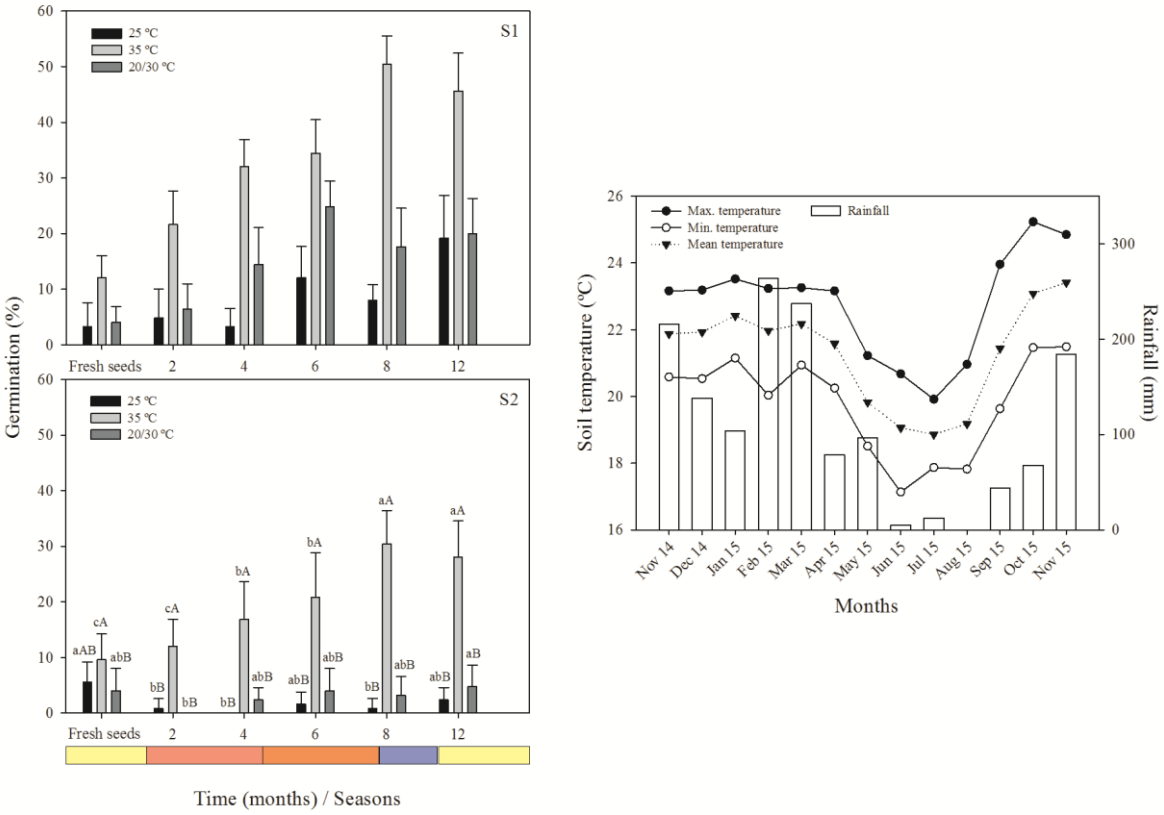

Fig. 3. Germination (mean % ± s.d.) of intact S1 and S2 collections of *Senna multijuga* seeds at three temperatures after 0-12 months of burial and soil temperature and rainfall during the experimental period. Different uppercase letters indicate significant differences between temperatures within time and different lowercase letters significant differences between times for a temperature. Bars represent the seasons of the year: spring = yellow bar; summer = red bar; autumn = orange bar; winter = blue bar.

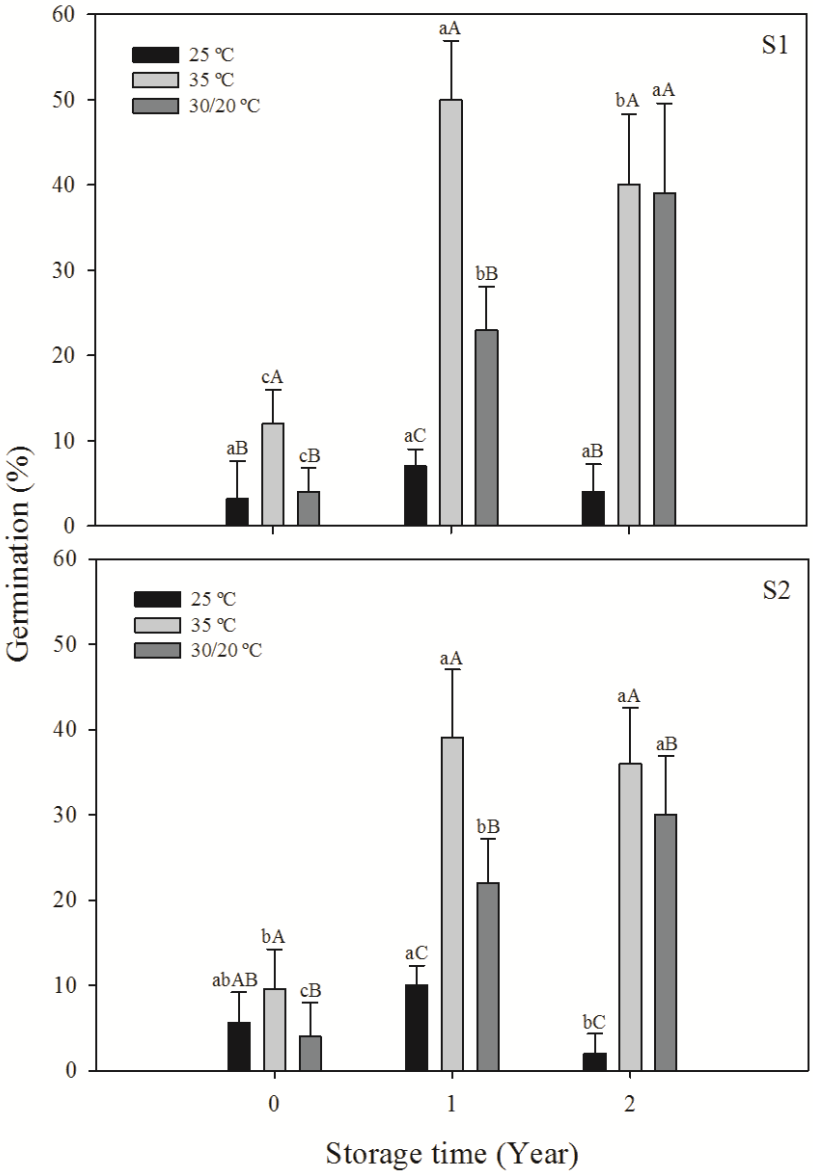

Fig. 4. Germination (mean % ± s.d.) of S1 and S2 collections of *Senna multijuga* seeds after dry storage in laboratory (25 ± 5 ºC) for 0 to 2 years. Different uppercase letters indicate significant differences between temperatures within a storage period and different lowercase letters significant differences between periods for a temperature.

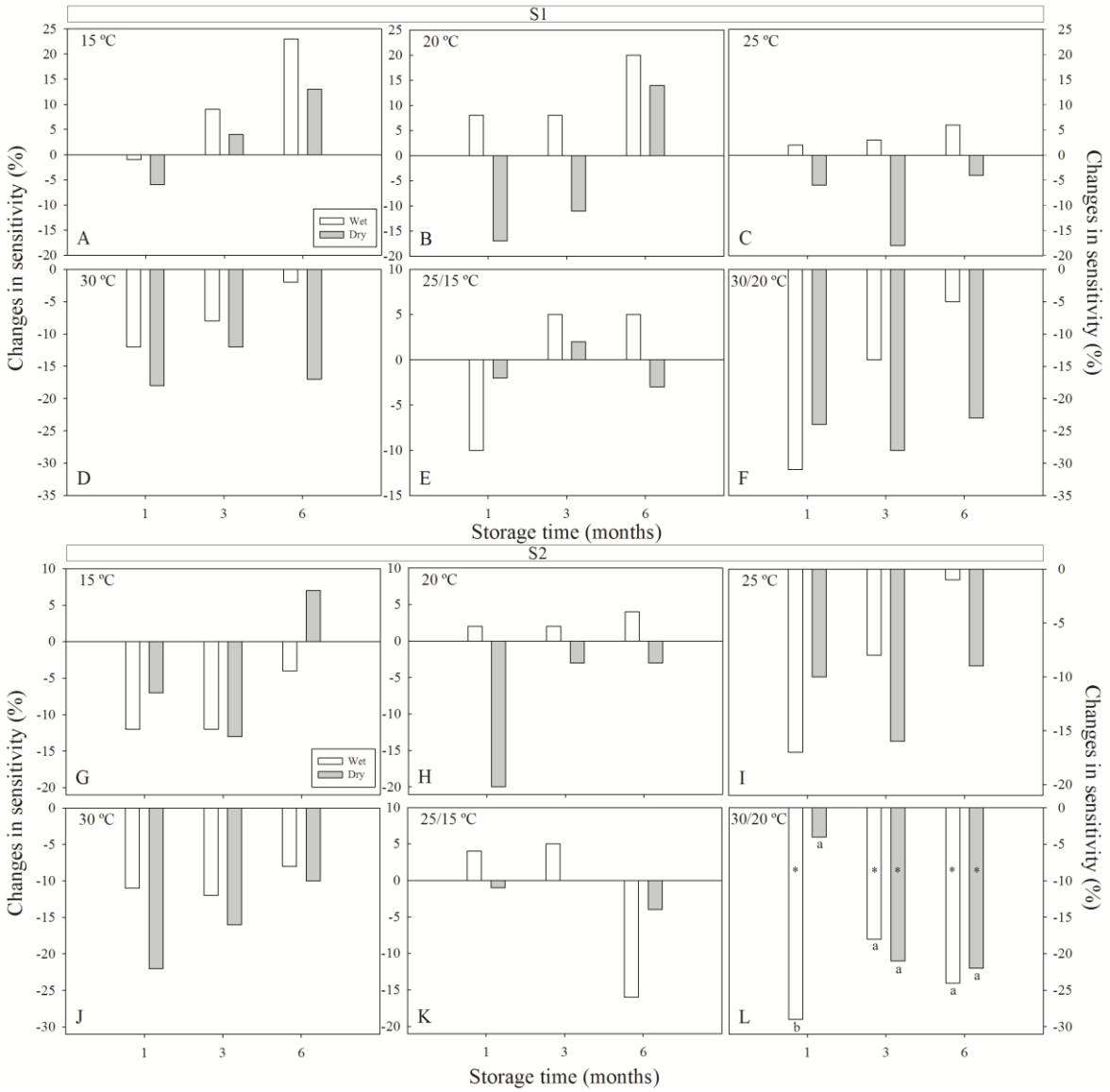

Fig. 5. Changes in sensitivity of *Senna multijuga* seeds at 35 ºC after wet or dry storage at different constant and alternating temperatures for 1, 3 and 6 months. Different lowercase letters indicate significant differences between moisture conditions within a storage time. Asterisks indicate significant differences from the control (P<0.05). Changes in sensitivity are denoted as the differences in germination in relation to control. There was an interaction between the factors only in panel L.

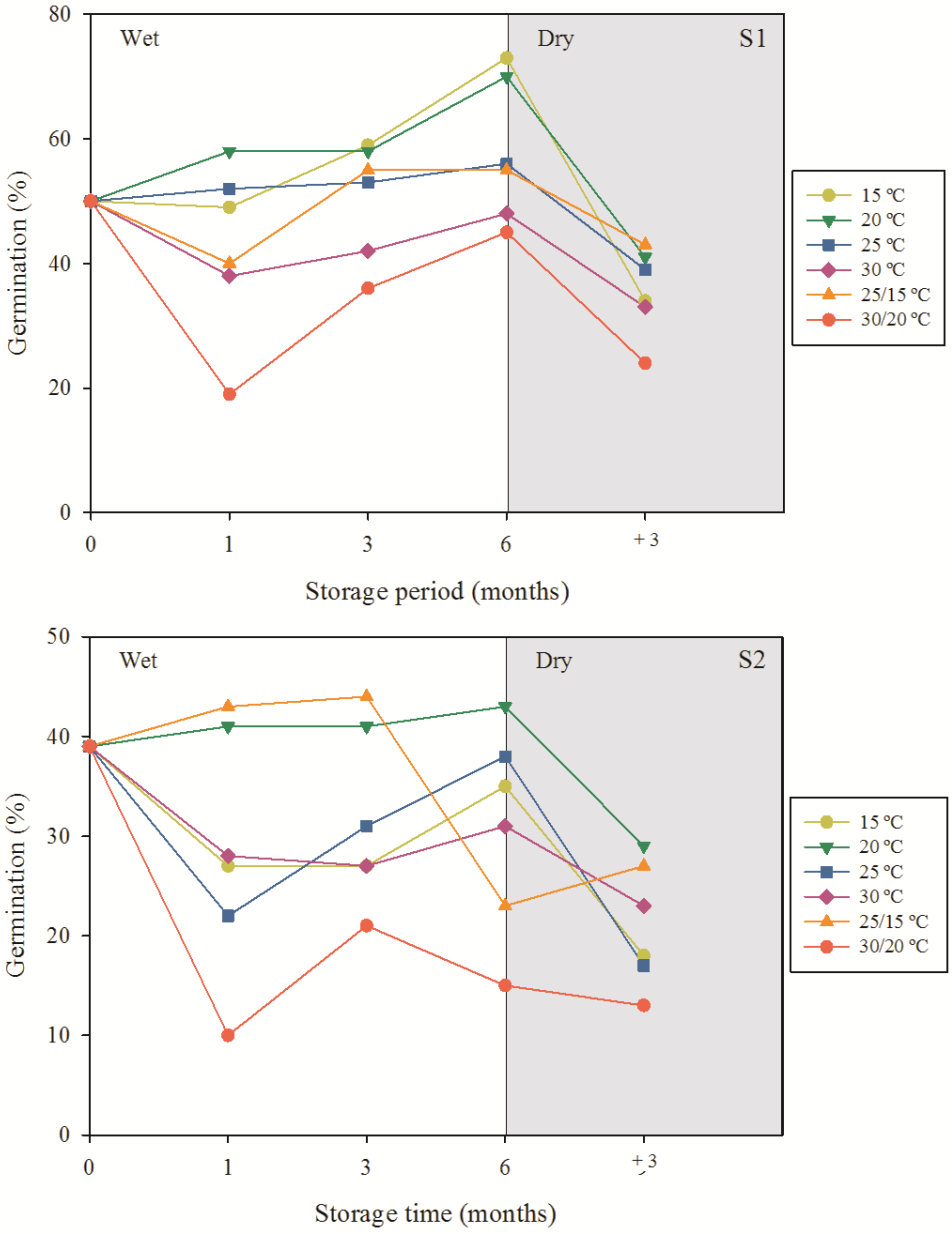

Fig. 6. Effect of dry storage at 30/20 ºC following wet storage at different temperatures on germination of S1 and S2 collections of *Senna multijuga* seeds at 35 ºC.

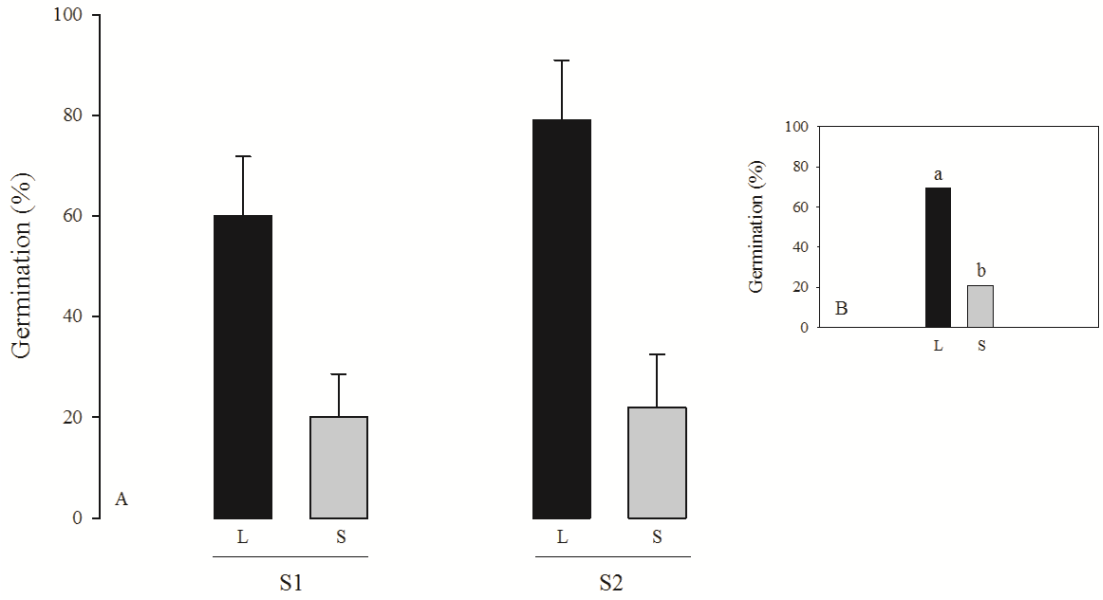

Fig. 7. Germination (mean % ± s.d.) at 35 ºC of large (L) and small (S) seeds after 1.5 years of dry storage in laboratory conditions, within each seed collection (A) and general mean, for seed size (B). Different letters in (B) indicate significant differences between large and small seeds.

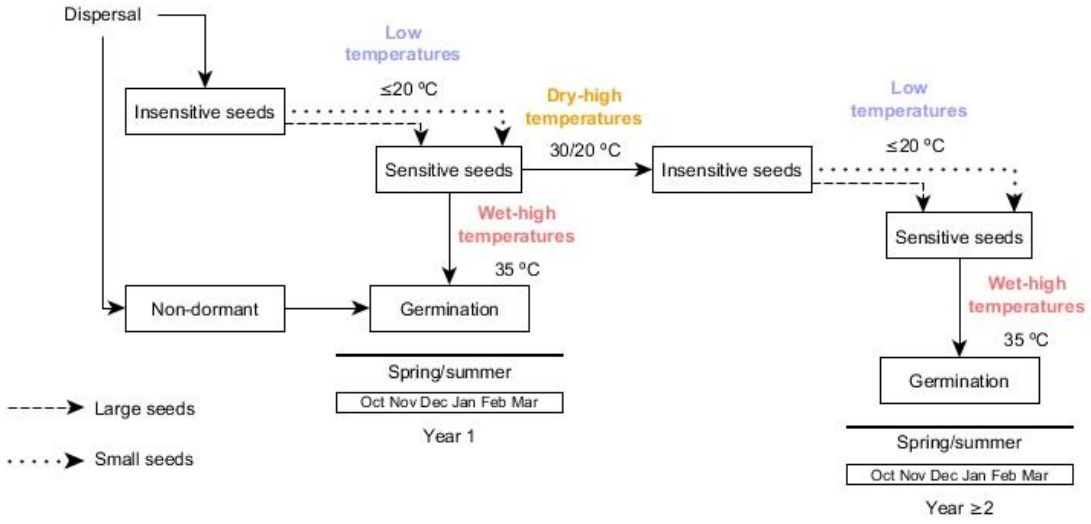

Fig. 8. Conceptual model of the steps in breaking dormancy of large and small seeds of *Senna multijuga*.

CAPÍTULO II

WHY LARGE SEEDS WITH PHYSICAL DORMANCY GERMINATE EARLIER THAN SMALL ONES

Manuscrito submetido à *PLOS ONE*

Research Article

Why large seeds with physical dormancy germinate earlier than small ones

Ailton G. Rodrigues-Junior[1,2*], Ana Caroline M. P. Mello[1], Carol C. Baskin[2,3], Jerry M. Baskin[2], Denise M.T. Oliveira[1] and Queila S. Garcia[1]

1 Departamento de Botânica, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil, **2** Department of Biology, University of Kentucky, Lexington, KY, USA, **3** Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY, USA

*ailtongrodrigues@gmail.com (AGR-J)

Short tittle: Mechanism of physical dormancy break

Abstract

Under natural conditions, large seeds with physical dormancy (PY) may become water permeable earlier than small ones. However, the mechanism for this difference has not been elucidated. Thus, our aim was to evaluate the traits associated with PY in seeds of *Senna multijuga* and to propose a mechanism for earlier germination in large than in small seeds. Two seedlots were collected and each separated into large and small seeds. Seed dry mass, water content, thickness of palisade layer in the hilar and extra-hilar regions and the ratio between palisade layer thickness (P) in the lens fissure and seed mass (M) were evaluated. Further, the correlation between seed mass and seed dimensions was investigated. Large seeds had higher dry mass and water content than small seeds. The absolute thickness of the palisade layer in the different regions did not show any trend with seed size; however, large seeds had a lower P:M ratio than small seeds. Seed mass correlated positively with all seed dimensions, providing evidence for a substantially higher volume in large seeds. Since wet, but not dry, high temperatures break PY in sensitive seeds of *S. multijuga*, the data support our prediction that internal pressure potential in the seed and palisade layer thickness in the water gap (lens), which is related to seed size, act together to modulate the second step (dormancy break) of the two-stage sensitivity cycling model for PY break. In which case, large seeds are predetermined to become water-permeable earlier than small ones.

Keywords: dormancy control mechanism; Fabaceae; palisade layer thickness; physical dormancy; seed mass; seed volume; seed water content

Introduction

Water-impermeable seeds/fruits have physical dormancy (PY) and specialized structures that can open in response to environmental cues, thereby creating a 'water-gap' whereupon dormancy is broken [1-4]. The intensity of PY can vary between and within species, which may be related to (1) seed coat thickness, wherein a thick seed coat confers higher resistance to dormancy break [5-8]; (2) seed size, wherein large seeds become sensitive to environmental cues that break PY earlier than small ones [9-12]; and (3) seed water content during the acquisition of PY, which may result in differences in dormancy intensity [13-15]. However, the role of these features of the seeds in the dormancy breaking process is unclear.

A two-stage model for PY break was proposed by Taylor [16, 17] and Jayasuriya et al. [18, 19], wherein seeds cycle between insensitive and sensitive states (i.e. sensitivity cycling) [18, 19]. In the first step, seeds become sensitive to dormancy-breaking conditions, but they remain water-impermeable. If sensitive seeds are exposed to the appropriate dormancy-breaking conditions, they become water permeable, i.e. water-gap opens. On the other hand, if sensitive seeds are exposed to unfavorable dormancy-breaking conditions they revert to the nonsensitive condition [18, 19]. However, it is not known how seed coat thickness, seed size and seed water content are related to induction of sensitivity or to dormancy break in seeds with PY.

Baskin and Baskin [3, 14] did not subdivide the *class* PY into lower hierarchial categories in their seed dormancy classification scheme but suggested that it probably should be subdivided. Thus, more detailed information is needed to separate PY into *levels* and *types* [3, 19]. Based on seed water content during dispersal, Jaganathan [15] divided PY into two groups: shallow and absolute. The first group included seeds with a relatively high water content and a low intensity of PY, and the second group included

seeds with a relatively low water content and a high level of dormancy. In addition to seed water content, seed size could affect PY. Rodrigues-Junior et al. [12] proposed a model for PY-break mediated by seed size, with large seeds being more sensitive to dormancy break than small seeds. Schutte et al. [8] suggested a possible trade-off between seed size and seed persistence in soil for species with PY, with persistence being directly related to seed coat thickness. Indeed, it is rather difficult to detect a relationship between seed coat thickness and level of dormancy, and Russi et al. [6] argued that measurements of the seed coat thickness in relation to seed size, as also evaluated in Schutte et al. [8], gives a more robust understanding of PY.

As hypothesized by Russi et al. [6], the increase in seed volume accentuates the tension transmitted mechanically to the weak region (e.g. lens on legume seeds) of the seed coat in seeds with PY during expansion and contraction induced by environmental changes. Hence, a thinner seed coat is more susceptible to disruption than a thicker seed coat [6]. These authors suggested that the volume of seeds with greater mass varies more widely than that of seeds with less mass when exposed to temperature fluctuations during the year and thus dormancy break would occur more quickly in large than in small seeds. Furthermore, seeds of some legume species at different positions within the fruit may exhibit a sequence of PY-break that is associated with the seed size [9, 10]. Based on differences in seed mass before and after approximately 2 years on bare soil, Smith et al. [11] suggested that small seeds persist in soil longer than large ones. However, no study has investigated in detail the effects of seed size and mass in relation to thickness of the palisade layer on the susceptibility to break PY.

Thus, we hypothesized that seed size and mass are correlated with seed water content and thickness of the water-impermeable palisade layer in the seed coat and that these features are related to PY break. We predicted that large seeds have higher water

content and a thinner palisade layer than small seeds. To test our hypothesis, we used seeds of *Senna multijuga*, a species with physically dormant seeds in which seed size mediates the time of response to the environmental cues during the PY-breaking process [12]. In this species, seeds made sensitive after exposure to temperatures ≤ 20 ºC become water-permable at a high temperature (35 ºC) on a moist substrate. However, large seeds need a shorter period of time to complete these two steps in PY-break, and thus they germinate earlier than small ones during the growing season. Thus, our aims were to (1) identify the features of large and small seeds of *S. multijuga* that may be involved in the breaking dormancy process, and (2) discuss these features in relation to the second-step of PY-break in seeds in response to summer habitat conditions.

Materials and Methods

Seed collection and processing

Seeds were manually collected from dry fruits at two locations on the campus of the Universidade Federal de Lavras, Brazil [seed collection 1 (S1) (21° 13′ 39,34″ S, 44° 58′ 11,85″ W; seed collection 2 (S2) (21° 13′ 30,53″ S, 44° 58′ 27,12″ W)] in September 2014 from 12 individuals for each collection. Nonfilled seeds were discarded after flotation in water. Seeds were then blotted dry and placed in plastic trays in ambient room conditions [25±5 ºC, 40-60% relative humidity (RH)] for 24 h. Then, the seeds were stored in sealed semipermeable plastic bags in the same conditions until the beginning of the experiments 1 year later. This storage period does not break PY in *S. multijuga* seeds. These two seed lots also were used in the study by Rodrigues-Junior et al. (in press), which found that seeds in the S1 collection were larger than those in the S2 collection. Further, large S1 and S2 seeds became sensitive faster than small S1 and S2 seeds, and thus large seeds germinated earlier in the field than small seeds [12].

Seed dry mass and water content

Firstly, S1 and S2 seeds were separated into two groups: (1) large seeds and (2) small seeds (Fig 1). These two groups were separated since the difference between these two sizes in relation to breaking PY and consequent germination is quite clear (Rodrigues-Junior et al., in press). To determine seed water content and dry mass, 25 seeds from each of the four groups (two seed sizes from two seed collections) were scarified with sandpaper to allow water loss during drying, weighed individually using a Shimadzu AUX220 analytical balance (0.00001 g) oven-dried at 103 ºC for 17 hours and then weighed again. The data for seed water content were expressed as percentage of water on a fresh weight basis [20].

Relationship between thickness of palisade layer and seed size and mass

Thickness of the palisade layer in the hilar and in the extra-hilar (in the middle third of the lateral part) regions was measured for large and small S1 and S2 seeds. Seeds were made water-permeable by immersing them in hot water (80 ºC for 15 min) [21]. Then, seeds were fixed with FAA for 48 h, dehydrated in a graded ethanol series and infiltrated with and embedded in 2-hydroxyethyl-methacrylate. Seed material was sectioned (8 µm) transversally using a Zeiss Hyrax M40 microtome, stained with 0.05% toluidine blue, pH 4.7 (modified from O'Brien et al. [22]) and mounted in synthetic resin. Sections were observed using a Leica DM500 optical microscope and photographs taken with a Leica ICC50 HD digital camera. Five measurements were made on each seed, using 10 replicates for each of the two sizes of S1 and S2 seeds. Thickness of the palisade layer was measured on the lateral part of the hilar region (M1), in the middle of the lens (M2) and on the two sides (lateral position) of the lens where a split had occurred (M3, M4). Thickness of the palisade layer was also measured in the extra-hilar region (M5) (Fig 2). The average for M3 and M4 was used to

determine the mean thickness of the palisade layer in the lens split. The ratio between thickness of the palisade layer in the lens fissure and seed fresh mass (P:M ratio) was calculated based on Russi et al. [6] and Schutte et al. [8].

Relationship between seed mass and seed dimensions

To assess the relationship between seed mass and seed length, width and thickness, S1 and S2 seeds were randomly sampled and these three dimensions and seed mass were measured for individual seeds. For this assay, we used seeds of all sizes, i.e. large, small and those on the gradient between large and small, in the two collections. The three dimensions were measured using Mitutoyo 500-144B digital calipers, and fresh mass of each seed was determined using the Shimadzu AUW220D analytical balance. One-hundred seeds (50 S1 and 50 S2) were used. Then, the relationship between each seed dimension and seed mass was analysed.

Statistical analyses

The data for seed dry mass and water content, palisade layer measurements and P:M ratio were firstly tested for normality (Shapiro-Wilk test) and homoscedasticity (Barlett test) ($P \geq 0.05$) to verify that they fit the assumptions of ANOVA. Since the data were nonparametric, they were analysed with a generalized linear model (GLM), and the means were compared by the post-hoc LSD test at 5% probability using R software for Windows [23]. The statistical model included the effects of seed collection and seed size as well as their interactions. Regression analyses were applied to evaluate the correlation between seed mass and seed dimensions. All graphs were designed using SigmaPlot® software (Systat Software Inc., San Jose, California, USA).

Results

Seed dry mass and water content

Small S1 and large S1 seeds had higher dry mass than small S2 and large S2 seeds, respectively (P<0.001) (Fig 3A). Small S1 and large S1 seeds had higher water content than small S2 and large S2 seeds, respectively (P<0.001) (Fig 3B).

Relationship between thickness of palisade layer and seed size and mass

Only seed size was related to thickness of the palisade layer in the hilar region (P=0.03), with large seeds having a thicker palisade layer than small seeds (Fig 4A). There was an interaction between seed size and seed collection for thickness of the palisade layer in the lens (in middle region) (P=0.02), but no trend was found for these measurements. Large S1 seeds had a thicker palisade layer than small seeds, and small S2 seeds had a thicker palisade layer than large seeds (Fig 4B). For thickness of the palisade layer in the slits in the lens, there was an interaction between seed size and seed collection (P<0.001). In the slit region, large S1 seeds had a thicker palisade layer, whereas small S2 seeds were thicker in this region. Small S2 had a thicker palisade layer in the slit region than small S1 seeds. S1 large seeds had a thicker palisade layer in the lens slit than S2 large seeds (Fig 4C). Seed size (P<0.001), and seed collection (P<0.001) affected thickness of the palisade layer in the extra-hilar region. Large seeds had a thicker palisade layer in the distal region than small ones in both seed collections, and S1 seeds had a thicker palisade layer in the extra-hilar region than S2 seeds (Fig 4D).

There was an interaction between seed size and seed collection (P<0.001) for the P:M ratio, and both S1 and S2 small seeds had a higher ratio than large seeds. S1 large and small seeds had a lower P:M ratio than S2 large and small seeds, respectively (Fig 5).

Relationship between seed mass and seed dimensions

Seed dimensions increased with seed mass (Fig 6A-C). All of these seed parameters were strongly and positively related to seed mass (P<0.0001). That is, with an increase in seed length, width or thickness, there was an increase in seed mass. Also, S1 seeds had more mass than those of S2 (Fig 6A-C).

Discussion

Large seeds of *S. multijuga* had higher dry mass and higher water content than small seeds, which is what we predicted. However, contrary to our predictions, there was no trend in the relationship between thickness of palisade layer and seed size. On the other hand, large seeds had a low P:M ratio, while small seeds had a high P:M ratio. Also, S2 (collection with smallest seeds) had a higher P:M ratio than S1 seeds. There is a direct relationship between seed mass and seed dimensions in *S. multijuga*, and thus an increase in seed volume occurs with an increase in seed mass. All of these results support the seed size-mediated model for PY-break proposed by Rodrigues-Junior et al. [12]. In this model, small seeds need more time to complete the two steps to break PY than large seeds, spreading germination over time.

According to Jayasuriya et al. [24], sensitive seeds of *Ipomoea lacunosa* (Convolvulaceae) can absorb water vapour through the fissure formed in the hilum, which closes after water vapour absorption. Thus, hilar closure prevents loss of internal water, and thus vapour pressure increases with an increase in temperature. The hilum does not open in insensitive *I. lacunosa* seeds [24]. The mechanism of opening and closing of the hilum are similar to that proposed by Hyde [25] for seeds of Fabaceae, subfamily Papilionoideae. *Senna multijuga* seeds require the same conditions (wet

substrate, 35 ºC) [12] as those of *I. lacunosa* to break dormancy [24], and the fissures formed in the hilum could act in the same way, thus contributing to an increase in seed internal pressure when subjected to high temperatures. High temperatures may elevate the internal energy and exert force on the seed coat, which disrupts in the weakest region of the seed coat, namely the lens. *Senna multijuga* seeds remain water-impermeable at temperatures lower than 35 ºC [12]. Therefore, if the model proposed by Jayasuriya et al. [24] fits *S. multijuga* seeds, why is dormancy in large seeds broken earlier/faster than that in small ones? The higher water content of large than of small seeds and thus more water per volume may increase internal vapour pressure more in large than in small seeds. Therefore, with exposure to high temperatures enough force is generated in large seeds to move the palisade layer outward in the weak region in the lens. In fact, the role of internal pressure on the mechanism to break PY was first mentioned by Hanna [26], but this author suggested that a possible increase in pressure caused by heat treatment could be due to an increase in the number of vascular bundle below the lens for *Acacia kempeana* seeds.

Hanna [26] and Serrato-Valenti et al. [27] found an evident weak region in the lens in *Acacia kempeana* and *Leucaena leucocephala* seeds (Fabaceae, Mimosoideae). This weak region was related to a decrease in height of cells in the palisade layer, but it was not determined if changes in cell height were related to the dormancy level in these species. We also found a decrease in height (thickness) of cells in the palisade layer on the two sides of the lens where a split occurs. However, in *S. multijuga* thickness of the palisade layer is related to the propensity for breaking dormancy only when seed mass is taken into account. Thus, in addition to the internal pressure in the seeds, thickness of the palisade layer may affect the PY-breaking mechanism by providing physical

resistance to the force exerted by internal pressure, and seed size can modulate this mechanism.

In addition, during their development on the mother plant seeds need to dehydrate to a certain water content to become physically dormant (see table 6.2 in Baskin and Baskin [14]). Relative humidity is closely linked to PY, and it affects both the onset and release of dormancy [24, 28, 29, 30]. After they become water-impermeable, seeds with PY continue to lose moisture through the hilum until moisture inside the seed equilibrates with the relative humidity outside the seed [25]. Seed water content at the onset of dormancy in *S. multijuga* is related to seed size, and it determines whether dormancy is broken earlier or later in the growing season. Large seeds may lose less moisture than small ones because they accumulate a larger amount of dry mass and the distance from the distal region of the seed to the hilum (region where the moisture moves out of the seed) is greater than that in small seeds. Consequently, large seeds tend to have higher water content at equilibrium with the surrounding environment than small seeds. This conclusion agrees with Hyde's [25] statement that "The duration of the impermeable condition increased with the degree of desiccation brought about by loss of water through the hilum". Hyde [25] also demonstrated that exposing dormant seeds to gradually increasing humidity can manipulate the mechanism of water control by the hilum and increase seed water content. In the conditions tested, an increase in water content was associated with an increase in germination [25].

The consequent increase in seed volume in large seeds could affect the rate of water loss during the acquisition of PY. That is, in large seeds there is a reduction in the amount of water lost since the cell-to-cell water transport towards the hilum requires more time in large than it does in small seeds. Furthermore, Hyde [25] demonstrated that the water content of dormant seeds (with PY) equilibrates with the lowest relative

humidity in the environment surrounding the seed. However, we found a difference in water content between large and small *S. multijuga* seeds collected from the field. A greater resistance to further dehydration in large than in small seeds allows the maintenance of a higher water content in large than small seeds. This also may be attributed to the area of the fissure in the hilum in proportion of the size of seed, which may be greater in small than in large seeds. Thus, we propose a conceptual model for the differences in water loss in relation to seed size (Fig 7).

A relationship has been found between seed size and PY, wherein small seeds tend to be more dormant than large ones [6, 8, 10, 12]. However, since PY is coat-imposed the differences in dormancy are caused by variation in the ability to open the water gap. Resistance of the water gap to disrupt (seed coat thickness) plus internal force (pressure) act during the process of breaking physical dormancy. With an increase in the thickness of the seed coat, the force required to open the water gap increases. This relation is true in the case of physically dormant seeds that need moisture to break dormancy. The role of internal pressure in breaking PY was hypothesized by Jayasuriya et al. [24, 31], who observed a distinct response to wet and dry conditions for seeds of congeneric species of Convolvulaceae to become permeable. Similarities are shared by *S. multijuga* and *I. lacunosa* seeds during the second step of dormancy break, as evidenced by Rodrigues-Junior et al. [12], and both species require summer habitat conditions to become water-permeable. The requirement for wet-high temperatures to break PY in *S. multijuga* seeds and the relationship between seed traits of this species support the role of internal pressure in the PY-breaking mechanism in seeds proposed by Jayasuriya et al. [24, 31].

Large *S. multijuga* seeds have a higher water content and a lower P:M ratio than small seeds. That is, the impermeable barrier can be broken in the weak region of the

lens earlier in large seeds than in small ones, which explains why large seeds germinated earlier than small ones in the study by Rodrigues-Junior et al. [12]. Therefore, the PY-breaking mechanism is much more complex than a simple retraction and expansion of the seed coat. The relationship between internal pressure potential and palisade layer thickness in the water gap (lens in this case) is related to seed size, and jointly they modulate the second step of the two-stage model for PY break proposed by Taylor [16, 17] and Jayasuriya et al. [18, 19].

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References

1. Baskin JM, Baskin CC, Li X. Taxonomy, anatomy and evolution of physical dormancy in seeds. Plant Species Biology. 2000; 15: 139-152.
2. Baskin CC. Breaking physical dormancy in seeds – focusing on the lens. New Phytologist. 2003; 158: 229-232.
3. Baskin JM, Baskin CC. A classification system for seed dormancy. Seed Science Research. 2004; 14: 1-16.
4. Gama-Arachchige NS, Baskin JM, Geneve RL, Baskin CC. Identification and characterization of ten new water gaps in seeds and fruits with physical dormancy and classification of water-gap complexes. Annals of Botany. 2013; 112: 69-84.
5. Tran VN. Effects of microwave energy on the strophiole, seed coat and germination of *Acacia* seeds. Australian Journal of Plant Physiology. 1979; 6: 277-287.
6. Russi L, Cocks PS, Roberts EH. Coat thickness and hard-seededness in some *Medicago* and *Trifolium* species. Seed Science Research. 1992; 2: 243-249.

7. Venier P, Funes G, García CC. Physical dormancy and histological features of seeds of five *Acacia* species (Fabaceae) from xerophytic forests in central Argentina. Flora. 2012; 207: 39-46.

8. Schutte BJ, Davis AS, Peinado Jr. SA, Ashigh J. Seed-coat thickness data clarify seed size–seed-bank persistence trade-offs in *Abutilon theophrasti* (Malvaceae). Seed Science Research 2014; 24: 119-131.

9. Halloran GM, Collins WJ. Physiological predetermination of the order of hardseededness breakdown in subterranean clover (*Trifolium subterraneum* L.). Annals of Botany. 1974; 38: 1039-1044.

10. McComb JA, Andrews R. Sequential softening of hard seeds in burrs of annual medics. Australian Journal of Experimental Agriculture and Animal Husbandry. 1974; 14: 68-75.

11. Smith FP, Cocks PS, Ewing MA. Short-term patterns of seed softening in *Trifolium subterraneum*, *T. glomeratum*, and *Medicago polymorpha*. Australian Journal of Agricultural Research. 1996; 47: 775-785.

12. Rodrigues-Junior AG, Baskin CC, Baskin JM, Garcia QS. Sensitivity cycling in physically dormant seeds of the neotropical tree *Senna multijuga* (Fabaceae). Plant Biology. in press.

13. Quinlivan BJ. 1971. Seed coat impermeability in legumes. The Journal of the Australian Institute of Agricultural Science. 37; 283-295.

14. Baskin CC, Baskin JM. Seeds: ecology, biogeography and evolution of dormancy and germination. 2nd ed. San Diego: Elsevier/Academic Press; 2014.

15. Jaganathan GK. Influence of maternal environment in developing different levels of physical dormancy and its ecological significance. Plant Ecology. 2016; 217: 71-79.

16. Taylor GB. Effect of constant temperature treatments followed by fluctuating temperatures on the softening of hard seeds of *Trifolium subterraneum* L. Australian Journal of Plant Physiology. 1981; 8: 547-558

17. Taylor GB. 2005. Hardseededness in Mediterranean annual pasture legumes in Australia: a review. Australian Journal of Agricultural Research. 56; 645-661.

18. Jayasuriya KMGG, Baskin JM, Baskin CC. Cycling of sensitivity to physical dormancy-break in seeds of *Ipomoea lacunosa* (Convolvulaceae) and ecological significance. Annals of Botany. 2008; 101: 341-352

19. Jayasuriya KMGG, Baskin JM, Baskin CC. Sensitivity cycling and its ecological role in seeds with physical dormancy. Seed Science Research. 2009a; 19: 3-13.

20. International Seed Testing Association. International rules for seed testing. Bassersdorf: ISTA; 2004.

21. Rodrigues-Junior AG, Faria JMR, Vaz TAA, Nakamura AT, José AC. Physical dormancy in *Senna multijuga* (Fabaceae: Caesalpinioideae) seeds: the role of seed structures in water uptake. Seed Science Research. 2014; 24: 147-157.

22. O'Brien TP, Feder N, McCully ME. Polychromatic staining of plant cell walls by toluidine blue. Protoplasma. 1964; 59: 368-373.

23. R Development Core Team. R: A language and environment for statistical computing. 2014. Vienna: R Foundation for Statistical Computing. Available from: http://www.R-project.org.

24. Jayasuriya KMGG, Baskin JM, Geneve RL, Baskin CC. A proposed mechanism for physical dormancy break in seeds of *Ipomoea lacunosa* (Convolvulaceae). Annals of Botany. 2009b; 103: 433-445.

25. Hyde EOC. The function of the hilum in some Papilionaceae in relation to the ripening of the seed and the permeability of the testa. Annals of Botany. 1954; 18: 241-256.

26. Hanna PJ. Anatomical features of the seed coat of *Acacia kempeana* (Mueller) which relate to increased germination rate induced by heat treatment. New Phytologist. 1984; 96: 23-29.

27. Serrato-Valenti G, De Vries M, Cornara L. The hilar region in *Leucaena leucocephala* Lam. (De Wit) seed: structure, histochemistry and the role of the lens in germination. Annals of Botany. 1995; 75: 569-574.

28. Lebedeff GA. Studies on the inheritance of hard seeds in beans. Journal of Agricultural Research. 1947; 74: 205-215.

29. Van Klinken RD, Goulier J-P. Habitat-specific seed dormancy-release mechanisms in four legume species. Seed Science Research. 2013; 23: 181-188.

30. Tozer MG, Ooi MKJ. Humidity-regulated dormancy onset in the Fabaceae: a conceptual model and its ecological implications for the Australian wattle *Acacia saligna*. Annals of Botany. 2014; 114: 579-590.

31. Jayasuriya KMGG, Baskin JM, Geneve RL, Baskin CC. Sensitivity cycling and mechanism of physical dormancy break in seeds of *Ipomoea hederacea* (Convolvulaceae). International Journal of Plant Sciences. 2009c; 170: 429-443.

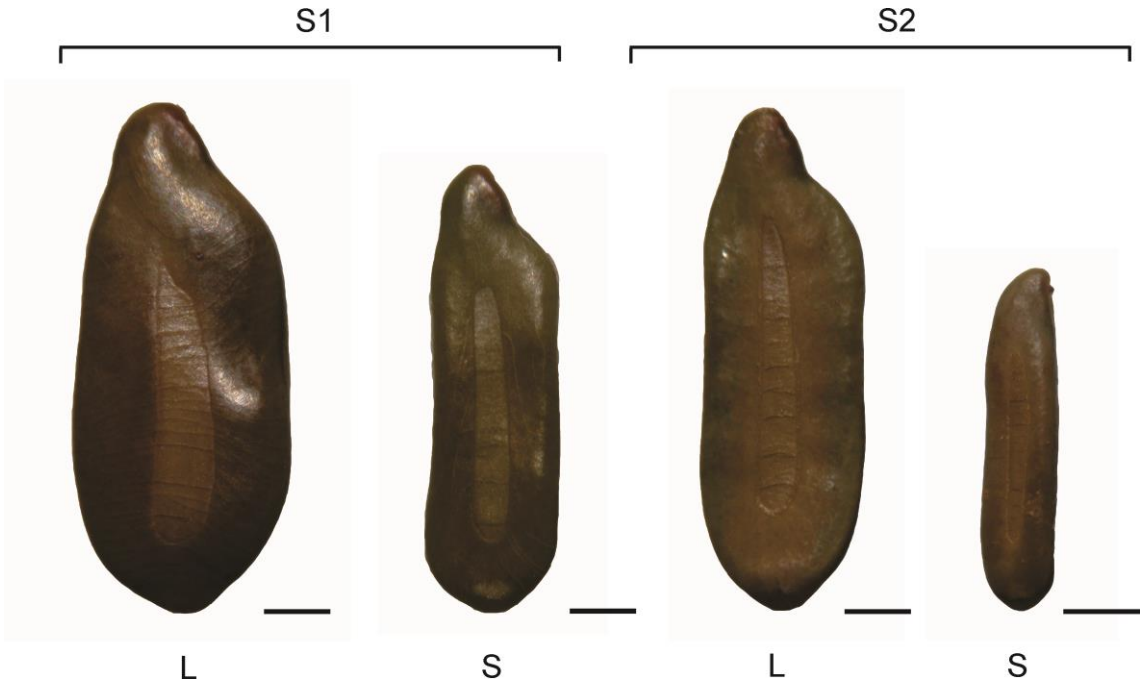

Fig 1. Large (L) and small (S) *Senna multijuga* seeds from collections S1 and S2. Bars = 1 mm.

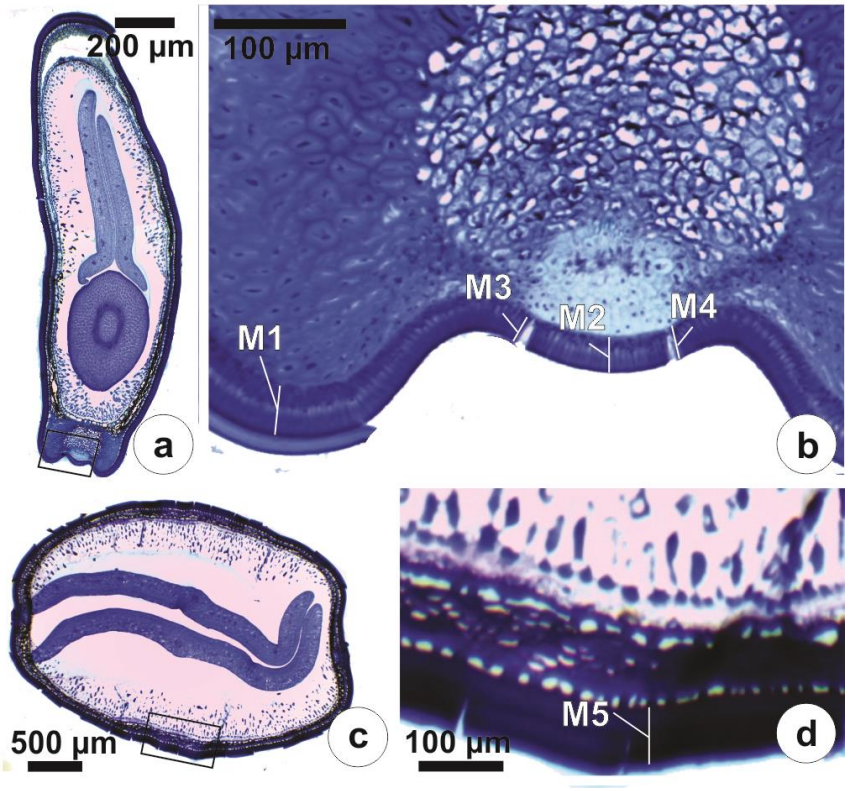

Fig 2. Sections of *Senna multijuga* seeds showing locations where palisade layer was measured. (A) Cross-section in hilar region. (B) Detail of region indicated by rectangle in A. (C) Cross-section in distal region (lateral position of the median third). (D) Detail of the region indicated by the rectangle in C. M1–M5 = locations where measurements were made in each seed.

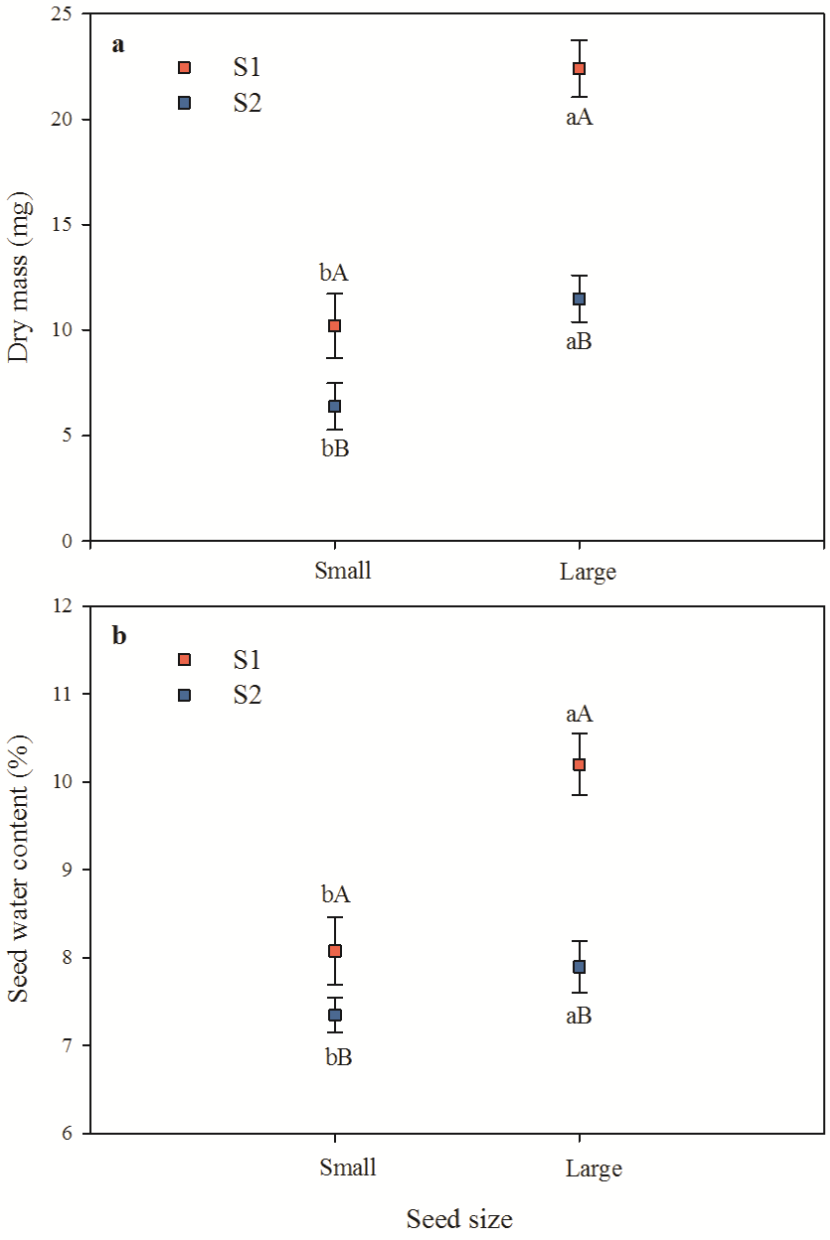

Fig 3. Mean (± s.e.) dry mass (A) and water content (B) of small and large S1 (seed collection 1) and S2 (seed collection 2) seeds. Different lowercase letters indicate significant differences between seed sizes within a collection. Different uppercase letters indicate significant differences between seed collections within a seed size, according to Fisher's test ($P \leq 0.05$).

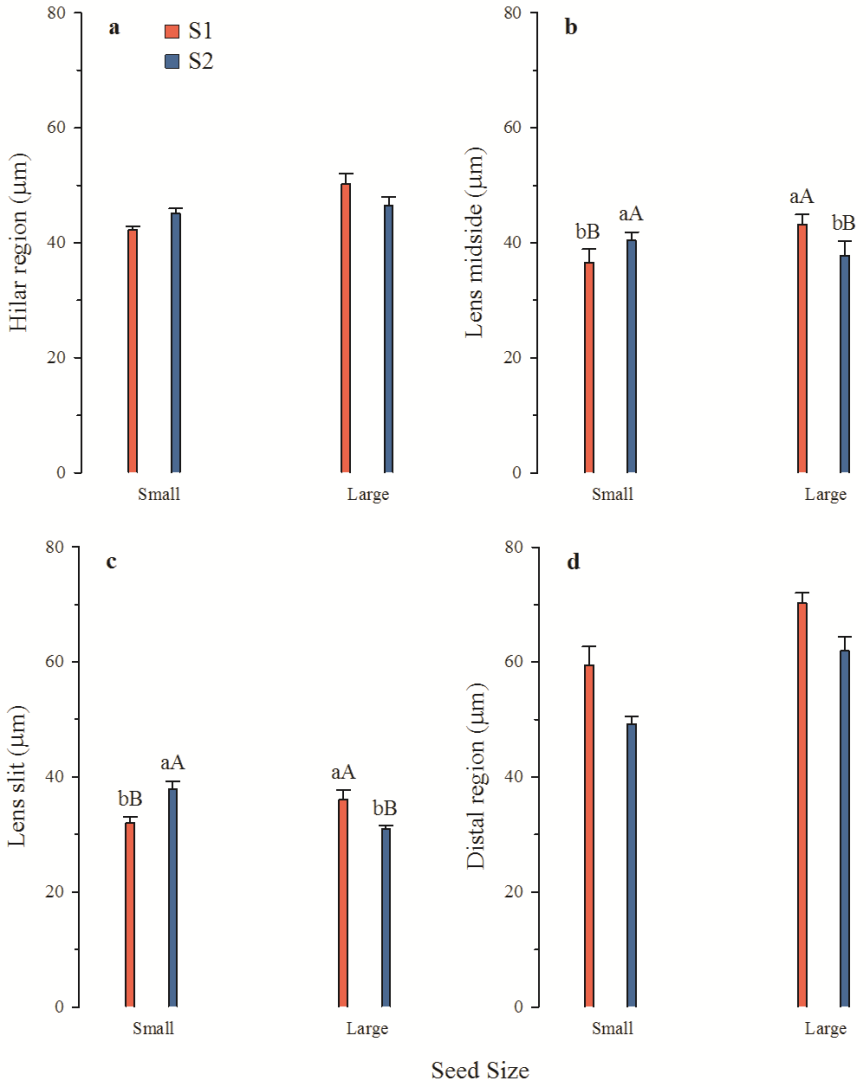

Fig 4. Thickness of palisade layer (mean ± s.e.) in different parts of S1 (seed collection 1) and S2 (seed collection 2) seeds. Different lowercase letters indicate significant differences between seed sizes within a collection. Different uppercase letters indicate significant differences between seed collections within a seed size, according to Fisher's test ($P \leq 0.05$). There is no interaction between seed size and seed collection in A and D.

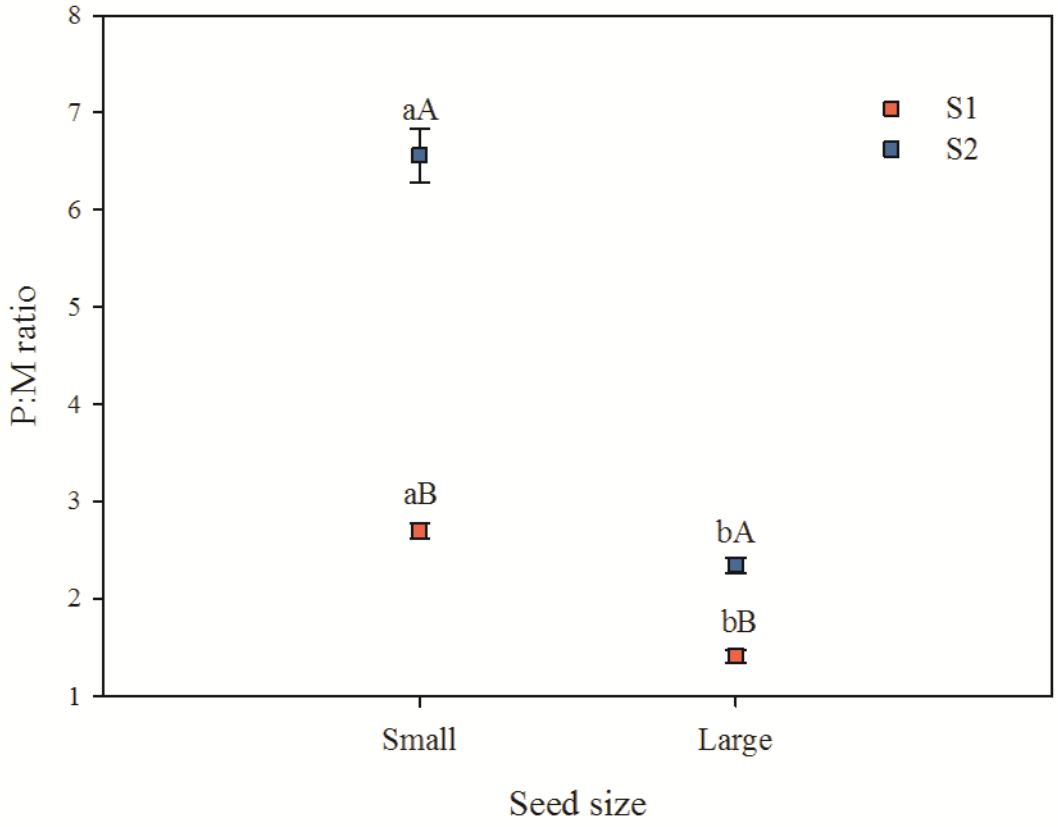

Fig 5. Relationship between P:M ratio and seed size for S1 (seed collection 1) and S2 (seed collection 2) seeds (mean ± s.e.). Different lowercase letters indicate significant differences between seed sizes within a collection. Different uppercase letters indicate significant differences between seed collections within a seed size, according to Fisher's test ($P \leq 0.05$).

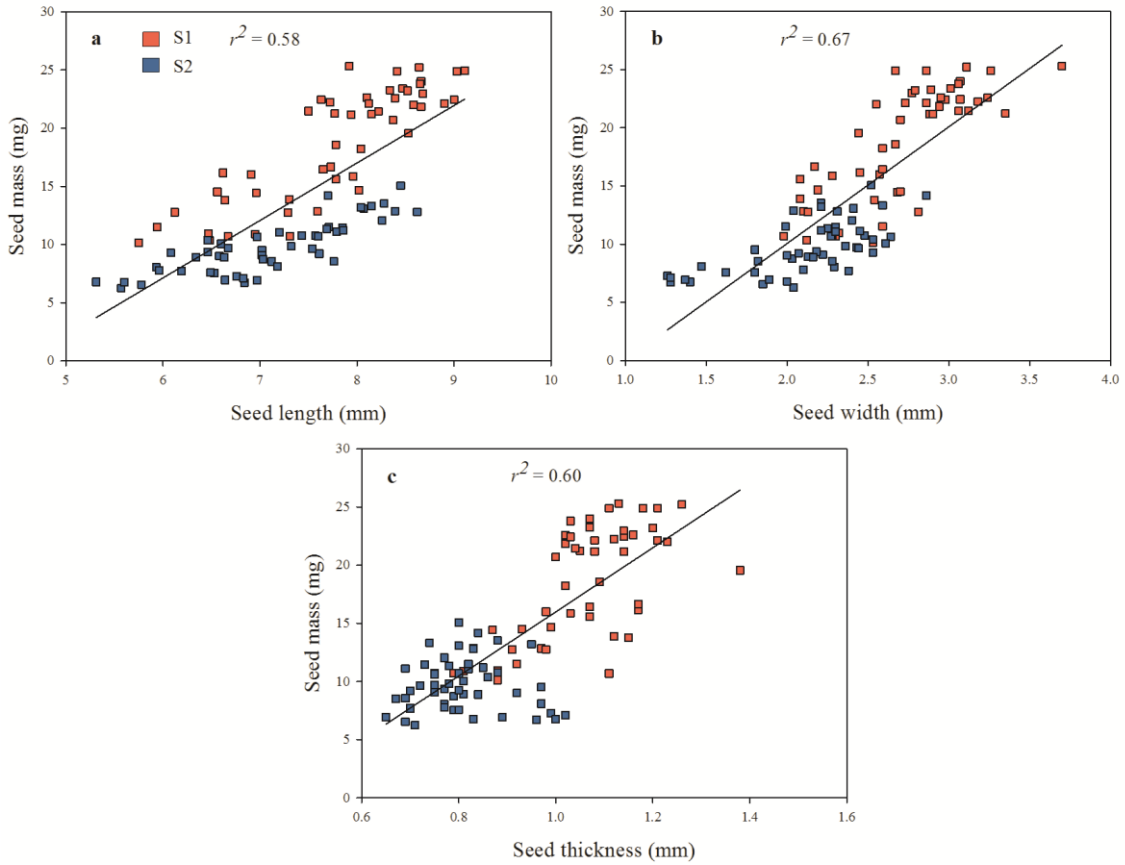

Fig 6. Relationship between seed mass and (A) seed length, (B) seed width and (C) seed thickness. (n = 100, all P < 0.0001).

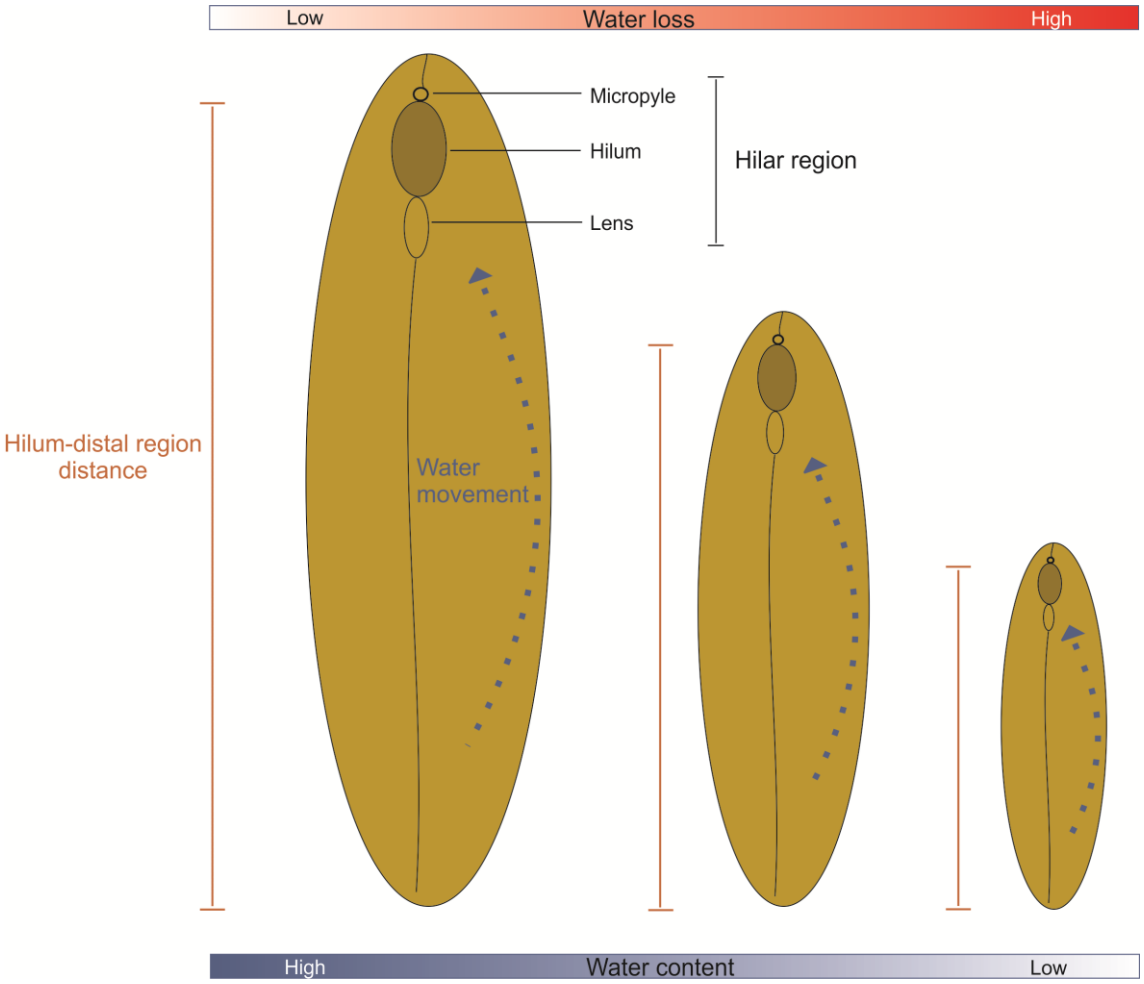

Fig 7. Conceptual model of differences in water loss during onset of PY in relation to seed size. Blue dotted arrows indicate path of water inside the seed towards the hilar region. Red scale represents the variation in water loss among the sizes of seeds. Blue scale represents the variation in water content among the sizes of seeds.

CAPÍTULO III

A FUNCTION FOR THE PLEUROGRAM IN THE PHYSICALLY DORMANT SEEDS OF FABACEAE

Manuscrito submetido à *Annals of Botany*

Original Article

A function for the pleurogram in the physically dormant seeds of Fabaceae

Ailton G. Rodrigues-Junior[1,2*], Ana Caroline M. P. Mello[1], Carol C. Baskin[2,3], Jerry M. Baskin[2], Denise M.T. Oliveira[1] and Queila S. Garcia[1]

[1]Departamento de Botânica, ICB, Universidade Federal de Minas Gerais, Caixa Postal 486, 31270-901, Belo Horizonte, MG, Brazil, [2]Department of Biology, University of Kentucky, Lexington, KY 40506, USA, [3]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546, USA

[*]For correspondence. E-mail: ailtongrodrigues@gmail.com

Running title: Function of the pleurogram

ABSTRACT

- **Background and Aims** Different structures have been shown to act as a water gap in seeds with physical dormancy (PY), and in Fabaceae they are commonly located in the hilar region. However, the function of the pleurogram, a structure located in the extra-hilar region that is common on seeds of caesalpinioid legumes, remains unknown. The aims of this study were to identify the water gaps in 11 *Senna* species (Fabaceae, Caesalpinioideae), determine if water can enter the seed through the pleurogram and compare the functional morpho-anatomy of water gaps in the hilar and extra-hilar regions of the seed.
- **Methods** Imbibition tests on intact seeds showed that all 11 species had PY. Structural features of the hilar and extra-hilar regions of the seeds were investigated using light and scanning electron microscopy, and dye-tracking experiments were performed to trace the pathways of water through the seed coat.
- **Key results** Water gaps differ among the species, with lens, hilum, micropyle and pleurogram taking up water after PY is broken. In *S. alata* seeds, only the pleurogram acted as a water gap, whereas in *S. reniformis* and *S. silvestris* water enters the seed through both the pleurogram and hilar region. In the pleurogram of *S. alata* and *S. reniformis*, the palisade layer is moved outward, exposing the hourglass cells, whereas in *S. silvestris* the palisade layer is broken. The other eight species have hilar water gaps only, with lens, hilum and micropyle functioning in water uptake.
- **Conclusions** Structural disruption of the pleurogram diverged in the species this structure opens. However, the mechanism for opening the pleurogram did not

differ among the species, which is caused by formation of a linear slit in the palisade layer, thus creating a pathway for water entry. This is the first report of the pleurogram functioning as a water gap.

Key words: Extra-hilar water gap, hilum, lens, micropyle, pleurogram function, physical dormancy, *Senna*.

INTRODUCTION

Seeds with physical dormancy (PY, water impermeable) have a specialized structure (the 'water gap') that opens during dormancy break, thereby allowing water to enter the seed (Baskin *et al*., 2000; Baskin, 2003). In seeds of Fabaceae, structures such as the hilum, lens and micropyle or a combination thereof function as the water gap, depending on the species (Gama-Arachchige *et al*., 2013). Gama-Arachchige *et al*. (2011) referred to the region of a physically-dormant seed where water enters as the 'water-gap complex', and Gama-Arachchige *et al*. (2013) identified three types of water-gap complexes. Type-I has a linear opening obstructed by modified palisade cells, Type-II an opening obstructed by a lid-like structure of palisade cells and Type-III an opening occluded by a plug-like structure of sclerenchyma cells. These three types were further classified as simple if one opening is formed and compound if more than one is formed (Gama-Arachchige *et al*., 2013). However, in some species of Fabaceae the exact site where water enters the seed has not identified, and Morrison *et al*. (1998) suggested that the water gap may not be located in the hilar region in seeds of some of them.

In seeds of some members of the Fabaceae subfamilies Caesalpinioideae and Mimosoideae and *Trichosanthes anguina* (Cucurbitaceae), a visibly demarcated structure called the pleurogram is present in the lateral region of the seed coat (Corner, 1976; Werker, 1997). In Fabaceae, a pleurogram is more common in seeds of

Mimosoideae than in those of Caesalpinioideae (Gunn, 1981). The pleurogram is a depression in the seed coat. It occurs on both sides of the seed and can be distinct from the rest of the seed coat (Corner, 1976; Werker, 1997). In some cases, more than one pleurogram occurs on each side of the seed, as detailed for *Chamaecrista* species (Caesalpinioideae) by De-Paula and Oliveira (2008). Morphology of the pleurogram varies in the Mimosoideae and Caesalpinioideae, and in some species of both subfamilies it covers most of the seed coat (Gunn, 1981).

Gunn (1981) hypothesized that the pleurogram acts as a hygroscopic valve, similar to that of the hilum (Hyde, 1954), due to the presence of a fissure similar to the hilar groove. However, there is no experimental evidence to support this hypothesis. Furthermore, Rodrigues-Junior *et al*. (2014) confirmed that although there are multiple fissures in the pleurogram of *Senna multijuga* seeds they were not deep enough to allow water to penetrate the water-impermeable palisade layer of cells. Kelly *et al*. (1992) and Werker (1997) showed a wide fissure in the pleurogram region of seeds of the legumes *Prosopis farcta* and *Dichrostachys cinerea* (both Caesalpinioideae); however, they did not determine if the fissure was deep enough to allow water to enter the seed. The presence of fissures in the pleurogram of *S. multijuga*, *P. farcta* and *D. cinerea* seeds leads to the general hypothesis that the pleurogram, at least in some species, may serve as a water gap. However, with the exception of *S. multijuga*, the function (or lack thereof) of the pleurogram has not been investigated.

Senna (Fabaceae, Caesalpinioideae, Cassiinae) is a large and diverse genus of ca. 350 species that occurs in various habitats throughout the world. It is one of the largest genera in Fabaceae and includes herbs, shrubs and trees (Irwin and Barneby, 1982; Randell and Barlow, 1998; Marazzi and Sanderson, 2010). All *Senna* species whose dormancy has been investigated have PY (De Paula *et al*., 2012; Baskin and

Baskin, 2014; Rodrigues-Junior *et al*., 2014; Erickson *et al*., 2016), and the vast majority of them produce seeds with a pleurogram (Irwin and Barneby, 1982). The pleurogram of Caesalpinioideae including that of *Senna* is classified as closed (Gunn, 1981), i.e. the line demarcating the seed coat is complete.

The lens is the only seed structure described as a water gap in *Senna* (De Paula *et al*., 2012; Rodrigues-Junior *et al*., 2014; Erickson *et al*., 2016). However, this genus has great seed morphological diversity, varying widely in hilum and pleurogram morphology and in some cases with a wide pleurogram bordering the hilar region, as in *S. alata* seeds. Thus, *Senna* is an appropriate genus with which to test our hypothesis that the pleurogram can function as a water gap in some species. As such, then, we addressed the following questions using seeds of 11 *Senna* species from Brazil as the study material: (1) Are there anatomical differences in the pleurogram among *Senna* species? (2) If the pleurogram acts as a water gap, are there morpho-anatomical differences between it and sites of water entry in the hilar region?

MATERIALS AND METHODS

Plant species

Seeds of the 11 *Senna* species were collected in various biomes in Brazil (Table 1). Nine species are shrubs, and two are trees. All species are native to Brazil, and *S. cana var. hypoleuca, S. reniformis* and *S. trachypus* are narrow endemics (Souza and Bortoluzzi, 2015). Seeds were placed in semipermeable plastic bags and stored at laboratory conditions (25 ± 5 ºC, 40-60% relative humidity) until used. Predated seeds were discarded before initiation of experiments.

Seed traits

Length, width and thickness were measured for 50 seeds of each species using digital calipers. Mass was determined for 50 individual seeds of each species by

weighing them with a Shimadzu AUX220 precision balance (0.0001g). Seed moisture content was measured by weighing four replications of 10 seeds of each species, drying them in an oven at 103 ºC for 17 hours and then reweighing. Percentage of water was expressed on a seed fresh weight basis (ISTA, 2004).

Presence of physical dormancy

To determine if seeds had PY, 25 individual intact or manually scarified seeds of each of nine species were placed in Petri dishes on germination paper moistened with distilled water and incubated at 25 ºC (light/ dark, 12/12 h). Light was provided by cool white fluorescent tubes (40 µmol m^{-2} s^{-1}). Seeds were individually weighed at different intervals for 48 h with a Shimadzu AUX220 precision balance. Prior to each weighing, seeds were removed from Petri dishes and blotted dry, weighed and then returned to the moistened paper. Changes in seed mass were calculated.

Dormancy break

Preliminary tests were performed prior to this experiment, and treatments that increased germination to the highest percentage were then used for each species. The following treatments were used: immersion in hot water (80 ºC and 100 ºC) for 15 min; immersion in sulphuric acid for 15 or 30 min; and dry heat (100 ºC) for 15 min. There were four replicates of 25 seeds for each species. Treated and non-treated (control) seeds of each species were placed in Petri dishes on moistened germination paper and incubated at 25 ºC (light/ dark, 12/12 h). Germinated seeds were scored at 3-d intervals for 30 d, and the criterion for germination was emergence of the radicle.

Structural changes during dormancy break

Dormant and non-dormant [made water-permeable by the best method for each species (see Fig. 2)] seeds were mounted directly on stubs using a double-sided carbon

tape and sputter-coated with gold (10 nm) (Robards, 1978). Then, the samples were observed with a scanning electron microscope (Quanta 200 FEI).

Dye tracking

Seeds were made water-permeable and then immersed in 0.1% methylene blue (modified from Johansen, 1940) for 15 and 30 min and 1, 3 and 6 h. Fifteen seeds were evaluated for each immersion period, after which they were rinsed with tap water, blotted dry and sectioned longitudinally and transversely to observe the presence of the dye and its route in the seed tissues. Seeds were observed under a stereomicroscope (Zeiss Stemi 2000-C) and pictures taken with a digital camera (Canon A650 IS).

Anatomy of pleurogram and water-gap complexes

Seeds were made water-permeable and then fixed with a formalin-acetic acid-50% ethanol solution for 48 h, dehydrated in a graded ethanol series and embedded in (2-hydroxyethyl)-methacrylate (Paiva *et al*., 2011). Then, seed material was sectioned (8 µm) longitudinally and transversally in the hilar and pleurogram regions using a microtome (Zeiss Hyrax M40), stained with 0.05% toluidine blue pH 4.7 (modified from O'Brien *et al*., 1964) and mounted in synthetic resin. Sections were observed with an optical microscope (Leica DM500) and photomicrographs taken with a digital camera (Leica ICC50 HD).

Pleurogram of Senna alata seeds

Senna alata seeds have a wide and dark green pleurogram located close to the hilar region, due to an unusual compression of seeds during maturation. Thus, we investigated changes that occurred during dormancy break of this species. Seeds were immersed in sulphuric acid (98%) or hot water (80 ºC) for 30 and 15 min, respectively. Then, morphological changes in the pleurogram were observed and seeds placed in

germination conditions (described above) to evaluate differences between dormant and non-dormant seeds. Images were taken using a digital camera (Canon A650 IS) coupled to a stereomicroscope (Zeiss Stemi 2000-C).

Statistical analyses

The experimental design was completely randomized. Seed imbibition and germination data were analysed with a generalized linear model (GLM) and a post-hoc comparison of means using LSD Test. A 5% interval of confidence was applied in these tests. The data were analysed using the software R for Windows (R Development Core Team, 2014). Graphs were designed using SigmaPlot® software (Systat Software Inc., San Jose, California, USA).

RESULTS

Seed traits

Senna alata seeds were the longest (7.23±0.37 mm) (Fig. S1A), widest (6.17±0.49 mm) (Fig. S1B) and heaviest (0.06±0.007 g) (Fig. S1D); *S. hirsuta* seeds the shortest (2.19±0.12 mm) (Fig. S1A); *S. trachypu*s seeds the narrowest (1.05±0.16 mm) (Fig. S1B), thinnest (0.95±0.12 mm) (Fig. S1C) and lightest (0.005±0.0007 g) (Fig. S1D); and *S. obtusifolia* seeds the thickest (2.22±0.23 mm) (Fig. S1C).

Presence of physical dormancy

Mass of scarified seeds of the nine species tested increased 260% to 360% following 48 h imbibition, whereas that of intact (non-scarified) seeds did not increase (Fig. 1, all $P<0.001$). Thus, the seeds of all species have PY.

Dormancy break

The most effective treatment(s) for breaking dormancy was (were) *S. alata*, immersion in hot water (80 ºC - 15 min) and in sulphuric acid (30 min) (Fig. 2A); *S. cana var. hypoleuca*, immersion in hot water (Fig. 2B); *S. cernua* (Fig. 2C) and *S. obtusifolia* (Fig. 2E), immersion in boiling water for 10 s; *S. hirsuta* (Fig. 2D), *S. silvestris var. guaranitica* (Fig. 2I), *S. spectabilis* (Fig. 2J) and *S. trachypus* (Fig. 2K), immersion in sulphuric acid; *S. occidentalis* (Fig. 2F) and *S. pendula* (Fig. 2G), immersion in hot water; and *S. reniformis*, immersion in hot water and in sulphuric acid (Fig. 2H).

Structural changes during dormancy break

Dormant *S. alata* seeds had well-defined and wide pleurograms with several superficial fissures (Fig. 3A). After the hot-water treatment, the palisade layer in the pleurogram was lifted, exposing the hourglass cells below it. The palisade layer remained attached to the seed coat only at the margins of the pleurogram (Fig. 3B). When sulphuric acid was used to break PY in *S. alata*, the pleurogram showed several cracks where the palisade layer was uplifted (Fig. 3C).

The lens in dormant *S. cana* seeds was at the level of the hilum and micropyle, but it was uplifted during dormancy break. This displacement in the lens created a gap beneath it, but the other structures in the hilar region remained unchanged (Fig. 3D, E). In *S. cernua* seeds, the lens was moved outward during dormancy break, and a groove was created in its margin (Fig. 3F, G). Similarly, in *S. hirsuta* seeds the lens moved outward during PY break (Fig. 3H, I). The lens expanded in non-dormant *S. obtusifolia* seeds, creating spaces around it; however, the micropyle and hilum remained intact after dormancy was broken (Fig. 3J, K). A thick mucilaginous stratum covered *S. occidentalis* seeds, except for the wide hilum and micropyle, which did not change

during PY break; however, the lens expanded and increased in size (Fig. 3L, M). Fissures in the lens region were due to flaking of the mucilaginous stratum (Fig. 3L).

In *S. pendula*, the lens was large in relation to the hilum and micropyle, and it was uplifted in non-dormant seeds, creating a gap around it (Fig. 4A, B). The lens was the only structure altered in *S. reniformis* seeds when hot water was used to break PY (Fig. 4C, D). However, the pleurogram also was disrupted in seeds of this species when dormancy was broken using sulphuric acid. The palisade layer moved outward in different parts in the pleurogram, exposing the hourglass cells (Fig. 4E). In *S. silvestris* seeds, the entire hilar region was corroded during dormancy break, but the micropyle was disrupted, forming a gap (Fig. 4F, G). Also, several cracks were formed in the pleurogram in non-dormant *S. silvestris* seeds (Fig. 4H). In dormant *S. spectabilis* seeds, the hilum and lens were morphologically separated (Fig. 4I). However, after dormancy was broken the space between them disappeared. The lens remained unchanged in all samples of *S. spectabilis*, but the hilum and micropyle sometimes were corroded, with an evident hole in the hilum (Fig. 4J). The micropyle and a space demarcating the hilum were evident in dormant *S. trachypus* seeds (Fig. 4K), and the hilar region was corroded during dormancy break. In some cases, this region changed completely (Fig. 4L), and in others only the hilum and micropyle were damaged (Fig. 4M). Visible openings were present only on the hilum and micropyle.

Dye tracking

The dye only penetrated the seed coat in *S. alata* seeds via the pleurogram in both dormancy-breaking treatments. Following immersion in hot water or sulphuric acid, hypodermal cells of the pleurogram had stained blue after 3 h and 30 min in the dye, respectively (Fig. 5A, B). Following immersion in hot water, all seed coat layers in the lens of *S. cana* seeds were stained blue after 6 h in the dye (Fig. 5C). After 3 h in the

dye, the cells beneath the lens in *S. cernua* seeds were stained; however, the dye also moved through the palisade layer in the hilum (Fig. 5D). In *S. hirsuta*, the lens and the cells under it had stained blue after 3 h (Fig. 5E). After 3 h, the dye stained the lens and spread through the cells beneath it in *S. obtusifolia* seeds (Fig. 5F). The palisade layer in the lens and in the hypodermis was stained blue in seeds of *S. occidentalis* and *S. pendula* after 30 min and 3 h, respectively (Fig. 5G, H).

For *S. reniformis* seeds treated with hot water, the lens and the cells beneath it were stained after 3 h (Fig. 6A). However, for seeds treated with sulphuric acid both the lens and pleurogram had stained blue after 3 h (Fig. 6B, C). In the extra-hilar region, only cell layers in the pleurogram were stained in water-permeable seeds of *S. reniformis* after 3h (Fig. 6C). The dye passed through the hilum and micropyle after 1 h, and staining was observed in both the outer and inner layers of the seed coat in *S. silvestris* (Fig. 6D). The palisade layer and hypodermis were stained in the pleurogram region after 3 h (Fig. 6E). After 3 h, the palisade layer in the hilum and the cells beneath it were stained in *S. spectabilis* seeds (Fig. 6F). In *S. trachypus* seeds, imbibition occurred promptly, and after 15 min different regions had stained blue, mainly in the hilar region, following dormancy break with sulphuric acid (Fig. 6G).

Anatomy of pleurogram and water-gap complexes

The pleurogram was a weak region in some of the *Senna* species and had several disruptions in it after PY was broken (Fig. 7A). An opening across the palisade layer that reaches the hourglass cells in seeds of *S. alata* (Fig. 7B) and *S. reniformis* (Fig. 7C) indicates that the pleurogram acts as a water gap. The complete disruption in the palisade layer of the pleurogram formed a linear fissure that allowed contact of the inner tissues with the moisture substrate (Fig. 7B, C). The linear fissure in the pleurogram was similar to the disruption in the palisade layer in the lens, as evidenced in the lens of

S. obtusifolia (Fig. 7D, E). However, a decrease in palisade layer thickness in the lens slit was observed, unlike what occurred for the slits in the pleurogram region.

Pleurogram of Senna alata seeds

Dormant seeds of *S. alata* are slightly wrinkled and have two pronounced dark green pleurograms without cracks near the hilar region (Fig. 8A, B). During both dormancy-breaking treatments the pleurogram was disrupted (Fig. 8C). After immersion in sulphuric acid, fissures were formed, and apparent tissue corrosion appeared in the pleurogram (Fig. 8D). The pleurogram also contained fissures after immersion in hot water, and some of the outer layers became detached (Fig. 8E). After 24h, water-permeable seeds were completely imbibed and the radicle protruded (Fig. 8F, G). There were no visible changes in the hilar region during the dormancy-breaking treatments.

DISCUSSION

Seeds of all 11 species of *Senna* included in this study have PY, which was broken by various treatments. We documented for the first time PY in seeds of nine species, and it previously was reported in *S. obtusifolia* (Baskin *et al*., 1998) and *S. silvestris* (LF Daibes, AT Fidelis, unpubl. res.). A diversity of water gaps was identified in our study, with the lens, hilum, micropyle and pleurogram opening during the dormancy breaking process, depending on the species. Seeds of *S. alata*, *S. reniformis* and *S. silvestris* had a pleurogram that acted as a water gap (Table 2). Thus, our hypothesis that the pleurogram acts as a water gap in some species was supported. This is the first demonstration that the pleurogram can function as a water gap. In *S. alata* seeds, the pleurogram was the only water gap; the lens was non-functional (did not open). For the other two species in which water entered the seed through the pleurogram (*S. reniformis* and *S. silvestris*), a hilar water gap was also present.

Hyde (1954) showed that the hilum in Fabaceae subfamily Faboideae acts as a hygroscopic valve, which opens when the external humidity is low and closes when it is high. Furthermore, Gunn (1981) suggested that the pleurogram acts in the seed dehydration process in a similar way to that of the hilum. The function of the pleurogram as a hygroscopic valve needs to be investigated, but it cannot be explained by structural features of the pleurogram. Unlike the Faboideae hilum, the pleurogram does not have a tracheid bar, a specialized vascular structure most likely involved in the hilum movements (Hyde, 1954; Lersten, 1982), and presence of multiple fissures in the pleurogram is different from the single groove in the hilum. On the other hand, the pleurogram can open during PY break and thus allow passage of water into seeds. However, unlike the hilum in Faboideae, the open pleurogram cannot be reclosed, since its palisade is completely disrupted during opening.

The lens is the most common water gap in seeds of Fabaceae species (Rolston, 1978; Karaki *et al*., 2012; Baskin and Baskin, 2014; Rodrigues-Junior *et al*., 2014). Also, the hilum and micropyle often are reported as secondary openings in legume seeds from tropical environments (De Paula *et al*., 2012; Delgado *et al*., 2015; Geisler *et al*., 2017), with only one report for a species (*Sophora alopecuroides*) from the temperate region (Hu *et al*., 2008). However, we found that the hilum and/or micropyle function as water gaps in three *Senna* species (*S. silvestris*, *S. spectabilis* and *S. trachypus*). In these three species, the lens was non-functional. In most cases, the lens, along with other structures, acts as the water gap (Hu *et al*., 2008; Delgado *et al*., 2015; Geisler *et al*., 2017).

Different treatments to break dormancy can act in different ways on seeds with PY. For example, Hu *et al*. (2009) showed that sulfuric acid and hot-water treatments disrupted the hilum in *Vigna oblongifolia* seeds, but the extra-hilar regions were also

disrupted when hot water was used. The same pattern was shown for *Koelreuteria paniculata* (Sapindaceae) seeds, in which wet heat and dry heat treatments opened the hilum and extra-hilar region, respectively (Gama-Arachchige *et al*., 2013). This treatment-specific effect could make it difficult to investigate how dormancy in a species such as *S. alata*, in which the water gap occurs only via the pleurogram, is broken in nature. However, structural features such as narrow palisade cells may indicate weak regions (i.e. where slits can occur) in the seed coat where water enters the seed (Rodrigues-Junior *et al*., 2014). In the present study, weak regions (indicated by breaks in the palisade cells) were seen in seeds of all 11 *Senna* species, but they were in different places, e.g. lens, hilum and pleurogram. In the case of the pleurogram, the weak regions were not indicated by a clear decrease in the palisade layer thickness. The weakness in the palisade layer was related to the reduction in the thickness where the slits occur at the lens region (Rodrigues-Junior *et al*. 2018).

The various water gaps found in seeds of *Senna* species can be fitted into the classification scheme of water gaps proposed by Gama-Arachchige *et al*. (2013) (Table 2). Most of the 11 *Senna* species have Type-I simple/compound, wherein the lens acts as the water gap (*S. cana*, *S. hirsuta*, *S. obtusifolia*, *S. occidentalis*, *S. pendula*, *S. cernua* and *S. reniformis*). Indeed, the lens was the main functional water gap in seven of the 11 species studied. The hilum and/or micropyle acted as a water gap when the lens was non-functional in *S. silvestris*, *S. spectabilis* and *S. trachypus*, and this was a type-I (simple or compound), while in *S. alata* a hilar water gap was absent. For *S. alata*, the palisade cells are dislodged only in the pleurogram, creating a pathway for water into the seed. Most palisade cells in the pleurogram are detached from the seed coat after PY is broken, but a minor part of the pleurogram remains attached to the seed. This structure is analogous to Type-II in the classification scheme. In seeds of *S.*

reniformis, two water gaps were found. The lens creates a linear opening that breaks the palisade layer (Type-I), while the palisade cells are ejected from the pleurogram, forming holes along this structure (Type-II). In fact, the pleurogram also can act by forming linear breaks in the palisade cells, as in *S. silvestris*, which is analogous to Type-I.

Heretofore, water gaps in seeds with PY have been described as small channels that direct the water to the inner tissues of the seed (Gama-Arachchige *et al*., 2013). In contrast, our study shows that the pleurogram, for which no function previously has been reported, can open, thereby creating a wide path for entrance of water into seeds of some leguminous species.

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LITERATURE CITED

Baskin CC. 2003. Breaking physical dormancy in seeds – focusing on the lens. *New Phytologist* **158**: 229–232.

Baskin CC, Baskin JM. 2014. *Seeds: ecology, biogeography and evolution of dormancy and germination*, 2nd edn. San Diego: Elsevier/Academic Press.

Baskin JM, Baskin CC, Li X. 2000. Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Species Biology* **15**: 139–152.

Baskin JM, Nan X, Baskin CC. 1998. A comparative study of seed dormancy and germination in an annual and a perennial species of *Senna* (Fabaceae). *Seed Science Research* **8**: 501–512.

Corner EJH. 1976. *The seeds of dicotyledons*, vol. I. Cambridge: Cambridge University Press.

Delgado CML, de Paula AS, Santos M, Paulilo MTS. 2015. Dormancy-breaking requirements of *Sophora tomentosa* and *Erythrina speciosa* (Fabaceae) seeds. *Revista de Biologia Tropical* **63**: 285–294.

De Paula AS, Delgado CML, Paulilo MTS, Santos M. 2012. Breaking physical dormancy of *Cassia leptophylla* and *Senna macranthera* (Fabaceae: Caesalpinioideae) seeds: water absorption and alternating temperatures. *Seed Science Research* **22**: 259–267.

De-Paula OC, Oliveira DMT. 2008. Multiple pleurograms in Chamaecrista Moench (Leguminosae, Caesalpinioideae). *Botanical Journal of the Linnean Society* **157**: 487–492.

Erickson TE, Merritt DJ, Turner SR. 2016. Overcoming physical seed dormancy in priority native species for use in arid-zone restoration programs. *Australian Journal of Botany* **64**: 401–416.

Esau K. 1964. *Anatomy of seed plants*. New York: Wiley.

Gama-Arachchige NS, Baskin JM, Geneve RL, Baskin CC. 2010. Identification and characterization of the water gap in physically dormant seeds of Geraniaceae, with special reference to *Geranium carolinianum*. *Annals of Botany* **105**: 977–990.

Gama-Arachchige NS, Baskin JM, Geneve RL, Baskin CC. 2011. Acquisition of physical dormancy and ontogeny of the micropyle–water-gap complex in developing seeds of *Geranium carolinianum* (Geraniaceae). *Annals of Botany* **108**: 51–64.

Gama-Arachchige NS, Baskin JM, Geneve RL, Baskin CC. 2013. Identification and characterization of ten new water gaps in seeds and fruits with physical dormancy and classification of water-gap complexes. *Annals of Botany* **112**: 69–84.

Geisler GE, Pinto TT, Santos M, Paulilo MTS. 2017. Seed structures in water uptake, dormancy release, and germination of two tropical forest Fabaceae species with physically dormant seeds. *Brazilian Journal of Botany* **40**: 67–77.

Gunn CR. 1981. Seeds of the Leguminosae. In: Polhill RM, Raven PH, eds. *Advances in Legume Systematics, Part. 2*. Kew: Crown Copyright, 913–926.

Hyde EOC. 1954. The function of the hilum in some Papilionaceae in relation to the ripening of the seed and the permeability of the testa. *Annals of Botany* **18**: 241–256.

Hu XW, Wang YR, Wu YP, Baskin CC. 2009. Role of the lens in controlling water uptake in seeds of two Fabaceae (Papilionoideae) species treated with sulphuric acid and hot water. *Seed Science Research* **19**: 73–80.

Hu XW, Wang YR, Wu YP, Nan ZB, Baskin CC. 2008. Role of the lens in physical dormancy in seeds of *Sophora alopecuroides* L. (Fabaceae) from north-west China. *Australian Journal of Agricultural Research* **59**: 491–497.

Irwin HS, Barneby RC. 1982. The American Cassiinae. *Memoirs of the New York Botanical Garden* **35**: 1–918.

Jayasuriya, KMGG, Baskin, JM, Geneve, RL, Baskin CC. 2007. Morphology and anatomy of physical dormancy in *Ipomoea lacunosa*: identification of the water gap in seeds of Convolvulaceae (Solanales). *Annals of Botany* **100**: 13–22.

Jayasuriya KMGG, Baskin JM, Geneve RL, Baskin CC. 2009. A proposed mechanism for physical dormancy break in seeds of *Ipomoea lacunosa* (Convolvulaceae). *Annals of Botany* **103**: 433–445.

Johansen, DA. 1940. *Plant microtechnique*. New York: McGraw-Hill.

Karaki T, Watanabe Y, Kondo T, Koike T. 2012. Strophiole of seeds of the black locust acts as a water gap. *Plant Species Biology* **27**: 226–232.

Kelly KM, Van Staden J. 1987. The lens as the site of permeability in the papilionoid seed, *Aspalathus linearis*. *Journal of Plant Physiology* **128**: 395–404.

Kelly KM, Van Staden J, Bell WE. 1992. Seed coat structure and dormancy. *Plant Growth Regulation* **11**: 201–209.

Lersten NR. 1982. Tracheid bar and vestured pits in legume seeds (Leguminosae: Papilionoideae). *American Journal of Botany* **69**: 98–107.

Manning JC, Van Staden J. 1985. The development and ultrastructure of the testa and tracheid bar in *Erythrina lysistemon* Hutch. (Leguminosae: Papilioinoideae). *Protoplasma* **129**: 157–167.

Marazzi B, Endress PK, Queiroz LP, Conti E. 2006. Phylogenetic relationships within *Senna* (Leguminosae, Cassiinae) based on three chloroplast DNA regions: patterns in the evolution of floral symmetry and extrafloral nectaries. *American Journal of Botany* **93**: 288–303.

Marazzi B, Sanderson MJ. 2010. Large-scale patterns of diversification in the widespread legume genus *Senna* and the evolutionary role of the extrafloral nectaries. *Evolution* **64**: 3570–3592.

Morrison DA, McClay K, Porter C, Rish S. 1998. The role of the lens in controlling heat-induced breakdown of testa-imposed dormancy in native Australian legumes. *Annals of Botany* **82**: 35–40.

O'Brien TP, McCully ME. 1981. *The study of plant structure: principles and selected methods*. Melbourne: Termarcarphi.

Paiva EAS, Pinho SZ, Oliveira DMT. 2011. Large plant samples: how to process for GMA embedding? In: Chiarini-Garcia H, Melo RCN, eds. *Light microscopy: methods and protocols*. New York: Springer/ Humana Press, 37–49.

R Development Core Team. 2014. *R: A language and environment for statistical computing*. Viena: R Foundation for Statistical Computing. http://www.R-project.org (accessed July 2016).

Randell BR, Barlow BA. 1998. Senna. *Flora of Australia* **12**: 89–138.

Robards AW. 1978. An introduction to techniques for scanning electron microscopy of plant cells. In: Hall JL, ed. *Electron microscopy and cytochemistry of plant cells*. New York: Elsevier, 343–403.

Rodrigues-Junior AG, Mello ACMP, Baskin CC, Baskin JM, Oliveira DMT, Garcia QS. 2018. Why large seeds with physical dormancy germinate earlier than small ones. *under review*.

Rodrigues-Junior AG, Faria JMR, Vaz TAA, Nakamura AT, José AC. 2014. Physical dormancy in *Senna multijuga* (Fabaceae: Caesalpinioideae) seeds: the role of seed structures in water uptake. *Seed Science Research* **24**: 147–157.

Rolston MP. 1978. Water impermeable seed dormancy. *The Botanical Review* **44**: 365–396.

Souza VC, Bortoluzzi RL. 2015. *Senna* in Lista de espécies da flora do Brasil. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB23149

Turner SR, Cook A, Baskin JM, Baskin CC, Tuckett RE, Steadman KJ, Dixon KW. 2009. Identification and characterization of the water gap in the physically dormant seeds of *Dodonaea petiolaris*: a first report for Sapindaceae. *Annals of Botany* **104:** 833–844.

Werker E. 1997. *Seed anatomy*. Berlin: Borntraeger.

Table 1. Collection and ecological data on species of *Senna* used in this study.

Species	Habit	Biomes*	Collection
S. alata	Shrub	AF/Am/Ca/Ce/Pa	August, 2013; 19º34'53"S 46º57'37"W
S. cana var. hypoleuca	Shrub	Ca/Ce	October, 2014; 13 º 42'02"S 47 º27'56"W
S. cernua	Shrub	AF/Ce	October, 2015; 23°58'54"S 48°54'58"W
S. hirsuta	Shrub	AF/Am/Ca/Ce/Pm	August, 2015; 23°58'52"S 48°55'66"W
S. obtusifolia	Shrub	AF/Am/Ca/Ce/Pa	October, 2015; 23°59'52"S 48°54'66"W
S. occidentalis	Shrub	AF/Am/Ca/Ce/Pa	October, 2015; 23°58'53"S 48°55'7"W
S. pendula	Shrub	AF/Am/Ca/Ce/Pa	July, 2015; 19°57' 59"S 43°55' 09"W
S. reniformis	Shrub	Ca/Ce	August, 2015; 19º57' 52"S 43º55' 02"W
S. silvestris var. guaranitica	Tree	AF/Am/Ca/Ce/Pa	October, 2014; 13º37'07"S 48º06'10"W
S. spectabilis	Tree	AF/Am/Ca/Ce	August, 2015; 14°53'36"S 44°41'24"W
S. trachypus	Shrub	Ca/Ce	July, 2014; 07°06'34,2"S 038°39'40,1"W

AF = Atlantic Forest, Am = Amazon Forest, Ca = Caatinga, Ce = Cerrado, Pa = Pantanal, Pm = Pampa.
*Data available on <http://floradobrasil.jbrj.gov.br/>.

Table 2. Features of the pleurogram and hilar water gaps in *Senna*.

Species	Pleurogram			To break PY	Water gap in hilar region	Water gap type*
	Features	Contrast	Functional			
S. alata	oblong, large, on the margins	texture, colour	yes	HW, SA	none	II-simple
S. cana	oblong, small, on lateral sides	colour	no	HW	lens	I-simple
S. cernua	oblong, large, on the margins	texture	no	BW	lens/hilum	I-compound
S. hirsuta	rounded, small, on lateral sides	texture	no	SA	lens	I-simple
S. obtusifolia	oblong, large, on lateral sides	texture	no	BW	lens	I-simple
S. occidentalis	oblong, large, on lateral sides	texture	no	HW	lens	I-simple
S. pendula	absent	none	none	HW	lens	I-simple
S. reniformis	oblong, large, on lateral sides	texture	yes	HW, SA	lens	I/ II-compound
S. silvestris	oblong, large, on lateral sides	texture	yes	SA	hilum/ micropyle	I-compound
S. spectabilis	oblong, small, on lateral sides	texture	no	SA	hilum	I-simple
S. trachypus	oblong, small, on the lateral sides	texture	no	SA	hilum/micropyle	I-compound

*According to Gama-Arachchige et al. (2013). BW, boiling water; HW, hot water; SA, sulphuric acid.

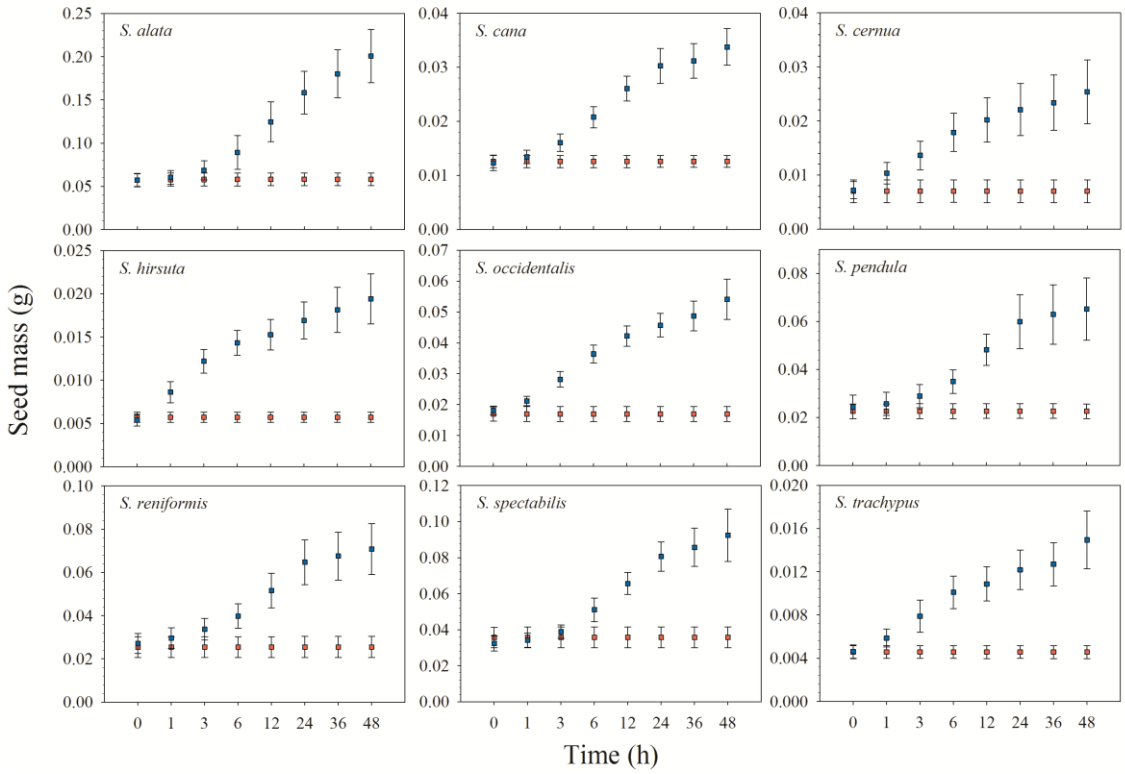

Fig. 1. Seed mass (mean ± s.d.) of intact ( ) and manually scarified ( ) seeds of nine *Senna* species incubated under moist condition at 25 ºC for 1 h to 48 h.

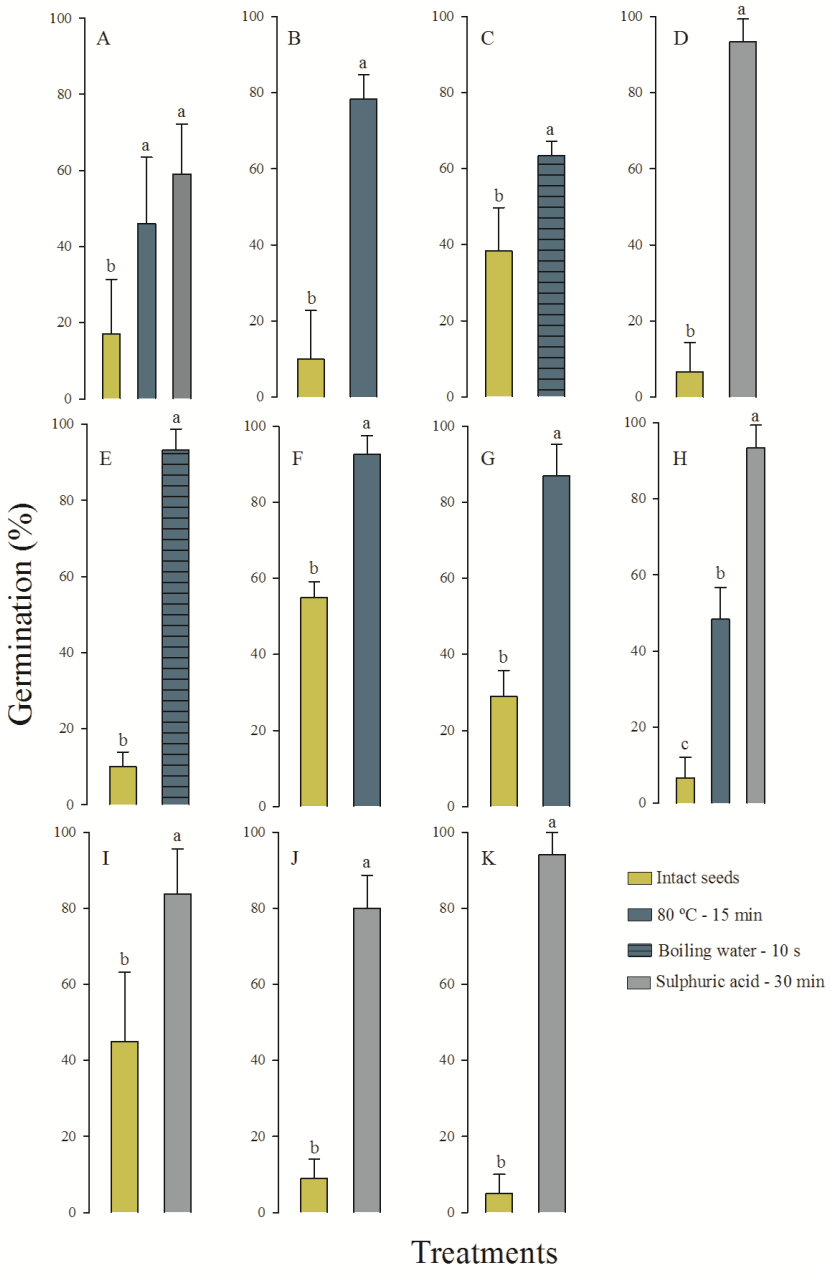

Fig. 2. Germination (mean ± s.d.) of *Senna* seeds following different dormancy-breaking treatments. A) *S. alata*; B) *S. cana*; C) *S. cernua*; D) *S. hirsuta*; E) *S. obtusifolia*; F) *S. occidentalis*; G) *S. pendula*; H) *S. reniformis*; I) *S. silvestris var. guaranitica*; J) *S. spectabilis*; K) *S. trachypus*. Different letters indicate significant differences among treatments within species according to Fisher's test ($P \leq 0.05$).

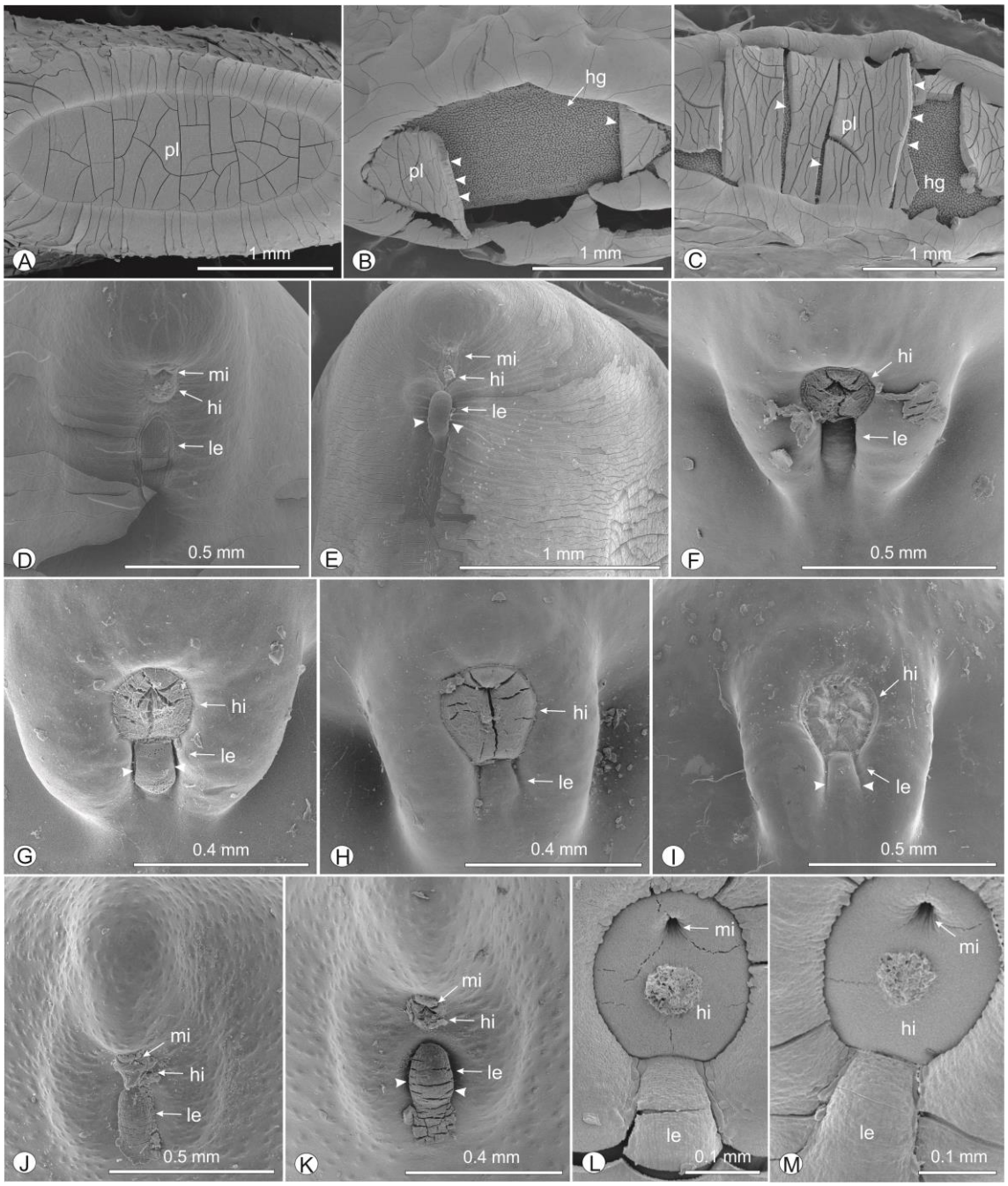

Fig. 3. Structural changes during dormancy break in seeds of *Senna*. A-C) *S. alata*. A) Pleurogram of dormant seeds. B) Pleurogram of non-dormant (using hot water) seeds showing lifting of the palisade layer (arrowheads). C) Pleurogram of non-dormant (using sulphuric acid) seeds with several cracks and the palisade layer lifted up (arrowheads). D-E) *S. cana*. D) Hilar region of dormant seeds. E) Hilar region of non-dormant seeds with the lens lifted up. F-G) *S. cernua*. F) Hilar region of dormant seeds. G) Hilar region of non-dormant seeds with slits formed around the lens (arrowheads). H-I) *S. hirsuta*. H) Hilar region of dormant seeds. I) Hilar region of non-dormant seeds with an elevated lens and stretched groove around the lens (arrowheads). J-K) *S. obtusifolia*. J) Hilar region of dormant seeds. K) Hilar region of non-dormant seeds with displacement of the lens creating spaces around it (arrowheads). L-M) *S. occidentalis*. L) Hilar region of dormant seeds. M) Hilar region of non-dormant seeds with the lens lifted up. hi, hilum; hg, hourglass cells; le, lens; mi, micropyle; pl, pleurogram.

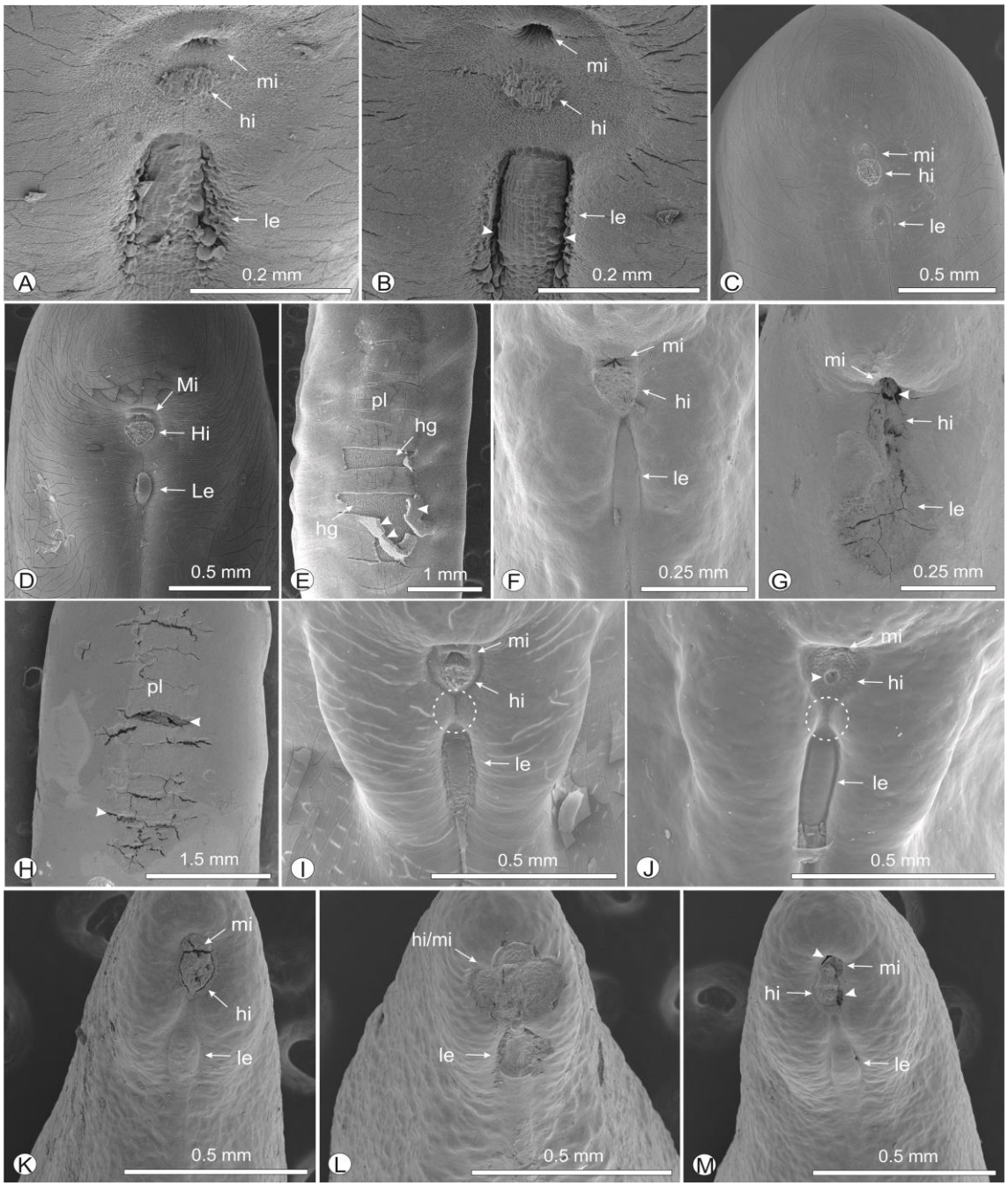

Fig. 4. Structural changes during dormancy break in seeds of *Senna* species. A-B) *S. pendula*. A) Hilar region of dormant seeds. B) Hilar region of non-dormant seeds with displacement of the lens creating slits around it (arrowheads). C-E) *S. reniformis*. C) Hilar region of dormant seeds. D) Hilar region of non-dormant seeds with the emerged lens. E) Pleurogram of non-dormant seeds with the palisade layer lifted up (arrowheads). F-H) *S. silvestris*. F) Hilar region of dormant seeds. G) Hilar region of non-dormant seeds with disruption of micropyle creating a gap (arrowhead). H) Pleurogram of non-dormant seeds with several cracks (arrowheads). I-J) *S. spectabilis*. I) Hilar region of dormant seeds with a separation between hilum and lens (circle). J) Hilar region of non-dormant seeds with a hole in the hilum (arrowhead) and absence of separation between hilum and lens (circle). K-M) *S. trachypus*. K) Hilar region of dormant seeds. L) Hilar region of non-dormant seeds with disruption in the entire hilar region. M) Hilar region of non-dormant seeds with disruption of hilum and

displacement of micropyle creating gaps (arrowheads). hi, hilum; hg, hourglass cells; le, lens; mi, micropyle; pl, pleurogram.

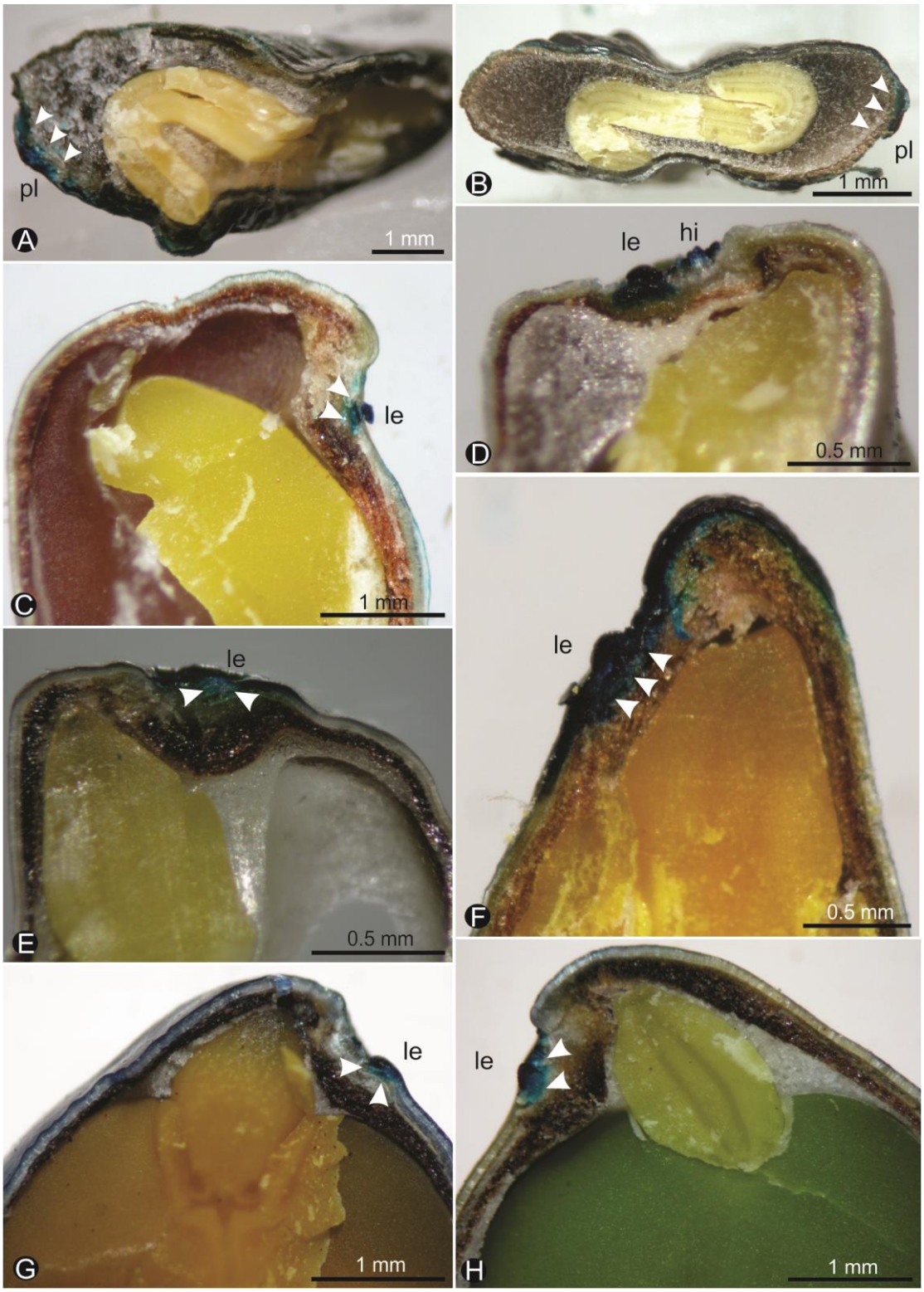

Fig. 5. Non-dormant seeds of *Senna* species stained with methylene blue to show pathways of water into the seeds. A-B) *S. alata*. A) Seed following immersion in hot water, with arrowheads indicating cells stained in the pleurogram. B) Seed following immersion in sulphuric acid, with cells in the pleurogram region stained. C) *S. cana* seed with the cells in the lens region stained. D) *S. cernua* seed with the cells in lens

region stained and with hilum stained E) *S. hirsuta* seed with lens stained. F) *S. obtusifolia* seed with lens stained. G) *S. occidentalis* seed with palisade layer in lens stained. H) *S. pendula* seed with the lens stained. hi, hilum; le, lens; pl, pleurogram.

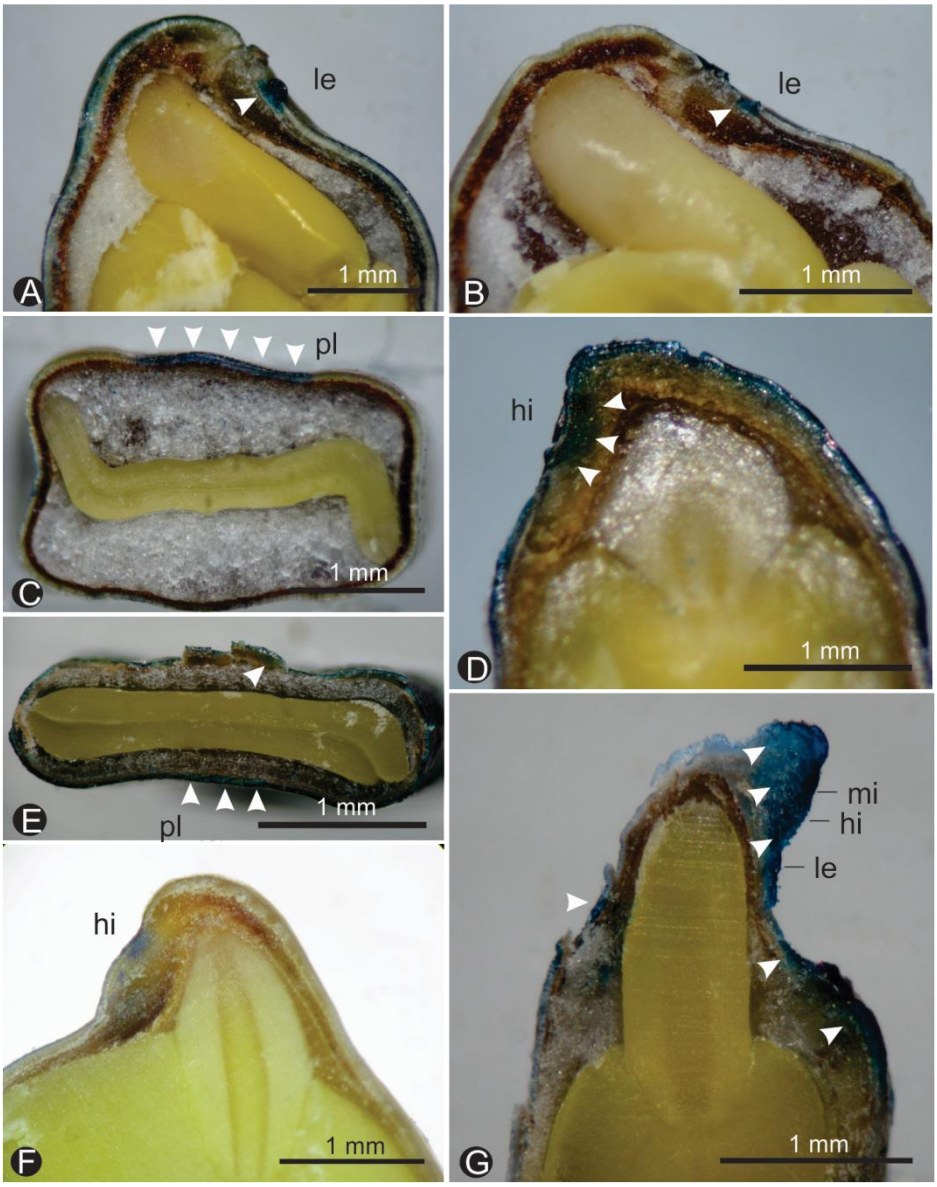

Fig. 6. Non-dormant seeds of *Senna* species stained with methylene blue to show pathways pf water into the seeds. A-C) *S. reniformis*. A) Seed following immersion in hot water, with the lens stained. B) Seed following immersion in sulphuric acid, with the lens stained. C) Seed following immersion in sulphuric acid showing the dye penetrating through the pleurogram only. D-E) *S. silvestris*. D) Seed with dye entering through the hilum. E) Seed with dye entering through the pleurogram. F) *S. spectabilis* seed with the hilum stained. G) *S. trachypus* seed with different regions stained. hi, hilum; le, lens; mi, micropyle; pl, pleurogram.

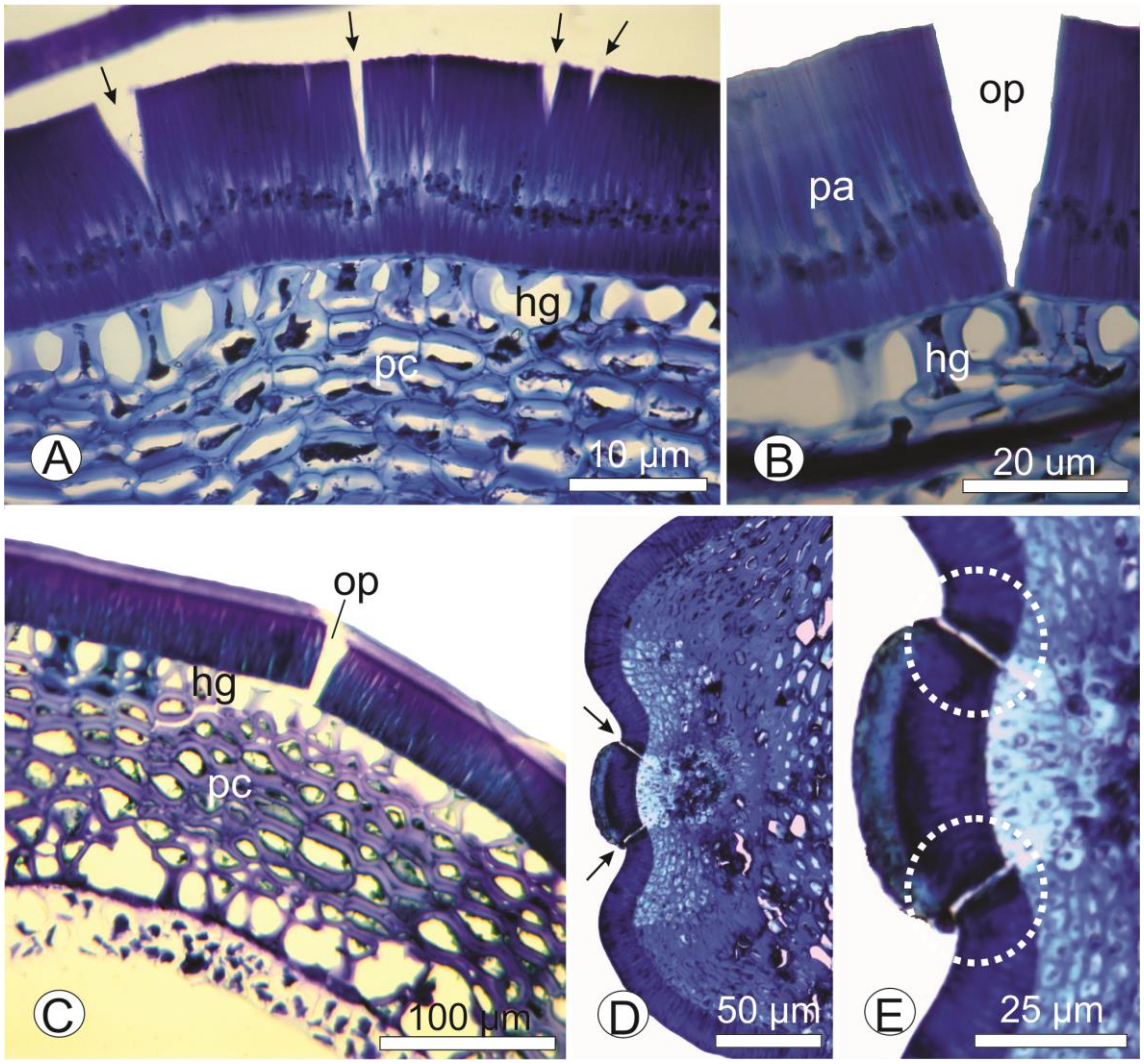

Fig. 7. Transversal sections of the water gaps. A) Pleurogram region showing several fissures in the palisade layer indicating the weak regions (arrows). B) Detail of an opening in the pleurogram of *S. alata* seeds. C) Pleurogram of *S. reniformis* seeds showing an opening. D) Lens of *S. obtusifolia* seed showing linear slits (arrows). E) Detail of the water pathway in the lens (dotted circle). hg, hourglass cells; op, opening; pa, palisade layer; pc, parenchyma cells.

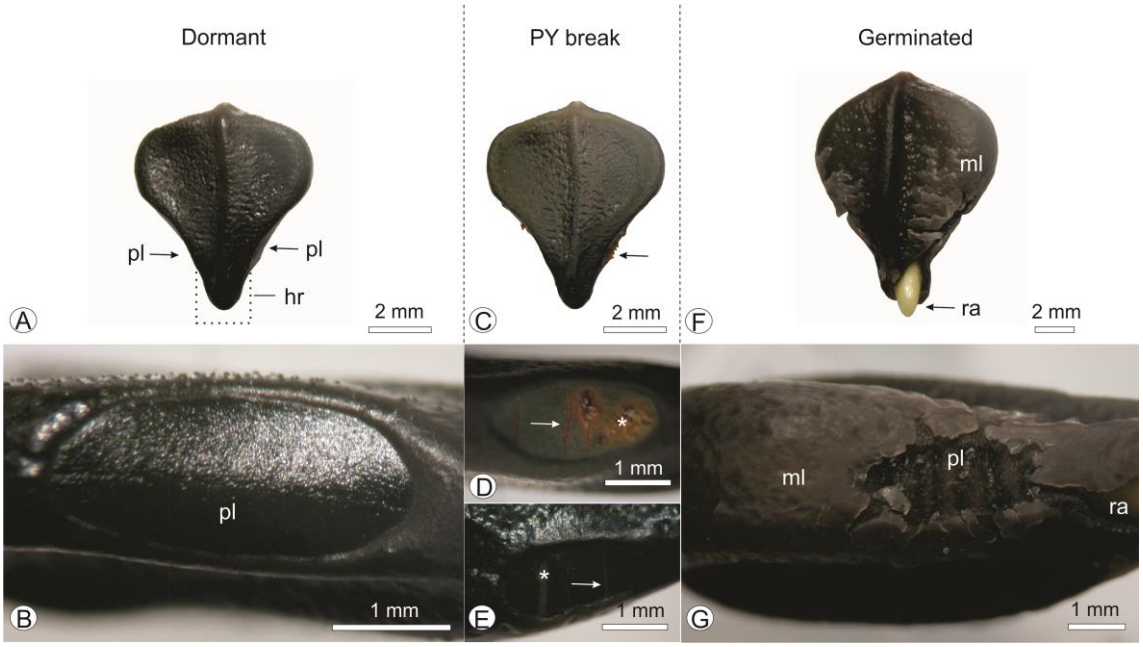

Fig. 8. Morphological changes in *Senna alata* seeds during dormancy break and germination. A-B) Dormant seed. A) General view. B) Detail of the intact pleurogram. C-E) Seed following dormancy break. C) General view. Arrow indicates the disruption of pleurogram. D) Details of the pleurogram after exposure to sulphuric acid. Arrow indicates the presence of fissures and asterisk the corrosion in some parts. E) Pleurogram after immersion in hot water. Arrow indicates the detachment of palisade layer and asterisk the exposure of inner tissues. F-G) Germinated seed after 24 h of imbibition. F) General view. G) Details of the pleurogram covered by a mucilaginous layer. hr, hilar region; ml = mucilaginous layer; pl, pleurogram; ra, radicle.

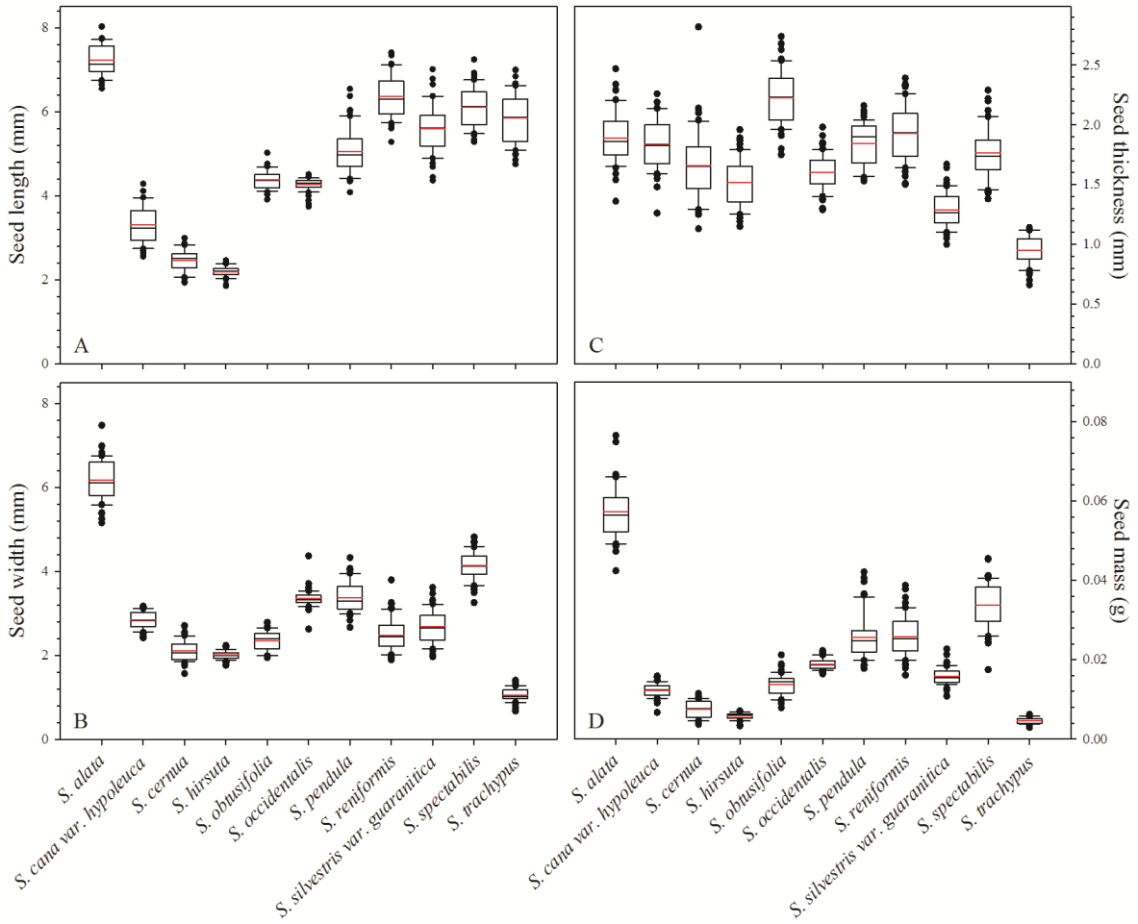

Fig. S1. Seed length (A), width (B), thickness (C) and mass (D) of 11 *Senna* species. Boxes represent the interquartile range (IQR) between first and third quartiles, and black and red lines are the median and the mean, respectively. Black dots are the outliers.

CONSIDERAÇÕES FINAIS

A dormência física (PY) é um mecanismo de controle da germinação recorrente em espécies distribuídas pelo globo. No entanto, apenas recentemente os estudos investigando esta classe de dormência incluíram espécies tropicais. A complexidade da PY, como mecanismo de controle da germinação de sementes, tornou-se evidente com a diversidade de fatores e características que afetam esta dormência. A função principal da PY em sementes foi referida em alguns trabalhos como sendo um mecanismo de escape da predação, no entanto, esta dormência demonstrou-se extremamente eficaz no controle da germinação. Uma característica essencial para o controle do tempo da germinação em sementes impermeáveis, o ciclo de sensibilidade, até então restrito às espécies herbáceas de zonas temperadas, foi demonstrado neste trabalho como sendo uma estratégia também presente em espécies tropicais. O requerimento de umidade para a superação da PY se mostrou determinante para o sincronismo da germinação com a estação chuvosa. Esta exigência também foi descrita para outras espécies de *Senna* e de gêneros correlatos, como *Cassia* e *Chamaecrista*. A história evolutiva destas espécies pode explicar esta similaridade. Assim, este atributo remete a um fator de seleção semelhante nestes grupos, excluindo a necessidade de fatores abióticos como o fogo para a continuidade do ciclo de vida nestas espécies.

Outra característica importante da PY está relacionada ao controle do tempo da germinação. O tamanho da semente, investigado nesta tese, regula a distribuição da germinação no tempo, aumentando as chances de sobrevivência das espécies na natureza. Outra contribuição inédita refere-se ao pleurograma, estrutura até então com função desconhecida, que foi demonstrado aqui atuando como *water gap* em algumas espécies do gênero *Senna* que produzem sementes impermeáveis. Nestas espécies, o pleurograma é uma estrutura que se rompe, criando canais para a passagem da água. A

variação na funcionalidade desta estrutura no controle da dormência sinaliza para uma diversidade de estratégias das espécies ao longo da evolução da dormência. A investigação do papel das estruturas na regulação da PY sugere um caráter ancestral relacionado à atuação do pleurograma, sendo uma estrutura presente e funcional em espécies primitivas e ausente em espécies mais derivadas dentro do gênero *Senna*, o que indica que a lente pode ser um *water gap* mais recente durante a evolução da PY.

Tendo em vista as características da PY descritas, os resultados desta tese permitiram concluir que a PY é um mecanismo complexo de regulação da germinação. A investigação da PY em espécies tropicais, que apresentam grande diversidade morfológica de sementes, possibilitarão uma maior compreensão deste tipo de dormência, como evidenciado neste trabalho. Portanto, estudos com espécies de regiões tropicais tendem a gerar uma maior compreensão da PY e das estratégias de sobrevivência relacionadas a ela.