

RESEARCH ARTICLE

Seed dormancy in relation to predation and pathogenicity of four tropical Fabaceae species from Sri Lanka

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Abstract: According to the seed defense theory, predation and pathogenicity should be lower in physically dormant (PY) seeds than those of no-dormant (ND). Thus, a study was conducted to determine the predation and pathogenicity of four tropical Fabaceae species to test the above hypothesis. Imbibition and germination of manually scarified (MS) and untreated (UT) seeds were tested using locally collected seeds. Pathogenicity and predatory rates of MS and UT seeds of each species was observed by keeping them separately in a woodland and a grassland for four weeks. Nutrient content and production of volatile compounds in seeds were determined. Germination and imbibition experiments confirmed the physical dormancy of two *Senna* spp., non-dormancy of *Bauhinia variegata* and physiological dormancy in *Pterocarpus indicus* seeds. Predation and pathogenicity were higher in the woodland than in the grassland. Lowest seed predation and pathogenicity were recorded in *P. indicus* and the highest in *B. variegata*. Pathogenicity and predation of MS seeds of *Senna* spp. were higher than that of UT seeds. No correlation was revealed between nutrient content and pathogenicity or predation. MS seeds released more volatile compounds than intact seeds as they were in imbibed state. Pathogenicity and predation depended on seed dormancy classes.

Keywords: Non-dormancy, Nutrient content, Physical Dormancy, Physiological Dormancy, Seed defense theory, Seed volatile compounds.

INTRODUCTION

The 'plant defense theory' explains the survival of seedlings and mature plants from pathogens and predators (McKey, 1974 and Herms and Mattson, 1992). However, this theory does not explain how seeds defend against pathogens and predators and survive until the seedlings are established. However, lack of understanding on seeds defense was a significant impediment in understanding the life cycle of a seed plant (Dalling *et al.*, 2011). Further, seeds are among the most heavily defended plant parts (Zangerl and Bazzaz, 1992) and for many plant species seeds determines the fitness of the species (Blake, 1935). Seed survival mainly depends on the seed size, nutrition and the structural or chemical traits that influence predation rates (Janzen, 1969; Grubb *et al.*, 1998 and Moles *et al.*, 2003). Considering

all these facts, Dalling *et al.* (2011) have put forward the 'seed defense theory' which explains the mechanisms of seed defense against pathogens and predators in the micro habitats of seeds. These mechanisms are termed as seed defense syndromes and are closely link with the seed dormancy classes. There are three types of seed defense syndromes; physical, chemical and physical defense continuum and microbial. Physical defense syndrome is mainly adopted by seeds with physical dormancy, chemical and physical defense continuum by seeds with physiological dormancy, and microbial defense syndrome by non-dormant seeds (Dalling *et al.*, 2011).

However, the seed defense theory has not been tested in the field. According to the seed defense theory, physically dormant seeds should be predated and attacked by pathogens in a slower rate than those of non-dormant seeds, when the seeds are in a moist environment. Further, physically dormant seeds which were made non-dormant may be predated and attacked by pathogens in a higher rate than dormant seeds in moist environments (Dalling *et al.*, 2011). Thus, the main objective of this research was to compare the seed pathogenic and predatory rates of four tropical Fabaceae species from Sri Lanka to test the above two hypotheses derived from the 'seed defense theory'. Thus, following sub-objectives were fulfilled in the current study; 1) pathogenicity and predatory rates of untreated intact seeds of *Bauhinia variegata*, *Senna spectabilis*, *Senna bacillaris* and *Pterocarpus indicus* were determined, 2) pathogenicity and predation of untreated *S. spectabilis*, *S. bacillaris* seeds were compared with that of manually scarified seeds of the same species, 3) nutrition contents of seeds of *B. variegata*, *S. spectabilis*, *S. bacillaris*, and *P. indicus* was analyzed and 4) effect of production volatile compounds by seeds on predation was studied.

Paulsen *et al.* (2013, 2014 and 2015) have shown that seeds with physical dormancy play a major role in predator escape by preventing the production of volatile compounds. Paulsen *et al.* (2013) suggested that physical dormancy in seeds have evolved due to the predatory pressure from rodents; however, there are contradictory opinions by Jayasuriya *et al.* (2015) and Jaganathan (2018). Further, it has been shown that many of the seeds produce higher

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amount of volatile compounds when they are in the imbibed state (Johnson and Jorgensen, 1981; Vander-Wall, 1998; Jorgensen, 2001 and Colville *et al.*, 2012). Thus, Paulsen *et al.* (2013) argued that seeds with physical dormancy produce lesser amount of volatile compounds in the soil seed bank as the seed coat is impermeable to water. Further, they have shown that hamsters predate non-imbibed physical dormant seeds less than those with permeable seed coats. Jayasuriya *et al.* (2015) further suggested that, as a result of water impermeability in seeds coats, it maintains lower moisture content inside the seeds that do not support microbial growth. Supporting this argument, seeds with physical dormancy have been observed as seeds with high longevity (Toole and Brown, 1946; Shen-Miller *et al.*, 1995 and Telewski and Zeevaart, 2002).

MATERIALS AND METHODS

Study species

Four introduced legume species were used in the study; *Bauhinia variegata* L., *Senna spectabilis* (DC.) H.S. Irwin & Barneby, *Senna bacillaris* (L.f.) H.S. Irwin & Barneby and *Pterocarpus indicus* Willd. Seeds of these selected species mature, disperse and germinate under approximately similar environmental conditions. The seeds of the two *Senna spp.* are physically dormant and seeds of *B. variegata* are nondormant, while seeds of *P. indicus* are physiologically dormant (Jayasuriya *et al.*, 2013). These test species produce seeds in the same season and represent three dormancy classes.

Mature seeds were collected from at least five individuals growing in the premises of the University of Peradeniya, Peradeniya, Sri Lanka during October 2011 and October 2012. Seeds were placed in brown paper bags and stored at ambient laboratory temperature and RH conditions, until they were used for the experiment. The treatments were initiated within 2-3 days from the collection. Two predatory trials were conducted from each collection, where 1st trial was initiated after 1 month and 2nd trial after 3 months from 2011 October collection and 3rd trial after 1 week and 4th trial after 2 months from 2012 October collection. Pathogenicity trials were conducted in the same way.

Confirmation of seed dormancy classes of test species

Testing seed dormancy

Two samples with three replicates of 15 untreated fresh and manually scarified seeds were incubated on moistened filter papers in 9 cm diameter Petri dishes at ambient laboratory temperature (~27 °C) and light conditions (cool white fluorescent light + diffused sunlight from windows). Samples were watered as required. Seeds were checked for germination at 2 day intervals, where the radicle emergence was noted as the criterion for germination.

Testing for physical dormancy

If seeds are physically dormant, untreated seeds will not imbibe water while, manually scarified seeds will increase

mass > 100 % during imbibition of water. Two samples of 15 untreated fresh and manually scarified seeds were weighed individually with a digital analytical balance to nearest 0.001 g. Incubated on moistened filter papers in Petri dishes with 9 cm diameter. Seeds were retrieved in time intervals as shown in Figure 1 and reweighed and return to the Petri dish. This procedure was continued until all the manually scarified seeds imbibed completely.

Seed predation

Two sets of samples with five replicates of ten intact (unscarified/nontreated) seeds from each species were placed on moistened filter papers in 9 cm diameter open plastic petri dishes, separately. Each sample was kept in two sites *viz.*, in an open environment (lawn in the Department of Botany, University of Peradeniya) and in an adjacent closed canopy environment (a small woodland). Replicates of each seed samples were kept in five different locations less than five meter distance from each other. Instead of intact seeds of two *Senna spp.* manually scarified seed samples were also used in the predation experiments. No mature trees of any tested species were in the vicinity of the study site though the sites were within the introduced range of the species.

These seeds were observed daily for a week and then weekly for another 3 weeks. Number of remained, germinated and damaged seeds was counted separately to calculate the rate of predation. Percentage predation of seeds of each species and treatments were calculated using the following equation;

Percentage prediction =

$$\frac{\text{Initial seed count} - (\text{Final seed count} + \text{Germinated seeds})}{\text{Initial seed count}} \times 100$$

Seed predation experiment was repeated two times in November, 2011 and November, 2012 using freshly collected seeds and two times with 2 months dry stored seeds. Two months dry stored seeds were used as under field conditions most of the fresh untreated seeds of two *Senna sp.* showed signs of imbibition.

Seed pathogenicity

Two samples with five replicates of 10 intact seeds from each species were inserted into net bags separately and buried under 15 cm deep soil in two sites described in the predatory experiment. This experiment was also conducted for manually scarified seeds of two *Senna spp.* Seeds were retrieved in one day intervals for a week and then once a week for another 3 weeks. Seeds were reburied after observation. Number of infected, germinated and remained seeds was counted and percentage pathogenicity of each seed type was calculated using the following equation,

Percentage pathogenicity =

$$\frac{\text{Number of seeds infected}}{\text{Initial seed count}} \times 100$$

Seed pathogenicity experiment was also repeated four times during the same period as mentioned above.

Nutrient content of seeds

Total lipid, carbohydrate and protein contents of the seeds were determined using the standard protocols by Bligh and Dyer (1959), Snell and Snell (1970) and Layne (1957) respectively. Three samples of five replicates of 10 % seed extracts were used to analyze carbohydrate, protein and lipid contents.

Release of volatile compounds by seeds

Three replicates with ten seeds of each species were incubated on moistened filter paper in 9 cm diameter Petri dishes at ambient laboratory temperature for 12 hrs. These samples were transferred into sample bottles and kept for 6 hrs for saturation. Presence of volatile compounds was determined using a gas-chromatography (Aligent 4890D). Determination of volatile compounds of manually scarified seed samples of two *Senna* spp. was also performed.

Analysis of data

Imbibition, germination, predation and pathogenicity data were percentage data and thus, were arc sign transformed and analyzed using ANOVA and T-test procedures. Nutrient content and release of volatile compounds data were analyzed directly with one-way ANOVA procedure. Effect of volatile compounds on predation was analyzed using a binary logistic regression procedure. Data analysis was conducted using MINITAB statistical software (version 14.1, Minitab Inc., State College, PA, USA).

RESULTS

Seed dormancy classes of test species

Seed germination

Both manually scarified seeds and untreated (intact) fresh seeds of *Bauhinia variegata* germinated > 90 %. None of the manually scarified or untreated fresh seeds of *Pterocarpus*

indicus germinated. Further, none of the untreated *Senna bacillaris* and 10 % of the untreated *S. spectabilis* seeds germinated. Over 90 % of the manually scarified seeds of two *Senna* species were germinated.

Imbibition of seeds

No significant mass increment was observed in the untreated fresh seeds of two *Senna* spp. during the imbibition whereas, manually scarified seeds of these two species increase in mass > 120 % (Figure 1). Mass increment of manually scarified seeds was significantly higher than those of untreated seeds of the two *Senna* spp. Both untreated fresh seeds and manually scarified seeds of *B. variegata* and *P. indicus* increased > 80 % in mass and the mass increment of untreated seeds and manually scarified seeds were not significantly different.

Seed predation

Significant differences of predation were observed between trials. Especially the predation pattern of trial one was different from those of 2nd, 3rd and 4th trials ($p = 0.009$, $F = 3.9$) (Figures 2a and 2b, and Table 1). Specially, untreated seeds of the two *Senna* sp. were highly predated compared to the other trail. Further, predatory rates in grassland was significantly higher than that in the woodland ($p < 0.09$, $F = 391.0$).

Predatory rates between species ($p < 0.001$, $F = 121.1$) were significantly different. *P. indicus* seeds had lowest rate of predation in both sites in all the trials. Manually scarified seeds of *S. spectabilis* and *B. variegata* seeds had the highest rate of predation except in the 1st trial. Except in the 1st trial, manually scarified *S. spectabilis* and *S. bacillaris* seeds predated more than the untreated seeds of the same species. In the first trial, manually scarified seeds and untreated seeds of the two *Senna* sp. had higher predatory rates than that of *B. variegata*. However, predatory rates between manually scarified seeds and untreated seeds of

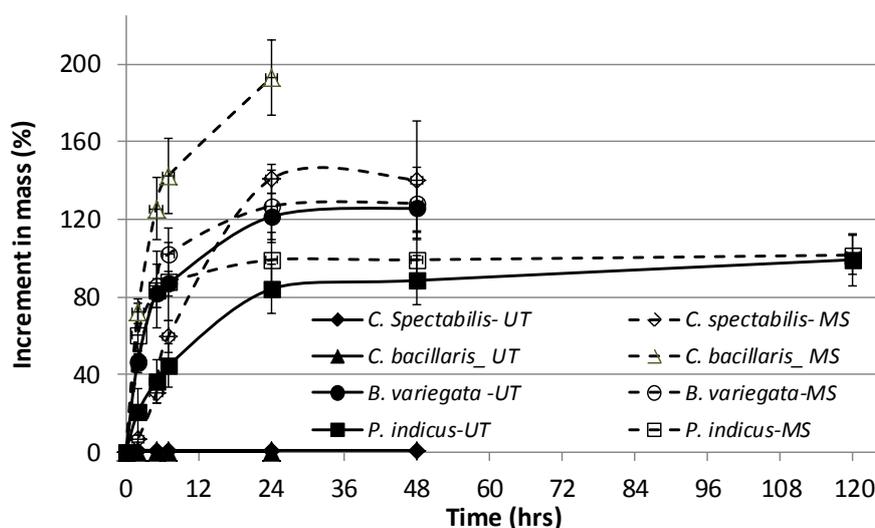


Figure 1: Mass increment of untreated fresh and manually scarified seeds of *Senna spectabilis*, *S. bacillaris*, *Bauhinia variegata* and *Pterocarpus indicus* placed on moistened filter papers in 9 cm diameter Petri dishes at ambient laboratory temperature (~ 27 °C) and light conditions. Error bars are \pm SEM.

Table 1: Overall average of percentage predation and pathogenicity of seeds of study species in two studied habitats.

Species	Treatment	Predation		Pathogenicity	
		Grassland	woodland	Grassland	Woodland
<i>Pterocarpus indicus</i>	UT	6.2 ± 3.4 ^a	19.4 ± 6.8 ^a	14.6 ± 9.6 ^a	22.5 ± 12.5 ^a
<i>Bauhinia variegata</i>	UT	82.5 ± 8.6 ^b	83.7 ± 17.4 ^b	72.5 ± 10.2 ^b	93.0 ± 6.5 ^b
<i>Senna spectabilis</i>	UT	56.2 ± 10.4 ^c	71.7 ± 15.6 ^{bd}	35.1 ± 16.4 ^c	68.0 ± 11.5 ^{cc}
	MS	73.7 ± 8.5 ^b	80.6 ± 13.4 ^b	52.1 ± 11.4 ^d	82.5 ± 7.1 ^{bd}
<i>Senna bacillaris</i>	UT	60.0 ± 23.4 ^c	66.2 ± 14.1 ^{cd}	19.1 ± 5.6 ^a	63.5 ± 13.4 ^c
	MS	88.1 ± 9.8 ^b	83.1 ± 13.0 ^b	45.5 ± 14.9 ^d	76.5 ± 8.1 ^{dc}

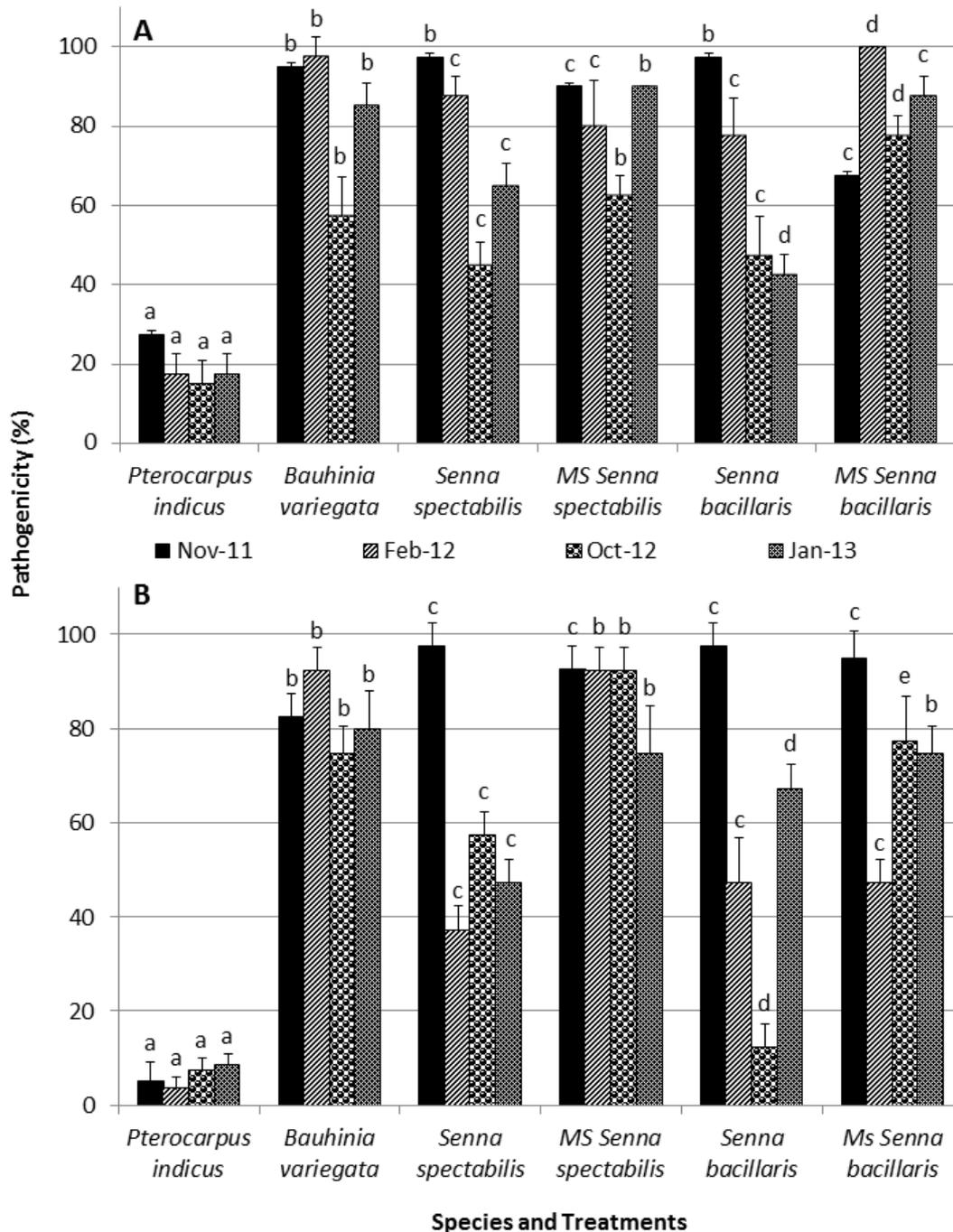


Figure 2: Predation of untreated *P. indicus*, *B. variegata*, *S. spectabilis* and *S. bacillaris* seeds and manually scarified *S. spectabilis* and *S. bacillaris* seeds kept in the grassland (A) and the woodland (B) of the Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka during different time periods. Different uppercase letters indicate significant differences between species and treatments within a single trial. Error bars are + SEM.

the two *Senna* sp. were not significantly different from each other. When the data of two *Senna* species were analyzed separately, no significant differences found between the predatory percentages among two species ($p = 0.054$, $F = 3.9$). Further, the predatory percentage of untreated seeds were significantly lower than the manually scarified seeds ($p < 0.001$, $F = 18.2$) and the predatory percentage of the 1st trial was higher than the other three trials ($p < 0.001$, $F = 23.4$).

Pathogenicity of seeds

Significant differences of pathogenicity levels were observed between trials ($p < 0.001$, $F = 10.4$). However, all the trials showed the same pattern of pathogenicity (Figures 3a and 3b, and Table 1). No significant differences were detected between the pathogenicity in two sites ($p = 0.126$, $F = 2.36$). However, the pathogenicity between species and between treatments within species were significantly different ($p < 0.001$, $F = 84.2$). *P. indicus* seeds had the lowest rate of pathogenicity in both sites in all the trials. Manually scarified *S. spectabilis* seeds and untreated *B. variegata*

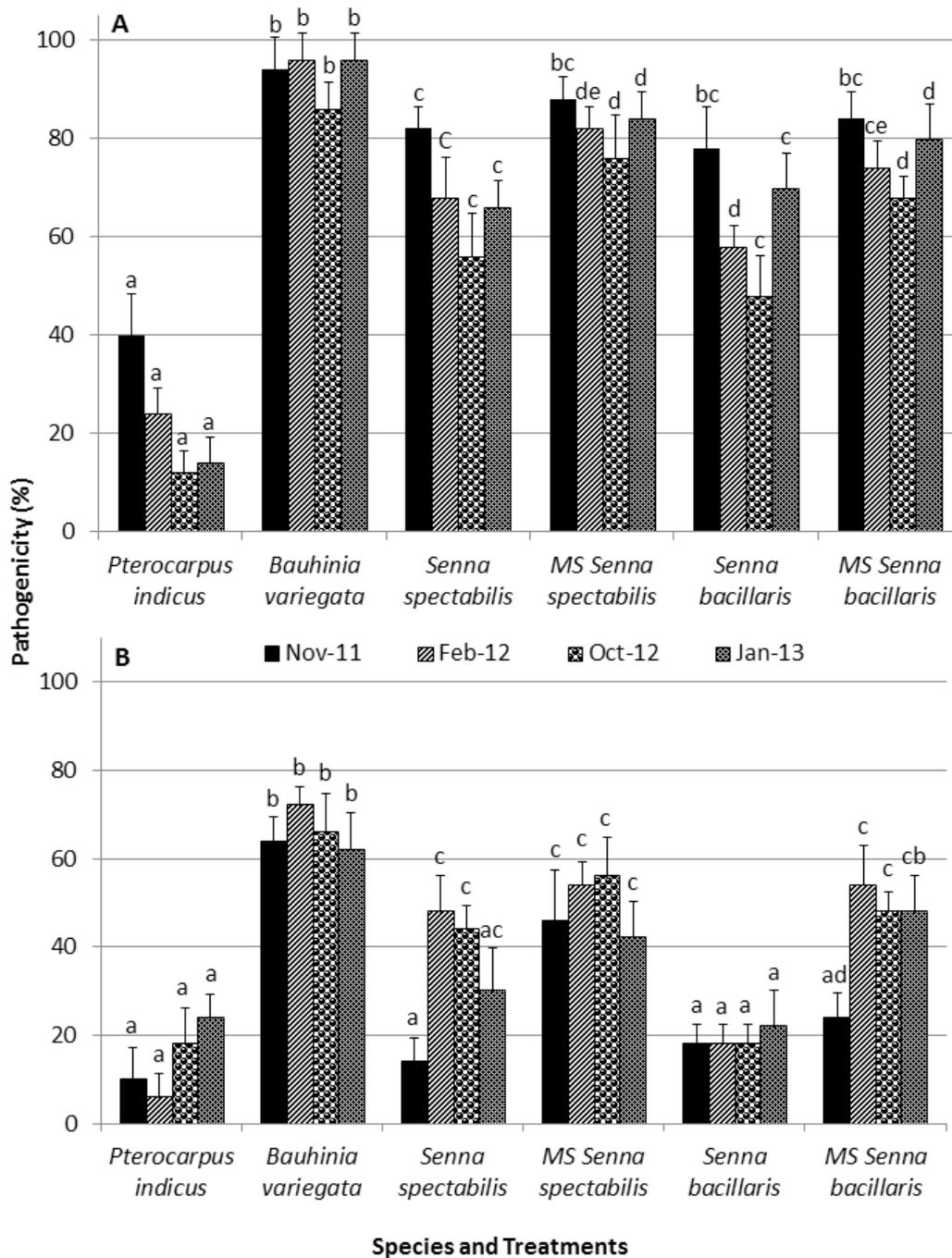


Figure 3: Pathogenicity of untreated *P. indicus*, *B. variegata*, *S. spectabilis* and *S. bacillaris* seeds and manually scarified *S. spectabilis* and *S. bacillaris* seeds kept in the grassland (A) and the woodland (B) of the Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka during different time periods. Different uppercase letters indicate significant differences between species and treatments within a single trial. Error bars are + SEM.

seeds had the highest rate of pathogenicity. When the data of the *Senna* species were analyzed separately, manually scarified seeds had significantly higher pathogenicity than untreated seeds ($p = 0.002$, $F = 11.01$) while there were no significant differences in pathogenicity among the two species ($p = 0.49$, $F = 0.49$).

Nutrient content of seeds

B. variegata seeds had the highest starch content while, *S. bacillaris* had the lowest (Table 2, $F = 14.09$, $P < 0.001$). *Senna bacillaris* seeds had the highest protein content while *S. spectabilis* had the lowest ($F = 178.07$, $P < 0.001$). Fat content was highest in *P. indicus* while the other three species have similar fat contents ($F = 154.6$, $P < 0.001$).

Release of volatile compounds by seeds

Bauhinia variegata seeds release higher amount of volatile compounds compared to other species (Table 3, $F = 60.61$, $P < 0.001$). Lowest amount of volatile compounds were released by the intact *S. bacillaris* seeds. Volatile

compounds released by manually scarified seeds of both *Senna* spp. were significantly higher than that of the intact seeds [for *S. bacillaris*, ($T = 5.09$, $P = 0.036$); for *S. spectabilis*, ($T = 5.75$, $P = 0.029$)].

Release of volatile compounds and predation

Binary logistic regression results revealed that there is a significant correlation between predation and the amount of volatile compounds released ($p < 0.001$, $G = 3240.4$). Low coefficient values obtained for amount of volatile compounds released by *P. indica*, untreated seeds and manually scarified seeds of *S. bacillaris* and untreated seeds of *S. spectabilis* (Table 4) indicated a low amount of volatile compounds released by them and have a comparatively low predatory rates, while high coefficient value for manually scarified seeds of *S. spectabilis* and seeds of *B. variegata* indicated a higher predation and higher amount of volatile compound release.

Table 2: Nutrient content (gg^{-1}) of seeds of the study species.

	<i>Pterocarpus indicus</i> (gg^{-1})	<i>Bauhinia variegata</i> (gg^{-1})	<i>Senna spectabilis</i> (gg^{-1})	<i>Senna bacillaris</i> (gg^{-1})
Starch	0.24 ± 0.01^{ab}	0.25 ± 0.01^a	0.23 ± 0.01^{bc}	0.21 ± 0.01^c
Protein	0.38 ± 0.02^b	0.43 ± 0.02^{bc}	0.05 ± 0.04^c	0.45 ± 0.03^a
Fat	0.40 ± 0.03^a	0.10 ± 0.02^b	0.10 ± 0.02^b	0.10 ± 0.02^b

Starch, ($F = 14.09$, $P < 0.001$); Protein, ($F = 178.07$, $P < 0.001$); Fat, ($F = 154.6$, $P < 0.001$)

Different lowercase letters indicate significant differences between species.

Table 3: Occurrence of the volatile compounds given by area under the peak.

<i>Pterocarpus indicus</i>	<i>Bauhinia variegata</i>	<i>Senna spectabilis</i>		<i>Senna bacillaris</i>	
		MS	UT	MS	UT
44.6 ± 3.3^a	481 ± 83.5^b	250 ± 46.3^c	93.3 ± 8.6^d	64.3 ± 19.6^d	5.3 ± 4.0^e

Different lowercase letters indicate significant differences between treatments ($F = 60.61$, $P < 0.001$)

Table 4: Results of the binary logistic regression (release of volatile compounds vs. predation).

Treatment and species	Predictor	Coefficient	SE of coefficient	Z	P	odds Ratio
	Constant	0.25	0.05	4.99	<0.001	
<i>Senna bacillaris</i> _UT	5.3	-3.51	0.14	-32.7	<0.001	0.07
<i>Pterocarpus indicus</i> _UT	44.6	-2.96	0.11	-25.75	<0.001	0.05
<i>Senna bacillaris</i> _MS	64	0.78	0.08	10.29	<0.001	2.19
<i>Senna spectabilis</i> _UT	93	0.15	0.07	2.15	0.032	1.17
<i>Senna spectabilis</i> _MS	250	1.75	0.09	19	<0.001	5.77
<i>Bauhinia variegata</i> _UT	481	1.29	0.08	15.68	<0.001	3.67

DISCUSSION

None of the *Pterocarpus indicus* seeds germinated during the 30 days of incubation period indicating that these seeds are dormant. However, both manually scarified and untreated seeds increase in mass > 90 %. Thus, seed coat of *P. indicus* is permeable. As the *P. indicus* seeds have fully developed embryos (data not shown), we conclude that seeds of *P. indicus* have physiological dormancy. Manually scarified *Senna spectabilis* and *S. bacillaris* seeds showed > than 90% germination even though only a significantly low percentage of untreated intact seed germinated, revealing that seeds of these two species show physical dormancy. Further, untreated intact seeds of the two *Senna* species imbibed significantly low amount of water while, manually scarified seeds imbibed > 120 %. Thus, it confirmed that seeds of two *Senna* species have physical dormancy. Observations on both untreated and manually scarified seeds of *Bauhinia variegata* (imbibed and germinated) revealed that they are non-dormant. These observations confirm the conclusions made by Jayasuriya *et al.* (2013), who reported that *P. indicus* seeds have physiological dormancy, *S. spectabilis* and *S. bacillaris* seeds have physical dormancy and *B. variegata* seeds have no-dormancy.

Physiologically dormant *P. indicus* seeds showed low predation in our experiments. Moreover, seeds of nondormant *B. variegata* and manually scarified seeds of *S. spectabilis* and *S. bacillaris* showed the highest predation in all experiments except in the 1st trial. Untreated (*i.e.*, physically dormant) seeds of *S. spectabilis* and *S. bacillaris* showed similar predatory percentages to that of manually scarified seeds in the first trial while in all the other trials they had moderate predation. It was observed that untreated seeds of the two *Senna* species imbibed during the 1st trial when they were at field conditions although the seeds had PY. It may be because that the PY seeds of these two species have relief dormancy under field conditions quicker than under laboratory conditions. Thus, high predation percentage among untreated seeds of *Senna* species may also be because of the dormancy relief of the PY species. However, the second trial was conducted with two-month stored seeds and it seems that dry storage have made them insensitive (*sensu* Jayasuriya *et al.*, 2009) to field conditions which relief dormancy of non-stored seeds. When the results of 2nd, 3rd and 4th trials were considered, it reveals that dormancy, is needed after condition more precisely the condition of the seed coat (water impermeability/permeability, hard/soft) is a significant factor that determines the predation of seeds. These results are in favor with the results of Paulsen *et al.* (2013). They found a higher predation rates by desert hamsters on manually scarified *Vicia sativa* and *Robinia pseudoacacia* seeds than that of untreated seeds of the same species.

A similar trend was observed in the seed pathogenicity experiment. A relatively low number of *P. indicus* seeds were attacked by pathogens, while a higher pathogenicity was observed in *B. variegata* seeds and manually scarified *S. spectabilis* and *S. bacillaris* seeds. These observations have

shown that the tested hypotheses are true *i.e.*, nondormant seeds are predated and attacked by pathogens compared to physically dormant seeds. Further, our results showed that the physiological dormant seeds *i.e.*, seeds of *P. indicus*, have lower predation and pathogenicity compared to seeds with physical dormancy or with no-dormancy. According to the seed defense hypothesis by Dalling *et al.* (2011), *P. indicus* seeds should have a chemical and physical defense continuum. Thus, depending on the species and their habitats, seeds show syndromes that enable them with varying degrees of defense ability against predators and pathogens. *Pterocarpus indicus* seeds with physical and chemical defense syndromes seem to be in a higher position in this continuum. In most cases, the relative effectiveness of chemical defenses against different classes of seed predators and pathogens has not been assessed. Phenolic compounds (Scalbert, 1991 and Picman *et al.*, 1995), anti-fungal proteins (Selitrennikoff, 2001) and alkaloids (Veldman *et al.*, 2007) in seeds considered to be effective against microbial infection. Consistent with an antimicrobial role, chemical defenses often are allocated primarily to seed coats rather than embryo or endosperm tissue (Berenbaum and Zangerl, 1986 and Suzuki and Waller, 1987), and also may be present in fruit tissue (Cipollini and Stiles, 1992 and Tewksbury *et al.*, 2008). Physical defense syndrome is most common among the species with 'hard seeds', a term synonymous with physical dormancy, where the seed is impermeable to water until germination commences. Thus, *S. spectabilis* and *S. bacillaris* seeds seem to have physical defense mechanisms. *B. variegata* seeds are non-dormant and according to Dalling *et al.* (2011), non-dormant seeds rely on fast germination and contain favourable microorganisms to escape from pathogens and predators.

Viable, asymptomatic seeds harbour pathogenic microbes that colonize internal tissues (Gallery, *et al.*, 2007; Clay and Schardl, 2002 and Schardl *et al.*, 2004) or in the seed surface (Kremer, 1986), thus seeds may not be infected from soil-borne pathogens. Sometimes microbes that colonize internal tissues of seeds can also be effective in preventing pathogen infections when seeds are incubated in soil (Kremer 1986). Seeds also could be infected by beneficial fungi after dispersal (Kirkpatrick and Bazzaz, 1979).

Nutrient content of seeds is one of the important factors that determine the amount of predator and pathogenic preferences on seeds (Janzen, 1969; Grubb *et al.*, 1998 and Moles *et al.*, 2003). It is assumed that the predators and pathogens prefer seeds with high nutrient contents. According to the result, out of the tested species *B. variegata* had a higher overall nutrient content (Table 2). *Senna spectabilis* seeds have the lowest nutritional value. In favour of the prior assumption, the highest predation and pathogenicity was observed with *B. variegata* seeds. Although the nutrient content of *P. indicus* is fairly high lowest predation and pathogenicity was reported in *P. indicus* seeds. Further, although *S. bacillaris* seeds have relatively high nutritional value, untreated seeds have been predated or attacked by pathogens in a lower rate. Thus, it seems that other factors rather than nutrition itself affecting

the predatory and pathogenicity rates at least in these two species. Predators and pathogens high preference on higher nutrient content is evident where manually scarified seeds of *S. bacillaris* were predated and attacked by pathogens in higher rate. Seeds of *S. spectabilis* have a low nutrient content but is predated and attacked by pathogens in a higher rate compared to *S. bacillaris* seeds.

The study revealed that *B. variegata* seeds release high amounts of volatile compounds when they are at the imbibed stage. Manually scarified seeds of the two physically dormant species also released a higher amount of volatile compounds than the untreated seeds. It seems that physical barriers in the physically dormant seeds prevent releasing of volatile compounds to the environment, indicating a main feature in physical defense. Several other researchers have also given a similar explanation (Johnson and Jorgensen, 1981; Vander-Wall, 1998; Jorgensen, 2001; Colville *et al.*, 2012; Paulsen *et al.*, 2013). Manually scarified seeds of *S. spectabilis* released a higher amount of volatiles than manually scarified seeds of *S. bacillaris*. Thus, predators may find *S. spectabilis* seeds more easily than *S. bacillaris* seeds and that may be the reason why manually scarified *S. spectabilis* seeds predated in a higher rate than manually scarified *S. bacillaris* seeds. Physical dormancy has already been suggested to play a defensive role, as rodents that rely on olfactory cues cannot detect buried seeds that remain impermeable (Vander-Wall 1998; Paulsen *et al.*, 2013).

As revealed in the experiments, *P. indicus* shows physiological dormancy. It has a hard covering which protect the nutrient rich embryo and endosperm. This hard covering seems to be acting as a barrier for releasing volatile compounds, thus predators cannot detect the seeds. As a result, the seeds of *P. indicus* show low predation. Seeds of *B. variegata* are non-dormant. The seed coat is not hard. Nutrient content is higher and the amount of volatile compounds released during imbibition is also high. Therefore, *B. variegata* seeds have a high rate of predation as well as high rate of pathogenicity. Seeds of both of the *Senna* species show physical dormancy. Thus, seeds do not imbibe and retain in the soil seed bank. As they stay in dry state in the soil seed bank they do not produce volatile compounds and escape from predation. However, when seed coat become permeable seeds imbibe and produce volatile compounds increasing the probability of exposure to predators. Thus, seeds need to germinate faster to escape from predators, which most of the PY seed do after relief from seed dormancy.

Same pattern of seed predation and pathogenicity were observed in the woodland and in the grassland. However, the amount of predation on all the species in the woodland was higher than that of the grassland. Two sites are about 100 m away from each and temperature between sites may not have differed. However, light intensity of the grassland is higher than that in the woodland (personal observations). Similarly, a higher amount of pathogenicity on all the tested species was observed in the woodland than in the grassland. This result was expected, as woodland creates a more favorable environment for seed predators and pathogens.

Woodland provide close environment for predators as even in the daytime seed predators can be active without any disturbances compared to more open grassland. Further, woodland is moist than the grassland and these conditions are very favorable for the microbial growth too. So the existence seed pathogens are also higher in the woodland. However, in two studies in moist tropical forest in Panama (Gallery *et al.*, 2007; Kluger *et al.*, 2008) have shown the same rates in both grassland and woodland pathogenicity.

CONCLUSION

The study can conclude that the non-dormant seeds have higher predation and pathogenicity compared to physically dormant seeds under moist environmental conditions. Further, physically dormant seeds made non-dormant have shown higher predation and pathogenicity than that of untreated seeds. Thus, our research reveals that the hypotheses made in the seed defense theory are true. This is perhaps the first study providing evidence to confirm the 'seed defense theory'.

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