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Short Communication
Germination and Growth of Selected Tropical Pioneers in Brunei Darussalam: Effects of Temperature and Seed Size

N.M.J. Manjul and F. Metali

Group of Environmental and Life Sciences, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, BE1410, Brunei Darussalam

Abstract
The effects of temperature and seed size on germination of tropical pioneer species have received little attention. This study aims to investigate the effects of temperature and seed size on germination of five common tropical pioneer species in Brunei Darussalam; the native *Melastoma malabathricum*, *Melastoma beccarianum*, *Rhodomyrtus tomentosa*, *Dillenia suffruticosa* and the invasive *Acacia mangium*. The species were grouped into small-seeded (seed mass ≤1 mg) and large-seeded (seed mass >1 mg) species and we determined the germination responses (number of days for seeds to start germination and reach 50% germination), final germination percentages and Relative Growth Rates (RGR) in different temperature using incubator chambers with a 12 h photoperiod. All species were treated with constant temperatures of 20, 25, 30, 35 and 40°C and alternating temperatures of 30/25, 35/25 and 40/25°C. Results demonstrated the possible influence of temperature on the germination of large-seeded *A. mangium* (13.86±1.17 mg) as revealed by its fastest time to germination and 50% germination across all temperature treatments, highest mean cumulative germination percentage (88±2.8%) and fastest RGR at constant temperature of 30°C. The abilities of *A. mangium* to have faster germination and higher RGR than other native plants are the possible traits that made non-native *A. mangium* invasive in the secondary and degraded forests of Brunei Darussalam. This study also shows potential effects of various environmental factors on seed germination that might contribute towards establishment and growth of pioneers in a habitat.

Key words: Germination, seed size, temperature, tropical pioneers, *Acacia mangium*

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Corresponding Author: F. Metali, Group of Environmental and Life Sciences, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, BE1410, Brunei Darussalam Tel: +6732463001

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In tropical rain forests, almost all tree regeneration originates from seeds (Raich, 1990). Production of seeds ensures exchanges of genetic material and preservation of plant populations and communities (Bradbeer, 1988; Leck *et al*., 1989). Seeds persist in the soils and becomes dormant as to delay seed germination especially when environmental conditions are not permissive (Baskin and Baskin, 2001). Even if the seeds transform into seedlings, their chances of survival and establishment will likely to be very low (Fenner and Thompson, 2005). Delaying germination helps to increase the probability of seedling growth and survival.

Temperatures (constant or alternating temperatures) are one of the factors that can alter the dormancy period of a seed (Fenner and Thompson, 2005) as they control seed germination by either influencing their germination rates or capabilities (Bewley and Black, 1994). According to Fenner and Thompson (2005), the stimulation of germination by alternating temperature is common for small-seeded species. The small-seeded seedlings are poor competitors and cannot emerge with an increase in soil depth, therefore, the alternating temperatures could be used by seeds to distinguish shade or gap in the forests. A study by Pearson *et al*. (2002) showed that the germination of small-seeded pioneers in alternating temperature was not significantly different from those in constant temperature. In contrast, the large-seeded species showed a positive germination response towards an increasing degree of temperature fluctuation (Pearson *et al*., 2002). The large temperature fluctuations assist the breakdown of hard, impermeable, waterproof testa of many tropical, subtropical and Mediterranean seeds (Bewley and Black, 1994; Probert, 2000).

The main aim of this study was to investigate the effects of temperature and seed size on germination of five common tropical pioneer species in Brunei Darussalam. It was hypothesized that germination percentages and rates of tropical pioneers with different seed sizes (large seeded-species and small-seeded species) differ in both constant and alternating temperature treatments.

MATERIALS AND METHODS

Study sites and species: Five common tropical pioneer species were used: the native *Melastoma malabathricum*, *Melastoma beccarianum*, *Rhodomyrtus tomentosa*, *Dillenia suffruticosa* and the invasive *Acacia mangium*. Species selection was based on the availability of ripe and mature fruits during the time of fruit collection. Mature fruits of each species were collected from the roadside areas and secondary forests in Brunei-Muara, Tutong and Belait districts (4°53.417`N 114°56.533`E). The seeds were extracted and stored in sealed plastic bags at room temperature until further use. Three subsamples of 100 seeds were weighted, following methods by Pérez- Fernandez *et al.* (2006). Seed mass of the three subsamples per species were averaged and the value was used as a mean seed mass of the species (Table 1).

Pre-germination treatments: Prior to the germination experiments, seeds with impermeable coats were treated. Various pre-germination treatments were as follows: (a) Soaking in 100°C water for 10 min for *Acacia mangium* seeds (following Bowen and Eusibio, 1981), (b) Soaking in 90°C water for 10 min for *Rhodomyrtus tomentosa* seeds and (c) Mechanical scarification using sandpaper for *Dillenia suffruticosa* seeds (following Besar, 1995).

The seeds were surface-sterilized with 5% solution of sodium hypochlorite (bleach) for 10 min. After sterilization, 25 seeds of each of the pioneer species were placed on sterilized filter papers wetted with distilled water in petri dishes. The petri dishes were sealed with parafilm to reduce water loss from evaporation.

Experimental procedure: Two temperature experiments were conducted in this study; constant and alternating temperature. In constant temperature experiments, there were five different temperature treatments as follows: 20, 25,30, 35 and 40°C. Four replicates of 25 seeds for each species were placed in plant growth incubator at a constant temperature for 24 h. The five temperature treatments could not be carried out at the same time due to limited number of growth incubators.

Table 1: Details of the five species of tropical pioneer species studied. All species are native pioneers to Brunei Darussalam, except for *Acacia mangium*, which is a non-native tropical pioneer species

Species	Family	Seed mass (mg)	Method of seed dispersal
Melastoma malabathricum L.	Melastomataceae	0.02±0.003	Animal
Melastoma beccarianum Cogn.	Melastomataceae	0.03±0.01	Animal
Acacia mangium Wild.	Fabaceae	13.86±1.17	Animal
Dillenia suffruticosa (Griff.) Martelli	Dilleniaceae	9.12±0.05	Animal
Rhodomyrtus tomentosa (Aiton) Hassk.	Myrtaceae	2.63±0.14	Animal

Similarly in alternating temperature experiments, four replicates of 25 seeds of each species were placed in plant growth incubators at the following alternating temperature treatments: 30/25°C, 35/25°C and 40/25°C over 12-h day (high temperature) and 12-h dark (low temperature) periods.

In both experiments, 12 h light period was supplied by fluorescent lamps with an irradiance of about 32.3±2.7 μM m^{-2} sec^{-1}. Irradiance was measured using photosynthetically active radiation sensors (LI-250, LI-COR Inc., Nebraska, USA). The seeds were moistened with 2 mL distilled water for every two days. Seed germination was scored and recorded daily until the end of the experiment (30 days for each temperature treatment). Radicle emergence of at least 1 mm was used as the criterion for seed germination (Jankowska-Blaszczuk and Daws, 2007). The germinated seeds were removed from the petri dishes to avoid confusion in scoring germination.

Relative growth rates of seedlings: During the constant temperature experiments, there was an apparent different in the early seedling growth rates between the native and introduced pioneer species. In a separate study, two replicates of 25 seeds of one native (*Melastoma malabathricum*) and one non-native (*Acacia mangium*) species were placed in a plant growth incubator at a constant temperature of 30°C. For each species, 10 seeds that had germinated (with radicle length of at least 1 mm) at day 1 and 10 after the start of the experiment were randomly chosen. Elongation lengths (hypocotyl and radicle) of the chosen germinated seeds at day 1 (initial measurements) and day 10 (final measurements) were measured using a piece of string and a ruler. Relative growth rate (RGR, mm mm^{-1} d^{-1}) of elongation length over a period of time was calculated (Hunt, 1982):

$$RGR = (\log_e EL_2 - \log_e EL_1)/(t_2 - t_1)$$

where, EL_2 and EL_1 are final and initial mean elongation lengths (mm), respectively and t_2-t_1 was 10 days.

Data analyses: The mean final germination percentage (±standard error of mean, SE) was calculated for each treatment per species. Final percentage germination was relatively expressed to initial seed numbers used in the experiment. Prior to analysis, germination percentages were arcsine-square root transformed, while relative growth rates were $\log_{10}$-transformed if residuals were not normally distributed after fitting models to the untransformed data. The germination percentages between treatments within a species and between species within a treatment were analyzed using a one-way analysis of variance (ANOVA) and Tukey's honest significant difference (Tukey's HSD) tests. The relative growth rates (RGRs) of elongation length between species were analyzed using independent samples t-test. Non-parametric data were analyzed using Mann-Whitney-Wilcoxon test. However, for convenience in interpretation, untransformed data appear in all tables and figures. All statistical analyses were conducted using R version 3.1.2 (R Development Core Team, 2014).

RESULTS

Germination responses to constant temperature: In all the temperature treatments, *Acacia mangium* (seed mass>1 mg) started to germinate on day 5 (day 4.8±1.0). *Melastoma malabathricum* and *M. becarrianum*, whose seed masses ≤1 mg, germinated later than *A. mangium*, which is between day 13 (12.9±2.6) and day 17 (16.8±3.3), respectively. The seeds of *Rhodomyrtus tomentosa* germinated between day 19 (18.5±6.2), while none of *Dillenia suffruticosa* seeds germinated in all temperature treatments.

There were significant differences in the mean final germination percentages among species ($p<0.001$) and constant temperature treatments ($p<0.001$) and interactions between them ($p<0.001$). Only seeds of *M. malabathricum*, *M. beccarianum* and *A. mangium* germinated across all constant temperature treatments (Fig. 1). Among the five pioneer species at 25 and 30°C *A. mangium*, whose mean seed mass >1 mg, had the highest mean final germination percentages (ranges from 57±13.3-88±2.8%) compared to other species ($p<0.05$, Fig. 1b and c). The mean germination percentage in *A. mangium* was the highest at temperature treatment of 30°C with 88±2.8% (Fig. 1c). Both *A. mangium* and *M. malabathricum* seeds had significantly similar mean germination percentages at 20 and 40°C ($p<0.05$, Fig. 1a and d). Both *M. malabathricum* and *M. beccarianum*, whose mean seed masses ≤1 mg, germinated in all constant temperature treatments except at 35°C (Fig. 1). The mean final germination percentages of *M. malabathricum* were significantly higher than that of *M. beccarianum* at 20°C (34% vs. 1%, respectively) and 40°C (87 vs. 58%, respectively) ($p<0.05$, Fig. 1a and d). *Rhodomyrtus tomentosa* only germinated at temperature treatment of 25°C. The seeds of *D. suffruticosa* did not germinate across all temperature

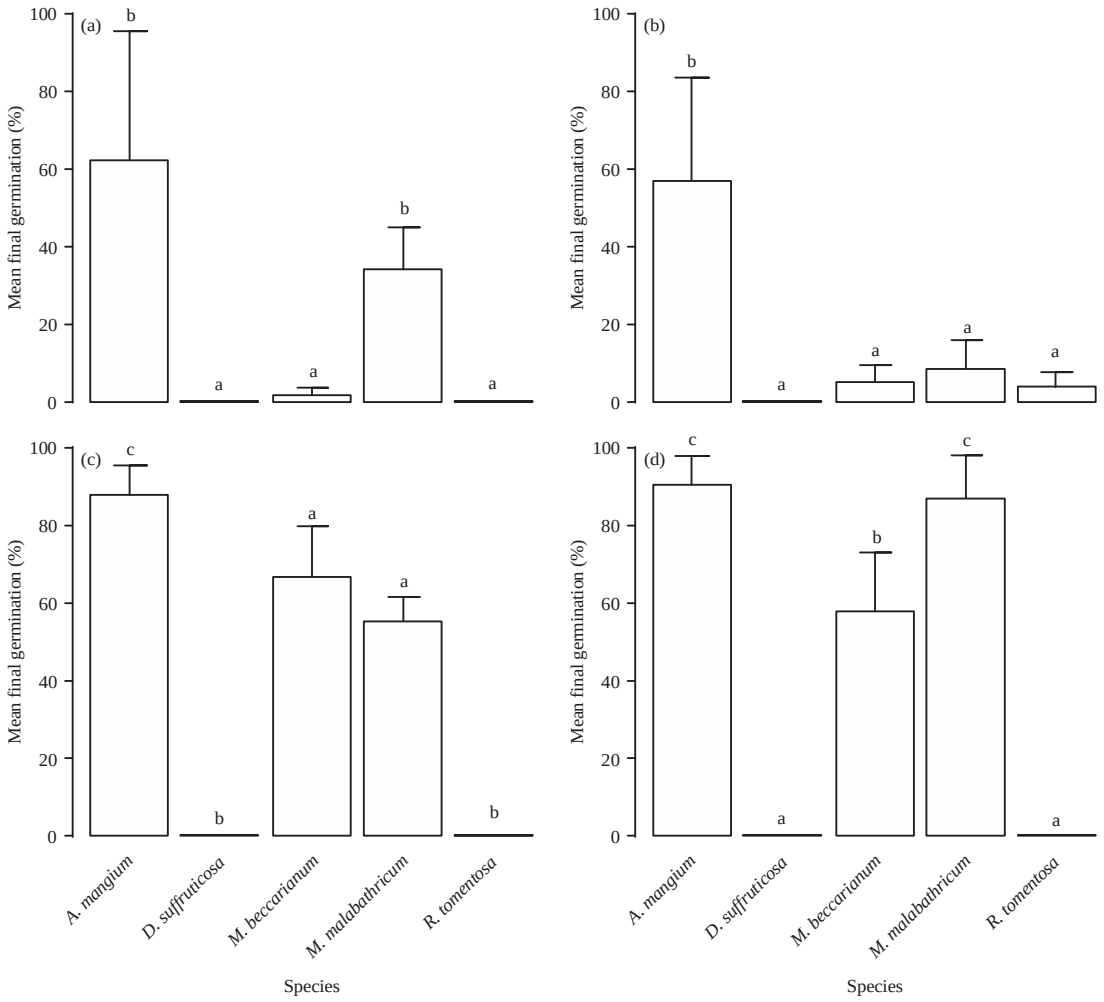

Fig. 1(a-c): Mean final germination percentages (±standard error of mean, SE) of *Acacia mangium*, *Dillenia suffruticosa*, *Melastoma beccarianum*, *Melastoma malabathricum* and *Rhodomyrtus tomentosa* at different constant temperature treatments, (a) 20°C, (b) 25°C, (c) 30°C and (d) 40°C. Each bar represents mean final germination percentage of four replicates of 25 seeds. *Melastoma beccarianum* and *Melastoma malabathricum* have mean seed mass ≤1 mg, while *A. mangium*, *D. suffruticosa* and *R. tomentosa* have mean seed mass >1 mg. The different letters within a temperature treatment indicates significant differences among the species mean values ($p<0.05$). Data for temperature treatment of 35°C were not shown here because none of the seeds germinated over 30 days

treatments. None of the species germinated at 35°C, so the data were not presented and were excluded from analysis.

Germination responses to alternating temperature: Since none of *M. malabathricum*, *M. beccarianum* and *D. suffruticosa* seeds germinated in this experiment, a two-way analysis of variance was not conducted. Similar to constant temperature experiments, only *A. mangium* germinated in all three alternating temperature treatments. Its mean final germination percentage was highest in fluctuating temperature of 30/25°C (60±10.9%) and lowest in 40/25°C (29±4.1%) but these differences were not significant when tested with ANOVA. The *R. tomentosa* only germinated at fluctuating temperature of 30/25°C with mean final germination percentage of 6±2.6%.

DISCUSSION

Germination responses to environmental factors: As predicted, our results showed that as temperature increases from 20-40°C, increased germination percentages and fast germination for all studied species (except *Dillenia suffruticosa*

in which none of the seeds germinated) were observed. The effect of temperature on rate of germination can be treated mathematically and similarly as an enzyme reaction. Temperature controls the rate of enzyme reaction during seed germination (Bewley and Black, 1994) and thus, increased in temperature leads to increase in rate of germination.

Previous studies (Thompson and Grime, 1983; Ekstam and Forseby, 1999) showed that alternating temperature is more favourable for seed germination than constant temperature on the basis that seeds are exposed to alternating temperature and not constant temperatures in natural habitats. Alternating temperature helps the seeds to recognize the presence of canopy gaps, especially for seeds found near the soil surface. Therefore, it is expected that germination percentage of the studied species is higher in alternating than that in constant temperature treatments. It is also expected that large-seeded species (>1 mg) germinates better in alternating temperatures than constant temperatures because the former may assist in the breakdown of their large and probably hard seed-coats (Bewley and Black, 1994; Probert, 2000).

However, alternating temperature treatments conducted in our study did not show any stimulative effects on germination when compared to constant temperature treatments. Only *A. mangium* germinated in our alternating temperature experiment but its highest germination percentage was low (60±10.9%), when compared to that in constant temperature treatments (for e.g., 88±2.8% at 30°C). Pearson *et al.* (2002) reported that germination responses to increasing degree of alternating temperature was negative in the smaller seeded species (<2 mg) such as *Piper peltatum*, *Piper dilatatum* and *Cecropia insignis*. This might offer an explanation as to why none of the seeds with mass <2 mg germinated in the alternating temperature treatments in this study.

Another possible explanation for the inhibitory effects of alternating temperature treatments is due to the innate condition of the seed lots used in this study. In this study, we have used seeds that were stored for 6 months at room temperature of 25-28°C. Olivier (2009) pointed out in his study that storage temperature significantly affected the number of seeds germinated. He has reported that commercial big bluestem (*Andropogon gerardii*) seeds that are stored at an average room temperature (25°C) for 120 days had lower germination percentages than low storage temperature (<25°C). Long storage period at room temperature (25°C) in this study may cause most of stored seeds (especially the small-seeded species) to lose their viability. Length of seed storage might also explain the zero germination percentages of small-seeded *Melastoma* species at constant temperature treatment of 35°C.

To confirm assumption that long storage period at room temperature affects the germination performance of small seeds (particularly, Melastoma seeds) and cause the seeds to desiccate and loss their viability, we conducted a Tetrazolium (TTZ) test on the seeds at the end of germination experiments. Although most of the seeds were stained pink, which indicated that they are viable, it was difficult to distinguish if the pink tissues were the embryos. As Melastoma seeds have very small sizes, it was difficult to assess the TTZ test, even with a microscope. We also conducted another experiment, in which we collected *M. malabathricum* seeds from fresh-opened fruits and germinated them at room temperature (about 30°C) with the seed lots that were 6 months earlier. After a week, it was found that few seeds from fresh collected seed lot but none from old seed lot had germinated. This finding further supports the possibility of long stored seeds losing their viability.

CONCLUSION

This study demonstrates that temperature plays a significant role in stimulating germination of pioneer species. There were differences in germination percentages of studied species between constant and alternating temperature. In addition to the fastest time to germination across all temperature treatments for the large-seeded *A. mangium* (13.86±1.17 mg), it had the highest mean cumulative germination percentage in constant (88±2.8% at 30°C) than in alternating temperature (60±10.9% at 30/25°C) treatment. However, due to limited data, the differential effect of constant and alternating temperature treatments between other large seeded and small seeded species was inconclusive. However, it was clearly presented that *A. mangium* outcompetes other native species in terms of final germination percentages, germination rates and relative growth rates. These traits might contribute to its successfulness in becoming an invasive species in Brunei Darussalam. In conclusion, it is essential to understand how different environmental factors affect seed germination for us to fully comprehend the establishment and growth of pioneers.

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