HIGHLAND SPECIES AND TEMPERATURE REQUIREMENT FOR GERMINATION: A CASE FROM TWO ENDEMIC PAPUAN *Pittosporum* (PITTOSPORACEAE) SPECIES

Spesies Dataran Tinggi dan Suhu yang Diperlukan Untuk Perkecambahan: Studi Dua Jenis *Pittosporum* (PITTOSPORACEAE) Endemik Papua

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Abstrak

Perubahan iklim, termasuk suhu yang semakin panas dan kekeringan merupakan kendala utama bagi regenerasi tumbuhan. Percobaan tentang pengaruh suhu terhadap regenerasi tumbuhan dilakukan pada dua jenis endemik pegunungan tengah Papua yaitu *Pittosporum pullifolium* dan *P. spicessens*. Suhu malam hari di habitat *P. pullifolium* adalah sekitar 8°C. Di habitat *P. spicessens*, suhu malam hari adalah sekitar 19°C, dan pada siang hari dapat mencapai 26°C. Percobaan pertama dilakukan untuk memahami pengaruh cekaman dingin terhadap perkecambahan *P. pullifolium.* Hasil penelitian ini menunjukkan bahwa *P. pullifolium* bergantung pada stratifikasi dingin untuk perkecambahannya. Perkecambahan tidak dapat berlangsung tanpa stratifikasi dingin walaupun suhu perkecambahan relatif rendah, yakni antara 13–26°C (di Kebun Raya Cibodas). Perkecambahan *P. pullifolium* berlangsung setelah dilakukan stratifikasi dingin pada suhu 6–8°C secara konstan selama satu bulan. Persentase perkecambahan biji yang mengalami stratifikasi dingin dengan cahaya lebih tinggi dibandingkan dengan biji yang diberi perlakuan stratifikasi dingin tanpa cahaya. Selanjutnya, masa stratifikasi dingin diperpanjang selama satu bulan lagi, dan yang menarik, perkecambahan *P. pullifolium* tetap berlangsung namun kali ini persentase perkecambahan yang lebih baik terjadi pada stratifikasi dingin dengan kondisi gelap. Perkecambahan *P. spicessens* baik pada rentang suhu di habitatnya (Wamena) maupun di suhu yang lebih hangat (Kebun Raya Bogor), berlangsung setelah lebih dari dua minggu penyemaian.

Kata kunci: ekologi biji, perkecambahan biji tumbuhan alpina, *Pittosporum pullifolium, Pittosporum spicessens*, stratifikasi dingin

Abstract

Climate change, including warming and drying, is currently the biggest challenge for plant regeneration. We conducted two experiments on how temperature affected the germination of *Pittosporum pullifolium* and *P. spicessens*, both endemic to Central Papua highlands. *P. pullifolium* habitat temperature at night could reach 8°C whereas *P. spicessens* habitat temperature ranged from 19°C early in the morning up to 26°C at midday. The first experiment was to understand the effect of chilling on *P. pullifolium* germination initiation. Our study showed that *P. pullifolium* was dependent on cold stratification for its germination. Without cold stratification the germination was absent even though the temperature range of sowing environment is at ca. 13–26°C (Cibodas Botanic Gardens). With a cold stratification at 6–8°C (constant) for more than a month, germination of *P. pullifolium* occurred, with better germination rate under a light. Subsequently we carried out extended cold stratification for a month and interestingly, the germination still occurred but now it is better under dark condition. For *P. spicessens*, the germination at its habitat temperature range (Wamena) and in the warmer environment (Bogor Botanic Gardens), both occurred at more than two weeks after sowing.

Keywords: alpine seed germination, cold stratification, *Pittosporum pullifolium, Pittosporum spicessens*, seed ecology

INTRODUCTION

Seed germination is a critical stage in the life of plants, particularly when considering the effects of global warming on high–altitude species due to the dependence of these species on specific temperature regimes to stimulate germination and ensure seedling development coincides with favourable growing conditions (Mondoni *et al.*, 2008; Milbau *et al.*, 2009; Martyn *et al.*, 2011). Understanding germination response to temperature has the potential to improve models of species response to climate change (Mondoni *et al.*, 2009; Milbau *et al.*, 2009; Ooi *et al.*, 2009; Martyn *et al.*, 2011), particularly as a large proportion of species regenerate from seed (Venn and Morgan, 2009). Information about germination can also improve the success rate of using the seed for rehabilitation (Kaye 1997; Giménez–Benavides *et al.*, 2005), which is critical for restoration (Martyn *et al.*, 2011).

The distribution and general ecology of Papuan highland species are increasingly studied, but little is known about the seed biology of individual shrubs. It is not known, for example, which species require low temperatures and/or cold stratification and dormancy break, and whether these requirements would be fulfilled in a warmer climate.

Dormancy in seeds of alpine and sub–alpine plants may be considered an adaptation to the extreme environment where controlled germination can be vital for seedling survival (Kaye, 1997). In this study, we investigated temperature requirement for germination and response to dormancy alleviating treatments of cold stratification for two *Pittosporum* species endemic to central highlands of Papua.

METHODS

Study Species

Two endemic Papuan species, *Pittosporum pullifolium* Burkill and *P. spicessens* Utteridge (Pittosporacea), were chosen as study species. The first species, *P. pullifolium* generally grows in the margin of alpine shrubbery (Johns *et al.*, 2006), in a form of a shrub or small tree reaching a height of 12 m. The inflorescence is terminal, racemose (Utteridge, 2000). *P. spicessens*is is a shrub or small tree to 5 m. The inflorescense is axillary, cymose. Fruits obovoid (9.5 -) 12–18 × 2.3–3 mm, yellow–orange, seeds c. 10–17, dark reddish–brown, 3.5–5 mm long, compressed and irregularly reniform (Utteridge, 2000).

Germination experiments

Two separate germination experiments were conducted at different times for *P. pullifolium* and *P. spicessens*. Specifically, for *P. pullifolium*, we addressed the following questions: (i) does cold stratification facilitate dormancy alleviation in this species, (ii) does light promote dormancy alleviation during cold stratification period. Subsequently, for *P. spicessens*, the research question addressed was whether temperature requirement for germination is similar to the developing (maternal) temperature. We intended to also carry out cold stratification treatment for *P. spicessens* but due to seed number limitation this was not done.

Pittosporum pullifolium **germination**

Preliminary experiment

The seeds were collected in June 2013 from Helalimo District, Mt Jaya (within the territory of Lorentz National Park) at an elevation of 3300 m. Fresh seeds (c. 10 fruits or equal to 200 seeds) were then sown in Wamena, Papua at an elevation of 1700 m, and daily temperature ranging from 19–26°C. Subsequently, seeds were transported to West Java in intact fruit or infructescense to eliminate seed desiccation and viability loss. Another 200 seed–lot was also sown in Cibodas–West Java 20 days after the first sowing in Wamena. Cibodas is situated at 1300 m asl with temperature ranges from 13 to 26°C (first half of the year). This experiment was adopted from the methodology of a *common garden experiment* (Molles, 2002). In both study sites, seeds were sown on top soil and rice husk ash. Seeds did not germinate until five months after sowing, but the embryo was still viable. Therefore, we assumed that *P. pullifolium* seeds were dormant.

Experiment set–up

As the information of seed biology, in particular for Papua alpine and subalpine plants are absent, we suspect that chilling may play a role in dormancy alleviation in tropical alpine species as also found for other temperate or continental alpine species. Hence, cold stratification (hereafter referred as CS) at 6–8 (± 2)°C were chosen for alleviating dormancy. Besides CS, light treatment was also applied. Half of treatment used was completely covered with black plastic and aluminium foil and thus, seeds in this part were complete without light exposure. In the field, germination likely occurs on natural *Sphagnum* cushions (pers. obs.), and therefore, *Sphagnum* moss was chosen as germination substrate. CS was done for two periods, one and two months. Three replicates were used for each combination of treatments, each contained 30 seeds. We had 2 CS × 2 light conditions × 3 containers × 30 seeds each, leading to 360 seeds involved in this experiment. Light conditions employed were either complete darkness or occasional light, mimicking light exposure to seeds in its natural habitat on *Sphagnum* cushions. After the CS period completed the seeds were brought to room temperature and germination was subsequently recorded.

Pittosporum spicessens **germination**

We carried out a common garden experiment similar to the *P. pullifolium* germination to observe the influence of temperature (habitat or maternal vs *ex–situ* environment) for its germination.

Experiment set–up

In order to reduce the desiccation that might reduce the viability, seeds were still in intact fruit (infructescence) when transferred from Wamena to Bogor. Thirty fresh seeds of *P. spicessens* were sown on *Sphagnum* moss substrate in Wamena and within subsequent two weeks 90 seeds were sown in Bogor Botanic Gardens on rice husk ash. In Wamena, seeds were germinated under semi–open shade house and in Bogor germination was carried out in a closed glass house. Photoperiods in both sites were not modified, and thus, seeds were exposed to a slightly different day length. Germination rate and average final shoot length were measured in the two sites.

Data analysis

The data from *P. pullifolium* experiment was analyzed using a non–parametric Kruskall – Wallis test because the requisites for univariate analysis could not be met. As for *P. spicessens* a more qualitative observation was employed due to the number of available seeds and lack of replicates.

RESULTS AND DISCUSSIONS

Pittosporum pullifolium germination

CS compulsorily needed for *P. pullifolium* to germinate. However, the CS period did not affect the final germination percentage (χ^2 = 1.095, p = 0,295, Kruskall–Wallis test). For a shorter period of CS, germination was better under light and vice versa for extended CS (Figure 1).

The germination of *P. pullifolium* under shorter CS first occurred about three weeks after they are transferred from CS temperature to room temperature (27–29°C). Under an extended CS, germination was earlier about two weeks after transfer. *P. pullifolium* germination was epigeal, sometimes with the seed coat still intact for more than three weeks limiting the phanerocotylar cotyledons to fully open. The eophylls were produced more than three weeks after cotyledons fully opened (Figure 2).

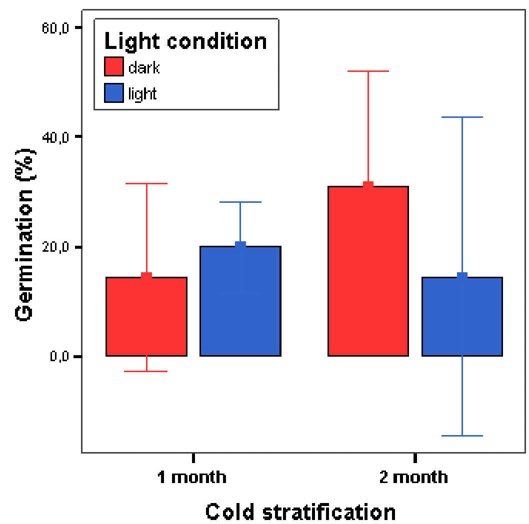

Figure 1. Germination percentage of *Pittosporum pullifolium* at different period of Cold Stratification and light.

Figure 2. Germination phase in *Pittosporum pullifolium*, from the elongation of radicle and emergence of cotyledon (left), the opening of photosynthetic cotyledonary leaves (middle), and eophyll flushes (right).

Pittosporum spicessens germination

Germination of *P. spicessens* was slightly faster for seeds sown in the natural environment in Wamena than those sown in Bogor. Germination percentage was also higher when seeds exposed under its optimum temperature range in Wamena Figure 3). However, the seedling vigor was more uniform and eophyll production was slightly more abundant for seeds germinated in Bogor (Table 1). There was a difference in the shoot length and eophylls produced by seedlings grown in both sites. In Bogor, seedling shoots, i.e. the length between cotyledon and eophyll axis, were relatively short

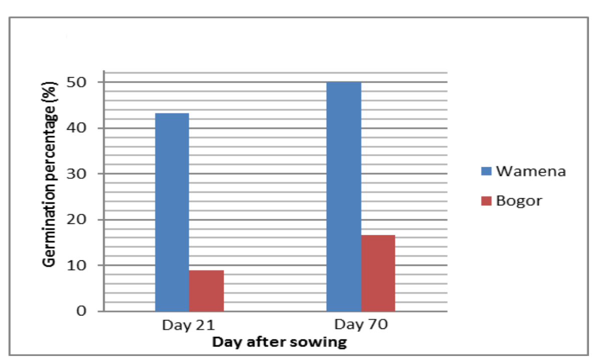

Figure 3. Germination percentage of *Pittosporum spicessence* sown in Wamena, where maternal/origin site and germination/

sowing temperature are very similar, and in Bogor, its non–native range.

Table 1. The comparison of germination start and seedling vigor of *Pittosporum spicessens* endemic Papuan species, sown under in situ maternal temperature (Wamena) and ex situ temperature (Bogor)

Pittosporum spicessens	**Wamena (colder temperature, lower RH)**	**Bogor (warmer temperature, higher RH)**
Germination started	19 days	21 days
Seedling length (2.5 months)	2.5–5.5 cm	4.1–5 cm
Eophyll	2 leaves	2–4 leaves

Figure 4. *Pittosporum spicessens* seedlings, 70 days after sowing, in Wamena with a slightly more slender profile (left) and those grown in Bogor (right).

compared to the shoots produced by seedlings in Wamena (Figure 4). This was potentially caused by the intensity of light and relative humidity in the shade house they experienced, that was higher for seedlings in Bogor.

Pittosporum pullifolium dormancy alleviation by Cold Stratification

Apparently *P. pullifolium* seeds possess non–deep physiological dormancy and cold stratification at 6–8°C was useful for alleviating the dormancy. An effective CS temperature was reported to be 0–10°C with 5°C to be optimum for most species. CS was also known to be effective for non–deep physiologically dormant seeds (Baskin and Baskin, 2001). We included only one population of *P. pullifolium* seeds in this study. Even though it was apparent that *P. pullifolium* experienced seed dormancy, types of dormancy and the percentages of seeds that are dormant in any species may vary from population to population, year to year and even season to season (Baskin and Baskin, 2000; Giménez–Benavides *et al.*, 2005; Mondoni *et al.*, 2008). Thus, the further experiment that includes seed collection from different populations, years, and seasons may be necessary to be done.

Climate change and alpine seed ecology of *Pittosporum pullifolium*

With the changing climate, seed germination of alpine species may face a bottleneck (Walck *et al.*, 2011) and may also change floristic composition in this ecosystem (Pickering and Green, 2009; Martyn *et al.*, 2011). In its natural habitat, *P. pullifolium* seeds may be dispersed and sit on a *Sphagnum*, litter or soil surface. This is a crucial step for regeneration, as soil temperatures are the main determinant of alpine vegetation (Martyn *et al.*, 2011).

Global warming projections forecast changes in temperature and precipitations (IPCC, 2007). On the other hands, temperature and water supply from precipitations also drive seed dormancy (initiation, break) and germination (Walck *et al.,* 2011). Alpine seed dormancy may have evolved from the temporally unpredictable environment, and germination in these areas can be limited to soil water availability and the period of precipitation (Kaye, 1997). In Mt. Jaya alpine regions, the occurrence of drought were increasingly more frequent recently and natural fires, a phenomenon rarely can be seen in alpine regions, sometimes occurs. Another experiment to investigate the water limitation to seedling growth and subsequently plant competitive ability for Papua alpine regions may be able to help us to predict the impact of climate change to this unique ecosystems, as well as to plan a restoration programme.

Understanding the germination response to temperature has the potential to improve models of species response to pressures such as climate change and improve seed utilization for rehabilitation, as well as clarify seed germination requirements for conservation seed banks and restoration (Martyn *et al.*, 2011). The results from this study support recommendations for shallow or surface planting of seeds to satisfy light requirements and to improve chances for natural stratification and scarification (Kaye, 1997).

***Pittosporum spicessens* temperature requirement for germination, and climate change**

P. spicessens seeds, unlike its congeneric *P. pullifolium*, have no dormancy. They germinate at 19 and 21 days after sowing in both sites. It was apparent, however, that seeds responded to specific temperature cues to germinate. *P. spicessens* seeds germination rate was higher under temperature they experience while they were still attached to their mother trees.

Temperature and water stress imposed on mother plants influence the phenotypic expression of seed mass, seed longevity, seed dormancy, seed germination percentage and rates and early growth (Kochanek *et al.*, 2010, Walck *et al.,* 2011). Thus, parental environments can facilitate the evolutionary divergence of life history patterns among plant populations (Walck *et al.*, 2011). Our finding with germination requirement of alpine *P. spicessens* is relevant to this. Plants tend to have its optimal germination temperature close or similar to its maternal condition in order to ensure its offspring are able to survive in a suitable environment, in this case temperature.

Climate change will likely affect population dynamics by altering several aspect of plant life history. The sexual reproductive phase may be particularly vulnerable to climate change (Adler and HilleRisLambers, 2008; Hedhly *et al.*, 2009).

CONCLUSIONS

The seed of *Pittosporum pullifolium* was physiologically dormant and its close relative *P. spicessens* seeds possess no dormancy. Cold stratification for at least one month was able in the facilitation of seed dormancy alleviation in *P. pullifolium*. Seed exposure to light during this period may affect the germination rate as well. Moreover, the temperature was found to be a key factor in the germination of *Pittosporum*. *P. spicessens* germination was higher under similar or same temperature regime of their developing time on their mothers.

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