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Research Note

Changes in the moisture and germination of recalcitrant *Hopea mollissima* seeds (Dipterocarpaceae) in different desiccation regimes

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Summary

This paper studied the changes in moisture and germination of whole seeds of *Hopea mollissima* using graded temperatures and relative humidity to mimic changes in the microclimate in rainforest fragments. *H. mollissima* seeds were highly sensitive to high temperatures and dehydration, with storage at temperatures $\geq$ 30°C or relative humidity $\leq$ 60% significantly decreasing the germination percentage. An increase in the surrounding temperature and decrease in relative humidity prevented *H. mollissima* seeds from germinating during storage, accelerated moisture and germination loss, and shortened seed lifespan. The results are discussed in relation to the potential for the fragmentation and disturbance of native habitats resulting in difficulties in seed germination and seedling establishment for this species in tropical rainforests.

Experimental and discussion

Hopea mollissima (Dipterocarpaceae), native to China, is listed as a rare and endangered species. A previous study (Wen *et al.*, 2009) has found that this species produces recalcitrant seeds (Roberts, 1973). *Hopea mollissima* grows only in the *Dipterocarpus tonkinensis* and *Hopea mollissima* forest, its native habitat in South Yunnan, China. South China is located on the boundary of the tropics where Dipterocarp forests are restricted to the moist valleys. These forests are now being seriously fragmented and disturbed. This paper tested the hypothesis that changes in the environment might influence seed germination and seedling establishment and investigated the changes in moisture and germination of *H. mollissima* seeds under different temperature and relative humidity regimes.

Mature fruits of *Hopea mollissima* C.Y. Wu were shaken off trees growing in Xishuangbanna Tropical Garden (for climate see Liu *et al.*, 2004). According to a previous report (Sasaki, 1980), the wing colour of Dipterocarp fruits is a good indicator of maturity; mature fruits have wings dried down to their base with distinct dark stain demarcation.

After collection, fruits were selected, dewinged (thereafter dewinged *Hopea* fruits are referred to as seeds throughout this paper), and kept in polyethylene bags at 15°C until used. Only mature fruits were used in the following experiments and all experiments were set up within 3 d after seed collection.

To investigate the influence of temperature on seed moisture and germination, incubators were used to provide various drying regimes, as used by Pritchard *et al.* (1995). Seeds stored in small cloth bags were placed in incubators set at a range of temperatures between 10-40°C and sampled for moisture and germination determination every second day at each temperature. The relative humidity recorded in these incubators ranged from 56% to 78%.

The influence of relative humidity (RH) on moisture and germination was examined by placing seeds at 11%, 30%, 58%, 79% and 100% RH, regulated by saturated solutions of LiCl, $CaCl_2$, $BaBr_2$, NH_4Cl and distilled water respectively, in desiccators at 20°C in a temperature-controlled room. Seeds were laid in a monolayer in these desiccators and sampled for moisture and germination determination once a week.

Moisture contents, expressed on a fresh weight basis, were determined gravimetrically on five replicates of single seeds before and after drying for 17 ± 1 h in a ventilated oven at 103 ± 2°C, according to the recommendations for oily seeds (International Seed Testing Association, 2006).

For germination assessment, seeds were sown on 1% distilled water agar in Petri dishes and placed at 25°C in temperature-controlled incubators, with 14 h light (20 μmol/ $m^{2} \cdot s$)/10 h dark per day provided by white fluorescent tubes. Five Petri dishes containing 10 seeds each were used for each treatment. They were monitored every second day and seeds with at least 5 mm long radicle protrusion were considered as germinated. According to a previous experiment (Wen *et al.*, 2009), no more seeds germinated after 10 d following sowing under this condition, so all germination trials continued to the 15th day at least. On the termination of the germination test, shoot and root length of seedlings were measured and the ungerminated seeds were carefully cut open to check if the embryos were healthy. Seed germination percentage and G · S value (a measure of vigour) were calculated.

G · S value = germination percentage × (root length + shoot length)

Seed Moisture Loss Rate Index defined by Samarah *et al.* (2009) was used to quantity the drying rate under different regimes. Meanwhile days to 50% germination (T50) were calculated by probit analysis.

The seeds used in this study had a moisture content of 41.40 ± 0.97%, a 1000-seed weight of 1,522.69 ± 33.75 g and an initial germination level of 96 ± 1.7%, while a moisture content of 40.22 ± 0.88% and a 1000-individual weight of 1,734.31 ± 42.12 g were recorded for intact fruits. The cotyledon is green and the embryonic axes pink inside viable seeds.

Temperature influenced seed moisture and germination loss significantly. Generally, the higher the surrounding temperature, the more rapidly the seeds lost moisture and germination (figure 1). Seeds at 10°C retained the highest moisture and germination for the longest duration among all temperatures investigated. Seeds at 20°C and 25°C lost moisture and germination more quickly than those at 10°C and 15°C, whilst at 30°C and 35°C seeds germination fell markedly to < 20% after 5 d storage. High temperature of 40°C was lethal and *H. mollissima* seeds lost half their germination ability within 3 d and germination fell to 0% within 6 d. The drying rate provided partial explanation for variation in seed lifespan under different temperatures, as shown by T50 co-plotted against Seed Moisture Loss Rate Index (figure 1e).

The relationship between seed moisture and germination varied at different temperatures (figure 1d). From the co-plot between moisture content and germination percentage, the seeds at high temperatures lost most germination sharply as moisture fell from 40% to 30%, with the curve having a convex slope. In contrast at low temperatures seeds retained germination at a higher level until the moisture fell below 30% and the curve had a concave slope.

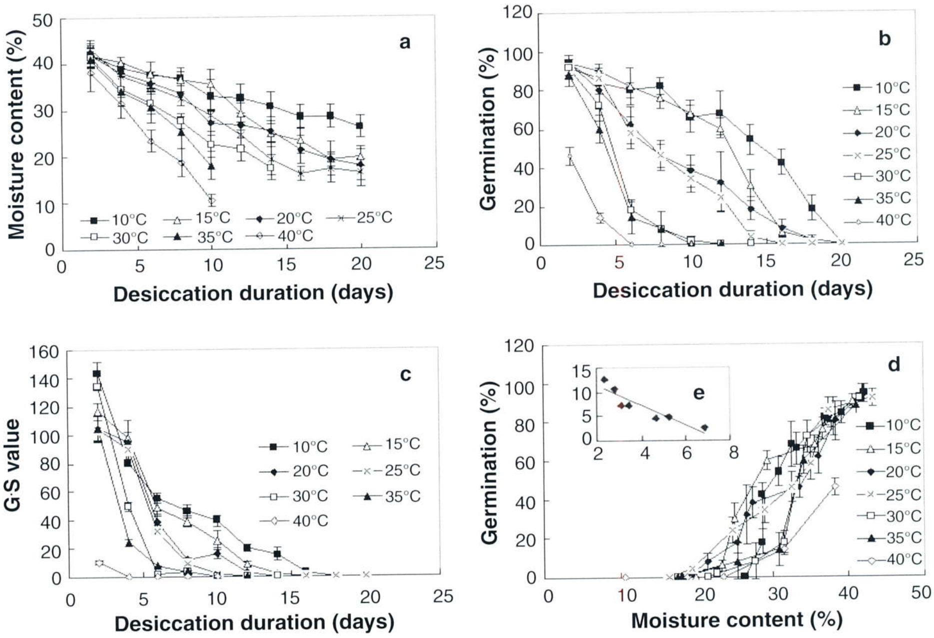

Figure 1. Moisture and germination loss of *Hopea mollissima* seeds at different temperatures in temperature-controlled incubators. Moisture contents were expressed on fresh basis as means ± SD of five replicates of single seed, germination percentages and G·S values were means ± SD of five replicates of ten seeds. The inset graph (e) demonstrates T50 (x-axis) co-plotted against Seed Moisture Loss Rate Index (y-axis).

Relative humidity also significantly influenced seed moisture and germination loss. The higher the relative humidity, the more slowly the seeds lost moisture and germination. Seeds held at 79% and 100% RH were similar, but below 79% every reduction in relative humidity from 79% to 11% RH accelerated moisture loss, depressed seedling vigour (G · S value) and curtailed the seed lifespan (figure 2). When relative humidity fell below 60%, most seeds lost their germination within 2-3 wk. At 100% RH, seeds germinated during storage, with precocious germination after 2, 3, 4 and 5 weeks being 24%, 45%, 75% and 84%, respectively. At this point, not enough seeds remained to continue the trials under this humidity regime. Seeds that did not germinate during storage at 100%RH showed no essential change in moisture content, but lost germination gradually in spite of their high moisture levels. The drying rate was an important cause for variation in seed lifespan under different relative humidity (figure 2e).

A varying relationship was also found between moisture and germination under different relative humidity (figure 2d). As the co-plot of moisture content and germination percentage shows, seeds at low relative humidity lost their most germination sharply at higher moisture content; while those at high relative humidity retained their germination to lower moisture content and then lost sharply, with the curve having a wide shoulder.

The previous study (Wen *et al.*, 2009) reported that incubation of *H. mollissima* seeds at temperatures > 30°C clearly depressed germination and seedling vigour although this temperature regime is very common outside forests in the tropics. The present study indicated that storage at temperatures ≥ 30°C or relative humidity ≤ 60% significantly accelerated loss of seed moisture and germination. If the seeds fail to germinate after shedding, they can retain a relatively longer lifespan only under cool and humid conditions. This implies that *H. mollissima* seeds require moist and cool conditions to retain germination and vigour. *H. mollissima* disperses its seeds during the dry season, when fog drip is an important source of moisture in tropical rainforests and represents, on average, up to 49 and 33% of the total rainfall in the cool-dry and hot-dry season,

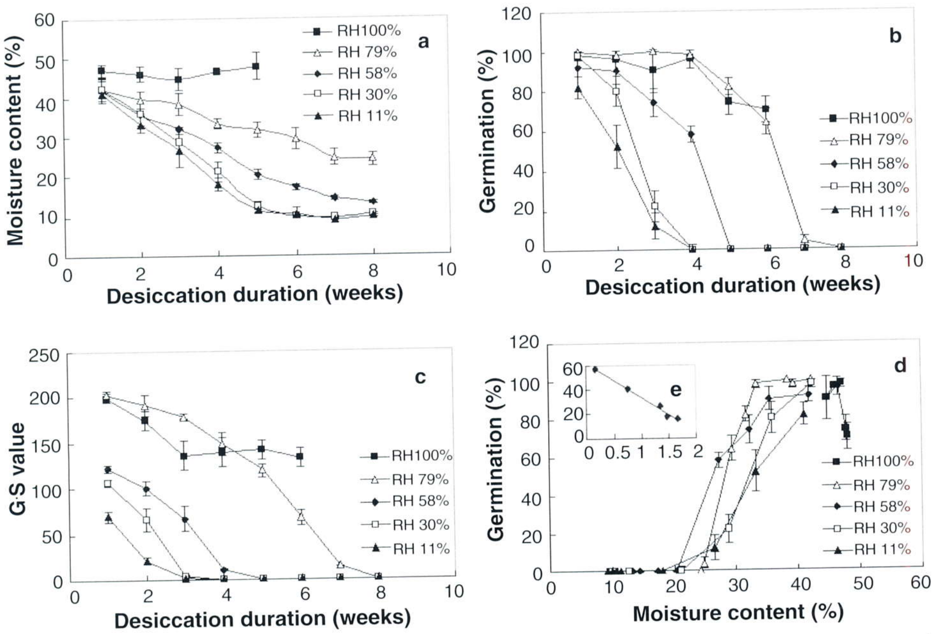

Figure 2. Moisture and germination loss of *Hopea mollissima* seeds under different relative humidity regulated by saturate solution of LiCl, $CaCl_2$, $BaBr_2$, NH_4Cl and distilled water respectively, in desiccators at 20°C. Moisture contents were expressed on fresh basis as means ± SD of five replicates of single seed, germination percentages and G·S values were means ± SD of five replicates of ten seeds. The inset graph (e) demonstrates T50 (x-axis) co-plotted against Seed Moisture Loss Rate Index (y-axis).

respectively (Liu *et al.*, 2004). These authors further reported that water from fog water dripping in the shallower soil horizons was also an important source for seedling growth during the dry season, especially at the peak of the dry season, for around 23-59% of the water used by seedling came from fog water (Liu *et al.*, 2010). Seeds in tropical rainforests may also use fog drip to complete their germination as this study demonstrated that up to 84% of *H. mollissima* seeds under 100% RH germinated during experiment period (figure 2). However, these forests have been being fragmented and disturbed since 1960's. Ma *et al.* (1998) reported that in these rainforest fragments the buffer effects to climate change have decreased, while the so-called edge effects resulting from human disturbance and deforestation have increased. This has reduced the variation between outside and inside rainforests, and made the inner forest environment drier and hotter. The sensitivity of *H. mollissima* seeds to high temperatures and dehydration suggests that they are highly dependent on the presence of an intact rainforest for survival and the increased temperatures and decreased relative humidity would unavoidably cause stress to their lifespan and germination in field.

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