Effects of heat on the germination of sclerophyllous forest species in the highlands of Madagascar.

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Abstract

The effects of fire on germination have been extensively studied in many ecosystems. Several studies have shown that plant species in ecosystems frequently exposed to fire can survive through two main mechanisms: vegetative regeneration (re-sprouts) and recruitment of new individuals from a fire-resistant seed bank. In Africa, an increase in temperature can break seed dormancy and stimulate germination of some herbaceous and woody species. In Madagascar, the once widespread highland ecosystems dominated by woody species are now highly fragmented and dominated by anthropic grasslands and fields, with a significantly reduced area occupied by sclerophyllous forests referred to as "tapia woodlands". Six species of this endemic vegetation type were studied: *Abrahamia ibityensis* (Anacardiaceae), *Aphloia theiformis* (Aphloiaceae), *Carissa edulis* (Apocynaceae), *Pentachlaena latifolia* (Sarcolaenaceae), *Uapaca bojeri* (Phyllanthaceae) and *Vaccinium secundiflorum* (Ericaceae). Germination tests were conducted 1) by soaking seeds in water for 24 hours (imbibition) or 2) by exposing the seeds to dry heat. Four different temperatures (40°C, 60°C, 80°C and 120°C), where seeds were exposed for 10, 30, 60 and 90 minutes. To simulate hotter-faster fires, two higher temperatures (100°C and 120°C) were also evaluated by exposing seeds to dry heat for 5 minutes. The results did not reveal any significant effect of water 24-hour imbibition on germination. For most species germination decreased with increasing temperature of treatment using dry heat. *Uapaca bojeri* did not germinate under any treatment. Further studies on the biological and ecological characteristics of tapia woodland species in response to fire are needed to help guide conservation, management and restoration activities focusing on this vegetation type.

Key words: Tapia woodland, Ibity mountain, conservation, *Abrahamia ibityensis*, *Aphloia theiformis*, *Carissa edulis*, *Pentachlaena latifolia*, *Uapaca bojeri*, *Vaccinium secundiflorum*.

1. Introduction

Fire is a natural disturbance element in many ecosystems, such as savannas and sclerophyllous woodlands in Africa (Kikula 1986; Campbell 1996; Kull 2000) and South America (Cerrado: Simon *et al.* 2009), and several Mediterranean-like ecosystems (Pausas *et al.* 1999, 2004; McCoy *et al.* 2002; Syphard *et al.* 2006; Schaffhauser *et al.* 2012). Fire determines the physiognomy and structure of vegetation and often influences species diversity (Bond and Keeley 2005). In fire-prone ecosystems, plant species can survive by using two main strategies: 1) resprouting, in which plants regenerate their above-ground biomass by growing from dormant buds on branches, trunks and underground; and 2) seedlings, which enable plants to recruit new individuals from a fire-resistant seed bank (Keeley and Zedler 1978; Pausas *et al.* 2004; Paula and Pausas 2008). Species are thus classified as 'resprouters' or 'seeders', according to the main mechanism involved in regeneration after fire, although both may be used. Other characteristics related to recruitment in fire-prone environments include post-fire seed dispersal and fire-induced flowering (Keeley and Fotheringham 1998).

The effects of fire on germination have been extensively studied in many ecosystems associated with this form of disturbance (Keeley 1987; Brown 1993; Mucunguzi and Oryem-Origa 1996; Gashaw and Michelsen 2002; Syphard *et al.* 2006; Chou *et al.* 2012). A large number of studies have concluded that temperature increase and smoke from burning, or their interaction, can break seed dormancy (Brown 1993; Keeley and Fotheringham 1998; Dayamba *et al.* 2010) and can stimulate germination of herbaceous and woody species (Keeley 1987; Mucunguzi and Oryem-Origa 1996; Keeley and Fotheringham 1998; Chou *et al.* 2012). These phenomena have been described for several families (e.g. Fabaceae, Rhamnaceae, Convolvulaceae, Malvaceae, Cistaceae, and Sterculiaceae) that have physically dormant fire-tolerant seeds (Baskin and Baskin 2000). In some species, germination is favored only when soil heating is low and time of heat exposure is short (Mucunguzi and Oryem-Origa 1996; Gashaw and Michelsen 2002). Other studies have shown that although fire increases the germination of some species, it may also inhibit germination, increase seed mortality and reduce seedling establishment (Moreno and Oechel 1991; Dayamba *et al.* 2008).

In the highlands of Madagascar, spatial and temporal mosaics of forests, savannas, woodlands and shrublands are the result of fire regimes that existed long before the arrival of humans ca. 2,000 years ago (Burney 1987; Dewar and Burney 1994). Currently, the highland ecosystems dominated by woody species are highly fragmented and dominated by anthropic grasslands and fields, with a significantly reduced area occupied by sclerophyllous forests referred to as "tapia woodlands" (Koechlin *et al.* 1974; Cornet and Guillaumet 1976), which have some similarities to the African "miombo" (Kull 2002). Tapia woodlands are dominated by the tapia tree *Uapaca bojeri* (Phyllanthaceae) associated with other woody species belonging to families endemic to Madagascar, such as Sarcocaulaceae and Asteraceae (Lowry II *et al.* 1997). The woody species present in tapia woodlands are fire tolerant: adult individuals typically have thick, spongy bark and weakly flammable leaves, and can re-sprout after fire (Campbell 1996). Previous studies have, however, shown that fire can be a significant threat to this distinctive vegetation type (Kull 2002; Birkinshaw *et al.* 2006) because it causes changes in structure and composition (Alvarado *et al.* 2014a) and reduces natural regeneration by killing seedlings (Perrier de la Bâthie 1921; Gade 1996).

The biological characteristics (e.g. seed dispersal, germination, phenology, etc.) of the species present in tapia woodlands, most of which are endemic to Madagascar, are still poorly known. Fruit production and dispersal of tapia woodland species occur mainly during the rainy season,

between November and May (Alvarado *et al.* 2014b), a pattern that is also observed in some other tropical ecosystems, such as the Cerrado (Batalha and Mantovani 2000; Weiser and Godoy 2001) and the Atlantic forests (Morellato *et al.* 2000) of Brazil. Germination thus also potentially occurs in the rainy season, when soil moisture is higher, which increases seed hydration.

Understanding the biological and ecological characteristics of tapia woodland species in response to fire is important for the conservation, management and restoration of this vegetation type, which is currently only partly protected within the Ibity and Itremo New Protected Areas and Isalo National Park. Restoration of degraded areas and the reinforcement of wild populations of threatened and/or ecologically important plant species requires the use of seeds and/or seedlings, and this must be based on sound knowledge of the processes involved and how best to manage storage and germination. The study of environmental drivers like fire is important to get an understanding of how this disturbance affects seed germination and in consequence how fire has the potential to affect reproduction rates/success. This information is important to better manage vegetation. In order to help address this need, an *ex-situ* study was thus performed on the main woody species of tapia woodlands to determine the effects of imbibition and fire on seed germination. Our goals were to determine whether 1) precipitation and thus imbibition alone stimulate their germination; and 2) if the seeds of these species can survive after being affected by different scenarios of fire, and thus whether fire could represent a barrier to natural regeneration in this vegetation type.

2. Methods

2.1. Seed material

Six species were studied: *Abrahamia ibityensis* (H. Perrier) Randrian. & Lowry, ined. (Anacardiaceae), *Aphloia theiformis* (Vahl) Benn. (Aphloiaceae), *Carissa edulis* (Forssk.) Vahl (Apocynaceae), *Pentachlaena latifolia* H. Perrier (Sarcocaulaceae), *Uapaca bojeri* Baill. (Phyllanthaceae) and *Vaccinium secundiflorum* Hook. (Ericaceae). The choice of species was based on two considerations: 1) the selected species were the dominant woody elements in the canopy and mid-storey (trees and shrubs with a DBH > 1cm): *U. bojeri*, represents 82% of the trees counted on Ibity (Alvarado *et al.* 2014a) and the other species are common in the lower canopy and mid-storey; and 2) these species are endemics to Madagascar and are potentially threatened by changes in fire regimes (Alvarado *et al.* 2014b). It is thus important to study their biology to increase knowledge within the framework of future conservation, restoration or reinforcement programs. Seeds used in this study were collected on the Ibity Massif, a New Protected Area, located in the Antananarivo province, in Madagascar's Central Highlands, 200km south of the capital city, Antananarivo, and 25km south of the town Antsirabe (47°07'S 20°01'E). The area's climate, classified as Cwb according to the Köppen system (Peel *et al.* 2007), is characterized by a well-defined dry season (June-October) and wet season (November to May). Average annual rainfall reaches 1583mm and average temperature is 17.5°C, with a mean maximum temperature of 27.0 °C and a mean minimum temperature of 9.1 °C in the wet season and a mean maximum temperature of 27.3 °C and a mean minimum temperature of 5.5°C in the dry season (based on data from 1961 to 1990; Meteorology Service of Ampandrianomby). Seeds were harvested randomly from 5-10 mother-plants of each species during the wet season (November-December) in 2010 and 2011, according to their availability; seeds were stored in a dry place at the laboratory; germination trials were run during the following 1-2 months.

2.2. Germination trials

Germination experiments, conducted using a series of treatments (Table 1), were performed at the Laboratory of Plant Physiology at the University of Antananarivo. One set of trials was done without pre-germination treatment on a set of “control” seeds, and a second set was performed to determine water requirements for germination (imbibitions) by soaking seeds in water for 24 hours prior to the germination tests. Using a third set of seeds, the effect of pre-germination dry heat was tested on four of the six species studied (*Aphloia theiformis*, *Carissa edulis*, *Uapaca bojeri* and *Vaccinium secundiflorum*) to simulate increased temperature produced by fires. Four different temperatures (40°C, 60°C, 80°C and 120°C) were used, following the protocol of Munyanziza and Msanga (1996). For the other two species (*Abrahamia ibityensis* and *Pentachlaena latifolia*), we did not have enough seeds to run the dry heat tests. Almost 50% of seeds of these two species germinated in the paper bags before to start the trial. Seeds were heated in a mufla oven with an external thermometer to verify the required temperatures; another thermometer was placed inside the oven to double-check the temperature. Seeds were introduced into the mufla after five minutes of constant temperature. For each temperature, seeds were separated in paper bags and exposed in Petri dish for 10, 30, 60 and 90 minutes in an oven. To simulate hotter-faster fires, the effects of two higher temperatures (100°C and 120°C) were also evaluated by exposing seeds to dry heat for 5 minutes. Seeds of all species were heated together for a same treatment, but replicates were heated separately in order to avoid pseudoreplication in the experimental design (Morrison and Morris 2000). Treatments were designed to cover the range of conditions that seeds could potentially encounter on the ground or in open sites during wild fires (Keeley 1987).

After treatment, seeds were sown on 90 × 15mm Petri dishes covered with Whatman 90mm diameter filter paper, and placed in a growth chamber at a constant temperature of 25°C and a 12 hour light regime, following the protocol of Baskin & Baskin (2000). The numbers of replicates (=petri dishes) and of seeds used for each species depended on their availability and on seed size (15, 25, 30 or 100 seeds per dish, Table 2).

The seeds were monitored every 2 days for 8 weeks. Germination was recorded when a 2 mm seed radicle had emerged. The effect of each treatment on seed germination was measured as the percentage of germination (PG) and mean germination time (MGT) (Munyanziza and Msanga 1996; Chou *et al.* 2012), calculated using the following formulas:

$$PG(\%) = \frac{\sum n_i}{N} \times 100 \quad \text{and} \quad MGT = \frac{\sum (t_i \times n_i)}{\sum n_i}$$

where n_i is the number of germinated seeds, N is the total number of seeds, and t_i is the day when n_i seeds had germinated.

2.3. Statistical analyses

The effects of treatments on germination were examined using generalized linear models with a binomial error structure (Zuur *et al.* 2009). Separate analyses were performed for each species. The response variable was the percent of germinated seeds after 8 weeks. First, the imbibition treatment and the control were used as factors and compared with one another. Then dry heat treatments were compared with the control. We used Akaike’s Information Criterion to select the best model(s) (Akaike 1973; Burnham and Anderson 2002).

To assess the difference in MGT between the pre-germination imbibition treatment and the control, t-tests were performed for each species. Two-way ANOVAs were conducted to determine the effects of temperature (40°C, 60°C, 80°C, 100°C and 120°C) and exposure time (5, 10, 30, 60 and 90 minutes) on MGT. For all ANOVA, MGT values were treated as variables

and temperature and exposure time as factors. The normal distribution of data values was verified with the Shapiro-Wilk test (<40 data) or the Lilliefors test (>40 data) and square root transformations were performed as necessary. The homogeneity of variances was confirmed with Bartlett's tests. When two-way ANOVA were significant ($P < 0.05$), post-hoc Tukey tests were performed. When the normal distribution of data was not confirmed, Kruskal-Wallis tests, followed by Wilcoxon tests, adjusted with the Bonferroni correction, were performed. All tests were performed using R software (The R Foundation for Statistical Computing, version 2.15.1, (R Development Core Team 2012)).

3. Results

3.1. Germination

The germination treatment by imbibition had no significant effect on any of the five species studied (Table 3). Germination percentages were similar between the control and the imbibition treatment: they are relatively high for *Abrahamia ibityensis* ($77.9 \pm 7.5\%$) and *Carissa edulis* ($97.6 \pm 1.1\%$), intermediate for *Aphloia theiformis* ($42.0 \pm 1.8\%$) and *Pentachlaena latifolia* ($43.9 \pm 3.74\%$), and comparatively low for *Vaccinium secundiflorum* ($26.2 \pm 3.47\%$). No germination was observed for *Uapaca bojeri*.

Only one species, *Carissa edulis*, had a significant difference in MGT between the two treatments: the imbibition treatment lowered MGT by one day (Table 3). *Pentachlaena latifolia* germinated in 3.98 ± 0.26 days (Table 3), whereas *Vaccinium secundiflorum* germinated in 19.7 ± 0.63 days (Table 3). MGT is intermediate for the other three species: *Abrahamia ibityensis* (5.9 ± 0.71 days), *Aphloia theiformis* (11.3 ± 0.37 days) and *Carissa edulis* (6.9 ± 0.20 days).

3.2. Effect of dry heat on seed germination

Temperature had a significant effect on PG and MGT for all tested species except *Uapaca bojeri* which did not germinate under any conditions. PG for *Aphloia theiformis* was significantly different between treatments ($P(>\text{Chi}) < 0.001$; Figure 1a). For this species, maximum PG was obtained from control seeds ($43.3 \pm 2.8\%$) which was not significantly different from seeds heated at 40°C or 80°C ; the PG was lower for seeds heated at 120°C for 10 minutes ($6.7 \pm 1.1\%$) and significantly the lowest for seeds heated at 120°C for 30, 60, 90 minutes ($0 \pm 0\%$, Table 4, Figure 1a). Heating increased MGT, which was maximum when seeds were treated at 120°C (27.7 ± 1.6 days; $F_{\text{Temperature}} = 49.9$, $P < 0.001$, Table 4). *Carissa edulis* PG were significantly different between treatments ($P(>\text{Chi}) < 0.001$, Figure 1b). For this species, maximum PG was obtained from control seeds and those heated at 40°C for 30 minutes ($96 \pm 1.95\%$ and $97.54 \pm 1.6\%$, respectively, Table 4, Figure 1b) and was not significantly different from seeds heated at 40°C for 10, 60 and 90 minutes or 60°C for 10, 30, 60 minutes; PG was intermediate for seeds heated at 60°C for 90 minutes ($73.6 \pm 4.1\%$) or 100 and 120°C for 5 minutes (81.6 ± 3.9 and 75.2 ± 4.08 respectively) and significantly the lowest for seeds heated at 80 and 120°C for more than 10 minutes ($4.8 \pm 1.96\%$, Table 4, Figure 1b); the MGT varied from 5.7 days to 7.9 days, depending on temperature (Kruskal-Wallis $\chi^2_{\text{Temperature}} = 38.23$, $P < 0.001$, Table 4). Finally, for *Vaccinium secundiflorum*, PG was significantly different between treatments ($P(>\text{Chi}) < 0.001$, Figure 1c). The maximum PG for *V. secundiflorum* was obtained from control seeds ($26.2 \pm 3.5\%$, Table 4, Figure 1c) and was not significantly different from seeds heated at 40°C for 10 or 90 minutes, at 80°C and at 120°C for 10 and 30 minutes; the PG was significantly the lowest for seeds heated at 60°C for 60 and 90 minutes, at 100°C for 5 minutes and at 120°C for 60 and 90 minutes (Table 4, Figure 1c). At 80°C , PG was higher (between 17.2 ± 0.23 and $20.2 \pm 0.79\%$) than at 60°C but did not reach the level obtained in the control. The MGT for this species varied depending on the temperature at which the seeds were heated and the time of exposure to heat ($F_{\text{Temperature} \times \text{Time}} = 5.52$, $P <$

0.001, Table 4). The highest value of MGT was observed when seeds were heated at 120°C for 5 minutes and at 60°C for 60 minutes (34.5 ± 1.2 and 32.93 ± 1.6 days, respectively), and the lowest when they were preheated at 120°C for 120 minutes (2.7 ± 2.7 days respectively).

4. Discussion

4.1. Germination

Two of the six species studied (*Carissa edulis* and *Abrahamia ibityensis*) exhibited percentages of germination (PG) over 84% under the control treatment, indicating that their seeds are mainly not dormant. The other four species examined had PG values under 45.5%. The water imbibition treatment did not significantly improve germination success in the species tested, but only resulted in a slight decrease in the mean germination time (MGT) of one species, *C. edulis*. The results show that none of the tapia woodland species considered in our study increase the percent of germination by imbibition alone. However, more germination tests including other combinations of factors (e.g. different incubation temperatures, ripening or stratification prior to germination, etc.) are needed to increase knowledge on seed physiology and ecology, and to confirm whether there is any kind of dormancy. In tropical dry woodlands and savannas, establishment from seeds has been reported as a rare event (Sarmiento and Monasterio 1983) and the seed ecology of germination for woody species is variable: some species exhibit seed dormancy (Brown 1993; Mucunguzi and Oryem-Origa 1996; Dayamba *et al.* 2008), some have non-dormant seeds, and others are recalcitrant (Oliveira and Valio 1992).

A phenology study performed on selected woody tapia woodland species (Alvarado *et al.* 2014b) shows that seed dispersal takes place during the rainy season for five of the species studied: *Abrahamia ibityensis*, *Aphloia theiformis*, *C. edulis*, *Uapaca bojeri* and *Vaccinium secundiflorum*. Seed germination of these and other woody species is potentially linked to rainfall because precipitation brings an increase in soil moisture, which promotes germination (Oliveira and Silva 1993; Baskin and Baskin 2000). This finding makes it difficult to interpret the results of the imbibition tests; we expected germination to be improved by water imbibition, as shown by Choinski and Tuohy (1991) for two African trees, *Acacia tortilis* (Forssk.) Hayne and *Acacia karroo* Hayne.

Concerning the dominant species of tapia woodlands, *Uapaca bojeri*, we did not observe any germination in our laboratory tests, regardless of the treatment. Kull *et al.* (2005) reported that *U. bojeri* seeds are recalcitrant, losing their viability within a few months after dispersal, a finding that was confirmed by Randrianavosoa *et al.* (2011). They also found that the percent of germination of *U. bojeri* in laboratory experiments was between 45% and 95%, when seed water content was 20-30%, regardless of whether lowered water content was obtained after 10-15 days of drying in the shade or 26 days storage at a relative humidity of 60-84%. On-site experiments in local soil, at the Ibity nursery, showed 17.8% germination after three months, with germination beginning six weeks after seeds were sowed (Alvarado *et al.* 2010). This could explain the absence of germination *U. bojeri* after eight weeks of observation in laboratory conditions. Moreover, since the viability of the seeds was not tested, it is not possible to conclude whether this result is due to the fact that the seeds were dormant or dead.

With the exception of *Abrahamia ibityensis* and *Carissa edulis*, we found that germination of the studied species was less than 50%. In *A. ibityensis* and *C. edulis*, PG values in the field nursery were lower (*A. ibityensis* 35.7% and *C. edulis* 22.4%, Alvarado *et al.* 2010) than in the laboratory (*A. ibityensis* 84.3% and *C. edulis* 96%). In *Aphloia theiformis*, PG in the nursery (26.9%; Alvarado *et al.* 2010) was just over half that in the laboratory (42.0%).

4.2. Effect of dry heat on seed germination

High temperatures failed to induce germination in the three species studied, suggesting that they have a germination strategy different from that of typical species found in most fire-adapted ecosystems, where fire at low intensities stimulates germination and at high intensities increases seed mortality (Keeley 1987; Moreno and Oechel 1991; Mucunguzi and Oryem-Origa 1996). Although the seeds of *Aphloia theiformis*, *Carissa edulis* and *Vaccinium secundiflorum* can tolerate moderate temperatures (60°C – 80°C depending of the species), they are not resistant to fire, as a reduction of PG was observed after both short or long exposure to high temperatures. Stimulation of germination by fire is common among species of Fabaceae (Portlock *et al.* 1990; Tarrega *et al.* 1992; Bradstock and Auld 1995; Mucunguzi and Oryem-Origa 1996; Auld and Denham 2006), Combretaceae (Dayamba *et al.* 2010) and Ericaceae, which do not have the ability to re-sprout after fire, and are thus totally dependent on the seed bank for reestablishment after burning (Keeley 1987). These species exhibit physical or physiological dormancy and germination is frequently stimulated by smoke or a combination of heat and smoke (Auld and Ooi 2008). However, we found that germination of the ericaceous species *V. secundiflorum* was not stimulated by increased temperature, a result contrary to the findings reported by Gonzalez-Rabanal & Casal (1995) for Mediterranean species, who noted that germination in the Ericaceae they studied was stimulated by heat shock, however, a wide range of responses can be detected in members of this family. Even if *V. secundiflorum* germination does not increase with high temperatures as expected, it is relevant to note that this species is at least fire tolerant. For the other two species examined in our study, exposure to moderate heat (<60°C for *A. theiformis* and <80°C for *C. edulis*), even for long periods, did not affect germination, whereas at temperatures >100°C, their seeds were sensitive to the time of exposure, which is similar to the response of certain woody species in Australia and Burkina Faso (Auld and O'Connell 1991; Zida *et al.* 2005). Fire temperature has been measured in several tropical ecosystems, ranging from 47°C (50 cm above the ground surface) to 537.5°C (at the surface) in Brazilian savannas (Fidelis *et al.* 2010), from 98-458°C in Australian savannas (Morgan 1999), and up to 150°C in Australian woodlands (Bradstock and Auld 1995). While some studies suggest that temperatures above 60°C as being fatal to plant tissues (Whelan 1995), but exposure to dry heat at 60°C and 100°C renders seeds of some species more permeable, thus promoting germination (Baskin and Baskin 2000).

Although tapia woodlands are a vegetation formation in which fire is a natural disturbance (Kull 2000, 2002), germination was not stimulated by dry heat in any of the species we studied. They showed varying levels of tolerance to different temperatures and duration of exposure to heat. *Carissa edulis* was the most sensitive, surviving in temperatures up to 60°C, regardless of the time of exposure to dry heat without reducing seed viability, but suffering significant reduction in PG (to less than 20%) at 80°C, although exposure for 5 minutes to 120°C did not kill seeds and only slightly reduced PG. *Aphloia theiformis* was less sensitive to dry heat. At 40°C, PG remained close to that observed in the control, but exposure to 40°C for 90 minutes and to 80°C, regardless of exposure time, PG decreased from 10-30%, and at 120°C all seeds died. In this species, higher temperatures reduced germination and increased MGT. *Vaccinium secundiflorum* had the most variable behavior faced with dry heat of the three species studied: high temperatures (80°C and 120°C) had a less negative impact on germination than low temperatures (40°C and 60°C).

5. Conclusion

Seed germination varied among the woody species we studied, but germination was low in untreated samples for 4 species out of 6 (exceptions: *Abrahamia ibityensis* and *Carissa edulis*) and decreased with increasingly hot shock treatment using dry heat for most species. Contrary

to our expectation, water imbibition did not stimulate seed germination. The reduced percentage of germination with increasing temperature suggests that high temperatures reduced viability. According with the literature and as suggested by our results, at least one of the species (*Uapaca bojeri*) has recalcitrant seeds and it is quite possible that some of the other species have similar (or at least intermediate) seeds. However, in order to conclude if seeds are dormant or recalcitrant, viability tests should be carry out to confirm if the lack of germination in the controls is due to seeds being dormant or dead. On Ibity Mountain, where the current fire regime has been modified by human activities, fires had a variable impact on seed germination in these species. Our results provide some useful insights, but further studies of the seed ecology (e.g. the role of smoke, the possible role of seed scarifications, etc.) of these and other species occurring in tapia woodlands are required in order to develop a better understanding of the relationship between fire and germination, and of the impacts of the current human-caused fire regime found on Ibity Mountain. Insights from additional studies would also be valuable for developing improved conservation and management strategies for the native species of tapia woodlands, especially with regard to efforts aimed at reintroducing species to areas where they have been extirpated and reinforcing populations that have been impacted in this localized and threatened ecosystem.

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References

- Akaike, H. (1973) Information theory and an extension of the maximum likelihood principle. *Second International Symposium on Information Theory*.
- Alvarado, S. T., Buisson, E., Rabarison, H., Rajeriarison, C., Birkinshaw, C., Lowry II, P. P. (2014a) Comparison of plant communities on two massifs in Madagascar (Ibity and Itremo) with contrasting conservation histories and current status. *Plant Ecology and Diversity* **7**, 497-508.
- Alvarado, S. T., Buisson, E., Rabarison, H., Rajeriarison, C., Birkinshaw, C., Lowry II, P. P., Morellato, L. P. C. (2014b) Fire and the reproductive phenology of endangered Madagascar sclerophyllous tapia woodlands. *South African Journal of Botany* **94**, 79-87.
- Alvarado, S. T., Buisson, E., Rabarison, H., Rajeriarison, C., Birkinshaw, C., Lowry II, P. P. (2010) Reintroduction and reinforcement of endangered woody species populations in Tapia woodlands, Mount Ibity, Madagascar: protocol and preliminary results. *SER-Europe Conference Proceedings* (<http://ser.semico.be/>, 4p.). [online]. Available from: http://ser.semico.be/ser-pdf/EA_SER2010_173.pdf [Accessed September 25, 2012].
- Auld, T. D., Denham, A. J. (2006) How much seed remains in the soil after a fire? *Plant Ecology*. **187**, 15–24.
- Auld, T. D., O'Connell, M. A. (1991) Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. *Australian Journal of Ecology*. **16**, 53–70.
- Auld, T. D., Ooi, M. K. J. (2008) Applying seed germination studies in fire management for biodiversity conservation in south-eastern Australia. *Web Ecology*. **8**, 47-54.
- Baskin, C. C., Baskin, J. M. (2000) *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Elsevier.
- Batalha, M. A., Mantovani, W. (2000) Reproductive phenological patterns of cerrado plant species at the Pé-de-Gigante Reserve (Santa Rita do Passa Quatro, SP, Brazil): a comparison between the herbaceous and woody floras. *Revista Brasileira de Biologia*. **60**, 129-145.
- Birkinshaw, C., Andriamihajarivo, T. H., Rakotoarinivo, C. R., Randrianaina, L., Randrianarivelo, C., Rasamimanana, V. N., Rasolondraibe, B., Ravoahangy, A., Razafindrasoa, R., Seing, S. T. (2006) *Le massif d'Ibity : Description, évaluation et stratégie de conservation*. Unpublished Report. Missouri Botanical Garden, Madagascar Program., Antananarivo, Madagascar.
- Bond, W. J., Keeley, J. E. (2005) Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology & Evolution*. **20**, 387–394.
- Bradstock, R. A., Auld, T. D. (1995) Soil temperatures during experimental bushfires in relation to fire intensity: consequences for legume germination and fire anagement in south-eastern Australia. *Journal of Applied Ecology*. **32**, 76–84.
- Brown, N. A. C. (1993) Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist*. **123**, 575–583.
- Burney, D. A. (1987) Late Quaternary stratigraphic charcoal records from Madagascar. *Quaternary Research*. **28**, 274–280.
- Burnham, K. P., Anderson, D. R. (2002) *Model selection and multi-model inference: a practical information-theoretic approach*. Springer. [online]. Available from **URL** <http://www.springer.com/statistics/statistical+theory+and+methods/book/978-0-387-95364-9>.
- Campbell, B. M. (1996) *The Miombo in transition: woodlands and welfare in Africa*. Centre for International Forestry Research (CIFOR), Bogor, Indonesia.
- Choinski Jr., J.S., Tuohy, J. M. (1991) Effect of water potential and temperature on the germination of four species of African savanna trees. *Annals of Botany*. **68**, 227–233.

- 419 Chou, Y. F., Cox, R. D., Wester, D. B. (2012) Smoke water and heat shock influence germination of shortgrass
420 prairie species. *Rangeland Ecology & Management*. **65**, 260–267.
- 421 Cornet, A., Guillaumet, J. L. (1976) Divisions floristiques et étages de végétation à Madagascar. *ORSTOM*,
422 *Paris*. **6**, 35–40.
- 423 Dayamba, S. D., Sawadogo, L., Tigabu, M., Savadogo, P., Zida, D., Tiveau, D., Oden, P. C. (2010) Effects of
424 aqueous smoke solutions and heat on seed germination of herbaceous species of the Sudanian savanna-woodland
425 in Burkina Faso. *Flora-Morphology, Distribution, Functional Ecology of Plants*. **205**, 319–325.
- 426 Dayamba, S. D., Tigabu, M., Sawadogo, L., Oden, P. C. (2008) Seed germination of herbaceous and woody
427 species of the Sudanian savanna-woodland in response to heat shock and smoke. *Forest Ecology and*
428 *Management*. **256**, 462–470.
- 429 Dewar, R. E., Burney, D. A. (1994) Recent research in the paleoecology of the highlands of Madagascar and its
430 implications for prehistory. *Taloha*. Vol 12, Available from URL
431 <http://www.taloha.info/docannexe.php=1249>
- 432 Fidelis, A., Delgado-Cartay, M. D., Blanco, C. C., Müller, S. C., Pillar, V. D., Pfadenhauer, J. (2010) Fire
433 intensity and severity in Brazilian Campos grasslands. *Interciencia*. **35**, 739–745.
- 434 Gade, D. W. (1996) Deforestation and its effects in highland Madagascar. *Mountain Research and Development*.
435 **16**, 101–116.
- 436 Gashaw, M., Michelsen, A. (2002) Influence of heat shock on seed germination of plants from regularly burnt
437 savanna woodlands and grasslands in Ethiopia. *Plant Ecology*. **159**, 83–93.
- 438 González-Rabanal, F., Casal, M. (1995) Effect of high temperatures and ash on germination of ten species from
439 gorse shrubland. *Plant Ecology*. **116**, 123–131.
- 440 Keeley, J. E. (1987) Role of fire in seed germination of woody taxa in California chaparral. *Ecology*. **68**, 434–
441 443.
- 442 Keeley, J. E., Fotheringham, C. J. (1998) Smoke-induced seed germination in California chaparral. *Ecology*. **79**,
443 2320–2336.
- 444 Keeley, J. E., Zedler, P. H. (1978) Reproduction of chaparral shrubs after fire: a comparison of sprouting and
445 seeding strategies. *American Midland Naturalist*. **99**, 142–161.
- 446 Khurana, E., Singh, J. s. (2001) Ecology of seed and seedling growth for conservation and restoration of tropical
447 dry forest: a review. *Environmental Conservation*. **28**, 39–52.
- 448 Kikula, I. S. (1986) The influence of fire on the composition of miombo woodland of SW Tanzania. *Oikos*. **46**,
449 317–324.
- 450 Koechlin, J., Guillaumet, J. L., Morat, P. (1974) *Flore et végétation de Madagascar*, J. Cramer. Gantner Verlag,
451 Vaduz, 687p.
- 452 Kull, C. A. (2000) Deforestation, erosion, and fire: degradation myths in the environmental history of
453 Madagascar. *Environment and History*. **6**, 423–450.
- 454 Kull, C. A. (2002) The ‘degraded’ tapia woodlands of highland Madagascar: rural economy, fire ecology, and
455 forest conservation. *Journal of Cultural Geography*. **19**, 95–128.
- 456 Kull, C. A., Ratsirarson, J., Randriamboavonjy, G. (2005) Les forêts de tapia des Hautes Terres malgaches.
457 *Terre Malgache*. **24**, 22–58.
- 458 Lowry II, P. P., Schatz, G. E., Phillipson, P. B. (1997) The classification of natural and anthropogenic vegetation
459 in Madagascar. In: *Natural change and human impact in Madagascar* pp. 93–123 .M. Goodman and B.D.
460 Patterson (eds), Washington and London.

461 McCoy, S., Jaffré, T., Rigault, F., Ash, J. E. (2002) Fire and succession in the ultramafic maquis of New
462 Caledonia. *Journal of Biogeography*. **26**, 579–594.

463 Morellato, L. P. C., Talora, D. C., Takahasi, A., Bencke, C. C., Romera, E. C., Zipparro, V. B. (2000) Phenology
464 of Atlantic rain forest trees: A comparative study. *Biotropica*. **32**, 811–823.

465 Moreno, J. M., Oechel, W. C. (1991) Fire intensity effects on germination of shrubs and herbs in southern
466 California chaparral. *Ecology*. **72**, 1993–2004.

467 Morgan, J. W. (1999) Defining grassland fire events and the response of perennial plants to annual fire in
468 temperate grasslands of south-eastern Australia. *Plant Ecology*. **144**, 127–144.

469 Morrison, D. A., Morris, E. C. (2000) Pseudoreplication in experimental designs for the manipulation of seed
470 germination treatments. *Austral Ecology*. **25**, 292–296.

471 Mucunguzi, P., Oryem-Origa, H. (1996) Effects of heat and fire on the germination of *Acacia sieberiana* D.C.
472 and *Acacia gerrardii* Benth. in Uganda. *Journal of Tropical Ecology*. **12**, 1–10.

473 Munyanziza, E., Msanga, H. P. (1996) Account of germination characteristics of the main commercial timber
474 trees of the Miombo woodlands in Tanzania [*Julbernardia globiflora*]. In: *Proceedings of the IUFRO*
475 *Symposium of the Project Group P Innovations in Tropical Tree Seed Technology*, Arusha (Tanzania), 7-10 Sep
476 1995. Olesen, K. (ed.).- Humlebaek (Denmark).

477 Oliveira, L. M. Q., Valio, I. F. M. (1992) Effects of moisture content on germination of seeds of *Hancornia*
478 *speciosa* Gom.(Apocynaceae). *Annals of Botany*. **69**, 1–5.

479 Oliveira, P. E., Silva, J. C. S. (1993) Reproductive biology of two species of *Kielmeyera* (Guttiferae) in the
480 cerrados of Central Brazil. *Journal of Tropical Ecology*. **9**, 67–79.

481 Paula, S., Pausas, J. G. (2008) Burning seeds: germinative response to heat treatments in relation to resprouting
482 ability. *Journal of Ecology*. **96**, 543–552.

483 Pausas, J. G., Bradstock, R. A., Keith, D. A., Keeley, J. E. (2004) Plant functional traits in relation to fire in
484 crown-fire ecosystems. *Ecology*. **85**, 1085–1100.

485 Pausas, J. G., Carbó, E., Neus Caturla, R., Gil, J. M., Vallejo, R. (1999) Post-fire regeneration patterns in the
486 eastern Iberian Peninsula. *Acta Oecologica*. **20**, 499–508.

487 Peel, M. C., Finlayson, B. L., McMahon, T. A. (2007) Updated world map of the Köppen-Geiger climate
488 classification. *Hydrology and Earth System Sciences Discussions*. **4**, 1633–1644.

489 Perrier de la Bâthie, H. (1921) La végétation malgache. *Annals du Musée Colonial de Marseille (Série 3)*.

490 Portlock, C. C., Shea, S. R., Majer, J. D., Bell, D. T. (1990) Stimulation of germination of *Acacia pulchella*:
491 laboratory basis for forest management options. *Journal of Applied Ecology*. **27**, 319–324.

492 Randrianavosoa, H., Andrianoelina, O., Ramamonjisoa, L. (2011) Tolérance à la dessiccation des graines
493 d'*Uapaca bojeri*, Euphorbiaceae. *International Journal of Biological and Chemical Sciences*. **5**. [online].
494 Available from: <http://www.ajol.info/index.php/ijbcs/article/view/68086> [Accessed August 20, 2012].

495 R Development Core Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for
496 Statistical Computing. ISBN 3-900051-07-0, Vienna, Austria.

497

498 Sarmiento, G., Monasterio, M. (1983) Life forms and phenology. In: *Ecosystems of the World* pp. 79–108
499 Bourlière F. (ed.), Amsterdam. [online]. Available from:
500 http://www.ciencias.ula.ve/icae/publicaciones/sabanas/pdf/sarmiento_g1983b.pdf [Accessed July 25, 2012].

501 Schaffhauser, A., Curt, T., Véla, E., Tatoni, T. (2012) Fire recurrence effects on the abundance of plants grouped
502 by traits in *Quercus suber* L. woodlands and maquis. *Forest Ecology and Management*. **282**, 157-166.

503 Simon, M. F., Grether, R., Queiroz, L. P. de, Skema, C., Pennington, R. T., Hughes, C. E. (2009) Recent
504 assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *PNAS*.
505 **106**, 20359–20364.

506 Syphard, A. D., Franklin, J., Keeley, J. E. (2006) Simulating the effects of frequent fire on southern California
507 coastal shrublands. *Ecological Applications*. **16**, 1744–1756.

508 Tarrega, R., Calvo, L., Trabaud, L. (1992) Effect of high temperatures on seed germination of two woody
509 Leguminosae. *Plant Ecology*. **102**, 139–147.

510 Weiser, V. L., Godoy, S. A. P.. (2001) Floristic composition in a hectare of cerrado stricto sensu hectare in the
511 ARIE-Cerrado Pé-de-Gigante, Santa Rita do Passa Quatro, SP. *Acta Botanica Brasilica*. **15**, 201-212.

512 Whelan, R. J. (1995) *The ecology of fire*. Cambridge University Press. Cambridge, UK.

513 Zida, D., Tigabu, M., Sawadogo, L., Oden, P. C. (2005) Germination requirements of seeds of four woody
514 species from the Sudanian savanna in Burkina Faso, West Africa. *Seed Science and Technology*. **33**, 581–593.

515 Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., Smith, G. M. (2009) *Mixed effects models and extensions*
516 *in ecology with R*. Springer. New York.

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519 Table 1: Summary of treatments applied to the seeds of six tapia woodland species (*Abrahamia*
 520 *ibityensis*, *Aphloia theiformis*, *Carissa edulis*, *Pentachlaena latifolia* *Uapaca bojeri* and
 521 *Vaccinium secundiflorum*. For additional information on the seeds, see some photos on
 522 *Electronic appendix 1*).

Treatment	Treatment details		Species tested
Control	No pre-germination treatment		All species
Imbibition	Seeds soaked in water for 24 hours prior to germination		All species except <i>V. secundiflorum</i>
Dry heat	40°C	10, 30, 60 or 90 minutes	All species except <i>A. ibityensis</i> and <i>P. latifolia</i>
	60°C	10, 30, 60 or 90 minutes	All species except <i>A. ibityensis</i> , <i>A. theiformis</i> and <i>P. latifolia</i>
	80°C	10, 30, 60 or 90 minutes	All species except <i>A. ibityensis</i> and <i>P. latifolia</i>
	120°C	10, 30, 60 or 90 minutes	All species except <i>A. ibityensis</i> and <i>P. latifolia</i>
Fast burnt	100°C	5 minutes	<i>C. edulis</i> , and <i>V. secundiflorum</i>
	120°C	5 minutes	<i>C. edulis</i> , and <i>V. secundiflorum</i>

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526 **Table 2:** Studied species and details of the experimental setup. Number of replicates, seeds/dish, total number of treatments and total number of
 527 seeds are indicated for each species.

Species	Family	Life-form/habit	Geographic distribution	Replicates (Petri dishes)	Seeds / dish	Total treatments	Total seeds
<i>Abrahamia ibityensis</i>	Anacardiaceae	Shrub	Endemic to Madagascar (Ibity and Itremo massifs only)	7	10	2	140
<i>Aphloia theiformis</i>	Aphloiaceae	Shrub or tree	Madagascar, Comoros, Mascarenes, Seychelles, Africa	5	30	14	2100
<i>Carissa edulis</i>	Apocynaceae	Shrub	Madagascar, Africa, Indian Ocean islands. Asia (India)	5	25	20	2500
<i>Pentachlaena latifolia</i>	Sarcolaenaceae	Shrub or tree	Endemic to Madagascar (Ibity only)	4	15	2	120
<i>Uapaca bojeri</i>	Phyllanthaceae	Tree	Endemic to Madagascar (widespread)	6	10	20	1200
<i>Vaccinium secundiflorum</i>	Ericaceae	Shrub	Endemic to Madagascar (widespread)	3	100	19	5700

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Table 3: Average percent of germination (PG) and mean germination time (MGT) of six woody species from tapia woodland (Madagascar) tested under laboratory conditions for water imbibition before the start if germinations tests. Germination was monitored for 8 weeks in a culture chamber at a constant temperature of 25°C and a 12 hours light regime. No water imbibition tests were carried out for *Vaccinium secundiflorum* (NT = not tested).

Species	PG (%)		Significance (PG)	MGT (days)		Significance (MGT)	
	Mean $\pm$ se			Mean $\pm$ se		t	P
	Imbibition	Control		Imbibition	Control		
<i>Abrahamia ibityensis</i>	71.4 $\pm$ 14.4	84.3 $\pm$ 4.8	0.07	7.0 $\pm$ 1.21	4.7 $\pm$ 0.48	t _{Treatment} =1.77,	0.12
<i>Pentachlaena latifolia</i>	42.3 $\pm$ 4.7	45.5 $\pm$ 6.8	0.71	3.8 $\pm$ 0.52	4.2 $\pm$ 0.13	t _{Treatment} =-0.84	0.46
<i>Aphloia theiformis</i>	40.7 $\pm$ 2.4	43.3 $\pm$ 2.8	0.64	10.8 $\pm$ 0.56	11.8 $\pm$ 0.41	t _{Treatment} =-1.48	0.18
<i>Carissa edulis</i>	99.2 $\pm$ 0.8	96 $\pm$ 1.95	0.08	6.3 $\pm$ 0.13	7.4 $\pm$ 0.12	t _{Treatment} =-6.09	<0.001 ***
<i>Uapaca bojeri</i>	0	0	-	0	0	-	-
<i>Vaccinium secundiflorum</i>	NT	26.2 $\pm$ 3.5	-	NT	19.7 $\pm$ 0.63	-	-

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538 **Table 4:** Average percent of germination (PG) and mean germination time (MGT) of three woody species from tapia woodland (Madagascar)
539 tested under laboratory conditions for control and dry heat treatments. Germination was monitored for 8 weeks in a culture chamber at a constant
540 temperature of 25°C and a 12 hours light regime. No germination was observed in *Uapaca bojeri* (NT = not tested). NC = not calculated. GLM
541 results for PG were significant for the interaction Temperature × Time for all species.

Temperature	Time	<i>Aphloia theiformis</i>		<i>Carissa edulis</i>		<i>Vaccinium secundiflorum</i>	
		PG Mean ± SE	MGT Mean ± SE	PG Mean ± SE	MGT Mean ± SE	PG Mean ± SE	MGT Mean ± SE
Control		43.3 ± 2.8	11.8 ± 0.4	96 ± 1.95	7.4 ± 0.1	26.2 ± 3.5	19.7 ± 0.6
40	10	38.7 ± 2.7	11.6 ± 0.9	95.3 ± 1.9	7.7 ± 0.1	13.4 ± 3.4	19.1 ± 0.3
40	30	39.9 ± 2.1	13.9 ± 1.0	97.5 ± 1.6	7.8 ± 0.3	9.6 ± 4.5	22.1 ± 2.2
40	60	43.3 ± 3.0	13.3 ± 0.9	94.3 ± 1.0	8.0 ± 0.3	9.7 ± 3.0	21.4 ± 1.1
40	90	28.7 ± 3.1	13.7 ± 0.7	91.2 ± 3.0	8.2 ± 0.4	12.6 ± 1.5	20.3 ± 1.2
60	10	NT	NT	91.2 ± 4.6	6.6 ± 0.1	6.3 ± 4.3	31.4 ± 2.5
60	30	NT	NT	88.8 ± 3.9	6.8 ± 0.2	3.0 ± 0.6	27.0 ± 3.8
60	60	NT	NT	83.2 ± 5.0	7.1 ± 0.2	3.7 ± 0.3	33.3 ± 1.8
60	90	NT	NT	73.6 ± 4.1	9.0 ± 0.7	5 ± 0.0	32.9 ± 1.6
80	10	26.7 ± 2.4	14.8 ± 0.8	6.4 ± 2.0	5.1 ± 1.3	18.8 ± 3.9	18.5 ± 0.7
80	30	17.3 ± 3.2	15.3 ± 0.9	8.8 ± 1.5	6.6 ± 0.9	17.8 ± 0.2	19.1 ± 0.6
80	60	14.0 ± 3.1	16.6 ± 0.6	10.4 ± 2.7	6.9 ± 0.5	20.2 ± 0.8	20.7 ± 0.04
80	90	17.3 ± 4.3	17.6 ± 2.2	8.8 ± 1.5	5.0 ± 0.6	17.4 ± 1.6	20.8 ± 0.2
120	10	6.7 ± 1.1	27.7 ± 1.6	4.8 ± 2.0	4.8 ± 1.7	18.2 ± 4.0	23.4 ± 0.4
120	30	0 ± 0	NC	17.6 ± 3.5	6.4 ± 0.5	14.7 ± 1.8	23.4 ± 0.6
120	60	0 ± 0	NC	20.8 ± 3.2	6.0 ± 0.4	---	NC
120	90	0 ± 0	NC	21.6 ± 3.7	5.6 ± 0.23	0.3 ± 0.3	2.7 ± 2.7
100	5	NT	NT	81.6 ± 3.9	7.4 ± 0.8	4.7 ± 1.5	21.9 ± 0.9
120	5	NT	NT	75.2 ± 4.08	7.4 ± 0.3	12 ± 0.6	34.5 ± 1.2
Statistic result		P(>Chi) Temperature x Time < 0.001	F _{Temperature} =49.9, P<0.001	P(>Chi) Temperature x Time < 0.001	Kruskal-Wallis chi ² Temperature = 38.23, p- value<0.001	P(>Chi) Temperature x Time < 0.001	F _{Temperature x Time} = 5.52, p-value<0.001

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Figure 1: Percent of germination of the three (out of five) species studied using various treatments to test the effect of dry heat pre-germination. Four temperatures (40°C, 60°C, 80°C and 120°C) were evaluated; for each temperature, seeds were exposed to heat for 10, 30, 60 and 90 minutes. To simulate a hot, fast-burning fire, seeds were exposed to dry heat at two temperatures (100°C and 120°C) for 5 minutes. a) *Aphloia theiformis*, b) *Carissa edulis*, c) *Vaccinium seccundiflorum*. White bars (control), light grey bars (seeds heated at 40°C), grey bars (seeds heated at 60°C), dark grey bars (seeds heated at 80°C),

551 **black bars (seeds heated at 120°C), barred bars (seeds heated at 100°C for 5 minutes).**
552 **Post-hoc results are showed by letters on the bar (a, b, c, etc.).**