Seed germination of the invasive species *Piper aduncum* as influenced by high temperature and water stress

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Summary

*Piper aduncum* is a common woody weed throughout the tropics and a successful invader in many areas. As this species most often dominates roadsides and forest margins, and these habitats frequently experience extreme changes in temperature and water availability, it is important to understand how the seeds adapt to these stresses. This study investigated the effect of high temperatures and water stress, continuous, periodic or transient, on *P. aduncum* seed germination before or after imbibition. It was found that *P. aduncum* seeds exhibited only intermediate tolerance and did not germinate at temperatures above 35°C or water potentials below –0.6 MPa. However, this species is well adapted to local conditions, with fruit ripening in the rainy season, seed desiccation tolerance to eRH 8%, rapid germination under wet conditions, higher temperature tolerance than the maximum ground temperature, germination of most seeds after 60 h continuous heat treatment or daily periodic heat treatments up to 5 h at 40°C and insensitivity to 12 h heat treatment at 40°C during the whole germination process. The intermediate tolerance to high temperature and water stress of *P. aduncum*, in combination with local environmental conditions in Xishuangbanna, makes *P. aduncum* an intermediate invader in this area.

Keywords: invader, seed germination, high-temperature tolerance, desiccation tolerance, heat shock, water stress.


Introduction

*Piper aduncum* L. (Matico tree) is native to the West Indies and mainland tropical America from Mexico to northern Argentina and has a number of uses, including traditional medicines and agroforestry. It was introduced as a fragrant plant into Xishuangbanna, south–west China, in the 1970s or 1980s, but has since escaped and is now common along roadsides and in thickets. Although this species has been thought as an important invader in Xishuangbanna, it is difficult to predict whether it will be a problem as serious as *Chromolaena odorata* (L.) R.M. King & H.E. Robins. in this area.

*Piper aduncum* has become invasive in many places across the tropics, including Fiji, Papua New Guinea, several parts of Indonesia, Singapore and Malaysia (Lepš et al., 2002; Siges et al., 2005; Hartemink, 2010; Padmanaba & Sheil, 2014; Global Invasive Species Database, 2005). In these areas, *P. aduncum* sometimes
replaces native pioneer species to alter the early succession on abandoned fields and may also compete with crops for water, nutrients and light. The seeds are widely dispersed by both birds and fruit bats in tropical Asia, but it has not been reported to invade closed-canopy forests.

Despite the successful spread of *P. aduncum*, very little is known about its basic biology. In its invasive range, *P. aduncum* grows only in open or semi-shaded sites, suggesting that it has specific adaptations to these conditions. These habitats are usually characterised by high light intensity and enhanced fluctuations in temperature and water availability. High light intensity favours the germination of *P. aduncum* seeds, which are positively photoblastic and do not germinate in the dark (Rocha *et al.*, 2005; Dousseau *et al.*, 2011), but how seeds of this species respond to high temperature and water restriction remains unknown. The study reported here investigated the responses of *P. aduncum* seed germination to temperature and water potential, with the intention of improving our understanding of its advantages and limitations as an invasive species. For this purpose, two experiments were designed to assess the effects of relative humidity and high temperature on quiescent seeds, and then two experiments were carried out to investigate the temperature and water requirements for seed germination. Two more experiments were performed to assay the response of seed germination to high temperatures, and lastly, an experiment was conducted to determine the stages of seed germination that are sensitive to high temperature and water stress.

**Materials and methods**

**Seed collection**

*Piper aduncum* has a long seed maturing season in Xishuangbanna, with ripe infructescences available from May to October. Seeds used in the experiments came from three collections in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (21°55’N, 101°15’E) between July and September 2013. After collection, seeds were extracted and air-dried for half a day. Initial moisture content was then determined using the gravimetric method, after drying at 103°C for 17 h, and is reported on a fresh mass basis. Seed weight (100 seeds × 10 replicates) and initial viability (50 seeds × 6 replicates) were also assessed. The remaining seeds were kept at 15°C and used for experiments within 7 days after collection, with seeds in the same experiment from the same seed lot.

**Experiment arrangement**

High temperature and water stress can occur in different ways: briefly or over a long period, periodically or continuously, or before or during seed germination. We therefore designed a set of experiments to test the tolerance and response of *P. aduncum* seeds to these stresses.

**Effects of relative humidity on seed moisture content and viability**

Response to relative humidity was investigated by equilibrating seeds for 1 week at 25°C over ten saturated salt solutions (KOH, K acetate, K2CO3, NH4NO3, NaCl, NH4Cl, (NH4)2SO4, KCl, BaCl2, KNO3), providing an eRH spectrum between 8 and 95% according to Hor *et al.* (2005). After treatment, seed moisture content and viability were determined.

**High-temperature tolerance of quiescent seeds**

A temperature range from 30 to 95°C with steps of 5°C provided by a water bath was used to investigate the tolerance of quiescent seeds to high temperature. Around 400 air-dried seeds in each tube were heated for half an hour at each temperature. As both dry and imbibed seeds may experience this heat shock in the field, two such tubes were used for each temperature, with one of them having a few drops of water added half an hour before heat treatment. Then seeds were sown for viability assessment.

**Effects of incubation temperature on seed germination**

Seeds were sown on 1% plain agar in Petri dishes and placed in incubators set at 10, 15, 20, 25, 30, 35 and 40°C, with 12 h photoperiod of 25 μmol m−2 s−1 irradiance provided by a white fluorescent light. This is the range of temperature we were interested to check the seed germination characteristics, for 10°C is close to the mean minimum temperature in the coldest month in this area and 40°C to the maximum ground temperature recorded in a rainforest gap in summer (Liu *et al.*, 2000). In parallel, 300 seeds were sown on moistened filter paper incubated at 25°C to record the germination time course.

**Effects of water availability on seed germination**

A range of osmotic potentials from −0.05 to −0.6 mPa were created within 5.5-mm-diameter Petri dishes using PEG-8000 (polyethylene glycol). The equation described by Michel (1983) was employed to calculate water potentials of the solutions at 25°C. These seeds were placed in an incubator set at 25°C.
for germination. Meanwhile, equal osmotic potentials to the PEG treatment, calculated according to Lang (1967), were created by NaCl solutions of different concentrations to investigate the effects of salinity stress on seed germination. Deionised water was used as a control medium. Also, seeds were sown on filter paper discs moistened by the above solutions and incubated at 25°C.

**Effects of continuous heat treatment on seed viability**

This experiment was designed to investigate the response of *P. aduncum* seed germination to a continuous 40°C heat treatment, because this was the maximum ground temperature recorded in a rainforest fragment in Xishuangbanna (Liu et al., 2000). Seeds sown on 1% plain agar in Petri dishes were placed in an incubator at 40°C. After heat treatment for the indicated period of time, they were withdrawn from the incubator and placed under ambient conditions (air-conditioned at 25°C and normal light) to check seed viability. The heat treatment time included 0, 6, 12–72 h with step of 12 h, and then to 240 h with step of 24 h.

**Effects of periodic high-temperature treatment on seed germination**

Seeds sown on 1% agar were exposed to alternations of 1/23 h, 2/22 h, 3/21 h, 5/19 h, 7/17 h, 9/15 h, 12/12 h and 15/9 h between 40°C and ambient temperature (air-conditioned at 25°C) to investigate the tolerance of seed germination to daily heat shocks.

**Stress-sensitive stages during seed germination**

Seeds sown on filter paper discs moistened with deionised water in Petri dishes were incubated at 25°C. These Petri dishes were sampled once every 12 h until 168 h after incubation, when they were either transferred to 40°C for 12 h heat treatment and then put back to 25°C, or air-dried at 50% RH and 15°C for 72 h, then watered and incubated at 25°C for viability assessment.

**Seed viability and germination assessment**

For each treatment, 50 seeds × 6 replicates were used. Moistened filter paper was used as a germination medium, except where 1% agar is specified in the methods. For seeds sown on filter paper discs, the six Petri dishes with the same treatment were sealed in a resealable double-clear plastic bag to minimise moisture loss during the experiment. The filter paper disc was initially saturated with 1.5 ml of deionised water and remoistened when needed. In the water stress experiments, including the PEG and NaCl solution treatments, the solutions with corresponding water potential were used to moisten filter paper discs. Every other day, the Petri dishes were taken out to drain the old solutions and add fresh ones. These seeds were incubated at 25°C in light, except in the germination temperature range experiment. The seeds were scored once a week until all seeds germinated or decayed. Those with the radicle protruding to 5 mm were considered to have germinated, or survived for stress-treated seeds, and they were considered to have emerged when a normal seedling formed. Meanwhile, a crush test was employed to determine decay of non-germinated seeds at the end of the test. The only exception was that seeds assigned to 10°C in the germination temperature range experiment, which had not germinated by the time the experiment finished at the other temperatures, were removed to 25°C for viability assessment after 8 weeks' incubation at 10°C.

**Data analysis**

Data are presented as means and standard errors and subjected to one- or two-way ANOVA and Duncan's multiple comparison tests (α = 0.05) after arc-sine transformation, using SPSS 13.0 for Windows.

**Results**

*Piper aduncum* had small, black seeds, with a 100-seed weight of 18.05 ± 0.86 mg, an initial moisture content of 13.51 ± 0.85% (fresh weight basis), and had an initial germination of 89.67 ± 3.74%. The seeds germinated rapidly when incubated at 25°C on filter paper moistened by deionised water, for the first germination recording was documented after 96 h and most seeds germinated within 2 week.

**Effects of relative humidity on seed moisture content and viability**

One week's equilibration over the ten saturated salt solutions produced seeds with moisture contents ranging from 6% to 20%. Equilibrium relative humidity had highly significant effects on seed moisture content, but no significant effects on seed viability, with seed survival and emergence not reduced even after desiccation to the lowest moisture content (Fig. 1, Table 1).

**High-temperature tolerance of quiescent seeds**

Air-dried seeds had high tolerance of short-term high-temperature shock, with survival and emergence
showing essentially no reduction up to 70°C. A temperature of 75°C was a threshold, with 30 min heat treatment killing more than half of the seeds. No seeds germinated after treatment above 80°C (Fig. 2, Table 1). Imbibed seeds were more sensitive to high temperature, with significant effects when temperature was raised to 45°C, only 30% germination after exposure to 55°C, and no seeds surviving higher temperatures. Two-way ANOVA indicated that air-dried and imbibed seeds exhibited significantly different responses to short-term high-temperature treatment ($P<0.001$ for treatment temperature, material and treatment temperature $\times$ material, for both survival and emergence).

Effects of incubation temperatures on seed germination

*Piper aduncum* seeds had a wide temperature range for germination, with an emergence of at least 80% when incubated at 15–30°C. Variation analysis indicated that 25°C gave maximum germination, but emergence and germination percentages were not significantly different to those incubated at 15, 20 or 30°C (Fig. 3, Table 1). When incubated at 35°C, only about a quarter of seeds showed radical protrusion, but these failed to form seedlings. No germination was observed for seeds incubated at 10°C, but they germinated soon after transfer.

### Table 1 One-way analysis of variance for germination of *Piper aduncum* seeds

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Variable</th>
<th>d.f.</th>
<th>F-value</th>
<th>P-value</th>
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<tr>
<td>Relative humidity on seed viability</td>
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<td>9</td>
<td>0.94</td>
<td>0.499</td>
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<td>Survival</td>
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<td>1.31</td>
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<td>Heat shock on air-dried seeds</td>
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<td>13</td>
<td>83.84</td>
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<tr>
<td></td>
<td>Survival</td>
<td>13</td>
<td>131.39</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Heat shock on imbibed seeds</td>
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<td>13</td>
<td>290.79</td>
<td>$&lt;0.001$</td>
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<tr>
<td></td>
<td>Survival</td>
<td>13</td>
<td>373.53</td>
<td>$&lt;0.001$</td>
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<tr>
<td>Incubation temperatures</td>
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<tr>
<td></td>
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<td>$&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>Germination</td>
<td>10</td>
<td>109.39</td>
<td>$&lt;0.001$</td>
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<tr>
<td>Water stress by NaCl</td>
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<td>14</td>
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<td>Periodical high-temperature stress</td>
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</table>

Bold number indicates significant at the 0.05 level.
to 25°C, with an emergence percentage of 82.7%. Seeds incubated at 40°C also failed to germinate, but they went mouldy, unlike those incubated at 10°C.

**Effects of water availability on seed germination**

When using PEG as the osmoticum, a water potential of −0.2 mPa significantly reduced both germination and emergence compared with the control (deionised water, 0 mPa). Germination was completely inhibited at −0.6 mPa (Fig. 4, Table 1). Seeds treated with NaCl solutions had markedly lower germination for equal water potentials than those treated with PEG solutions. A water potential of −0.05 mPa significantly decreased both emergence and germination compared with the control and no seeds emerged at −0.25 mPa (Fig. 4, Table 1). Two-way ANOVA showed significant differences between the effects of water potentials created by PEG and NaCl solutions ($P < 0.001$ for water potential, reagent and water potential × reagent, for both survival and emergence), suggesting salt toxicity in addition to osmotic stress when NaCl solutions were used.

**Effects of continuous heat treatment on seed viability**

There was a significant difference in both survival and emergence among continuous heat treatments at 40°C (Table 1). Even a 6 h treatment had a significant effect and treatment for 72 and 96 h reduced seedling formation to 50% and 30%, respectively. Only a few seeds germinated when treated for more than 150 h (Fig. 5, Table 1).

**Effects of periodic high-temperature treatment on seed germination**

Daily heat treatments of 3 h or less affected neither germination nor emergence, but a daily 4 h treatment reduced both. With a daily 7 h treatment, more than half the seeds failed to form seedlings and few seeds emerged with a daily 9 h treatment (Fig. 6, Table 1).

**Stress-sensitive stages during seed germination**

Desiccation following 12 h imbibition had a significant effect on both survival and emergence, while desiccation after 72 and 120 h imbibition killed 60% and 90% of seeds respectively (Fig. 7, Table 1). This may be related to the initiation of seed germination. In contrast to the desiccation sensitivity after imbibition, imbibed *P. aduncum* seeds exhibited obvious tolerance to heat treatment at all stages investigated. Almost all seeds germinated and formed morphologically normal
seedlings, except those heated after imbibing for 108 to 120 h, whose seedling percentage had a slight reduction (Fig. 7, Table 1).

Discussion

A previous study showed that *P. aduncum* has an invasive advantage over other pioneer species because of its dominance in the soil seedbank, extremely rapid growth rate and high rate of biomass accumulation (Rogers & Hartemink, 2000). Although this shrub can spread locally by sprouts and suckers, forming large clumps, long-distance dispersal is by seeds (Lepš et al., 2002). *Piper aduncum* has a long seed maturation season in Xishuangbanna and produces numerous small seeds every year, but these must germinate for invasion to occur.

This study used only fresh seeds to conduct experiments, with seeds used in each experiment only from the same collection within 7 days after harvest. Although from three collections, these seeds should not differ in germination response because they developed and maturated under similar condition, hot and wet, and all were collected during the rainy season. Dormancy in seeds of this species has neither been reported in literature, nor found in this study. In this study *P. aduncum* had a maximum germination percentage above 80% in all experiments, which was immediate compared with previous reports (Rocha et al., 2005; Silva et al., 2007; Dousseau et al., 2011). Our results showed that *P. aduncum* seeds failed to germinate at temperatures above 35°C (Fig. 3) or water potentials below −0.6 MPa (Fig. 4). This is in agreement with Silva et al. (2007), who demonstrated the sensitivity to water shortage in seeds of this species.

*P. aduncum* seeds tolerate desiccation at maturity, but they lose this tolerance soon after imbibition (Fig. 7), as most orthodox seeds do (Daws et al., 2007). In comparison with other tropical plant species, the responses of *P. aduncum* seeds to high temperature and water stress are intermediate. Some weed seeds, such as *Urena lobata* L. (Wang et al., 2009), *Eriochloa villosa* (Thunb.) Kunth. (Bello et al., 2000), *Dactyloctenium aegyptium* (L.) Wild. (Burke et al., 2003) and *Solanum sarrachoides* Sendtner (Zhou et al., 2005), can germinate at a constant temperature of 40°C, although with reduced germination percentage, and a few can tolerate 45°C, such as *Cassia occidentalis* L. (Norsworthy & Oliveira, 2005) and *Senna obtusifolia* (L.) Irwin & Barneby (Norsworthy & Oliveira, 2006), and even 50°C, such as *Ipomoea lacunosa* L. (Oliveira & Norsworthy, 2009), *Cassia occidentalis* L. (Norsworthy & Oliveira, 2005) and *Senna obtusifolia* (L.) Irwin & Barneby (Norsworthy & Oliveira, 2006), had maximum germination at 40°C. Similarly for water stress, some species, such as *Eupatorium adenophorum* Sprengel (Li & Feng, 2009), *Eclipta prostrata* (L.) L. (Chauhan & Johnson, 2008a), *Echinochloa colona* (L.) Link (Chauhan & Johnson, 2009) and *Ougeinia dalbergioides* Benth. (Uniyal & Nautiyal, 1998), retained 20–70% seed germination under water stress up to −0.6 MPa, and some seeds germinated up to −1.0 MPa, such as *Solanum sarrachoides* (Zhou et al., 2005), *Mimosa invisa* Mart. ex Colla (Chauhan & Johnson, 2008b) and *Ceratocarpus arenarius* L. (Ebrahim & Esfandi, 2011). Invasive weeds *Emex spinosa* (L.) Campd. and *Emex australis* Steinh. had similar behaviour (Javaid & Tanveer, 2014). Thus, *P. aduncum* seeds did not exhibit a strong tolerance to high temperature and water restriction. Also, Luo and Cardina (2012) failed to prove that invasive species germinate better than non-invasives under various conditions. A
literature analysis revealed that physical environment mechanisms are less general, compared with enemy release mechanisms and resources mechanisms in invasive plant species (Ren & Zhang, 2009).

Colonisation success is determined by many factors, with seed germination only one of them. While the results reflect the response of seeds from a single location and thus possibly do not cover the global range of variation for the species, *Piper aduncum*’s seeds were not exceptional and were well adapted to local environmental conditions. Xishuangbanna has hot, wet summers followed by a cool, foggy and then a hot, dry season. *Piper aduncum* fruits ripen and their seeds are dispersed between May and October, in the hot, wet season. Previous studies documented a maximum ground temperature of 40.9°C in a rainforest gap in the dry season (Liu et al., 2000), and 70°C in a bare field, which do not exceed the extreme temperatures that can be endured by quiescent seeds, imbibed and air-dried seeds respectively (Fig. 2). Although the seeds cannot germinate in a constant 40°C, daily treatments up to 3 h at this temperature made no difference to their emergence (Fig. 6) and most seeds emerged after 60 h continuous heat treatment (Fig. 5) or 5 h daily treatments (Fig. 6). The rapid germination of *P. aduncum* seeds allows them to establish during wet periods. On the other hand, deforestation and habitat disturbance in this area (Li et al., 2009) provide many opportunities for the shade-intolerant *P. aduncum* to invade. It does best in habitats, such as roadsides and forested ridges with plentiful light, high but not extreme temperatures, and soils that stay moist after rain.

Xishuangbanna is situated on the northern margin of the Asian tropics and receives less annual rainfall compared with the typical tropics (Zhu, 2004). In Xishuangbanna, *P. aduncum* occurs only along roadsides and in thickets, although this species can grow in open sites and form monospecific stands in Malaysia and Kalimantan (Hartemink, 2010), while *Chromolaena odorata* can grow and form tickets in open fields in Xishuangbanna because it has a markedly higher tolerance to abiotic stress (Quan et al., 2011) than *P. aduncum*. Thus, the intermediate tolerance to high temperature and water stress of *P. aduncum*, in combination with local environmental conditions of Xishuangbanna, made *P. aduncum* an intermediate invader in this area. This species has been described as an aggressive invader in some countries (Hartemink, 2010), but has only limited success in invasion to Xishuangbanna, restricted to relatively wetter and cooler habitats, such as roadsides and forest ridges. We suggest that *P. aduncum* will remain as an intermediate invader in this area, and not become a problem as serious as *Chromolaena odorata*.

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References


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