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Sympatric species of *Hibbertia* (Dilleniaceae) vary in dormancy break and germination requirements: implications for classifying morphophysiological dormancy in Mediterranean biomes

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• *Background and Aims* Several ecologically important plant families in Mediterranean biomes have seeds with morphophysiological dormancy (MPD) but have been poorly studied. The aim of this study was to understand the seed ecology of these species by focusing on the prominent, yet intractably dormant Australian genus *Hibbertia*. It was hypothesized that the slow germination in species of this genus is caused by a requirement for embryo growth inside the seed before germination, and that initiation of embryo growth is reliant upon a complex sequence of environmental cues including seasonal fluctuations in temperature and moisture, and an interplay with light and smoke. Using the results, the classification of the MPD level in species of *Hibbertia* is considered.
• *Methods* Four species of *Hibbertia* in winter rainfall south-western Australia were selected. These species, whilst differing in geographic distributions, are variously sympatric, and all are important understorey components of plant communities. The following aspects related to dormancy break, embryo growth and germination were investigated: temperature and moisture requirements; effects of karrikinolide, gibberellic acid and aerosol smoke; and phenology.
• *Key Results* Following exposure to wet/dry cycles at low or high temperatures, embryo growth and germination occurred, albeit slowly in all species at low temperatures when moisture was unlimited, corresponding to winter in south-west Australia. Photo regime influenced germination only in *H. racemosa*. Aerosol smoke triggered substantial germination during the 1st germination season in *H. huegelii* and *H. hypericoides*.
• *Conclusions* Although the study species are con-generic, sympatric and produce seeds of identical morphology, they possessed different dormancy-break and germination requirements. The physiological component of MPD was non-deep in *H. racemosa* but varied in the other three species where more deeply dormant seeds required >1 summer to overcome dormancy and, thus, germination was spread over time. Embryos grew during winter, but future studies need to resolve the role of cold versus warm stratification by using constant temperature regimes. To include Mediterranean species with MPD, some modifications to the current seed-dormancy classification system may need consideration: (*a*) wet/dry conditions for warm stratification and (*b*) a relatively long period for warm stratification. These outcomes have important implications for improving experimental approaches to resolve the effective use of broadcast seed for ecological restoration.

Key words: Framework species, germination phenology, *Hibbertia*, karrikinolide, Mediterranean biome, morphophysiological dormancy, restoration, seeds, smoke, underdeveloped embryos.

INTRODUCTION

One of the most commonly accepted classification schemes for seed dormancy was developed originally by Nikoleava (1967, 1977) and subsequently modified and proposed as an international standard by Baskin and Baskin (2004). The system is hierarchical with five classes further divided into levels and types. Now used the world over, this scheme is based upon (*a*) innate properties of seeds including seed- (or fruit-) coat permeability to water, embryo morphology, and whole-seed physiology, and (*b*) the nature and length of environmental cues that alleviate dormancy [the primary environmental cues are moist-cold (0–10 °C) conditions (cold stratification) and moist-warm ($\geq$15 °C) conditions (warm stratification)]. These innate properties of seeds should be universally usable to classify dormancy in any biome, but a challenge to this premise is that specific ecological conditions and therefore dormancy-break requirements vary among and within biomes. Understanding these parameters is fundamental to improving the use of seed in ecological restoration, particularly broadcast seeding where seed wastage can be considerable (Merritt and Dixon, 2011).

Due to a high number of research papers originating from a relatively small region, study-location biases have entered into the classification scheme as is the case for morphophysiological dormancy (MPD). Seeds with MPD have tiny embryos that must develop considerably inside the seed before radicle emergence (hereafter, called underdeveloped) but they also have a

physiological block that must be overcome before, during, or after embryo growth. MPD is most prominent among species from temperate broad-leaved evergreen and deciduous forests and from boreal forests (Baskin and Baskin, 2003). As such, this class of dormancy has been studied best on species from these biomes and a classification system for levels of MPD has been developed mostly based on them (Nikolaeva, 1967, 1977; Baskin and Baskin, 1998, 2004). Although species presumably with MPD occur in Mediterranean biomes, very little work has been done on them (Baskin and Baskin, 1998).

Fire-stimulated germination is pronounced in Mediterranean biomes, and this is a unique environmental variable that needs to be considered for species with MPD. Seeds of many Mediterranean species persist in soil for many years and germinate synchronously after a fire (Dixon and Barrett, 2003). Heat and smoke are the main components of fire influencing germination (Dixon *et al.*, 2009). Karrikinolide (KAR_1) is a compound in smoke that elicits remarkable germination (Chiwocha *et al.*, 2009). It is similar to other plant growth regulators, being active at very low concentrations, and may influence gibberellin (GA) signalling for germination (Nelson *et al.*, 2009). However, smoke-responsive species also possess dormancy and germination is difficult even in the presence of smoke. Because of differing responses to temperature, moisture and fire-related cues, categorizing the level of MPD in Mediterranean species using the current classification system may be very different from that for temperate and boreal forest species.

To understand better the seed ecology of species with small embryos in a Mediterranean biome, the Australian framework (dominant and/or ecologically significant) family Dilleniaceae was selected. Within this family of predominantly woody shrubs, the genus *Hibbertia* contains the most species, with 100–200 species (Mabberley, 1997; Horn, 2005). Members are Gondwanan in origin, found from Madagascar and Malesia to New Caledonia and Fiji. The majority of species occur in Australia, particularly the south-west biodiversity hotspot, where they are often co-dominant shrubby components in various woodlands and kwongan vegetation communities (Stebbins and Hoogland, 1976; Grieve, 1998; Pate and Bell, 1999; Western Australian Herbarium, 2009).

The germination process in *Hibbertia* is slow – seeds of several species take on average 37–76 d for seedling emergence (Kullmann, 1981), a feature that means this genus is unreliable in propagation programmes and ecological restoration where broadcast seeding requires seed to be cued for germination (Merritt and Dixon, 2011). Germination is influenced by slow imbibition and seed ageing in the soil, and may be stimulated by exogenous application of GA_3 (Schatral, 1996; Schatral *et al.*, 1997; Roche *et al.*, 1997*a*; Allan *et al.*, 2004). Application of smoke, as smoke water or as an aerosol, occasionally increases germination under *ex situ* conditions and enhances seedling emergence in the field (Dixon *et al.*, 1995; Roche *et al.*, 1997*b*, 1998; Clarke *et al.*, 2000; Tieu *et al.*, 2001). Altogether, these data show a physiological barrier to germination in *Hibbertia* seeds. Additionally, seeds also have a rudimentary embryo (Schatral, 1995), which may need to grow as a prerequisite for radicle emergence indicating the presence of MPD.

Our hypothesis is that slow germination in *Hibbertia* species is caused by a requirement for embryo growth inside the seed before germination, and that initiation of embryo growth is reliant upon a complex sequence of environmental cues including seasonal fluctuations in temperature and moisture, and an interplay with light and smoke. To test this hypothesis, firstly the following aspects were determined: (*a*) temperature and moisture requirements for dormancy break and germination; (*b*) conditions under which embryo growth, if any, occurs before radicle emergence; and (*c*) effects that the germination stimulants GA_3, KAR_1 and smoke have on dormancy break and germination. Secondly, dormancy break, embryo growth (if it occurs) and germination were considered in relation to annual *in situ* cycles of temperature and precipitation and how dormancy status interacts with smoke, a crucial germination cue in synchronizing the emergence of *Hibbertia* species in fire-prone natural habitats. Finally, we consider the classification of the MPD level in the four study species of *Hibbertia*. To the best of our knowledge, this study is the first analysis of embryo growth and dormancy and germination characteristics for species with small embryos in a Mediterranean biome with potential applications for species with this trait in the other four regions with distinctive Mediterranean floras [‘maquis’’ (Mediterranean basin), ‘fynbos’ (South Africa), ‘chaparral’ (California) and ‘matorral’ (Chile)].

MATERIALS AND METHODS

Study species

Four species of *Hibbertia* from south-west Western Australia were selected for the study: *H. commutata* Steud., *H. huegelii* (Endl.) F.Muell., *H. hypericoides* (DC.) Benth. and *H. racemosa* (Endl.) Gilg. The four species have slightly different distributions in Western Australia, but all overlap in the Perth region that experiences a typical (lowland) Mediterranean climate. *Hibbertia racemosa* occurs mostly along the west and south coasts of south-western Australia, whereas the other three species are found along the west and south-west coasts and more inland to the western portion of the Avon Wheatbelt with *H. huegelii* having the narrowest range (Western Australia Herbarium, 2009). *Hibbertia commutata* and *H. huegelii* grow in sandy or lateritic soils, *H. hypericoides* is found in a variety of upland habitats, and *H. racemosa* occurs in coastal areas on dunes, plains and limestone. All seeds were wild collected from natural bushland within 120 km of Perth, Western Australia when mature and ready to naturally dehisce: *H. commutata* near Boddington [32°48′1″S, 116°28′28″E, approx. 200 m above mean sea level (a.m.s.l.), *H. huegelii* and *H. hypericoides* near Wanneroo (31°45′07″S, 115°48′14″E, approx. 50 m a.m.s.l.) and *H. racemosa* near Yanchep (31°32′58″S, 115°37′29″E, approx. 20 m a.m.s.l.)]. Before experimentation, seeds were put through a zig-zag aspirator to remove debris and empty seeds, and then they were surface sterilized in 2 % (w/v) $Ca(OCl)_2$ for 30 min followed by thorough rinsing with sterilized distilled water. Viability of 50 seeds per species was determined by a cut-test with examination of the embryo (considered viable when firm and white or nonviable when soft and

grey) and tetrazolium testing (Grabe, 1970) of the embryo. Moreover, during incubation, the arils (Schatral and Fox, 1994) were gently removed to reduce fungal attack.

General procedures

Laboratory studies were done in temperature- and light-controlled incubators set at daily alternating (12/12 h) temperature regimes of 18/7, 26/13 and 33/18 °C, or constant 35 °C. The alternating regimes approximate the mean daily maximum and minimum air temperatures for Perth, from January to March (33/18 °C), in April and from October to December (26/13 °C), and between May and September (18/7 °C) (Bureau of Meteorology, 2009). The light source was 30-W cool white fluorescent tubes with a photon flux density (400–700 nm) delivering at seed level approx. 50 μmol m^{-2} s^{-1}.

Seeds were placed either on two glass microfibre 1.2-μm filter papers (embryo growth studies) or on white quartz sand (germination studies) in 9.0 × 1.5 cm (diameter × depth) plastic Petri dishes. Three replications of 50 seeds per dish were used in each treatment, unless otherwise stated. Either a solution of distilled water or of 0.67 μM KAR_1, synthesized from pyromeconic acid as described in Flematti *et al.* (2005), was added to the dishes. Dishes were wrapped with plastic film to reduce water loss, and those in dark treatments were additionally wrapped in aluminium foil. Protrusion of the radicle was the criterion for germination. At the end of each experiment, embryos were examined and viability determined.

Germination requirements of fresh seeds

Seeds of *H. commutata* and of *H. racemosa* were incubated on wet sand in light or in darkness at 18/7, 26/13 and 33/18 °C for 4 weeks. *Hibbertia huegelii* and *H. hypericoides* seeds were maintained moist with distilled water or with KAR_1 in light or in darkness at 18/7 °C and 35 °C for 4 weeks. At the end of week 4, the number of seeds that germinated, if any, and the number of viable seeds were determined.

Effects of GA_3 on dormancy break and germination

Seeds of *H. huegelii* and of *H. hypericoides* were placed on filter paper moistened with either distilled water (control) or 2.89 mM GA_3 in Petri dishes. Three replications, with 25 seeds each, were used in the control and in the treatment. Seeds were incubated on both solutions at 18/7 °C and at 35 °C for 28 weeks in light with germination being scored every 2 weeks.

Effects of a sequence of temperatures ('move-along') on dormancy break and germination

We modified the move-along experiment described by Baskin and Baskin (2004) to fit environmental conditions of a Mediterranean biome. Seeds of the four *Hibbertia* species were exposed either to continuous temperature regimes or to annual temperature cycles in light (see Fig. 1). In the continuous temperature regimes, seeds were kept at 18/7, 26/13 or 33/18 °C for 84 weeks. Annual cycles simulated the sequence (→) of natural temperatures beginning the 1st summer when seeds are dispersed and ending after the 2nd winter (totalling 84 weeks): 12 weeks of 1st summer (33/18 °C) → 4 weeks of 1st autumn (26/13 °C) → 20 weeks of 1st winter (18/7 °C) → 12 weeks of 1st spring (26/13 °C) → 12 weeks of 2nd summer (33/18 °C) → 4 weeks of 2nd autumn (26/13 °C) → 20 weeks of 2nd winter (18/7 °C). Six dishes in each continuous temperature regime and in the annual cycle were used for two moisture treatments: three dishes were wet and the other three alternating wet/dry (see Fig. 1). In continuous temperatures, seeds were wet/dry during weeks 0–16 and weeks 38–64, corresponding to the wet/dry period in the annual cycle, but they were wet for all other weeks. In the annual cycle, seeds were wet/dry during summer, autumn and spring but wet in winter to simulate moisture conditions in the species' habitats. The wet/dry treatment in this experiment followed the same protocol as that in the warm stratification experiment (see below). Dishes were examined every 2 weeks, and seedlings were counted and removed from the dishes. When needed, water was added to the dishes to keep substrates moist.

Effects of warm stratification on dormancy break and germination

Seeds of the four *Hibbertia* species were warm stratified at 33/18 or 35 °C for 4–16 weeks in light or in darkness and then incubated at 18/7, 26/13 or 33/18 °C in light or in darkness (Table 1). The incubation period was normally 12 weeks since germination progressively increased until weeks 10–12 (S. N. Hidayati, unpubl. res.). During stratification and incubation, seeds of *H. commutata* and of *H. racemosa* were moistened with distilled water but those of *H. hypericoides* and of *H. huegelii* with water or KAR_1. Regardless of solution, all seed sets were subjected to two moisture regimes: continuously wet or alternating wet/dry cycles (Table 1). In wet/dry cycles, seeds were moistened on the first day for 24 h, and then at 4-week intervals, within their respective temperature regime for 24 h with lids placed on top of the Petri dishes. Then, the dishes were uncovered for 1–2 d to let the substrate air-dry. When the substrate was dry, lids were put back onto the Petri dishes until the next wet period. Appropriate controls were maintained throughout the stratification experiment (Table 1). Seeds in darkness were not examined until the end of the stratification and/or incubation period, and those in light were examined every 2 weeks with seedlings, when present, being counted and removed from dishes. When needed, water or KAR_1 solution was added to the dishes to keep the substrate moist.

Temperature requirements for embryo growth

At the beginning of the experiment, 45 seeds of each species (20 seeds for *H. hypericoides*) were placed for 24 h on moist filter paper at room temperature. Under a dissecting microscope equipped with a micrometer, the seeds were bisected with a razor blade (which usually crumbled the seed coat) and the lengths of the endosperm (hereafter, called seed size) and of embryos were recorded. For each species, five additional Petri dishes consisting of 50 seeds (20 for *H. hypericoides*) each were incubated in light at 18/7 °C or

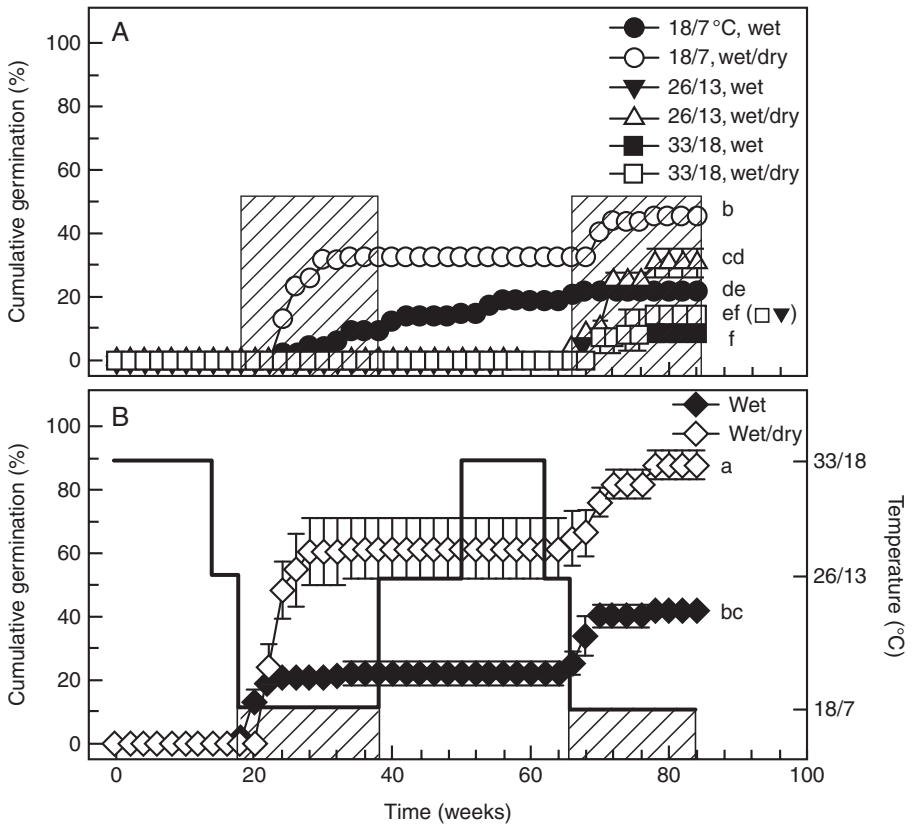

FIG. 1. Effects of incubation at continuous temperature regimes (A) or at a simulated sequence of annual temperature cycles (B) on mean (± s.e.) germination of *Hibbertia commutata* in light. Hatched areas indicate when seeds of both moisture regimes were wet; at all other times seeds were either wet or wet/dry. The continuous line in (B) indicates the sequence of temperature regimes. Means at week 84 with different letters are significantly different (PLSD, $P \leq 0.05$); those with the same letters are not. Where data points are clustered in (A), symbols specify the temperature-moisture regime associated with each letter. Three replicates of 50 seeds were used for each treatment.

at 33/18 °C for 20 weeks. At 4-week intervals, embryo lengths were measured from seeds at both temperature regimes. Coiled embryos were straightened before measurement.

Germination phenology

Eight replications of 100 seeds each of *H. huegelii* and of *H. hypericoides* were sown in 10-cm-diameter plastic pots on 29 February 2008 on a mixture consisting of four parts composted jarrah (*Eucalyptus marginata*) sawdust to two parts nursery sand to one part coarse river sand. To adjust the pH of the soil mix to 6.0, 1 kg of lime and 800 g of dolomite were added per 1 m^3 of soil. Before use, the soil mixture and pots were steam-pasteurized for 1 h at 60–70 °C. Pots were placed on a bench in a tunnel-house covered with fibreglass, located in Kings Park and Botanic Garden (Perth), with no heating or air-conditioning and only the roof and the top-half of its sides covered forming a rain-out shelter. Four pots of each species were exposed to aerosol smoke on 28 April 2008 and again on 21 April 2009; the other four pots (control) were not exposed to smoke. Smoke was produced by hay burnt in a metal drum and fan-forced through a pipe into a windowless smoke-shed for 1 h with doors closed (Dixon *et al.*, 1995). Following the smoke treatment, pots were returned to the tunnel-house. Soil was watered to maximum capacity and allowed to drain weekly from spring to early autumn (29 February 2008 to 31 March 2008, 1 September 2008 to 31 March 2009, 1 September 2009 to 2 October 2009), twice a week (Tuesday and Friday) in mid-autumn (first 2 weeks in April 2008 and in April 2009); and daily in late autumn and winter (rest of 2008 and 2009). Maximum and minimum daily air temperatures throughout the study (29 February 2008 to 2 October 2009) were determined from regional records at the Perth weather station (#009225; Bureau of Meteorology, 2009), approx. 5 km from Kings Park, and from a data logger (Tinytag) on top of the soil surface in a pot in the tunnel-house; daily precipitation was obtained from the Perth Metropolitan weather station. Scoring was done weekly with seedlings being removed until 2 October 2009 when the study terminated.

Statistical analyses

Germination percentages were calculated based on the number of viable seeds (laboratory tests) or on the number of seeds sown (phenology study), and they were arcsine square-root transformed for statistical analyses. Means of embryo lengths and of germination percentages from controls and/or treatments were compared by *t*-tests or by one-way analyses of variances (ANOVAs) followed with protected least

TABLE 1. *Duration and regimes of temperature and moisture and light conditions (L = light, D = darkness) for stratification and controls in experiments on four species of* Hibbertia

Stratification	Incubation after stratification	Controls
H. commutata and *H. racemosa*		
4, 8, 12, or 16 weeks wet; 33/18 °C; L*	12 weeks wet; 18/7, 26/13 or 33/18 °C; L	28 weeks wet; 18/7, 26/13 or 33/18 °C; L
4, 8, 12, or 16 weeks wet/dry; 33/18 °C; L*	12 weeks wet; 18/7, 26/13 or 33/18 °C; L	16 weeks wet/dry then 12 weeks wet; 18/7, 26/13 or 33/18 °C; L
16 weeks wet; 33/18 °C; L	4 weeks wet; 18/7, 26/13 or 33/18 °C; D	20 weeks wet; 18/7, 26/13 or 33/18 °C; D
16 weeks wet; 33/18 °C; D	4 weeks wet; 18/7, 26/13 or 33/18 °C; L	
16 weeks wet; 33/18 °C; D	4 weeks wet; 18/7, 26/13 or 33/18 °C; D	
H. hypericoides and *H. huegelli*		
4, 8, 12 or 16 weeks wet; 35 °C; L	12 weeks wet; 18/7 °C; L	28 weeks wet; 18/7 or 35 °C; L
4, 8, 12 or 16 weeks wet/dry; 35 °C; L	12 weeks wet; 18/7 °C; L	16 weeks wet/dry then 12 weeks wet; 18/7 or 35 °C; L
4, 8, 12 or 16 weeks wet; 35 °C; D	12 weeks wet; 18/7 °C; D	28 weeks wet; 18/7 or 35 °C; D
4, 8, 12 or 16 weeks wet; 35 °C; D	Scored and discarded following stratification	Ascertained amount of germination during stratification

* *Hibbertia racemosa* seeds were not stratified for 4 weeks.

significant difference tests (PLSDs, $P = 0.05$) (SAS, 2008). Initial size of embryos was compared among species by a one-way ANOVA, while that of seeds was done by Welch's ANOVA since no transformation corrected for heteroscedasticity.

RESULTS

Seed and embryo viability

Viability of embryos varied from 86 % to 94 % across all species.

Germination requirements of fresh seeds

Only 0–3 % of fresh seeds from all species germinated following 4 weeks of incubation regardless of temperature regime, light conditions or type of solution (data not shown).

Effects of GA_3 on dormancy break and germination

Seeds (with seed coats intact) of *H. huegelii* and of *H. hypericoides* germinated significantly higher on GA_3 (51 ± 5 % and 40 ± 5 %, respectively) than on water (3 ± 1 % and 4 ± 4 %, respectively) during 28 weeks at 18/7 °C (ANOVA, $P \leq 0.0002$). Seeds of neither species germinated at 35 °C regardless of treatment.

Effects of a sequence of temperatures ('move along') on dormancy break and germination

Seeds of all four species subjected to the simulated sequence started to germinate during winter (18/7 °C) following summer (33/18 °C) and autumn (26/13 °C) in both the 1st and 2nd cycles (Figs 1–4). Up to 75–96 % of *H. commutata*, *H. hypericoides* and *H. racemosa* seeds provided with annual cycles germinated under wet/dry conditions, compared with 42–62 % under constant moisture (ANOVA, $P < 0.0001$) (Figs 1, 3 and 4). In the continuous temperature regimes, seeds of *H. commutata* and of *H. hypericoides* germinated to 40–45 % at 18/7 °C with wet/dry and to 0–31 % over the other temperature–moisture conditions but those of *H. racemosa* germinated to 92 and 69 % at 18/7 and 26/13 °C, respectively, for wet/dry and to 25–49 % at other conditions. In contrast, up to 30 % of *H. huegelii* seeds germinated in the annual cycle under constant wetness and up to 8 % did so under wet/dry conditions (Fig. 2). In continuous temperatures, seeds of this species germinated only to 0–12 % regardless of temperature–moisture conditions.

Effects of warm stratification on dormancy break and germination

Under wet/dry conditions in light, incubation at 18/7 °C for 12 weeks following warm stratification for 16 weeks significantly increased germination of *H. commutata* seeds to 81 %, compared with 26 % in controls at 18/7 °C for 28 weeks and similarly to 28 % vs. 1 % under wet conditions in light (*t*-tests, $P \leq 0.0004$) (Fig. 5). Warm stratification significantly increased germination of *H. racemosa* seeds, compared with controls, that were incubated under wet ($P = 0.0001$), but not wet/dry conditions ($P = 0.5644$), reaching 71–78 % under both moisture conditions. Warm stratification did not increase germination of *H. huegelii* or *H. hypericoides* ($P \geq 0.0845$). Warm-stratified seeds of both species germinated higher (ANOVA, $P \geq 0.0127$) with wet/dry cycles on water and KAR_1, reaching 20–41 %, than with constant wetness. Germination of warm-stratified and control seeds of all species was ≤22 % at 26/13, 33/18 or 35 °C in light, regardless of the moisture regime or type of solution (data not shown).

Hibbertia racemosa seeds stratified in darkness germinated to 76–94 % over the range of temperatures during incubation regardless of the light regime, and those stratified in light germinated to 40–74 % in darkness (Table 2). Control seeds of this species germinated to 60–87 % over the range of temperatures in darkness. In contrast, ≤30 % of warm-stratified or

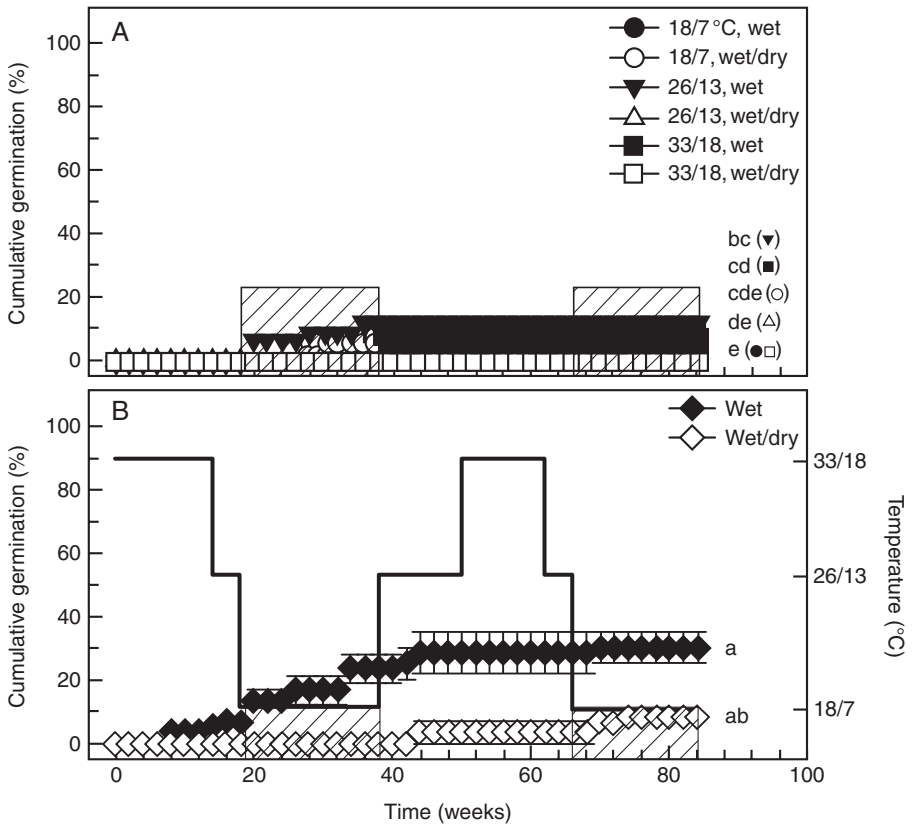

FIG. 2. Effects of incubation at continuous temperatures (A) or at a simulated sequence of annual temperature cycles (B) on mean (± s.e.) germination of *Hibbertia huegelii*. The details are as given for Fig. 1. Three replicates of 50 seeds were used for each treatment.

control seeds of the other three species germinated in darkness (Table 2).

Temperature requirements for embryo growth

Initial sizes of seeds and embryos varied significantly among the species (ANOVA, $P < 0.0001$): *H. huegelii* = *H. hypericoides* > *H. commutata* = *H. racemosa* (Fig. 6). Embryos in fresh seeds of all species were approx. 11–14 % of endosperm length. Growth of the embryo was found to be a prerequisite for radicle emergence (Fig. 6). As the embryo grew through the endosperm, it curved around the inside of the seed before the radicle emerged (Fig. 7). During 20 weeks of incubation, cooler temperatures (18/7 °C) promoted the relative growth of embryos more than warm temperatures (33/18 °C) in *H. huegelii* (46 % vs. 6 %, respectively; $P \geq 0.0525$), in *H. hypericoides* (332 % vs. 97 %; $P \geq 0.0639$), and in *H. racemosa* (513 % vs. 61 %; $P < 0.0001$). In contrast, the embryo growth of *H. commutata* was not sensitive to temperature over this range ($P = 0.9380$) but the embryos increased in length by 123–126 %).

Germination phenology

Although daily watering commenced in mid-April 2008 and smoking occurred in late April 2008, no seedlings of *H. huegelii* or of *H. hypericoides* started to emerge from the soil until late May 2008 and they continued doing so until mid-August 2008 (Fig. 8). Mean daily maximum and minimum temperatures between sowing and late May at the regional level were 25.5 °C and 13.2 °C, respectively, and between late May and mid-August were 19.1 °C and 7.1 °C, respectively; those in the tunnel-house were 31.8 °C and 15.8 °C, respectively, and between late May and mid-August were 27.6 °C and 10.2 °C, respectively. Our daily watering in the tunnel-house commenced at about the same time as the regional wet season during 2008, but it was earlier as compared with regional precipitation in 2009. Smoke promoted higher emergence of seedlings of both species (*t*-tests, $P \leq 0.0008$). Up to 21 % and 50 % of *H. huegelii* and of *H. hypericoides* seedlings, respectively, emerged in winters 2008 and 2009 from smoked conditions and only 0–4 % from non-smoked conditions.

DISCUSSION

Requirements for dormancy break and germination

Embryos in *Hibbertia* seeds are rudimentary, i.e. their width and length are about equal and they appear globular in shape (Martin, 1946; Schatral, 1995; Baskin and Baskin, 2007). In all four *Hibbertia* species, embryos needed to elongate considerably inside the seeds before radicle emergence (Figs 6–7; S. N. Hidayati *et al.*, pers. obs.). Fresh seeds took ≥6 weeks

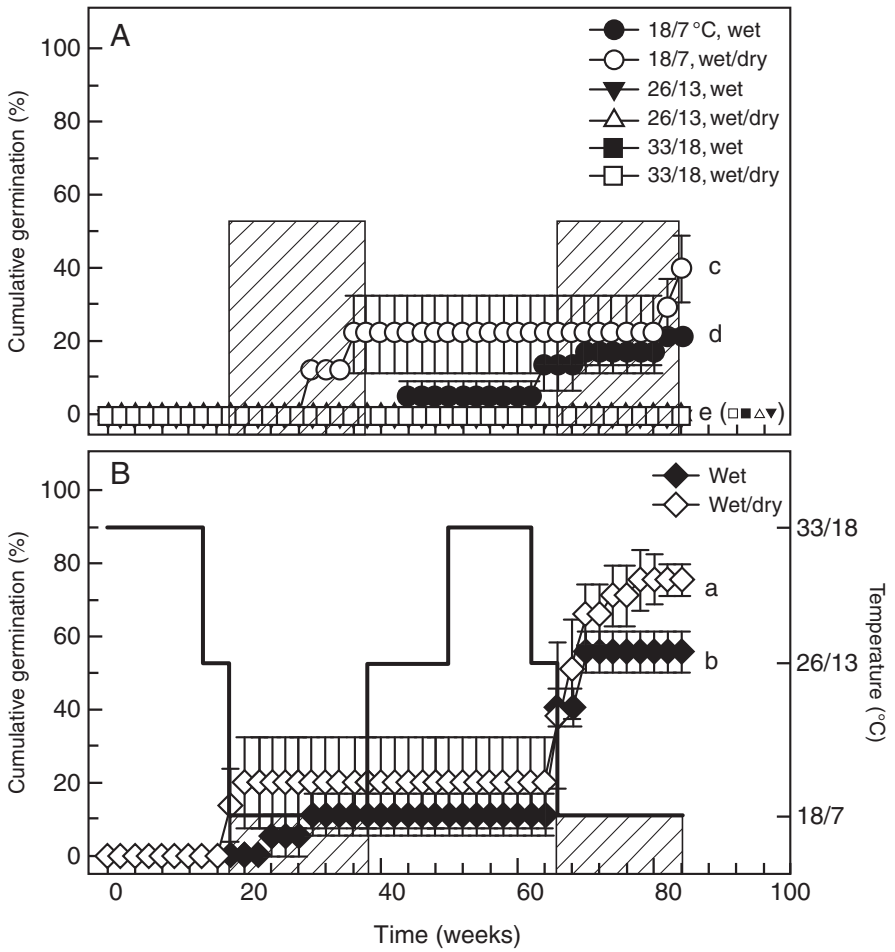

FIG. 3. Effects of incubation at continuous temperatures (A) or at a simulated sequence of annual temperature cycles (B) on mean (± s.e.) germination of *Hibbertia hypericoides*. The details are as given for Fig. 1.

to initiate germination (Figs 1–4), regardless of the wide range of incubation conditions. Apparently, a physiological block is present preventing embryo growth and the seeds of *Hibbertia* have MPD.

In nature, seeds of the four *Hibbertia* species are exposed to high summer soil temperatures (up to 65 °C; see Turner *et al.*, 2006) with little rainfall following dispersal. This type of environmental condition is conducive for overcoming dormancy in seeds of many species that require warm (20–35 °C) and (mostly) dry conditions (Baskin and Baskin, 1998; Merritt *et al.*, 2007). Seeds of *H. commutata*, *H. hypericoides* and *H. racemosa* germinated to higher percentages, especially at 18/7 or 26/13 °C, with alternating wet/dry substrate conditions than constantly moist (Figs 1, 3, 4 and 5). Similarly, wet/dry cycling resulted in higher levels for germination of the Australian semi-arid tropical species *Actinobole uliginosum* (Asteraceae), *Goodenia cycloptera* and *Velleia glabrata* (Goodeniaceae) when compared with constant wetness or dryness at warm temperatures (Hoyle *et al.*, 2008) and, additionally, for *Actinotus leucocephalus* (Apiaceae) a species sympatric with the *Hibbertia* species assessed in this study (Baker *et al.*, 2005). On the other hand, while seeds of *H. huegelii* germinated to the greatest degree under constant moisture in the move-along experiment (Fig. 2), they did so under wet/dry cycles in the warm stratification treatment (Fig. 5).

The effects of the warm, wet/dry conditions on dormancy break were mixed among the four *Hibbertia* species with four patterns observed. First, germination significantly improved for seeds of *H. commutata* with warm stratification as compared with controls in both the move-along (Fig. 1) and the stratification experiments (Fig. 5). Secondly, seeds of *H. hypericoides* exposed to warm stratification germinated equally well as controls (Fig. 5) but those exposed to a simulated sequence of natural temperatures germinated significantly higher than controls (Fig. 3). Thirdly, *H. racemosa* seeds germinated to high percentages regardless of whether they received a warm stratification treatment (Figs 4 and 5). Fourthly, warm stratification did not alleviate dormancy in seeds of *H. huegelii* (Figs 2 and 5). Thus, while *H. commutata* and *H. hypericoides* seeds require some degree of warm stratification to overcome dormancy it appears that a long incubation period at low temperature (i.e. not stratification *per se*) is needed for *H. racemosa* seeds.

There also appears to be an influence of photo regime on dormancy loss in *H. racemosa* seed – an interaction not evident in the other *Hibbertia* species (Table 2). *Hibbertia racemosa* seeds germinated in light to high percentages

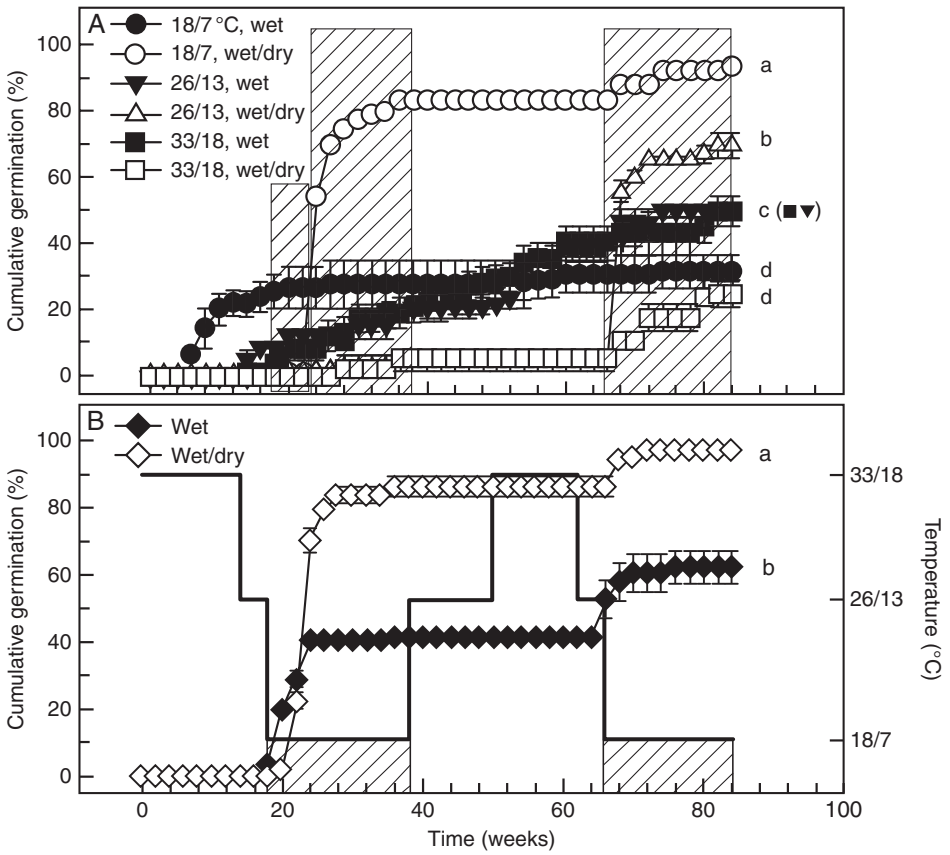

FIG. 4. Effects of incubation at continuous temperatures (A) or at a simulated sequence of annual temperature cycles (B) on mean (± s.e.) germination of *Hibbertia racemosa*. The details are as given for Fig. 1.

only at 18/7 °C following warm stratification in light, whereas they germinated in light or in darkness to high percentages at 18/7, 26/13 and 33/18 °C after stratification in darkness. Thus, the broadest range of temperatures for stimulating germination was achieved with dark conditions during stratification for *H. racemosa*. Schatral *et al.* (1997) found that 6- to 8-month-old seeds of *H. huegelii* germinated to 32 % in darkness when the seed coat was removed but to 2 % in alternating light/dark conditions. Seeds of this species also germinated to higher percentages in darkness than in light/dark when seed coats were cracked; no intact seeds germinated regardless of the light conditions. For *H. huegelii*, no differences between germination in darkness and in light were observed. Light inhibition of germination at warmer temperatures has previously been found for a wide number of native species from south-west Australia (Bell *et al.*, 1995; Turner *et al.*, 2005) and the Mediterranean (Thanos *et al.*, 1989).

Exposure to aerosol smoke stimulated emergence during the 1st germination season when seeds of *H. huegelii* and of *H. hypericoides* were in the tunnel-house (Fig. 8). Stimulation of germination solely by smoke application has been observed in other *Hibbertia* species: *H. amplexicaulis*, *H. lasiopus* and *H. quadricolor* under greenhouse, and *H. amplexicaulis* in an *in situ* habitat treatment (Dixon *et al.*, 1995; Roche *et al.*, 1998). In contrast, seeds of *H. huegelii* and of *H. hypericoides* germinated in the laboratory on water, i.e. KAR_1 did not promote germination (Figs 2, 3 and 5). The reason(s) for the discrepancy between laboratory (water, KAR_1) and greenhouse (smoke) results for these two species are poorly understood, though it is known that there is a complex interaction between seed dormancy status, which is modulated by incubation time, temperature and seed moisture content, and the smoke response (Merritt *et al.*, 2007; Turner *et al.*, 2009). The discrepancy also may have been caused by using only KAR_1 since there are other related karrikinolides and cyanohydrin compounds in smoke that stimulate germination (Flematti *et al.*, 2011).

During the 2nd germination season in the tunnel-house, the smoke effect was much reduced (Fig. 8). In fact, equal emergence of *H. hypericoides* occurred with and without smoke perhaps suggesting that seeds may become desensitized to smoke over time as seed dormancy is reduced, possibly as a bet-hedging strategy in the event a fire does not occur for some time. At the same time, a cohort of seeds may include some that are more dormant than others, and only the relatively less-dormant seeds respond to smoke. Seeds that lose dormancy under soil-based conditions may also suffer attrition through decay or predation, resulting in the loss of non-dormant seed from the soil seed bank, which may explain the low percentages of seedlings of *H. huegelii* and of *H. hypericoides* that emerged during the 2nd germination season.

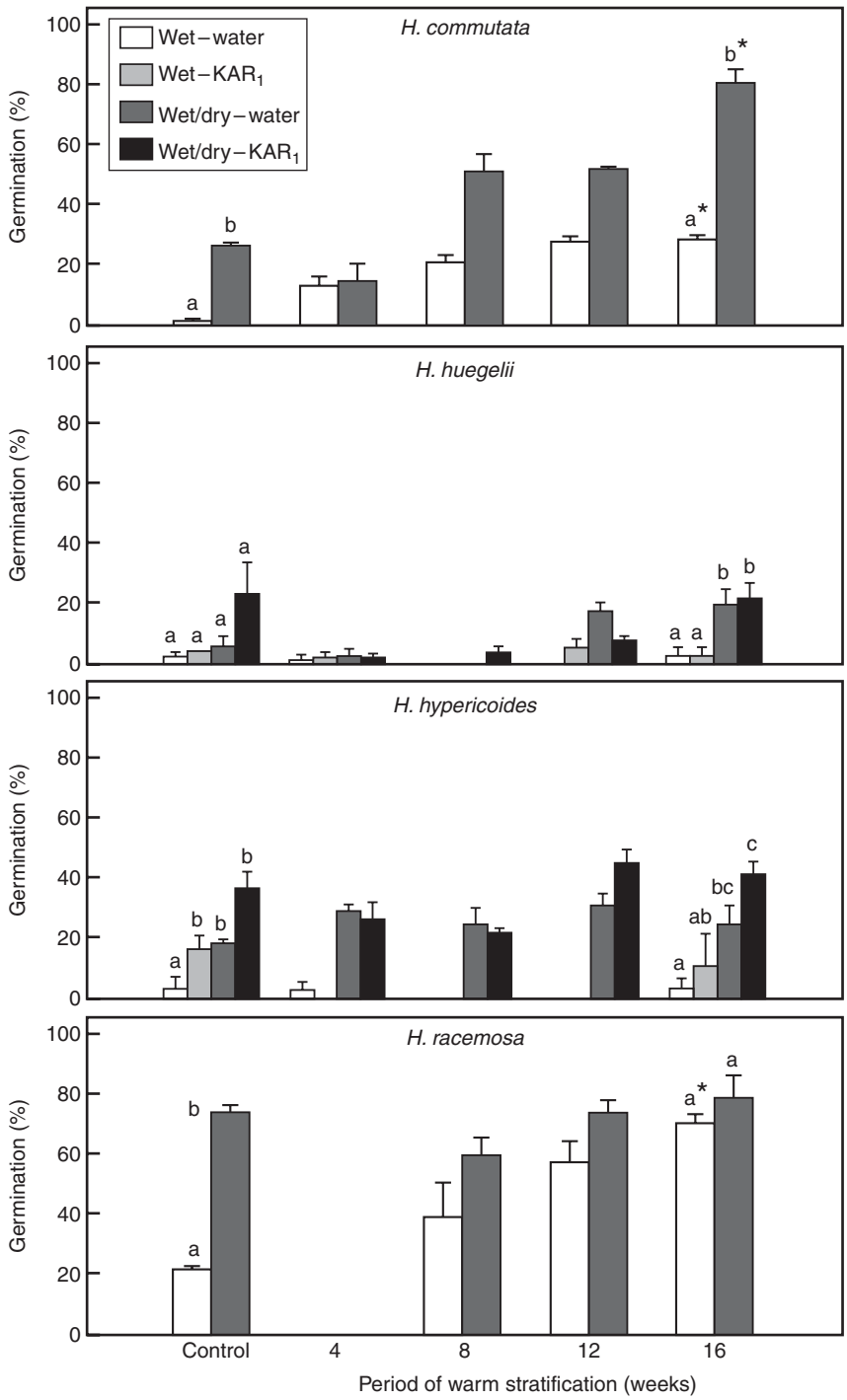

FIG. 5. Effects of warm stratification on mean (± s.e.) germination of four species of *Hibbertia*. Seeds were stratified at 33/18 °C (*H. commutata*, *H. racemosa*) or at 35 °C (*H. huegelii*, *H. hypericoides*) for 4–16 weeks in light and then incubated at 18/7 °C for 12 weeks in light. Stratification occurred under wet or wet/dry conditions but incubation was wet. Control seeds were incubated either wet at 18/7 °C for 28 weeks or wet/dry at 18/7 °C for 16 weeks followed by wet at 18/7 °C for 12 weeks. Substrates were moistened with water or with 0.67 μM karrikinolide (KAR_1). Within the control or 16-week stratification, means with different letters are significantly different (*t*-tests or PLSDs, $P \leq 0.05$) and those with the same letters are not. An asterisk indicates that the 16-week stratification significantly differs from the control within the same moisture-solution regime (*t*-tests, $P \leq 0.05$); bars without an asterisk do not differ. Three replicates of 50 seeds were used for each treatment.

Seedling emergence in nature

Our laboratory studies suggested that seeds of *H. huegelii* and of *H. hypericoides* are dormant when dispersed (Figs 2 and 3). Following an exposure to a wet/dry period either at low temperatures or at high temperatures, seeds of these two species germinate at cooler temperatures (<26/13 °C) when moisture is not limiting, that corresponds to autumn and winter conditions in south-west Australia. Indeed, onset of

TABLE 2. *Effects of warm stratification on mean (± s.e.) germination of four species of* Hibbertia

Light conditions		Water			KAR_1	
Stratified	Incubated	18/7	26/13	33/18 or 35*	18/7	35
H. commutata						
L (16)‡	D (4)	0	0	0	–†	–
D (16)‡	L (4)	0	0	0	–	–
D (16)	D (4)	0	0	0	–	–
	D (20)#	0	0	0	–	–
H. huegelii						
D (4)‡	D (12)	20 ± 4	–	–	6 ± 6	–
D (8)‡	D (12)	0	–	–	0	–
D (12)‡	D (12)	0	–	–	7 ± 4	–
D (16)‡	D (12)	0	–	–	0	–
	D (28)#	0	–	0	0	0
H. hypericoides						
D (4)‡	D (12)	0	–	–	0	–
D (8)‡	D (12)	0	–	–	0	–
D (12)‡	D (12)	0	–	–	0	–
D (16)‡	D (12)	0	–	–	0	–
	D (28)#	6 ± 6	–	0	30 ± 4	0
H. racemosa						
L (16)§	D (4)	74 ± 2	49 ± 4	40 ± 5	–	–
D (16)¶	L (4)	94 ± 2	92 ± 1	93 ± 2	–	–
D (16)	D (4)	76 ± 1	88 ± 2	83 ± 3	–	–
	D (20)#	60 ± 2	87 ± 1	80 ± 4	–	–

Seeds were stratified at 33/18 °C (*H. commutata*, *H. racemosa*) or at 35 °C (*H. huegelii*, *H. hypericoides*) in light (L) or darkness (D) and then incubated at various temperatures in L or D. Lengths (in weeks) of stratification and incubation are given in parentheses. Substrates during stratification and incubation were moistened with water or with 0.67 μM karrikinolide (KAR_1). Three replicates of 50 seeds were used for each treatment.

* Seeds of *H. commutata* and of *H. racemosa* were incubated at 33/18 °C and those of *H. huegelii* and of *H. hypericoides* at 35 °C.

† –, Not tested.

‡ No seeds germinated during the stratification period.

§ 2 % of seeds germinated in light during stratification.

¶ 92 % of seeds germinated in darkness during stratification.

Served as control, i.e. seeds were not stratified only incubated.

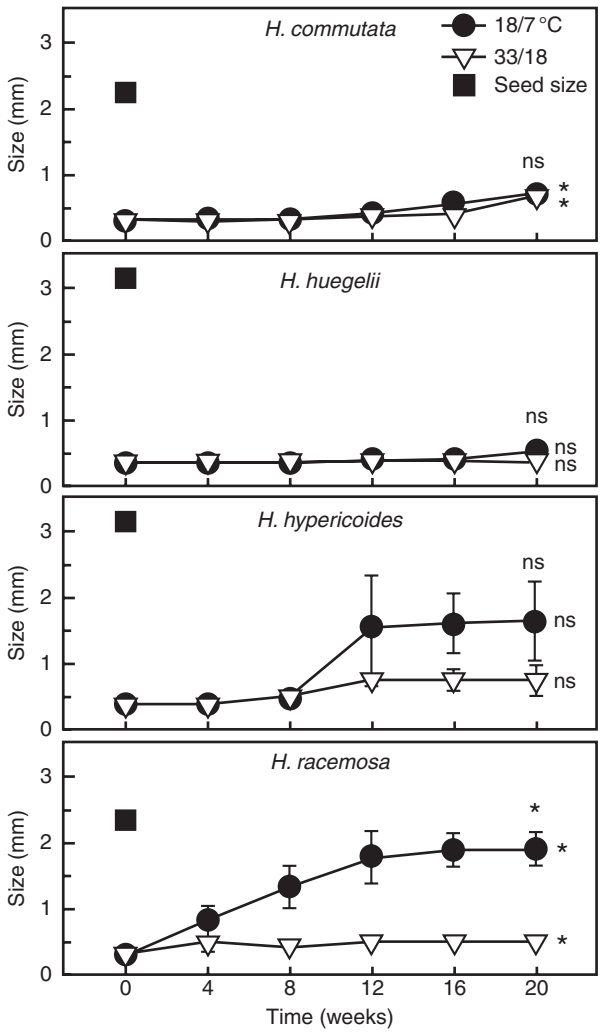

FIG. 6. Embryo growth and seed sizes (mean ± s.e.) among four species of *Hibbertia*. Seeds were incubated at 18/7 °C or at 33/18 °C for 20 weeks in light, and embryo lengths determined at 4-week intervals. An asterisk above the data points at week 20 indicates a significant difference in embryo size between 18/7 °C and 33/18 °C and that to the right of data points denotes a difference in size between week 0 and week 20 within each temperature regime (*t*-tests, $P < 0.05$); 'ns' designates no significant difference. Embryos from six (*H. hypericoides*) to 47 (other three species) seeds were assessed at each time.

seedling emergence for *H. huegelii* and for *H. hypericoides* from pots maintained under tunnel-house conditions occurred approx. 1 month (in late May) after the start of daily watering and exposure to aerosol smoke (Fig. 8). Our laboratory work suggests that seedling emergence for *H. commutata* and for *H. racemosa* would take place at a similar time as *H. huegelii* and as *H. hypericoides* (Figs 1–4).

Mass emergence did not occur for *H. huegelii* or for *H. hypericoides* in the phenology study, but gradually increased over a 2.5-month period. In the move-along experiment, emergence for *H. commutata*, *H. huegelii* and *H. hypericoides* occurred slowly but that in *H. racemosa* took place relatively faster. The gradual emergence observed for *Hibbertia* species might have been due to protracted periods of embryo growth and/or dormancy regulation. Dixon *et al.* (1995) also documented continued germination over several weeks in other smoke-responsive species, such as *Grevillea wilsonii*, *Andersonia lehmanniana* and *Tetratheca hirsuta*.

The move-along experiment also suggested that germination from a cohort of *Hibbertia* seeds could be spread over several years as was shown in the tunnel-house experiment. Germination occurred in all four *Hibbertia* species following the 1st summer period during the move-along, and then additional germination took place after the 2nd summer period (Figs 1–4). For *H. hypericoides*, the difference between germination following the 1st and 2nd summers was dramatic indicating that multiple summer periods are required to elicit more complete germination. Apparently, the degree of dormancy varies within the cohort. Seeds with less dormancy (requiring only one summer) germinate during the 1st germination season, whereas seeds with more dormancy may need a relatively long warm stratification period to overcome dormancy.

Classification of MPD in Hibbertia

The levels of MPD are first divided into two categories based on temperatures at the time of embryo growth: warm

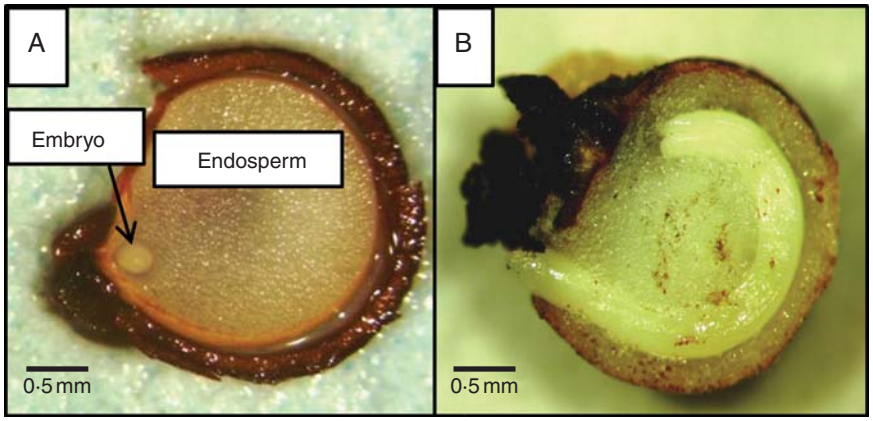

FIG. 7. Cross-section of a freshly matured seed of *Hibbertia commutata* showing the tiny embryo and copious endosperm (A) and of a seed of *H. racemosa* showing the curving of the embryo and radicle emergence (B). The non-elongated and elongated embryos in all four species of *Hibbertia* in our study looked similar.

temperatures (≥15 °C) are needed for embryo growth in seeds with simple levels of MPD and cold temperatures (0–10 °C) in those with complex levels of MPD (Baskin and Baskin, 2004). Ecologically, warm temperatures match spring, summer or autumn, and cold temperatures correspond to winter in temperate and boreal forests, biomes where the MPD classification system has been mostly developed (Nikolaeva, 1967, 1977; Baskin and Baskin, 1998). In south-western Australia, which has a Mediterranean climate, germination and seedling recruitment in most species, including *Hibbertia*, occurs predominantly during the cooler winter months when rainfall (and therefore soil water) is readily available. Indeed, temperatures during the time of emergence in the tunnel-house were 28/10 °C and regional temperatures were 19/7 °C, which approximated results from our laboratory studies.

At these alternating winter temperatures, seeds may receive some cold stratification at night and warm stratification during the day. Thus, we do not know for certain if cold or warm stratification promoted embryo growth and thus germination. Since embryo growth occurred at an alternating temperature regime that includes both cold and warm temperatures, there is no way to answer whether the seeds of *Hibbertia* have simple or complex MPD. Future studies need to resolve whether cold stratification occurs following warm stratification by using constant temperature regimes. On the other hand, we do not know the temperature ranges over which cold and warm stratification effectively breaks dormancy in Mediterranean species. In this respect, the current temperature ranges for cold and warm stratification to break dormancy are somewhat arbitrary and may not apply to species in south-west Australia. If the current temperature schemes for cold and warm stratification do not match conditions in south-western Australia, then the classification of the levels for MPD might be better based on seasonality rather than temperature *per se* to align with ecological cues in temperate and boreal forests.

The substrates during warm stratification are usually moist, simulating soil water conditions in temperate and boreal forests during summer. However, in Mediterranean biomes warm stratification with wet/dry conditions or after-ripening (dry storage) matches soil moisture during summer (the dormancy break period). In fact, seeds of the *Hibbertia* study species germinated to higher percentages with wet/dry conditions. The moisture definition of warm stratification in Baskin and Baskin (2004) should be expanded to include wet/dry (as well as dry after-ripening) conditions in the current classification system so as to include species in Mediterranean biomes.

The three levels of complex MPD are recognized based on the physiological component of their dormancy: non-deep physiological dormancy (PD), intermediate PD and deep PD. The length of dormancy-break treatment increases from <2 months for non-deep PD, to 2–3 months for intermediate PD, and 3–4 months for deep PD (Baskin and Baskin, 2004). Cold and/or warm stratification breaks non-deep PD and cold stratification does so for intermediate and deep PDs. However, Baskin *et al.* (2005) reported a case for deep PD broken by warm temperatures. Widening of the temperature range for germination as dormancy is broken occurs for non-deep and intermediate PDs but not for deep PD (Baskin *et al.*, 2005). Response of seeds to GA_3 also aids in differentiating the levels of PD: GA_3 overcomes dormancy in seeds with non-deep and intermediate but not in those with deep PD (Baskin and Baskin, 1998).

Seeds of *H. racemosa* required a comparatively short period (<2 months) of either cool or warm temperatures to overcome dormancy and they germinated over a broad range of temperatures (Table 2 and Figs 4 and 5). No data on the effects of GA_3 on *H. racemosa* seeds are available, but the relatively short stratification period for dormancy break and broadening of the temperature response to dormancy break approximate non-deep PD. In contrast, a long period (4 months) of warm temperatures followed by cool temperatures (18/7 °C) was required for dormancy loss and germination in *H. commutata* seeds, but requirements for these processes were less clear for *H. huegelii* and for *H. hypericoides* seeds. Germination in all three species was mostly confined to 18/7 °C. GA_3 overcame dormancy in about 48 % and 36 % of *H. huegelii* and of *H. hypericoides* seeds (with seed coats intact), respectively, during 196 d at 18/7 °C. Other studies have reported contrasting results for these two species. GA_3 did not affect germination of *H. hypericoides* seeds during 112 d at 18/9 °C (Schatral, 1996), but enhanced germination of *H. huegelii*

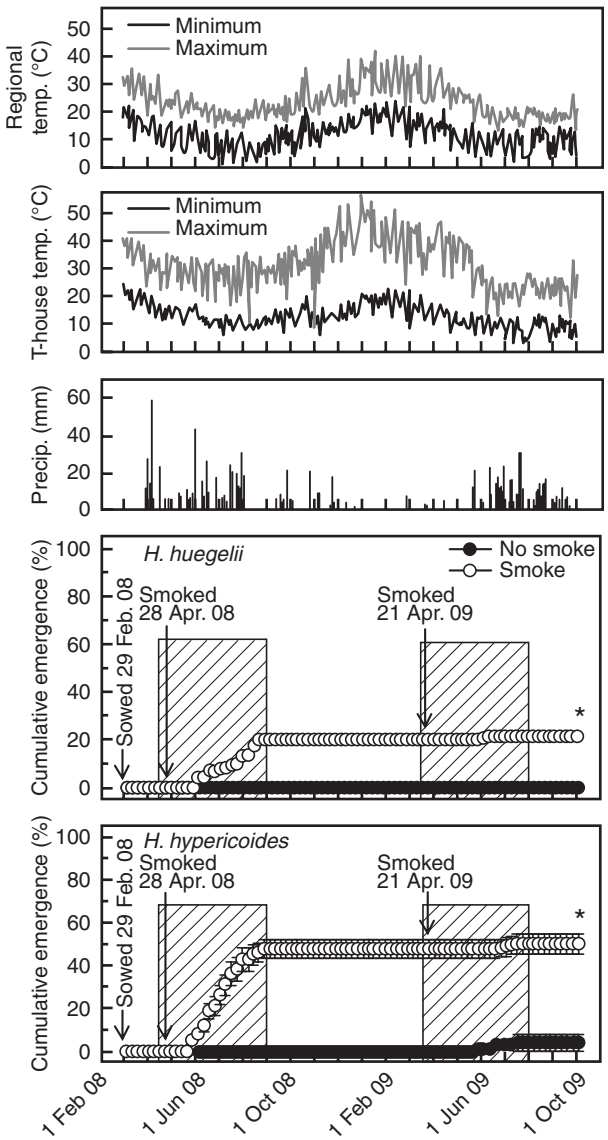

FIG. 8. Maximum and minimum daily temperatures from a regional weather station (Perth, Western Australia) and from the tunnel-house, regional total daily precipitation and emergence percentages (mean ± s.e) for two species of *Hibbertia*. Seeds of both species were collected at maturity, sown on soil in February 2008 in a tunnel-house with ambient temperatures and exposed to aerosol smoke in April 2008 and in April 2009. Soil was watered daily (hatched areas) or it was watered twice weekly (only in April) or weekly (rest of the year) (non-hatched areas). An asterisk indicates that the percentages at the end of the experiment significantly differed between smoke and non-smoke conditions within each species (*t*-tests, $P \leq 0.05$). Four replicates of 100 seeds were assessed for each treatment.

seeds if they were decoated at 90 d and 20 °C (Schatral *et al.*, 1997). Application of GA_3 to scarified seeds of *H. commutata* did not affect their germination at 18/9 °C as compared with scarified controls (Allan *et al.*, 2004). Thus, the physiological component of MPD in some seeds within a cohort of *H. commutata*, *H. huegelii* and *H. hypericoides* approximates deep PD but we cannot rule out that seeds responding to GA_3 may have non-deep or intermediate PD. Our study is the first to report that a relatively long warm stratification period was required to overcome dormancy in seeds with MPD, which was previously only reported for seeds with PD (Baskin *et al.*, 2005).

Conclusions

Here we show a remarkable level of variation in dormancy control in sympatric congeners with identical seed morphology, and highlight the difficulties, and caution required, in extrapolating dormancy regulation requirements in biodiverse regions such as the south-west Australian biodiversity hotspot. For such biodiverse, old and stable environments (Hopper, 2009), empirical research may be required to resolve dormancy requirements even when seed is similar phylogenetically and morphologically. However, the mix of species in this study did exhibit some potentially interesting trends. The least-dormant species *H. racemosa* has the most extensive geographic range and the most-dormant species *H. huegelii* has the narrowest range. Seeds of *H. racemosa* from the coastal dune environments (high wind, soil movement locations) germinated to higher percentages in darkness than in light (under some conditions), which is a feature common to some other dune species (Baskin and Baskin, 1998). In contrast, the other three *Hibbertia* species grow in wooded habitats and their germination responses were positively photoblastic. Lastly, *H. hypericoides* occurs commonly in highly fire prone environments (i.e. jarrah forest, banksia woodland, kwongan) and perhaps this may explain its more smoke responsive nature compared with the other species.

Presently, too few species with underdeveloped embryos from the biodiverse south-west Australia and, indeed other Mediterranean regions have been investigated with regards to determining whether they have MPD, and, if so, classifying the level. Much additional work is needed on seeds with underdeveloped embryos in south-west Australia, and in other Mediterranean regions, before a clear understanding of MPD can be achieved. Studies of this type undertaken in the systematic way presented here may help to resolve complexities with dormancy regulation and facilitate germination-on-demand for conservation taxa in reintroduction and restoration programmes and improve the success and use of seed, particularly where seed availability is limited for broadcast seeding.

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