Seed germination of *Solanum* spp. (Solanaceae) for use in rehabilitation and commercial industries

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Abstract. Effective methods for propagation of native *Solanum* species are required for mine rehabilitation and the native food industry in Australia. This study investigated seed germination of eight native *Solanum* species with respect to incubation temperature and the efficacy of germination promoting compounds gibberellic acid (GA_3), the butenolide isolated from smoke (karrikinolide, KAR_1) and smoke water (SW). Seeds of all species were tested under a temperature regime of 26/13°C or 33/18°C. In these conditions, seeds of only two species, *S. cunninghamii* Benth. and *S. phlomoides* Benth. germinated to high levels without treatment. Of the remaining six species, GA_3 alone promoted germination in *S. chippendalei* Symon, *S. diversiflorum* F.Muell. and *S. sturtianum* F.Muell., whilst GA_3, KAR_1 and SW were effective at promoting germination of *S. centrale* J.M.Black, *S. dioicum* W.Fitzg. and *S. orbiculatum* Dunal ex Poir. to varying degrees. Additional incubation temperatures (10, 15, 20, 25 and 30°C) were examined for *S. centrale* and *S. orbiculatum*. For both species, broadly similar patterns were noted in the response of seeds to GA_3, KAR_1 and SW across all temperatures. However, for *S. centrale* seeds, germination percentages were higher at 26/13°C than at any of the constant temperatures, and there was a trend of increasing germination with increasing constant temperature for *S. orbiculatum* seeds. Analysis of seed embryo type and imbibition characteristics and consideration of the subsequent germination results indicates that dormant *Solanum* seeds possess physiological dormancy.

Introduction

Solanum species occur across many ecosystems and in all continents. The genus includes economically important food crops such as potato (*Solanum tuberosum*) and eggplant (*Solanum melongena*). There are 47 native species of *Solanum* in Western Australia and 11 naturalised species (Paczkowsa and Chapman 2000). Many of the native species, commonly know as bush tomatoes, were used as a food source by indigenous Australians and a number of species are in commercial production or evaluation as bush tucker. Edible *Solanum* species, including *S. centrale* (Latz 1995; Stefaniski 1998; Ahmed and Johnson 2000) and *S. chippendalei* (Courtenay pers. comm.), are important food sources with fruits possessing high carbohydrate and vitamin C content.

While the fruit of *S. centrale* and *S. chippendalei* can be collected from the wild, commercial production of *S. centrale* is underway (Ahmed and Johnson 2000) and is planned for *S. chippendalei* (Courtenay pers. comm.). However, information about propagation is required. Also, propagation of *Solanum* species is required for minesite restoration in Australia, particularly as a result of a resurgence in mining activity in the arid zone where the genus most commonly occurs. Species required in restoration include *S. orbiculatum* and *S. diversiflorum* as both species are common and widespread components of the pre-mined vegetation. However, little is known about the seed germination biology of arid zone vegetation in Australia, particularly with respect to methods applicable to large scale propagation and restoration. Furthermore, poor seed germination and limited horticultural information available on *Solanum* species are hampering propagation and commercial production.

The two studies published on *S. centrale* seeds indicate that gibberellic acid (GA_3) and smoke may be useful germination promoting agents for *Solanum* spp. (Stefaniski 1998; Ahmed *et al.* 2005). Stefaniski (1998) found gibberellic acid increased germination from 7% to 20% while Ahmed *et al.* (2005) showed that a combination of seed-coat nicking and aerosol smoke improved germination. In particular, fire related cues warrant further investigation as disturbance by fire has been observed to encourage the spread of *Solanum* species in natural ecosystems (Latz 1995). Smoke products are well known to promote germination of a large number of Australian species (Dixon *et al.* 1995; Roche *et al.* 1997) and the newly discovered active chemical in smoke, the butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) (Flematti *et al.* 2004), now known as karrikinolide

(KAR_1) (Dixon *et al.* 2008), has proved highly effective at promoting germination of a broad range of Australian species, including arid zone species (Merritt *et al.* 2006). Gibberellins are similarly known to be efficacious across a broad range of Australian species (Bell *et al.* 1995; Plummer and Bell 1995) and are thought to act via mechanisms that include promoting the growth potential of the embryo (Kucera *et al.* 2005), weakening endospermic cells (Groot and Karssen 1987; Groot *et al.* 1988; Debeaujon and Koornneef 2000), and replacing after-ripening requirements (Baskin and Baskin 2004*a*).

Optimal germination temperatures for seed germination usually correspond to the time where water is non-limiting in the environment (Bell *et al.* 1993; Bell 1999; Bell *et al.* 1999). The distribution of the *Solanum* species in this study covers a range of environmental conditions from wet summers and dry winters (Pilbara, Great Sandy Desert and Dampierland regions), to an arid region with aseasonal rainfall (MacDonnell Ranges in central Australia) and finally to areas that receive sporadic winter rain and occasional summer cyclonic systems (Geraldton Sandplains and Murchison regions). As these regions receive summer rainfall, it is likely that incubation temperatures corresponding to the season of reliable rainfall may be higher than typically used in nursery propagation of Australian species in southern Australia (15-20^{o}C) (Bell 1999). For example, Jurado and Westoby (1992) found that germination of a *Solanum* species from arid Australia was higher at 28^{o}C compared with 12^{o}C and 20^{o}C.

Therefore, the aim of this study was to develop an understanding of germination and dormancy characteristics for an indicative range of eight *Solanum* species with restoration and commercial value from the arid and semi-arid zone of Australia. Specifically, for each species we determined (a) the seed and embryo morphology, (b) whether seeds were permeable and able to imbibe water (via imbibition studies) and (c) the effects and interactions of incubation temperature, gibberellic acid (GA_3), karrikinolide (KAR_1) and smoke water (SW) on seed germination.

Materials and methods

Seed collection

Table 1 shows the collection date, location and region for the eight *Solanum* species used in this study. The method of seed cleaning and storage conditions varied between species. Following collection of fruits of *S. cunninghamii*, *S. dioicum*, *S. phlomoides* and

S. sturtianum, seeds were extracted from fruits and air dried and stored at -18°C after collection. Seeds were retrieved from storage in June 2006 and used in experiments immediately.

Table 1. Collection date, location and Interim Biogeographic Regionalisation for Australia (IBRA region) of eight *Solanum* species

Species	Collection date	Location	IBRA region
S. centrale	Feb 2007	Napperby Station, north of Alice Springs (S 23° 38' 51'' E 133° 51' 50'')	Burt Plain
S. chippendalei	Aug 2005	The Great Sandy Desert near Punju Njamal	Great Sandy Desert
S. cunninghamii	1993	Between Millstream and Pannawonica	Pilbara
S. dioicum	1993	5.3 km on Shay Gap Road, near Marble Bar	Pilbara
S. diversiflorum	Feb 2007	Telfer mine (S 21° 43' 26'' E 122° 12' 33'').	Great Sandy Desert
S. orbiculatum	Nov 2004	Shark Bay Salt Lease (S 26° 07' 53.7'' E 113° 22' 58.5'')	Geraldton Sandplains
S. phlomoides	1993	15 km south of Meekatharra	Murchison
S. sturtianum	2004	Lake Carey (S 28° 50' 04'' E 122° 11' 10'')	Murchison

For *S. orbiculatum*, seeds were removed from freshly collected fruits using pectinase (1%) to dissolve the fleshy fruit. Seeds were then air dried and stored at ambient laboratory conditions (c. 22°C, 50% RH) for three months prior to use in experiments in 2005. *S. orbiculatum* seeds used for additional experiments at constant temperatures of 10, 15, 20, 25 and 30°C were collected in November 2005, cleaned as described above and stored at ambient laboratory conditions for four months prior to the experiment in 2006.

Fruits of *S. chippendalei* and *S. diversiflorum* were air dried then cracked open to remove the seeds. Seeds were stored at ambient laboratory conditions (c. 22°C, 50% RH) after collection for three months (*S. chippendalei*) and six weeks (*S. diversiflorum*) prior to use in experiments in 2005 and 2007 respectively.

Seeds of *Solanum centrale* were provided by Alice Springs Desert Park. Experiments were undertaken in April 2007. The method of cleaning is unknown.

Specimens of each species were lodged at the Kings Park and Botanic Garden Herbarium. Voucher numbers are as follows; *S. centrale* (LCOM4), *S. chippendalei* (LCOM2), *S. cunninghamii* (LSWE1488), *S. dioicum* (LSWE1429), *S. diversiflorum*

(LCOM3), *S. orbiculatum* (LCOM1), *S. phlomoides* (LSWE1348), *S. sturtianum* (LSWE6365).

Seed and embryo characteristics, viability testing and imbibition studies

Given the consistency of seed coat colour, the colour of the seed coat of each species was recorded from a simple observation. Seed diameter was determined for three replicates of 10 seeds. Seed weight was determined by weighing three replicates of 100 seeds and multiplied by 10 to estimate 1000 seed weight. A cut test was used to estimate the viability of the seeds prior to germination experiments. Three replicates of 20 imbibed seeds were cut in half and inspected for healthy embryonic tissue. Firm, white embryos were considered viable and shrivelled or black embryos were considered non-viable. Results of the cut test were confirmed by using tetrazolium chloride (Moore 1972) whereby seeds were cut in half and placed cut side down on germination test paper irrigated with 1% tetrazolium chloride buffered to pH 7 with a phosphate buffer (KH_2PO_4 and Na_2HPO_4). The embryos of dissected seeds were examined and classified according to Martin (1946) and described as fully developed or underdeveloped (Baskin and Baskin 2004*b*).

For each species three replicates of ≥0.03 g of seeds were weighed, placed on moist germination test paper in Petri dishes for five minutes, patted dry with paper towel to absorb water on the seed surface, then re-weighed. Seeds were returned to the moist germination test paper and each replicate was weighed again after 2, 4, 6, 24, 48, 72 and 96 h. Seeds were kept at ambient laboratory conditions (*c*. 22^{o}C, 50% RH) for the duration of the experiment. Percent water uptake was determined gravimetrically.

Germination

Seeds of all species were soaked for 24 h in solutions of 2.89 mM gibberellic acid (GA_3) (Sigma Aldrich, 90% GA_3), smoke water (SW) (1:10 v/v), 0.67 μM karrikinolide (the butenolide, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) or deionised water (control). SW was prepared with straw using the process described by Dixon *et al.* (1995). Karrikinolide was synthesised in pure form as described in Flematti *et al.* (2005). After soaking, seeds were surface sterilised in 2% (w/v) calcium hypochlorite ($Ca(OCl)_2$) for 30 mins, then rinsed three times with sterilised deionised water. Afterwards, four replicates of 25 seeds were placed in plastic Petri dishes (90mm) on water agar (0.7% w/v) and incubated at a 12/12 h alternating temperature regime of 33/18^{o}C or 26/13^{o}C. These two temperatures

approximate summer and winter temperatures in the arid environment of Western Australia where these plants commonly occur. In addition, three replicates of 10 seeds of all species were nicked by removing the portions of seed coat and endosperm covering the radicle tip. Nicked seeds were then incubated only at 33/18^{o}C as described above.

In a second germination experiment, additional incubation temperatures of 10, 15, 20, 25 and 30^{o}C were examined for *S. orbiculatum* and *S. centrale* seeds, but could not be performed on the other species due to limited seed numbers. For all experiments, Petri dishes were sealed with plastic (food grade cling film), then wrapped in aluminium foil to exclude light. Foil was removed each time germination was recorded in the laboratory under ambient light conditions. Germination of intact seeds was defined as the emergence of the radicle and germination of nicked seeds was defined as the elongation of the radicle tip, the production of root hairs and subsequent development into a normal seedling. Germination was assessed five days a week for 2 weeks, then weekly until germination had ceased. Final percentage germination data are presented for the first experiment, and both final percentage germination and time to 50% of the final germination data are presented for the second experiment.

Statistical analysis

Germination percentages were arcsine transformed prior to analysis. Data analysis was performed on individual species to determine temperature and treatment differences however, data from germination of nicked seeds were not included in this analysis. Germination data were analysed by analysis of variance (ANOVA) (P=0.05) using Genstat 8.1 (Copyright 2005, Lawes Agricultural Trust). If significant differences were detected by ANOVA, Fishers LSD was used to determine treatment differences. Due to missing values, the control treatment was not included in the analysis of time to 50% germination of *S. centrale*.

Results

Seed and embryo characteristics, viability testing and imbibition studies

Four species had dark (black/dark brown) seed coats including the larger massed species *S. chippendalei, S. diversiflorum* and *S. sturtianum* and the remaining four had light (white/cream) seed coats (Table 2). Seed diameter ranged from 2.1 – 4.7 mm. Seed viability was generally high with the three lower massed species exhibiting 100% viability. *S. chippendalei* had the lowest viability at 73% (Table 2). The seeds of all

eight species were endospermic and contained curved linear embryos. The curved embryo was longer than the seed and was fully developed. Seeds of all species readily imbibed water (Fig. 1). Increase in seed mass due to water uptake over 48 h ranged from 17% (*S. dioicum*) to 46% (*S. chippendalei*).

Table 2. Seed coat colour, seed diameter, seed weight and viability (Mean ± SE) of eight *Solanum* species.

Species	Seed coat colour	Seed diameter (mm)	Weight of 1000 seeds (g)	Viability (%)
S. centrale	Light	2.8 ± 0.04	2.4 ± 0.02	88 ± 5%
S. chippendalei	Dark	4.7 ± 0.06	7.8 ± 0.10	73 ± 3%
S. cunninghamii	Light	2.1 ± 0.02	1.1 ± 0.01	100 ± 0%
S. dioicum	Dark	2.1 ± 0.04	1.4 ± 0.02	100 ± 0%
S. diversiflorum	Dark	4.0 ± 0.01	8.1 ± 0.01	96 ± 3%
S. orbiculatum	Light	2.9 ± 0.04	2.2 ± 0.03	95 ± 3%
S. phlomoides	Light	2.3 ± 0.04	1.4 ± 0.02	100 ± 0%
S. sturtianum	Dark	3.0 ± 0.03	4.0 ± 0.00	78 ± 2%

Germination

Whilst untreated (control) seeds of *S. cunninghamii* and *S. phlomoides* had less than 20% germination when incubated at 26/13^{o}C, germination was 97% and 62% respectively when incubated at 33/18^{o}C (Fig. 2c,g). In contrast, untreated seeds of *S. centrale*, *S. dioicum* and *S. orbiculatum* had only 1% –27% germination at both 26/13^{o}C and 33/18^{o}C (Fig. 2a,d,f). Seeds of *S. diversiflorum* did not germinate at 33/18^{o}C, but demonstrated 2% germination when incubated at 26/13^{o}C (Fig. 2e). Untreated seeds of *S. chippendalei* and *S. sturtianum* failed to germinate at either temperature (Fig. 2b,h).

Treatment of seeds of all species with GA_3 significantly increased (P<0.05) germination, compared with the controls, at either one or both temperature regimes (Fig. 2). GA_3 promoted germination of *S. cunninghamii* at 26/13^{o}C, but when incubated at 33/18^{o}C germination of both control and GA_3 treated seeds was >95% (Fig. 2c). GA_3 significantly increased (P<0.05) germination of *S. phlomoides* at 26/13^{o}C, but suppressed germination at 33/18^{o}C (Fig. 2g). For the other six species, GA_3 significantly increased (P<0.05) germination at both 26/13^{o}C and 33/18^{o}C (Fig. 2a,b,d,e,f,h). For most species germination of GA_3 treated seeds was similar at both temperatures, although *S. orbiculatum* seeds germinated to a higher percentage at 33/18^{o}C than at 26/13^{o}C (P<0.05) (Fig. 2f) and *S. centrale* germinated to a higher percentage at 26/13^{o}C than at 33/18^{o}C (P<0.05) (Fig. 2a).

Unlike GA_3, SW promoted germination in some, but not all species. SW significantly increased ($P<0.05$) germination of *S. centrale*, *S. dioicum* and *S. orbiculatum* relative to the control at both temperature regimes (Fig. 2a,d,f). For seeds of *S. cunninghamii*, SW increased germination at 26/13°C but suppressed it at 33/18°C (Fig. 2c).
For *S. phlomoides* seeds, SW did not affect germination at 26/13°C, but suppressed germination at 33/18°C (Fig. 2g). For the remaining three species *S. chippendalei, S. diversiflorum* and *S. sturtianum*, germination of SW treated seeds was negligible (Fig. 2b,e,h).

Karrikinolide elicited higher germination than control seeds for five species at one or both incubation temperatures ($P<0.05$). Karrikinolide increased germination of *S. dioicum* and *S. orbiculatum* to at least the same level as GA_3 and SW at both incubation temperatures (Fig. 2d,f). For *S. centrale* seeds, germination of karrikinolide treated seeds exceeded that of control and SW treated seeds at both incubation temperatures (Fig. 2a). Germination of *S. cunninghamii* and *S. phlomoides* was promoted by karrikinolide at 26/13°C but not at 33/18°C (Fig. 2c,g). For the remaining three species (*S. chippendalei*, *S. diversiflorum* and *S. sturtianum*) germination in the presence of karrikinolide was <5% (Fig. 2b,e,h). Coincidently, these three species all had dark seed coats, and had larger seeds (1000 seeds ≥4.0 g) compared with the other five species (1000 seeds ≤2.4 g) (Table 2).

Nicking seeds did not elicit germination of *S. chippendalei*, *S. diversiflorum* or *S. sturtianum* (Fig. 2b,e,h). Nicking seeds of *S. centrale*, *S. dioicum* and *S. orbiculatum* increased germination relative to the control, and to similar levels as seeds treated with GA_3, SW or karrikinolide (Fig. 2a,d,f). Nicked seeds of *S. cunninghamii* germinated to the same percent as control seeds but those of *S. phlomoides* germinated to only half the percentage of control seeds (Fig. 2c,g).

Additional experiments were undertaken on *S. centrale* and *S. orbiculatum* to examine the effects of incubation temperature in greater detail. As in the first experiment, control germination of *S. centrale* seeds was very low (<2%) across all incubation temperatures. Germination of seeds treated with GA_3 was high (81-99%) between 10-25°C, but lower at 30°C (65%) (Fig. 3a). Similarly, germination of seeds treated with SW and karrikinolide was slightly higher at 10, 15 and 20°C (7-35%), compared with at 25 and 30°C (<5%) ($P<0.05$). Germination of seeds treated with karrikinolide was lower at the

constant incubation temperatures compared with the alternating temperatures of 26/13 and 33/18^{o}C (63-84%) (P<0.05). Although germination of GA_3, SW and karrikinolide treated seeds of *S. centrale* incubated at 10^{o}C was significantly higher (P<0.05) than at 30^{o}C, time to 50% germination was much longer (Fig. 3c). At 10^{o}C, time to 50% germination was around 22-24 days, compared with 2-6 days at 30^{o}C. Time to 50% germination did not differ from 15 to 30^{o}C.

For *S. orbiculatum*, germination percentage of control seeds increased as the temperature increased (Fig. 3b). All treatments significantly increased germination (P<0.05) relative to the control at each temperature. Germination of GA_3 and SW treated seeds was higher at 20, 25 and 30^{o}C compared with 10 and 15^{o}C (P<0.05), whereas karrikinolide treated seeds had high germination (90-98%) across all temperatures. These treatments also increased the rate of germination (P<0.05) (i.e. decreased the time to 50% germination) compared with the control at all temperatures (Fig. 3d). In addition, the time to 50% germination decreased as the incubation temperature increased, with the fastest germination observed at 20, 25 and 30^{o}C (Fig. 3d).

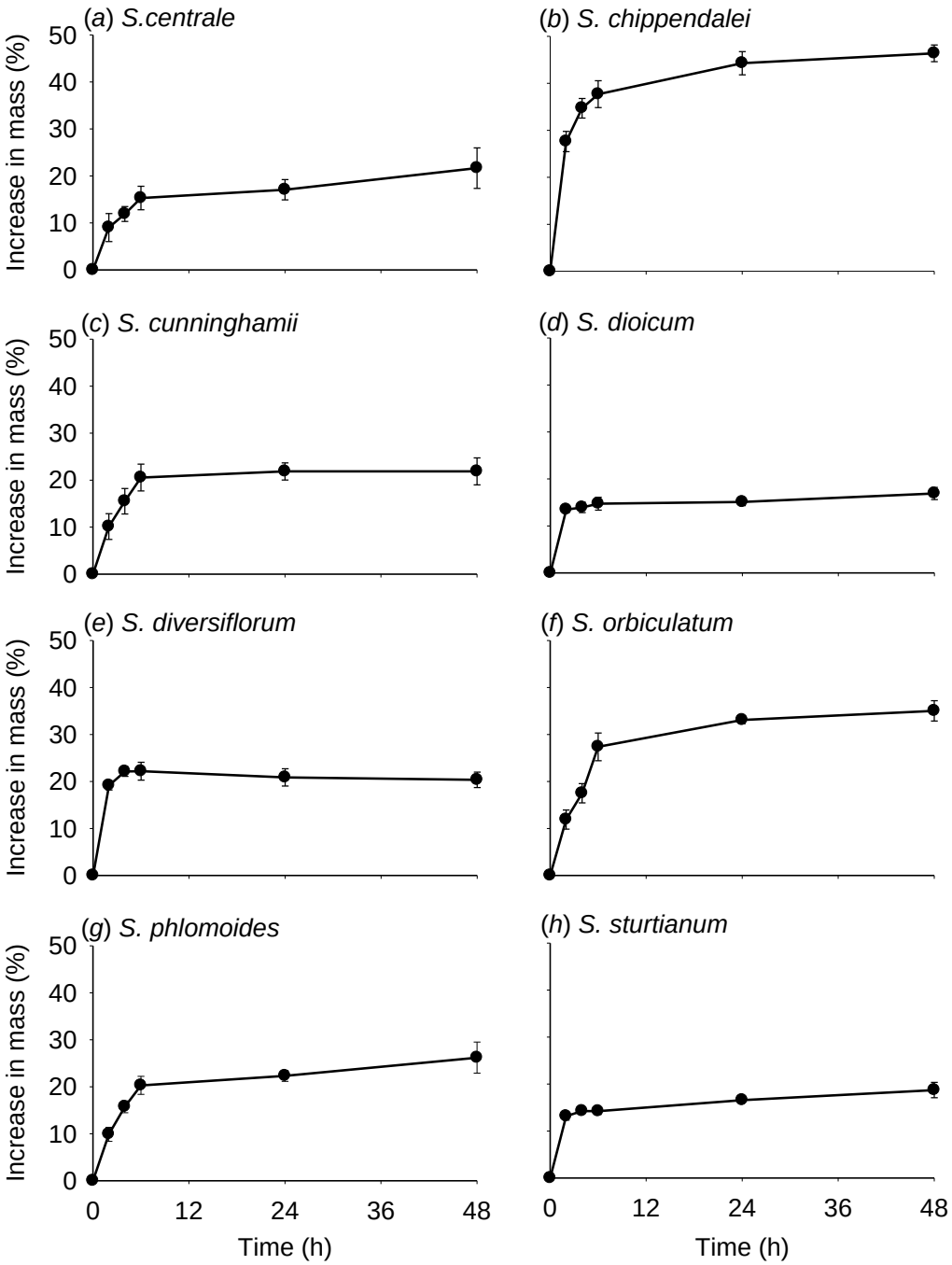

Fig. 1. Imbibition (% water uptake) of eight *Solanum* species over 48 h at room temperature (c. 22^{o}C) (*a*) *Solanum centrale*, (*b*) *S. chippendalei*, (*c*) *S. cunninghamii*, (*d*) *S. dioicum*, (*e*) *S. diversiflorum*, (*f*) *S. orbiculatum*, (*g*) *S. phlomoides*, and (*h*) *S. sturtianum*. Bars indicate standard error

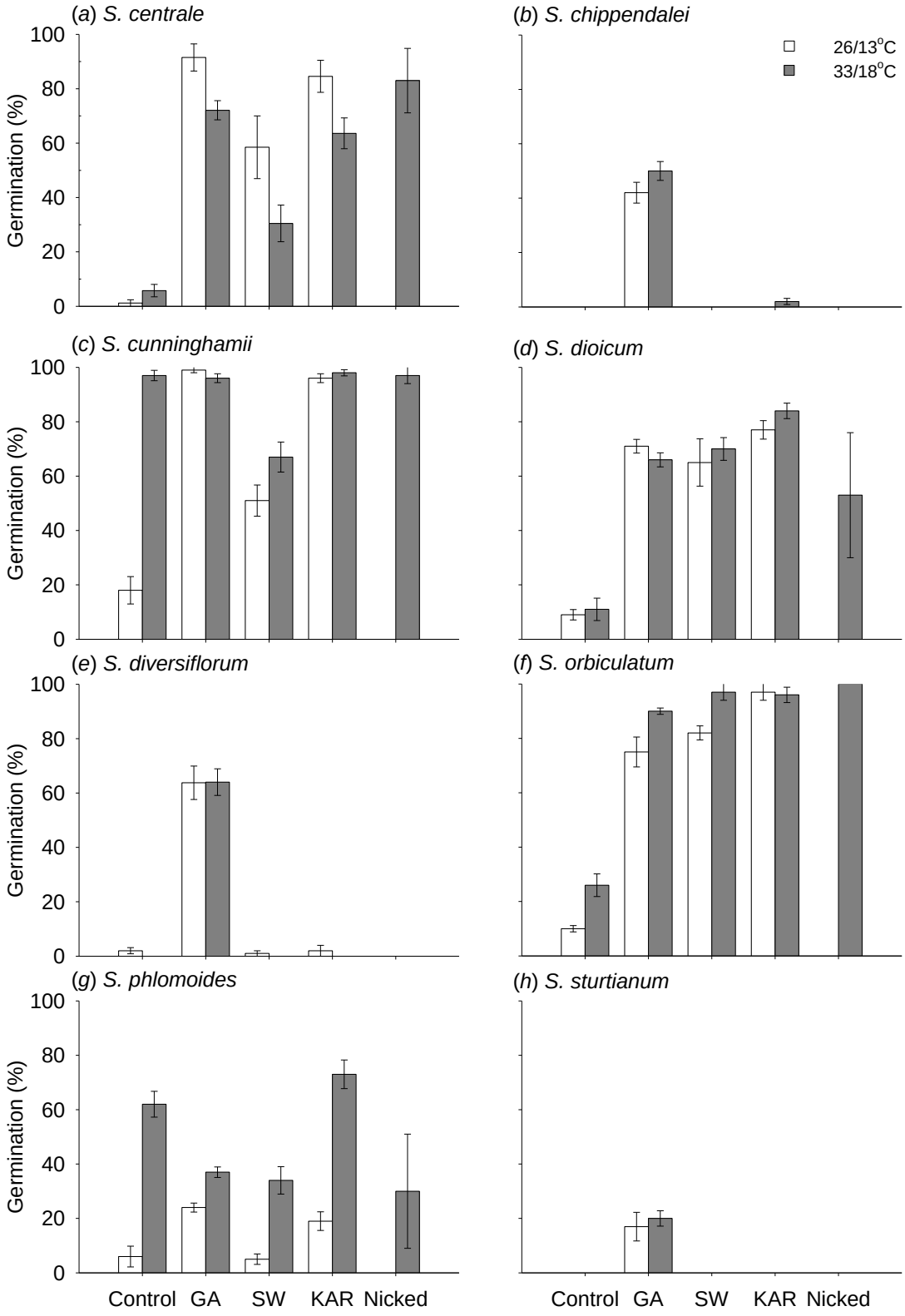

Fig. 2. Mean (± SE) germination (radicle emergence) of (*a*) *Solanum centrale*, (*b*) *S. chippendalei*, (*c*) *S. cunninghamii*, (*d*) *S. dioicum*, (*e*) *S. diversiflorum*, (*f*) *S. orbiculatum*, (*g*) *S. phlomoides*, and (*h*) *S. sturtianum*. Seeds were soaked for 24 h in water (Control), in gibberellic acid (GA), in smoke water (SW), karrikinolide (KAR) or nicked, and incubated at 12/12h alternating temperature regime of 26/13°C or 33/18°C (Nicked treatment only incubated at 33/18°C).

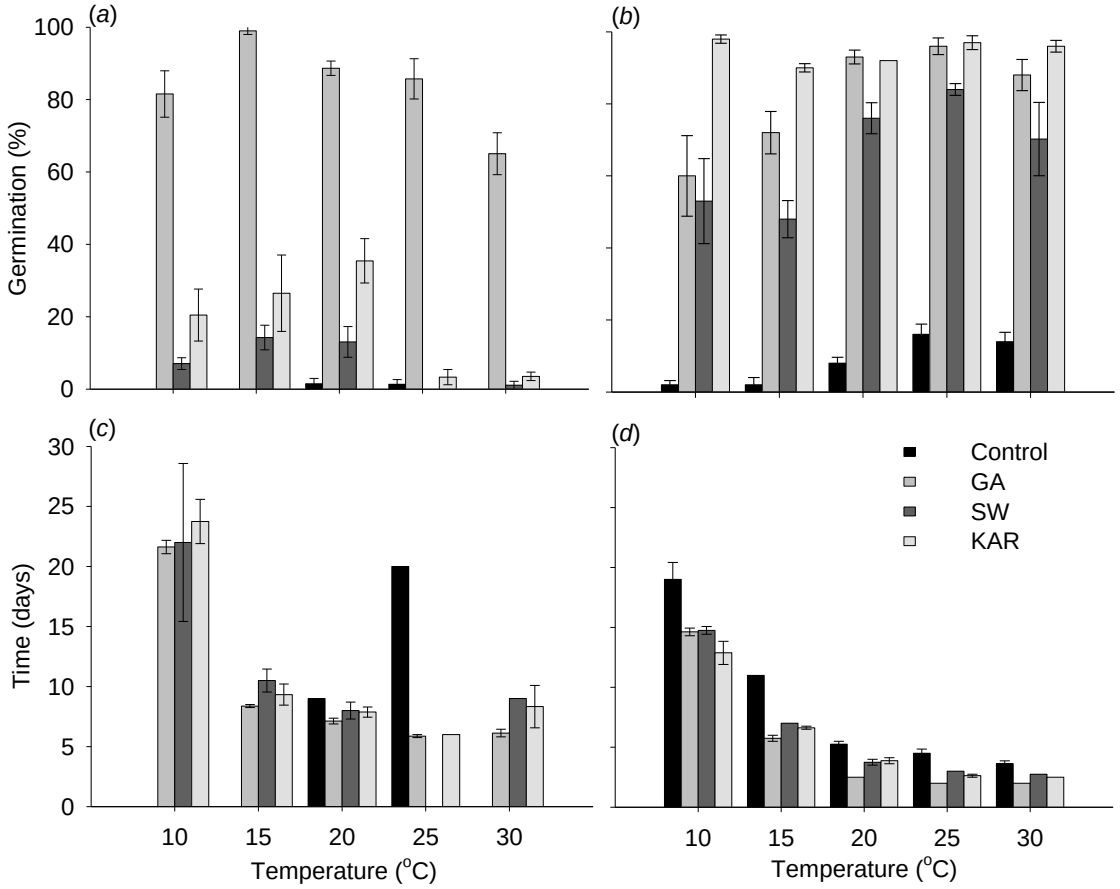

Fig 3. Mean (± SE) germination of (*a*) *Solanum centrale* and (*b*) *S. orbiculatum* and time to 50% of the final germination of (*c*) *S. centrale* and (*d*) *S. orbiculatum* seeds treated with water (control), gibberellic acid (GA), smoke water (SW) and karrikinolide (KAR) and incubated at constant temperatures of 10, 15, 20, 25 and 30°C.

Discussion

Germination was increased in all *Solanum* species at one or both incubation temperatures using germination-promoting compounds and these results provide some direction for more efficient methods for rehabilitation and commercial production. The degree to which each compound was effective varied somewhat between species, probably due to differing germination and dormancy characteristics and different seed ages and storage histories. Some species germinated without treatment, whereas germination in others was stimulated by SW, karrikinolide or GA_3. Firstly, germination of untreated seeds of two species (*S. cunninghamii* and *S. phlomoides*) was moderate to high at the incubation temperature 33/18°C. It is possible that these two species are either non-dormant or they may have after ripened between collection and storage (the time and conditions between collection and storage are unknown), hence dormancy may have been partly or fully overcome. Secondly, species that exhibited little or no germination of untreated seeds (*S. centrale, S. chippendalei, S. dioicum, S. diversiflorum, S. orbiculatum* and *S. sturtianum*) could be considered dormant (i.e. do not germinate within a period of time (30 days)

when provided with normal physical environmental factors (Baskin and Baskin 2004*b*)). However, species where control germination was low, but germination of SW or karrikinolide treated seeds was high (*S. centrale, S. dioicum* and *S. orbiculatum*), may not be dormant, if smoke products are considered as agents that promote germination independently of dormancy status as suggested by some studies (Baker *et al.* 2005; Merritt *et al.* 2007; Rokich and Dixon 2007). For the three species where germination of control, SW and karrikinolide treated seeds of *S. chippendalei, S. diversiflorum* and *S. sturtianum* was low or zero, but germination was promoted by GA_3, the presence of dormancy is likely, although this can not be concluded absolutely as germination was tested over limited temperature conditions and seed age varied.

If seeds are dormant, it is useful to know what type of dormancy they exhibit. Imbibition studies indicated that seeds of all species readily take up water thus do not exhibit physical or combinational dormancy. Observing seed morphology of all species showed that the embryos were differentiated and fully developed indicating that the seeds do not exhibit morphological or morphophysiological dormancy. As four classes of dormancy have been ruled out, dormant species must therefore exhibit physiological dormancy.

Germination promotion by smoke in the Australian flora is well established (Dixon *et al.* 1995; Roche *et al.* 1997) and the active compound in smoke, a butenolide, now know as karrikinolide, has been recently discovered to promote germination of a range of smoke responsive species from a wide variety of ecosystems including arid regions (Flematti *et al.* 2004; Merritt *et al.* 2006; Stevens *et al.* 2007). The results of the present study contrast with two other studies on *S. centrale*; one finding neither SW or aerosol smoke effective at promoting germination (Stefaniski 1998) and the other finding aerosol smoke only increased germination after seeds were nicked (Ahmed *et al.* 2005). A difference in smoke responsiveness could be due to collection of *S. centrale* at different locations and in different years. For example, Stevens *et al.* (2007) found a difference in butenolide (karrikinolide) response of *Brassica tournefortii* depending on collection year and location. In the present study both SW and karrikinolide increased germination of over half of the species (including *S. centrale*). Notably, germination of karrikinolide treated seeds of four species (*S. centrale, S. cunninghamii, S. orbiculatum* and *S. phlomoides*) was higher than that of SW treated seeds at one or both incubation temperatures. Increased germination in the presence of karrikinolide, as compared to SW, was also found in a study on Australian Asteraceae (Merritt *et al.* 2006) and this was explained by

the presence of possible toxic compounds in SW. Similar evidence for toxicity issues with SW have been noted by Flematti *et al.* (2004) who found that undiluted SW reduced germination of *Conostylis aculeata* and *Stylidium affine* compared with a 1 in 10 dilution.

For the three species where SW and karrikinolide failed to elicit germination (*S. chippendalei, S. diversiflorum* and *S. sturtianum* – which had dark seed coats and the largest seeds), the seeds are either not smoke-responsive, or dormancy must be overcome before the seeds become smoke-responsive. Seeds of two of these species were fresh when experiments commenced, and the other had been stored for two years at -18^{o}C, suggesting these seeds may not have been sensitive to the smoke cue. In some studies, freshly collected seeds have been found to be insensitive to smoke. For example, seeds of some species are more responsive to smoke after dormancy has been released by dry after-ripening (Tieu *et al.* 2001*a*) warm stratification (Merritt *et al.* 2007) or soil burial (Tieu *et al.* 2001*b*; Baker *et al.* 2005). Although germination of these three *Solanum* species was not stimulated by SW or karrikinolide, it was stimulated by GA_3. This observation indicates that seeds of the study species exhibit physiological dormancy, as GA has been observed to promote germination of other physiologically dormant seeds (Baskin and Baskin 1998; Baskin and Baskin 2004*b*). However, nicking (scarification) is also known to promote germination of seeds with non-deep physiological dormancy, as the embryos within these seeds lack the growth potential to emerge through their covering structures (Groot and Karssen 1987; Baskin and Baskin 1998; Baskin and Baskin 2004*b*). In this study, nicking did not promote germination of *S. chippendalei*, *S. diversiflorum* and *S. sturtianum* suggesting that germination control is not simply via mechanical restraint to embryo growth imposed by the seed coat. It is therefore possible that the seeds of these three species exhibit intermediate physiological dormancy as in these types of seeds scarification does not overcome dormancy, but GA promotes germination (Baskin and Baskin 2004*b*).

Dormancy of *S. centrale* was recently classified by Ahmed *et al.* (2005). Like our study, these authors found that germination of *S. centrale* seeds was promoted by nicking. They inferred from this result that the seeds had a water impermeable seed coat and that the species exhibited seed coat imposed dormancy. However, imbibition was not tested to determine whether or not the seeds imbibed water prior to nicking. As our study found all eight *Solanum* species readily imbibed, *S. centrale* seeds have a water permeable seed coat and do not possess physical dormancy. Two recent studies (Baskin and Baskin

2004*b*; Baskin *et al.* 2006) have emphasised that mechanical scarification promotes germination of both physically and physiologically dormant seeds, and that some studies have incorrectly identified physical dormancy based on increased germination of scarified seeds, highlighting the importance of imbibition testing for identification of dormancy states.

Although there were some subtle differences between germination at 26/13^{o}C and 33/18^{o}C, for most species broadly similar responses at these two temperatures were evident. In addition, karrikinolide treated seeds of *S. orbiculatum* germinated to a high percentage over the temperature range of 10 to 30^{o}C. This apparent broad temperature range for germination suggests that some *Solanum* species may be able to germinate throughout the year, responding to moisture cues rather than temperature cues (within their normal seasonal range), and enabling germination at any time during the year (Ahmed *et al.* 2005). In a study on germination of central Australian plants, Jurado and Westoby (1992) found that 30% of species tested did not show a preference for germination temperature, although *S. quadriloculatum* had higher germination at 28^{o}C compared with 20 and 12^{o}C. The range over which the *Solanum* species germinated in this study was generally higher than that of species from the south west of Australia which have optimal germination between 13 and 20^{o}C (Bell 1999). In addition, time to 50% germination of *S. orbiculatum* decreased as the temperature increased. These results will be important to those propagating *Solanum* species for restoration and commercial production, particularly if propagation is to occur in areas outside the normal range of the species.

In conclusion, this study has observed that SW, karrikinolide and/or GA_3 can promote germination of eight *Solanum* species, the degree to which differs between species. Seeds of some species may be dormant, and given that *Solanum* seeds have fully developed embryos and seeds readily take up water, it is likely that dormancy is physiological. This study also offers some insight into preferred germination temperatures. The information about germination will be useful for propagation of *Solanum* species for horticulture or restoration.

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