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Ecological niche and bet-hedging strategies for *Triodia* (R.Br.) seed germination

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• **Background and Aims** Regeneration dynamics in many arid zone grass species are regulated by innate seed dormancy mechanisms and environmental cues (temperature, moisture and fire) that result in infrequent germination following rainfall. This study investigated bet-hedging strategies associated with dormancy and germination in arid zone *Triodia* species from north-west Australia, by assessing (1) the effects of the mechanical restriction imposed by the indehiscent floral bracts (i.e. floret) covering the seed and (2) the impact of dormancy alleviation on florets and cleaned seeds (i.e. florets removed) when germinated under water stress.
• **Methods** The initial dormancy status and germination for six species were tested on intact florets and cleaned seeds, across temperatures (10–40 °C) with and without the fire-related stimulant karrikinolide (KAR_1), and under alternating light or constant dark conditions. Physiological dormancy alleviation was assessed by wet/dry cycling florets over a period of 10 weeks, and germination was compared against untreated florets, and cleaned seeds across a water potential gradient between 0 and –1.5 MPa.
• **Key Results** Florets restricted germination (<45 %) at all temperatures and, despite partial alleviation of physiological dormancy (wet/dry cycling for 8 weeks), intact florets germinated only at high water potentials. Cleaned seeds showed the highest germination (40–90 %) across temperatures when treated with KAR_1, and germinated at much lower water potentials (–0.4 and –0.9 MPa). *Triodia pungens* was the most responsive to KAR_1, with both seeds and florets responding, while for the remaining five species, KAR_1 had a positive effect for seeds only.
• **Conclusions** Only after seed dormancy was alleviated by removing florets and when KAR_1 was applied did germination under water stress increase. This suggests that seeds of these *Triodia* species are cued to recruit following fire and during periods of high precipitation. Climate change, driven by large shifts in rainfall patterns, is likely to impact *Triodia* recruitment further in arid zone grasslands.

Key words: Arid grass, floret, karrikinolide, KAR_1, light, recruitment bottleneck, seed dormancy, temperature, spinifex, water potential, water stress, wet/dry cycling

INTRODUCTION

Germination in arid systems is a high-risk event, as sufficient rainfall to initiate germination may not necessarily be enough to support subsequent seedling establishment, plant growth and reproduction (Schwinning and Sala, 2004; Gremer *et al.*, 2016; Liu *et al.*, 2017). As a consequence, there is a tendency for arid zone species to spread this recruitment risk through 'bet-hedging', i.e. by dispersing a high and variable proportion of dormant seeds to avoid depletion in the soil seed bank (e.g. Gremer *et al.*, 2016; Commander *et al.*, 2017). Bet-hedging allows a seed cohort to forego a current recruitment opportunity for potentially enhanced establishment in a future one, thus accepting a short-term reduction in fitness for maximizing long-term fitness by ensuring a seed cohort as a whole is not lost to establishment failure in an unfavourable year (Clauss and Venable, 2000; Tielbörger *et al.*, 2012). Through variably dormant seed cohorts, recruitment events therefore spread risk over several years, thus enabling survival in the soil seed bank.

Although risk-spreading via seed dormancy may provide a survival strategy in water-limited ecosystems, the regeneration of grasslands in arid ecosystems is often infrequent and slow, even under favourable rainfall conditions (Zimmermann *et al.*, 2008; Lewandrowski *et al.*, 2017). Furthermore, arid grasslands are particularly difficult to regenerate, because the recruitment of grass seedlings relies on the simultaneous alignment of several environmental factors (Muñoz-Rojas *et al.*, 2016; Lewandrowski *et al.*, 2017). For example, in fire-prone ecosystems, flushes of seedling recruitment are particularly evident after fire events following periods of high rainfall (Mott, 1978; Lewandrowski *et al.*, 2017). Despite our knowledge of ecological recruitment patterns in natural landscapes, understanding of the physiological interactions driving recruitment processes in the context of bet-hedging for arid grass species is still limited.

Bet-hedging is influenced by the depth of dormancy in any given seed cohort. In the Poaceae, seed dormancy is regulated by two separate, but interacting mechanisms: (1) the ancillary bracts of the floret surrounding the seed, which are thought to limit oxygen and water uptake (Mott, 1974; Huarte *et al.*, 2007) or modify sensitivity to light; and/or (2) the physiological restriction of embryo growth preventing germination

(Gallart *et al.*, 2008; Ma *et al.*, 2008; Bewley *et al.*, 2013). Consequently, seed germination from florets can be slow, resulting in delayed recruitment following favourable rainfall events or optimal moisture conditions. Many studies have shown that the removal of the covering floret structures from seeds promotes higher germination due to increased water imbibition and the reduction of mechanical pressure on embryo growth (Adkins *et al.*, 2002; Bewley *et al.*, 2013; Erickson *et al.*, 2016).

Physiologically dormant grass seeds require suitable temperature and water availability for dormancy alleviation, and certain environmental cues such as smoke and light stimulation for germination to proceed (Baskin and Baskin, 2014). Particularly in fire-prone ecosystems, a considerable number of grass species have a positive germination response after exposure to karrikinolide (KAR_1) (e.g. Long *et al.*, 2011) – a smoke-derived chemical stimulant that is formed through the burning of plant material (Flematti *et al.*, 2004). The overall sensitivity of seeds to KAR_1 varies depending on dormancy state. For example, intact florets (seeds enclosed by the ancillary bracts) that receive a period of controlled after-ripening or burial in field conditions are often more responsive to KAR_1 than freshly dispersed florets (Long *et al.*, 2011; Erickson *et al.*, 2016).

Wetting and drying events that mimic intermittent rainfall periods also influence seed dormancy in a range of arid zone species (Hoyle *et al.*, 2008; Lewandrowski *et al.*, 2017). For grasses specifically, four fortnight-long wetting and drying cycles increase germination performance of previously dormant material (Lewandrowski *et al.*, 2017). From an ecological perspective, this may suggest that rainfall followed by drying periods can potentially alter bet-hedging of any given seed cohort by reducing dormancy under a range of environmental cues. If the floret influences seed germination events, understanding the interaction of seed dormancy and various environmental conditions, such as temperature, water availability and exposure to smoke-related cues, will aid our knowledge of the recruitment dynamics of grasses in natural systems.

This study tested these concepts on seeds of six grass species in the widespread Australian genus *Triodia* (R.Br). *Triodia* grasslands represent one of the most widespread Australian habitats covering approximately one-third of the arid interior, and influence fire regimes that contribute to ecosystem functioning and composition over much of the continent (Nicholas *et al.*, 2009; Nano and Clarke, 2010). *Triodia* recruitment has often been observed following fire events, with germination stimulated by smoke-related cues (Wright and Fensham, 2017). While a considerable body of knowledge exists on the recruitment of *Triodia* species, little is known about how the floret interacts with environmental variables such as temperature, moisture availability and smoke-related cues. Given their extensive distribution throughout the arid zone of Australia, it is believed that the resident soil seedbank of *Triodia* may provide a mechanism for bet-hedging recruitment events during unpredictable fire events and subsequent rainfall periods in the wet season (Wright and Fensham, 2017).

With the combination of floret- and seed-imposed physiological dormancy mechanisms believed to restrict germination in arid grass species to a limited environmental range, this study tested the hypotheses that florets: (1) would restrict germination regardless of the addition of a germination stimulant (KAR_1) across a wide range of temperatures and available light; and (2) wet/dry cycling would improve germination in florets by alleviating physiological dormancy, but there would still be no advantage under water stress when compared with seeds with the floret removed (cleaned seeds), as the floret would restrict germination at decreasing water availability.

MATERIALS AND METHODS

Study region, study species, seed cleaning and quality control

Florets of six species [*Triodia basedowii* E. Pritz, *T. epactia* S.W.L. Jacobs, *T. lanigera* Domin, *T. pungens* R.Br., *T.* sp. Shovelanna Hill (S. van Leeuwen 3835) and *T. wiseana* C.A. Gardener] were commercially collected in March 2011 (Nindethana Seed Service and Native Bushland Seed Supply) from the Pilbara, a region characterized by cool (10–25 °C), dry winters and warm (25–45 °C) summers (BOM, 2016). Rainfall ranges between 300 and 350 mm per annum, and 60–70 % of this total average is received over summer (December–February). Rainfall events occur as small, infrequent and intermittent showers and thunderstorms, or as large events associated with tropical cyclones (Charles *et al.*, 2013). After collection, seeds were maintained in an air-conditioned [approx. 25 °C/50 % relative humidity (RH)] room for 6 months. While after-ripening of florets may have occurred under these warmer and more humid storage conditions (Erickson and Merritt, 2016), germination from florets was still low, ranging between 0 and 40 % across the species tested. Batches of uncleaned florets were transferred to a cool (15 °C), dry (15 % RH) storage facility. The florets in the seed collections had low fill, where only 10–42 % of florets contained a seed (see Supplementary Data Appendix S1; Table S1), and were processed to remove unfilled florets for experimental purposes (*sensu* Erickson and Merritt, 2016). Florets were often joined together as a spikelet, and were separated by gently rubbing through a 2 mm sieve using a rubber bung, followed by repeated passes through a vacuum aspirator ('Zig Zag' Selecta, Machinefabriek BV, Enkhuizen, The Netherlands), which separated light, unfilled floret fractions from heavy, filled floret fractions (i.e. florets that contained a seed). The final quality of the florets was assessed via X-ray analysis (Faxitron MX-20 x-ray cabinet, Tucson, AZ, USA), with most batches displaying 99 % fill (i.e. florets contained a healthy seed that possessed an intact and undamaged embryo and endosperm). In order to obtain pure seeds, florets were removed by gently rubbing them through a 1.4 mm sieve. Floret chaff and seeds were separated with the use of the vacuum aspirator. To remove any non-viable seeds damaged during the cleaning process, seeds were checked under a dissecting microscope and removed if any damage to the testa or embryo was visible. Once processed, batches of filled florets and cleaned seeds were stored at 15 °C and 15 % RH until experimental use.

Experiment 1: determining the effects of floret removal, temperature, light and karrikinolide on germination.

Intact florets and cleaned seeds were incubated at temperatures between 10 and 40 °C, under alternating light or constant dark, and in the presence or absence of the smoke-derived

germination stimulant KAR_1 synthesized following Flematti *et al.* (2004). Prior to germination testing, florets and cleaned seeds used in the experiment were surface-sterilized in a 2 % (w/v) calcium hypochlorite [$Ca(OCl)_2$] solution for 30 min, under 10 min cycles of alternating vacuum (e.g. on/off/on at –70 kPa), and then rinsed in sterile deionized water. After sterilization, four replicates of 25 florets or cleaned seeds were plated on 90 mm Petri dishes containing 0.7 % (w/v) water–agar that was prepared with or without KAR_1 at a 0.67 µM concentration (Long *et al.*, 2011). Petri dishes were transferred into individual incubators set to alternating 12 h light/dark conditions and constant temperatures of either 10, 15, 20, 25, 30, 35 or 40 °C (Contherm Scientific Limited, Wellington, New Zealand). The arrangement of Petri dishes was randomized within treatments, with further daily randomization of dishes occurring within and between treatments within incubators. To exclude light in the constant dark treatments, Petri dishes were wrapped in aluminium foil until scoring at the end of the experiment. Light emitted in incubators was measured at 45 µmol m^{-2} s^{-1}, from cool-white fluorescent lights. The germination test was conducted over 28 d to satisfy dormancy classification according to Baskin and Baskin (2014). Germination was defined as radicle emergence greater than one-third of the floret or seed coat length.

Experiment 2: improving germination in florets through wet/dry cycles

To improve germination of intact *Triodia* florets, a wet/dry cycling experiment was conducted following a modified method of the experiment described by Hoyle *et al.* (2008), under alternating light or constant dark conditions. Each 2-week cycle started with a 48 h wet exposure followed by a 12 d dry exposure at 50 % RH. Nylon mesh bags containing florets were placed onto 0.6 % water–agar during the wet exposure, to enable complete imbibition of the florets. After the conclusion of wet exposure, florets were dried and stored at 50 % RH maintained by a 370 g^{-1} L LiCl solution (Sigma-Aldrich Pty. Ltd, Sydney, NSW, Australia) in an airtight 270 × 270 × 130 mm polycarbonate electrical enclosure box (NHP Fibox, Australia) for 12 d. At the completion of each wet/dry cycle, four replicates of 25 florets were plated on to either water or KAR_1–agar for 28 d at 30 °C (12 h alternating light/dark) and assessed for total germination percentage. The sterilization procedure prior to plating and Petri dish arrangement in the incubators followed the same protocol as described in Experiment 1.

Experiment 3: determining germination responses of treated florets and cleaned seeds under water stress

To determine if the floret limits germination under water stress, a germination test was conducted on different dormancy states (untreated florets, wet/dry cycled florets and cleaned seeds) across osmotic potentials imposed by polyethylene glycol solutions (PEG-8000, Sigma-Aldrich Pty. Ltda). Osmotic potentials are well suited to simulate water stress in soil (Michel, 1983). The various PEG solutions were prepared as described by Michel (1983) for 30 °C incubator conditions, and validated using a dewpoint psychrometer (WP4C Dew

Point PotentiaMeter, Decagon Devices, Inc., Pullman, WA, USA). For this experiment, an 8-week wet/dry cycling (four 2-week cycles) treatment was used for all species, and was conducted as described in Experiment 2. Intact florets and cleaned seeds were plated on 90 mm Petri dishes containing two filter papers that were hydrated with 20 mL of PEG solutions for –0.01, –0.1, –0.2, –0.3, –0.4, –0.6, –0.8, –1 and –1.5 MPa, or a control that was hydrated with water only. All plates were incubated at 30 °C (12 h alternating light/dark). Filter papers maintained moisture for the total duration of the trial and did not require re-wetting. PEG concentrations were re-tested with the dewpoint psychrometer from plates at the conclusion of the experiment to ensure solutions were maintained at the target concentrations. The sterilization procedure prior to plating and Petri dish arrangement in the incubators followed the same protocol as described in Experiment 1. Germination was scored daily over 28 d.

Statistical analysis

Factor analysis. Germination data were analysed using binomial logistic regressions [general linearised models (GLMs)] in the statistical environment R (R Core Team, 2014); data were fitted with a logit-link function followed by a likelihood test. For each experiment, a full model including all main factor terms and their two-way interactions was undertaken for each species separately. In Experiment 1, temperature was analysed as a continuous factor, while dormancy state (intact florets and cleaned seeds), stimulant (water and KAR_1) and light (constant dark and alternating light/dark) were categorical factors. In Experiment 2, the number of wet/dry cycles was analysed as a continuous factor, while stimulant (water and KAR_1) was a categorical factor. Finally, in Experiment 3, water stress was continuous, while the dormancy state (untreated floret, wet/dry cycled floret and cleaned seed) was analysed as a categorical factor.

Parameterization and comparison of environmental thresholds for water stress. In order to determine differences in germination following dormancy alleviation treatment across water stress, non-linear functions were fitted to the germination response for water stress. The thresholds were calculated by fitting a three-parameter Weibull function (Weibull, 1951) available from the 'drc' package in R (Ritz and Streibig, 2005),

$$F(x, b, d, e) = c + (d - c) \exp \left(-\exp \left(b \left(\log(x) - \log(e) \right) \right) \right)$$

where *d* is the parameter for the upper limit or maximum germination on a germination curve, *c* the lower limit equal to 0, *b* proportional to the slope of the curve of *F* and water stress *x*, and the parameter *e* equal at the median response or curve inception point (Ψ_{b50}). Estimated water stress limiting germination at fractions at 95 % response (i.e. the initial reduction in germination by 5 % from the maximum germination response, Ψ_{b95}); median 50 % (50 % reduction in germination from the maximum germination response Ψ_{b50}); and the minimum response (i.e. the water potential reducing germination to a 5 % response close to the base, Ψ_{b5}) were calculated from

the fitted curve. These parameters were chosen to represent different germination fractions of intact florets and seeds that are limited by increasing water stress, assuming that the upper limit for germination represents the maximum response possible in the germination treatment. The three-parameter Weibull functions were most suitable for determining the responses over other non-linear functions, with the curve selection determined as described in Ritz and Streibig (2005). The best-fitting curve was selected based on the log-likelihood, Aikike information criterion (AIC) and lack-of-fit test. Comparison of calculated parameters was performed through pairwise comparisons of means. All analyses were conducted in R (R Core Team, 2014).

RESULTS

Experiment 1: determining the effects of floret removal, temperature, light and karrikinolide on germination

Overall germination responses were between 0 and 85 % and varied significantly for species, dormancy state (intact florets and cleaned seeds), temperature, light and KAR_1 (Table 1). For all species, germination after seed cleaning outperformed germination from florets (all $P < 0.001$), with KAR_1 further stimulating germination on average by 15 % in cleaned seeds ($P < 0.001$). While cleaned seeds germinated between 30 and 85 % on KAR_1, germination from florets even after exposure to KAR_1 was somewhat limited (0–45 %). Additionally,

germination was only significantly improved in florets in *T. pungens* on KAR_1 across temperatures between 20 and 40 °C, while only seeds were affected from most temperatures in the other species (Fig. 1; Table 1). From both intact florets and cleaned seeds, germination proceeded across a wide temperature range under alternating light conditions ($P < 0.001$), while showing a slightly reduced response under constant dark conditions (Fig. 1). Under alternating light, maximum germination responses were between 20 and 40 °C, while germination from constant dark conditions decreased at temperatures >30 °C (Fig. 1).

Overall, the lowest germination was observed in florets of *T. basedowii*, *T. lanigera* and *T.* sp. Shovelanna Hill, which never exceeded 2.5 %, while germination of cleaned seeds ranged between 35 and 45 % ($P ≤ 0.05$) on KAR_1. In contrast, KAR_1 more strongly affected germination in *T. epactia*, *T. pungens* and *T. wiseana*, with maximum germination under optimal temperature between 80 and 85 % for cleaned seeds (Fig. 1).

Experiment 2: improving germination in florets through wet/dry cycles

Out of the six *Triodia* species tested, only three responded significantly to wet/dry cycling (Table 1). In seeds of *T. epactia*, *T. pungens* and *T. wiseana*, germination improved up to 4-fold under optimal treatment of eight weeks of wet/dry cycling ($P < 0.01$), when compared with germination from control

TABLE 1. *Summary statistics (z-statistics and* P*-values) from binomial logistic regressions to determine the effects of dormancy state (untreated florets; seeds), temperature (10–40 °C), light (constant dark; alternating light/ dark) and germination stimulant (water; KAR_1) in Experiment 1; wet/dry cycles (0–10 weeks), germination stimulant (water; KAR_1) in Experiment 2; and, water stress (0 to –1.5 MPa) and dormancy state (untreated florets; wet/dry-cycled florets; cleaned seeds) in Experiment 3*

Factor terms	d.f.	*Triodia basedowii*	*Triodia epactia*	*Triodia lanigera*	*Triodia pungens*	*Triodia* sp. Shovelanna Hill	*Triodia wiseana*
Experiment 1							
Dormancy state	1	3.41***	7.26***	3.70***	7.01***	6.78***	6.87***
Temperature	1	2.80**	4.90***	2.58**	5.12***	2.63**	3.52***
Light	1	–2.01*	3.38***	–0.16	3.48***	1.60	5.54***
Stimulant	1	2.87***	3.33***	2.03*	4.12***	2.64***	3.10***
Dormancy state × temperature	1	–0.01	–1.22	0.25	–1.15	–1.76	0.65
Dormancy state × light	1	–1.01	–2.58**	2.08*	2.03*	–1.63	–6.89***
Dormancy state × stimulant	1	–1.02	–1.05	–0.90	3.12***	–2.22*	1.72
Temperature × light	1	1.55	0.95	–1.57	0.85	0.58	1.83
Temperature × stimulant	1	0.43	0.12	–0.44	0.34	0.42	–0.06
Light × stimulant	1	–1.18	–2.54*	–1.39	2.32*	–0.06	–2.79**
Experiment 2							
Wet/dry cycling	1	–0.13	4.52***	–1.36	4.12***	0.16	5.63***
Stimulant	1	–0.36	2.15	0.17	3.10***	1.56	2.08*
Wet/dry cycling × stimulant	2	–0.05	–0.81	–0.26	–1.49	–0.59	0.18
Experiment 3							
Water stress	1	4.64***	14.10***	9.06***	4.62	4.84***	12.00***
Dormancy state (seeds)	2	7.37***	5.45***	4.92***	4.62***	4.10***	4.41***
Dormancy state (wet/dry-cycled florets)	2	0.01	2.28***	–0.01	1.89	–0.02	1.84
Water stress × dormancy state (seeds)	2	–3.73***	–5.80***	–3.13**	–3.48***	–3.74***	–4.89***
Water stress × dormancy state (wet/dry-cycled florets)	2	–0.004	–0.97	–0.004	–0.21	–0.01	–0.66

Main factor effects and their two-way interactions are shown for each species separately.

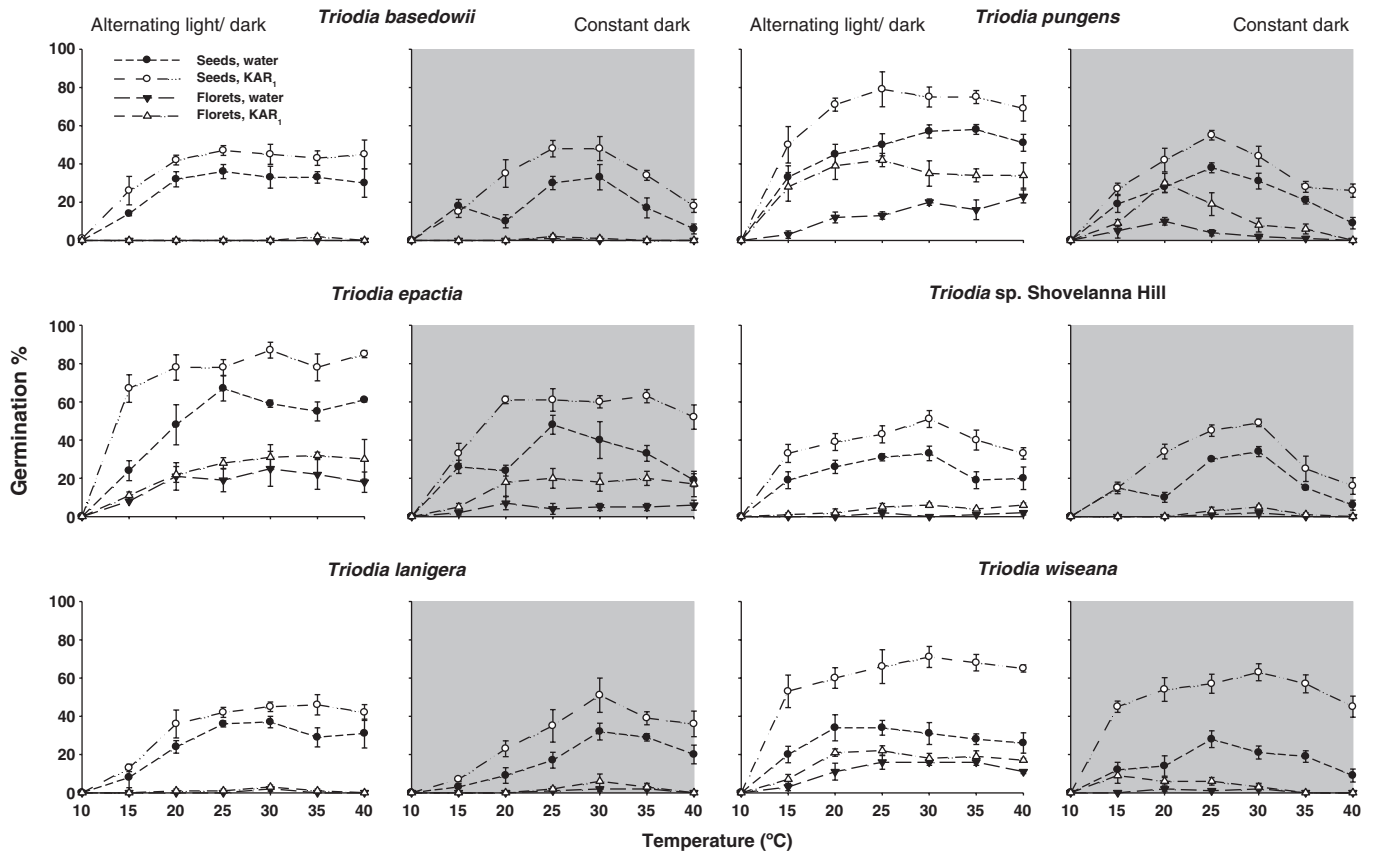Asterisks show the significance level at *$P < 0.05$, **$P < 0.01$, or ***$P < 0.001$.

FIG. 1. Effects of temperature, germination stimulant and alternating light/dark or constant dark treatments on mean germination (± s.e., *n* = 4) of intact florets and cleaned seeds of six Australian *Triodia* species. Intact florets or cleaned seeds were incubated at temperatures between 10 and 40 °C on either water or KAR$_1$ (0.67 μm) agar plates and exposed to 12 h alternating light/dark conditions, or constant dark by wrapping plates in aluminium foil for 28 d.

treatments (Fig. 2). Following eight weeks of wet/dry cycling, germination was 45 % in *T. epactia*, 40 % in *T. pungens* and 38 % in *T. wiseana*. Plating onto KAR$_1$ further enhanced germination, with maximum germination of 65 % in *T. epactia*, 60 % in *T. pungens* and 47 % in *T. wiseana* (all *P* < 0.001; Fig. 2).

Experiment 3: determining germination responses of treated florets and cleaned seeds under water stress

Under well-watered conditions, germination for all species was highest in cleaned seeds, although 8-week wet/dry-cycled florets demonstrated similar responses at 0 MPa in *T. epactia*, *T. pungens* and *T. wiseana* (*P* ≥ 0.131; Fig. 3). The highest germination in cleaned seeds was ≥40% in *T. epactia* and *T. pungens*, 31 % in *T. wiseana*, 21% in *T. basedowii* and *T. lanigera*, and 10 % in *T.* sp. Shovelanna Hill when compared to the two floret treatments (*P* < 0.001).

Overall, the reduction in germination due to water stress was always significantly greater (all *P* < 0.001) for intact florets, when compared with cleaned seeds in all species (Fig. 3; Table 2). The overall range for germination under water stress was also consistently greater for cleaned seeds when compared with intact florets in all species, as shown by lower water potential thresholds (Table 1; all *P* < 0.001). Regardless of wet/dry cycling, intact florets germinated between –0.05 and –0.49 MPa,

while cleaned seeds ceased to germinate between –0.4 and –0.9 MPa (Fig. 3; Table 2).

DISCUSSION

The floret restricts Triodia *germination to a narrow water stress envelope*

This study examined bet-hedging strategies for seed germination in *Triodia* species by testing how the floret impacted seed germination across temperatures and under water stress. While other studies demonstrate that the removal of the restrictive floret improves seed germination under optimal temperature and water availability (Adkins *et al.*, 2002; Ma *et al.*, 2008; Erickson *et al.*, 2016), we show that the floret influences seed germination performance under water-limiting conditions in arid zone grass species. Seeds contained within the florets of all six *Triodia* species showed limited germination under relatively low water stress conditions (i.e. high water potentials; see Fig. 3 and Table 1), suggesting that their seeds were overall highly sensitive to water stress during germination. For all species tested, the median and base water potential thresholds were consistently higher in florets when compared with cleaned seeds, further demonstrating how the floret restricts germination to a narrow range, under water-limited conditions. Such restrictions have significant implications for *Triodia* recruitment in arid zone grasslands (Lewandrowski *et al.*, 2017).
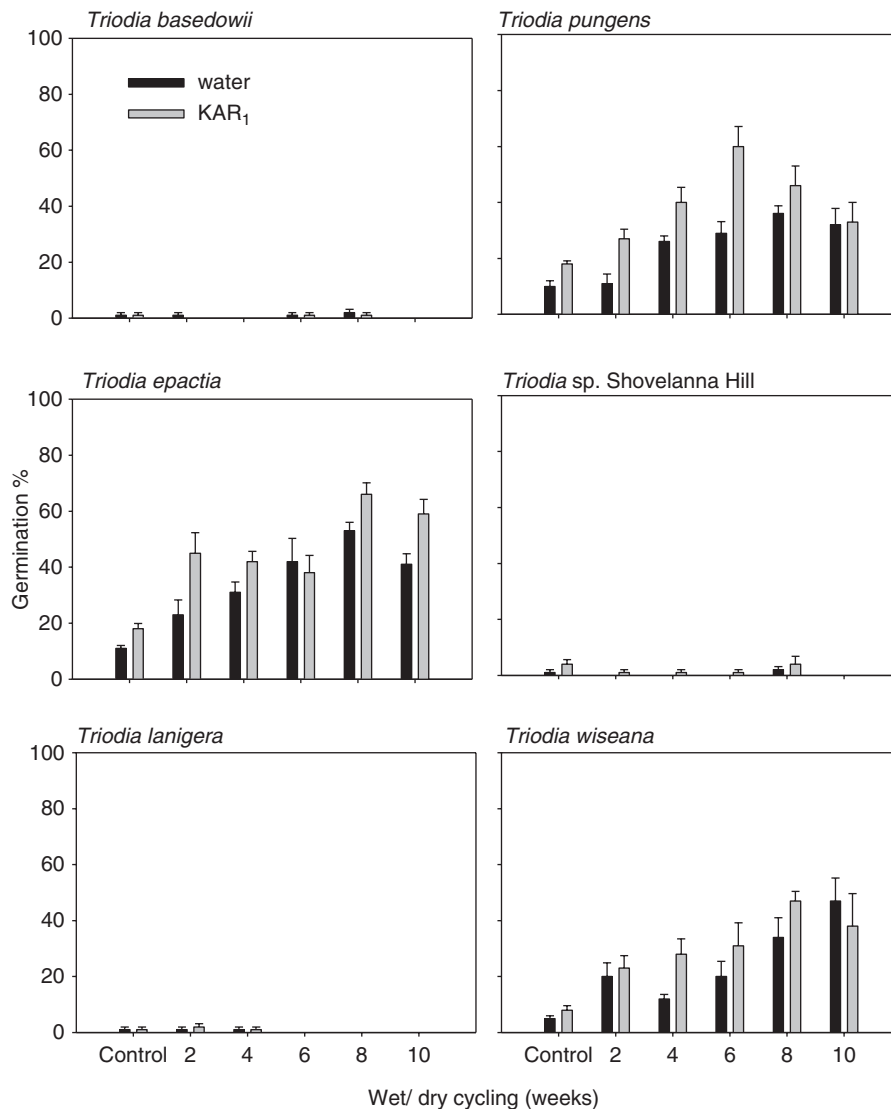
FIG. 2. Effects of wet/dry cycling on germination of intact florets in six Australian *Triodia* species. Florets were untreated (Control) or wet/dry cycled for 2–10 weeks. Following wet/dry cycling treatment, intact florets were incubated in Petri dishes containing either water or KAR$_1$ (0.67 µm) agar at constant 30 °C under 12 h alternating light/dark for 28 d.

Given that the seeds tested in this study were relatively small (approx. 0.7–1.6 mg; see Supplementary Data Appendix S1) and exhibited improved germination responses under alternating light and dark conditions compared with constant dark conditions, the germination niche appears to be close to the soil surface. This is due to smaller seeds lacking the penetrative strength to emerge from depth and they therefore are more frequently found in the upper soil layers (Fenner and Thompson, 2005). Additionally, the wide temperature range (20–40 °C) over which *Triodia* germination occurred is a common trait in many arid zone grass species (Mott, 1978; Jurado and Westoby, 1992; Erickson *et al.*, 2016), and one that may allow seeds to take advantage of prolonged rainfall events across seasons. While rainfall in arid zone *Triodia* grasslands occurs predominantly during summer, high evaporative water stress during summer may disadvantage establishing seedlings. A wide germination envelope for temperature may provide a window of

opportunity for germination to occur during cooler low evaporation periods, i.e. early/ late summer periods, although this remains to be tested.

The processes of spreading germination over time (i.e. as controlled by physiological dormancy) and restricting seedling emergence to periods of greatest water availability (i.e. high water potentials) probably represent a recruitment strategy to reduce seedling mortality in environments where rainfall is sporadic and unpredictable. Because of the different germination envelopes between intact florets and cleaned seeds, we suggest that the floret acts to limit *Triodia* germination when rainfall is low or sporadic. Furthermore, if the floret imposes a mechanical restriction on the seed under water stress, any compromise to floret structure by abrasion or scarification would influence moisture sensitivity, i.e. low water potentials (e.g. Ma *et al.*, 2008; Stevens *et al.*, 2015; Guzzomi *et al.*, 2016).
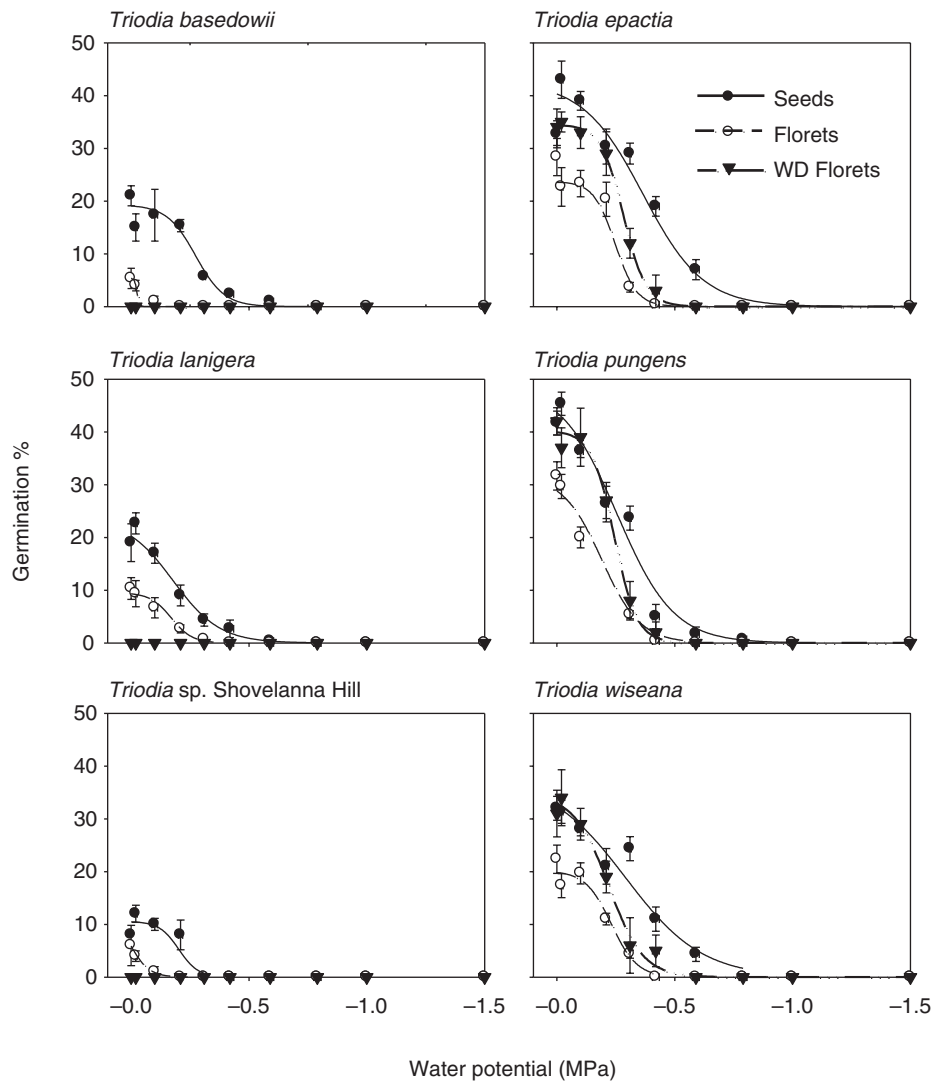
FIG. 3. Effects of water potential on mean germination (± s.e., *n* = 4) of untreated florets, wet/dry-cycled (WD) florets and cleaned seeds in six *Triodia* species. Untreated florets, WD florets and cleaned seeds were plated into Petri dishes containing temperature-corrected PEG solutions at 0, –0.01, –0.1, –0.2, –0.3, –0.4, –0.6, –0.8, –1.0, and –1.5 MPa concentrations, and incubated at constant 30 °C under alternating light/dark conditions for 28 d. Water potential thresholds limiting germination at the maximum (Ψ_{b95}), median (Ψ_{b50}) and minimum (Ψ_{b5}) are presented in Table 2.

Implications for regeneration of Triodia *grasslands in the arid zone*

Our results offer insight into why *Triodia* germination is often so unpredictable in degraded and/or arid landscapes (Bateman *et al.*, 2016; Sluiter *et al.*, 2016; Shackelford *et al.*, 2017). While positive responses to KAR_1 confirm the presence of a smoke-derived cue in *Triodia* seeds (Erickson *et al.*, 2016), we demonstrate that intact florets hamper germination when exposed to KAR_1, and that sensitivity to KAR_1 increases with dormancy loss through wet/dry cycling. Intact florets still seem to hamper seed germination (0–45 %) when exposed to KAR_1 in most *Triodia* species, and maximum germination rates were only achieved when florets were cleaned from seeds (germination 40–90 %). Out of all six species examined in this study, *T. pungens* florets were the most responsive to KAR_1, corroborating the high regeneration potential this species shows following exposure to smoke (Gamage *et al.*, 2014). With intact florets showing a reduced sensitivity to KAR_1 in comparison

with cleaned seeds, our findings suggest that the floret has a significant impact on seedling recruitment in fire-prone ecosystems. Although cued to germinate following fire (Wright and Fensham, 2017), slower germination speed (t_{50} rates >3 d) observed in intact florets (see Supplementary Data Appendix S2, Table S2) indicates that post-fire germination delay is contingent on a requirement for extended water availability after rainfall. Water retention in semi-arid ecosystems is typically short lived during summer periods, while high water stress through surface evaporation would also limit water availability to support seed germination. *Triodia* florets enable further bet-hedging, by preventing immediate post-fire depletion of the soil seed bank and facilitating the potential for some seeds to germinate only after later, extended rainfall events.

Although a number of studies have reported increased germination pulses following soil wetting and drying (Mott, 1978; Battaglia *et al.*, 2000; Fay and Schultz, 2009), our results indicate that natural short-term wetting and drying cycles may be a

TABLE 2. *Water potential thresholds describing the maximum (Ψ_{b95}), median (Ψ_{b50}) and minimum (Ψ_{b5}) (mean ± s.e., n = 4) responses of untreated florets, wet/dry-cycled (WD) florets and cleaned seeds in* Triodia

Triodia species	Dormancy state	Ψ_{b95} [MPa]	Ψ_{b50} [MPa]	Ψ_{b5} [MPa]
Triodia basedowii	Florets	0.0 ± 0.0	-0.01 ± 0.01	-0.05 ± 0.01
	Seeds	$-0.1 \pm 0.01^{t=6.31***}$	$-0.24 \pm 0.01^{t=6.31***}$	$-0.49 \pm 0.05^{t=6.31***}$
Triodia epactia	Florets	-0.14 ± 0.03	-0.27 ± 0.01	-0.36 ± 0.04
	WD Florets	$-0.12 \pm 0.03^{t=-0.21}$	$-0.30 \pm 0.02^{t=-1.06***}$	$-0.39 \pm 0.07^{t=0.65}$
	Seeds	$-0.11 \pm 0.01^{t=0.66}$	$-0.44 \pm 0.01^{t=-3.04***}$	$-0.88 \pm 0.1^{t=-7.14***}$
Triodia lanigera	Florets	-0.04 ± 0.03	-0.18 ± 0.03	-0.32 ± 0.07
	Seeds	$-0.03 \pm 0.01^{t=0.19}$	$-0.24 \pm 0.02^{t=-1.21}$	$-0.49 \pm 0.06^{t=2.20***}$
Triodia pungens	Florets	-0.12 ± 0.02	-0.24 ± 0.01	-0.36 ± 0.04
	WD Florets	$-0.13 \pm 0.02^{t=-0.21}$	$-0.27 \pm 0.01^{t=-1.06***}$	$-0.40 \pm 0.04^{t=-0.65}$
	Seeds	$-0.11 \pm 0.01^{t=0.34}$	$-0.37 \pm 0.01^{t=-6.78***}$	$-0.73 \pm 0.06^{t=-8.01***}$
Triodia sp. Shovelanna Hill	Florets	0.0 ± 0.0	-0.01 ± 0.01	-0.1 ± 0.1
	Seeds	$0.0 \pm 0.0^{t=0.03}$	$-0.18 \pm 0.02^{t=-5.81***}$	$-0.4 \pm 0.05^{t=-6.91***}$
Triodia wiseana	Florets	-0.09 ± 0.02	-0.23 ± 0.01	-0.38 ± 0.03
	WD Florets	$-0.07 \pm 0.03^{t=0.5}$	$-0.26 \pm 0.03^{t=2.59}$	$-0.41 \pm 0.03^{t=-0.46}$
	Seeds	$-0.07 \pm 0.01^{t=0.73}$	$-0.41 \pm 0.01^{t=-5.06***}$	$-0.78 \pm 0.07^{t=-8.32***}$

Untreated, WD florets and cleaned seeds were plated onto Petri dishes containing different PEG solutions simulating moisture stress. Experimental responses in *T. basedowii*, *T. lanigera* and *T.* sp Shovelanna Hill were omitted due to lack of germination following wet/dry cycling.

Water potentials were calculated from a three-parameter Weibull function for germination data across water potentials.

Summary statistics (t-statistics and *P*-values) are shown for WD florets and cleaned seeds for comparison with untreated florets only. Asterisks show the significance level at $P < 0.001$ (***).

significant ecological mechanism influencing recruitment bet-hedging in *T. epactia*, *T. pungens* and *T. wiseana*. As wetting and drying events occur through intermittent rainfall in the arid zone, an increasing number of florets are likely to respond following increasing rainfall in the season. Specifically, for the responsive species, optimal treatment consisted of four cycles of wetting and drying (treatment duration of eight weeks), with responses to KAR$_1$ improving significantly with an increasing number of cycles. For the species that did not respond to wet/dry cycles, other environmental conditions mimicking natural seed bank dynamics may be required to improve germination (Erickson *et al.*, 2016). Taken together, varying responsiveness following wet/dry cycling and exposure to KAR$_1$ indicates that the degree to which bet-hedging occurs varies between species. While this study only investigated six *Triodia* species, further research is required in order to understand whether species responses within the genus are environmentally and/or genetically driven (Anderson *et al.*, 2017; Dayrell *et al.*, 2017).

Implications for regeneration from the restoration perspective

Arid zone restoration success is significantly impaired by low and erratic rainfall, and causes slow and infrequent regeneration (Merino-Martín *et al.*, 2017; Shackelford *et al.*, 2017). With seeds being the primary means to restore vegetation, removing the floret and treating seeds with KAR$_1$ in conjunction with other seed enhancement technologies (e.g. Erickson *et al.*, 2017) may improve establishment success in smoke-responsive grass species; however, this remains untested at large scales. Additionally, we show that successful germination is limited to windows of high water availability. As such, targeted irrigation that mimics large rainfall events to raise soil water potentials for extended periods could enhance recruitment in restoration sites, as long as water potentials are held above critical thresholds for germination.

While active restoration through seed enhancements or irrigation of sites may promote plant regeneration in a restoration context, recruitment events in natural systems may become altered, if not compromised, under predicted climate change scenarios (Parmesan and Hanley, 2015). Climate change is anticipated to negatively influence the timing, duration and intensity of rainfall in many ecosystems worldwide (Millennium Ecosystem Assessment, 2005; IPCC, 2013), including the Australian arid zone (Charles *et al.*, 2013). As a consequence, if arid zone species such as *Triodia* demonstrate such tightly regulated recruitment patterns, shifts in rainfall patterns caused by climate change will significantly alter arid zone plant regeneration worldwide. Only a few studies highlight seedbank resilience to climate change (Parmesan and Hanley, 2015), and further research is required to understand the influence of climate change on seed dormancy, and subsequent plant recruitment in general.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Appendix S1: seed quality of commercial seed collections used for experimentation. Appendix S2: methodology and results for temperature and water stress effects on germination rate of treated *Triodia* seeds.

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