Seed germination biology of the Albany pitcher plant, *Cephalotus follicularis*

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Abstract

Cephalotus follicularis is an ecologically unique, taxonomically isolated and range-restricted carnivorous plant that occurs exclusively within vulnerable wetland habitats in coastal southwestern Australia. Very little is known about the reproductive biology of this iconic plant species, particularly in relation to seed dormancy and the specific requirements for germination. This knowledge gap must be filled to facilitate the establishment of conservation and management initiatives for the species, as *Cephalotus* is increasingly impacted by habitat loss, alteration to natural hydrological and fire regimes and, in recent times, climatic change. This study aimed to determine the type of seed dormancy that the seeds of *Cephalotus* possess, determine the optimum conditions required for seed germination, and examine the storage behaviour of seeds. The seeds of *Cephalotus* are small (1.0 × 0.5 mm), lightweight (0.1 mg) and remain indehiscent within a wind-dispersed hairy achene. Results suggest that the seeds may exhibit some sensitivity to desiccation and appear to be short lived (<12 months) when stored at 23 °C. Maximum germination was achieved after 16 weeks incubation at 15 °C for seeds removed from the protective outer layer of the achene, while seeds retained within the protective outer layer displayed lower germination success. The post-ripening morphological changes in the embryo, limited response to gibberellic

acid, and the long time period required for germination suggests that the seeds exhibit morphophysiological dormancy, with a fraction of seeds remaining dormant for a period of time post-dispersal. These results highlight the importance of limiting hydrological alteration within the few remaining habitats that continue to support *Cephalotus*, but to ensure its long-term protection, further research focusing on phenology and *in situ* recruitment is required.

Keywords: *Cephalotus*, germination, morphophysiological seed dormancy, Albany, Southwest Australia, narrow-range endemic, Oxalidales

Introduction

The Albany pitcher plant, *Cephalotus follicularis* Labill., is the only species in the monotypic Cephalotaceae (Conran 2004; Cross *et al.* 2018c; 2019) and represents an ancient and highly isolated flowering plant lineage that arose in the Late Cretaceous (Heibl and Renner 2012). The species is both long-lived and slow growing, taking up to five years to reach reproductive maturity and develop a woody rhizome from which it resprouts following fire (Cross *et al.* 2019). It also exhibits remarkable ecological specificity (Cross *et al.* 2019), unique seedling and vegetative development (Conran and Denton 1996), and complex biotic associations with invertebrates, including an obligate mutualistic relationship with the range-restricted endemic wingless stilt-legged fly *Badisis ambulans* McAlpine, 1990 (Micropezidae) (Yeates 1992; Cross *et al.* 2019).

Cephalotus follicularis only occurs in the Southwest botanical province of Western Australia (Fig. 1), where it is endemic to a very narrow area along a coastal belt from Nannup in the west to Manypeaks in the east (Lowrie 2014; Cross *et al.* 2018c; 2019). It occurs predominantly in *Homalospermum firmum* Schauer – *Callistemon glaucus* Sweet peat thicket swamp fragments, where it occupies a narrow, and highly specific ecological niche along the water table (Cross *et al.* 2019). Although the species was more widespread in the past, its distribution is now extremely localised, predominantly due to a combination of habitat loss to agriculture, weed and stock encroachment, as well as eutrophication. Consequently, *Cephalotus* has undergone significant population declines in recent decades throughout its range, with fewer than 25% of the 114 recorded historical localities now remaining (Sandiford and Barrett 2010; Cross *et al.* 2019).

Altered fire regimes and hydrological changes stemming from land use alteration and climatic change have also been implicated as major threatening processes (Clarke *et al.* 2018; Cross *et al.* 2019). Without urgent conservation action based on a robust understanding of the species' ecology and biology, *Cephalotus* faces an increasingly bleak future.

An important knowledge gap hindering the development of conservation initiatives is a poor understanding of how and when *Cephalotus* populations recruit from seed. Despite over two centuries of horticultural interest and botanical study of the species, its reproductive ecology has never been studied in detail and its seed germination and dormancy alleviation requirements remain poorly described. *Cephalotus follicularis* produce numerous achenes (unicarpellary single-seeded indehiscent fruit) which function as the diaspore (Cross *et al.* 2019). Achenes, despite being small and lightweight, apparently disperse poorly as populations are isolated to relatively small, disjunct wetlands and exhibit minimal genetic connectivity (Cross *et al.* 2019). The germination morphology of *Cephalotus* is also unusual, with a non-vascularised extension of the hypocotyl growing back into the achene prior to any root development (Fig. 2) (Conran and Denton 1996). Seedlings are rarely observed in natural populations (Cross *et al.* 2018c; Cross *et al.* 2019), and no studies have to date provided empirical data relating to seed ecology or germination biology in *Cephalotus*. Pietropaulo and Pietropaulo (1986) suggested anecdotally that cold stratification (2–3 months) was required to break dormancy and promote germination, and according to some authors germination may take up to a year (Pietropaulo and Pietropaulo 1986; Lecoufle 1990) and seeds often fail to germinate in cultivation unless they are removed from the enclosing protective outer layer of the achene (Lowrie 2014). However, removal of the protective outer layer is apparently not a prerequisite for germination (Conran and Denton 1996). Fire-related cues such as the smoke-derived karrikinolide (KAR_1) (Flematti *et al.* 2004) have been proposed as possible germination triggers for *Cephalotus* (Cross *et al.* 2018b), based upon the role of these cues in stimulating seed germination for other species from similar fire-prone habitats in southwest Western Australia (e.g., Baker *et al.* 2005; Cross *et al.* 2013; Downes *et al.* 2013; Cross *et al.* 2018b). However, there appears to be little evidence for significant post-fire seedling emergence in *Cephalotus* (Cross *et al.* 2019). Therefore, the reproductive ecology of this species needs to be understood before effective long-term conservation measures involving population maintenance, translocations, reintroductions or fire management can be developed and implemented.

This study represents the first detailed examination of seed dormancy and germination biology undertaken for *Cephalotus*. As part of this study we aimed to (i) define seed dormancy type by determining seed water uptake, classifying embryo morphology, determining whether embryo growth occurs within the seed prior to germination, and examining the germination response of freshly collected seeds; (ii) determine the dormancy break and germination responses to different light and temperature cues; (iii) test the effectiveness of the naturally occurring germination stimulant KAR_1; and (iv) examine the capacity of seeds to germinate following periods of desiccation and storage under different temperatures to investigate the seed storage behaviour.

Materials and methods

Seed collection

The seeds of *Cephalotus* are retained inside a small hairy achene (Fig. 2A) (Cronquist 1981; Cross *et al.* 2019). Mature *Cephalotus* achenes (brown and separating from each other) were collected from three natural populations in the Warren region of southwest Western Australia (Table 1). This region is characterised by a temperate Mediterranean coastal climate with generally cool, wet winters and warm summers (Fig. 1).

FIGURE 1

Achenes were collected in mid-late January of 2005 and 2006 (AP), 2017 (GR) and 2018 (MA) from ca. 25 individuals at each subpopulation, with collected achenes subsequently pooled from the individual plants for each population, resulting in four discrete seed accessions. After collection from AP, achenes were stored in a controlled environment room at 15 °C and 15% relative humidity prior to use in experiments. Subsequent accessions were hermetically sealed in laminated foil bags immediately following collection to prevent post-harvest drying as there was an observed decline in germination with post-harvest drying in accessions from AP. Seeds were removed from the protective layer of the achenes where required by gently rubbing achenes between rubber mats, and the seeds visually inspected to ensure the testa showed no indication of damage from the

extraction process. Germination and storage experiments were implemented and conducted, where possible, within four weeks of seed collection.

TABLE 1

Achene and seed characteristics

Achene, seed and embryo characteristics were determined for seeds from GR and MA, with the larger collections from these populations allowing for the examination of some additional traits. Mass was determined for 5 and 10 replicates of 100 achenes or seeds, respectively. Seed size (measured digitally) and seed fill were determined for five replicates of 20 seeds via light microscopy (Leica DFC495 camera with analysis in Leica application X, Leica Camera, Wetzlar, Germany) and X-ray analysis (MX-20 digital X-ray cabinet, Faxitron, Tucson, USA) for all populations. Seeds were scored as filled if the endosperm was fully developed, not shrunken or retracted from the testa, and showed no signs of internal damage.

To assess water permeability of the testa in *Cephalotus*, three replicates of 20 filled seeds from the MA accession were placed into a Petri dish lined with filter paper irrigated with deionised (DI) water. Seeds were weighed at time 0 and then again at 1, 2, 4, 8, 12, 16 and 24 h imbibition after being gently patted dry on paper towels before each measurement. Percentage water uptake by the seeds was determined gravimetrically, based on the fresh weight of non-imbibed seeds after subtracting the weight of the bags, with the percentage increase in seed mass calculated as:

$$[(W_1 - W_d)/W_d] \times 100,$$

where W_1 and W_d are the mass of imbibed and dry seeds, respectively (*sensu* Turner *et al.* 2009).

Embryo morphology and growth

To determine whether embryo growth occurs within *Cephalotus* seeds prior to emergence of the hypocotyl and, thus, if the seeds have underdeveloped embryos that grow and mature within the seed prior to germination (i.e., morphological or morphophysiological dormancy) (Baskin and

Baskin 2014), 100 seeds were incubated for 16 weeks on water agar at 20 °C with a 12-h photoperiod using the same approaches as outlined below for the germination trials. After this time seeds were assessed individually under a binocular microscope and 20 were selected which had signs of testa cracking and swelling (but no sign of radicle emergence or hypocotyl extension – Fig. $2E_{II}$) which was previously noted as a precursor to imminent germination. Each of these seeds was measured longitudinally under a dissecting microscope equipped with an ocular micrometre and then dissected to remove the intact embryo, which was also measured along the same axis. Data were used to determine the embryo to seed length (E:S) ratio for each of the 20 seeds. Fresh seeds were also measured using the same approach after 24 hours hydration at 20 °C under the same conditions; however, seeds were not assessed for cracking or swelling prior to measurement or embryo extraction. The measurements from these latter seeds and embryos were used for comparative purposes.

Germination biology

To assess the germination response to temperature, light, and germination stimulants, achenes and seeds of each collection were plated in 90mm Petri dishes containing 0.7% (w/v) water agar only (control) or water agar containing 2.89 mM gibberellic acid (GA_3; Sigma Aldrich Chemicals, Australia), or 0.67 µM (AP1 and AP2) or 0.99 µM (GR and MA) KAR_1. Achenes and seeds were plated as three replicates of 15 (AP) or five replicates of 25 (GR and MA) for each treatment. Achenes are generally not produced in large quantities by *Cephalotus*, as subpopulations are small and individual plants rarely produce more than a few inflorescences (Cross *et al.* 2019), so replication was constrained by seed availability among collections. Plated achenes and seeds were placed in incubators at each of four constant temperatures: 10, 15, 20 or 25 °C, either on a 12-h photoperiod, or in constant darkness (plated in darkness and wrapped in aluminum foil to exclude light) at 15 ºC for AP1 only.

Germination was defined as hypocotyl emergence to >1 mm, given that a non-vascularised extension of the hypocotyl emerges prior to radicle growth (Conran and Denton, 1996) (Figure $2E_V$). Germination was scored weekly for 16 weeks in light treatments, but once only after 16 weeks in dark treatments. On completion of each experiment, all non-germinated seeds were cut-

tested to determine viability, with seeds possessing a firm, white endosperm and embryo judged to be viable. Germination percentages are, therefore, based on the number of viable seeds.

Seed storage behaviour

To investigate the sensitivity of seeds to desiccation and to determine seed storage behaviour, freshly collected seeds (AP1) were dried at either 20% or 50% relative humidity (RH) at 15 °C for seven days. Seeds were dried to 20% RH as per international standards for gene banking of orthodox seeds (FAO 2014), and drying seeds to 50% RH allowed for examination of the potential for non-orthodox storage behaviour (i.e. desiccation sensitivity) (Hong and Ellis 1996). Following drying replicates of 15 seeds were plated on water agar as described previously. Seeds were then incubated in constant darkness at 10, 15, 20 and 25 °C. Additional replicates of freshly collected seeds were dried by suspending over a non-saturated solution of lithium chloride (640 g L^{-1} or 364 g L^{-1} to achieve 20% and 50% RH, respectively) (Hay *et al*. 2008) at 20 °C, inside a 270 × 190 × 100 mm polycarbonate electrical enclosure box (NHP Fibox, Perth, WA, Australia) prior to being plated on water agar as described previously. Additional replicates of seeds dried under the same conditions were sealed hermetically in laminated aluminum foil bags and stored at either 23, -18, or -80 °C for 12 months. The bags were then retrieved and warmed at ambient temperature (~23 °C) for 24 hours, after which replicates of 15 seeds were prepared for germination testing as described previously and incubated in constant darkness at 15 °C. Germination was scored weekly after 4 weeks and the experiment concluded after 64 days. Additionally, seed viability of non-germinating seeds was tested using tetrazolium (TZ) staining. Seeds were nicked gently to allow the TZ to be absorbed into the seed, placed on filter paper saturated with 1% TZ (2,3,5-Triphenyl-2*H*-tetrazolium chloride) solution and incubated for three days at 30 °C, before the embryos were extracted and analysed for staining under a binocular microscope (*sensu* Moore 1985). Embryos that stained a uniform red were considered viable.

Statistical analysis

A binomial generalized linear mixed model was fitted to assess the main and interaction effects of light, temperature, GA_3 and KAR_1 on the outcome of seed germination success (SPSS Statistics 25, IBM, USA). Population and collection date were included as random effects. One-way ANOVA with Tukey post hoc tests were used to test the effect of incubation duration on E:S ratio for the embryo growth experiment. Preliminary analyses of all data were conducted to test the assumptions of normality (Kolmogorov–Smirnov test), linearity and homoscedasticity (Levene's test), and consequently no data transformations were required. All statistical tests were conducted using the 95% confidence interval (CI), with significance determined by $P \leq 0.05$.

Results

Achene and seed characteristics

The diaspore in *Cephalotus follicularis* is a single, hairy achene with an expanded base (Fig. 2A) that is very light (achene mass 0.03 ± 0.003 mg). The single, permanently enclosed seed is spindle-shaped (Fig. 2B), small (1.0 ±0.07 mm long by 0.5 ±0.04 mm wide) and lightweight (mean seed mass 0.01 ± 0.001 mg) accounting for ~30% of the overall achene mass. Seed fill was high for freshly collected achenes from MA and GR (93 ± 3.4% and 91 ± 4.1%, respectively). Achenes were water-permeable, and seeds removed from the protective outer layer of the achene readily imbibed water, becoming ovoid in shape (Fig. $2E_{II}$) and increasing in mass by 56 ± 3.9% after 24 hours, although most of the mass increase occurred within the first 2 hours of exposure to moisture.

Embryo morphology and growth

The embryo is embedded in copious granulose endosperm and is small, linear, and underdeveloped, but still possesses distinctive cotyledons (Fig. 2C). Embryo length in freshly collected, recently mature seeds from MA was 404 ± 54.5 µm (E: S = 0.4 ± 0.06), which increased to 772 ± 113.5 µm (E: S = 0.6 ± 0.08; $F = 113.40$, d.f. = 1, $P < 0.001$) after 16 weeks of incubation at 20 °C (Fig. 5), just prior to radicle emergence (Fig. $2E_{II}$). Thus, embryo length increased by >90% in seeds prior to germination ($F = 170.56$, d.f. = 1, $P < 0.001$).

Seed germination

Germination is phanerocotylar, with emergence of the cotyledons (and seed coat remnant) from the persistent achene (Fig. 2D_I–D_{IV}) occurring after ca. 105 days on average (range 101–113). Germination was slow in all incubation temperatures, with observation of extracted seeds indicating that the first stage of germination, the emergence and expansion of the non-vascularised parenchymatous and fusiform hypocotyl extension from the seeds (Fig. 2E_{III}–E_V), occurred after ca. 90 days (range 59–120 days). This was followed by the emergence of the cotyledons (Fig. 2E_{VI}) and then the radicle (Fig. 2E_{VII}–E_{VIII}). The fully-expanded cotyledons are obovate, spreading, glabrous, ca. 2 × 0.8–1.0 mm, and the first true leaves are alternate and non-carnivorous, followed shortly by the first carnivorous leaves (Fig. 2D_{IV}).

FIGURE 2

The main effect of temperature on germination was highly significant (Wald χ^2 = 387.5, d.f. = 3, P <0.001). Average germination probability ranged from ca. 0.2–0.8, with significantly higher germination in seeds treated with gibberellic acid and incubated at 15 °C (Fig. 3). No germination occurred at 5 °C in any treatment, and few germinants were observed from extracted seeds or achenes incubated at 25 °C. Although no main effect of population on germination probability was evident (Wald χ^2 = 1.2, d.f. = 3, P = 0.249), germination probabilities were higher for seeds of AP1 (Fig. S1) compared with other collections across all temperatures tested (Wald χ^2 = 35.6, d.f. = 2, P <0.001). This was driven predominantly by higher germination probability for AP1 in control and KAR_1 treatments compared with seeds from other tested populations (Fig. S1).

FIGURE 3

No main effect of germination stimulation treatment was evident and germination probability was not improved markedly by exposure to either GA_3 or KAR_1 (Fig. 3). However, there was a significant interaction between treatment and the removal of the protective outer layer of the achene on germination probability (Wald χ^2 = 7.7, d.f. = 2, P = 0.021), with the germination

probability (across all temperatures) of seeds retained in achenes incubated on water agar containing KAR_1 significantly reduced compared to other treatments (Fig. 3).

Seeds removed from achenes collected from AP1 and GR did not require light to germinate at 15 °C, nor was germination probability light-suppressed. Germination probability did not vary significantly within collections between seeds incubated on a 12-h photoperiod and seeds incubated in constant darkness and the main effect of light on germination probability was not significant (Wald $\chi^2 = 1.3$, d.f. = 1, $P = 0.258$).

FIGURE 4

Seed storage behaviour

Drying of seeds of AP1 to 50% and 20% RH for seven days markedly reduced germination probability on both water agar and water agar containing GA_3, compared with freshly collected seeds (Fig. 4). Highly significant interaction effects were present between drying treatment and germination stimulation treatment (Wald $\chi^2 = 38.2$, d.f. = 2, $P < 0.001$), and between drying treatment and incubation temperature (Wald $\chi^2 = 33.9$, d.f. = 6, $P < 0.001$). Overall, drying reduced germination probability on water agar compared with fresh seeds at both at 20% RH and 50% RH (B = -2.35, $P < 0.001$ and B= -1.55, $P = 0.004$, respectively), although this reduction was less pronounced for seeds incubated on water agar containing GA_3 after drying at 50% RH (Fig. 4). The amplitude of reduced germination probability for seeds dried at 50% and 20% RH compared with freshly collected seeds was greatest for seeds incubated at 15 °C (B= 0.64, $P = 0.261$ versus B = 1.76, $P = 0.001$) and 20 °C (B= 0.69, $P = 0.226$ versus B = 1.52, $P = 0.004$).

FIGURE 5

Seeds stored for 12 months at 23 °C failed to germinate (Fig. 4). Supplementary TZ staining on these seeds indicated that relatively few survived the storage period, as only 40% and 15% of embryos stained for seeds after drying at 50% and 20% RH prior to storage, respectively. In contrast, around 20–40% of seeds germinated on water agar after storage for 12 months at -18 and

-80 °C, and germination probability was not reduced significantly compared with seeds incubated on water agar alone after equilibration for 7 days at 50% and 20% RH prior to storage (Fig. 4). No significant main effects for equilibration RH (Wald $\chi^2 = 0.69$, d.f. = 1, $P = 0.405$) or sub-zero storage temperature (Wald $\chi^2 = 0.20$, d.f. = 2, $P = 0.903$) on germination probability were evident, nor was there a significant interaction effect (Wald $\chi^2 = 0.45$, d.f. = 2, $P = 0.797$).

Discussion

This study provides the first empirical examination of seed dormancy type and the requirements for germination in the narrow range endemic and Gondwanan relict *Cephalotus follicularis*. Our data suggest that the seeds of *Cephalotus* possess morphophysiological dormancy (MPD) and that germination occurs within a relatively narrow thermal range (15–20 °C) after a long period (8–16 weeks) of post-dispersal embryo maturation and growth (Fig. 3) and that these processes are restricted by the presence of the protective outer layer of the achene (Fig. 3). Freshly collected seeds exhibit a degree of sensitivity to desiccation, with germinability and viability declining modestly after drying for 7 days at both 50% and 20% RH (Fig. 4). Seeds exhibited no response to the smoke-derived germination stimulant KAR_1, suggesting that fire does not play a direct role in the recruitment of the species from seed.

It has been previously concluded that seed dormancy within Cephalotaceae was likely to be physiological (PD), based in part upon the lack of under-developed embryos in Poales and their under-representation within Lamiales and Oxalidales (Baskin and Baskin 2014). Germination in this present study was preceded by a period of post-dispersal embryo maturation, with significant embryo growth (i.e., a ca. 2-fold increase in embryo length prior to germination) observed after 8–16 weeks of incubation. This indicates that the linear embryo of *Cephalotus* is underdeveloped at seed maturity (<40 % of seed length). Underdeveloped embryos can also occur in seeds with morphological dormancy (MD), but the sporadic positive response to GA_3 (Figs. 3, S1), the narrow thermal germination window (Fig. 3) and the fact that germination success remained low (<50%) for some populations even under optimal conditions (Fig. S1), suggests an additional physiological component to dormancy (Baskin and Baskin 2004; 2014). The evidence of embryo growth from the present study therefore suggests that the classification of Cephalotaceae seeds as possessing

PD is incomplete and that the seeds of *Cephalotus* instead show morphophysiological dormancy (MPD), with temperature signals required to increase embryo growth potential as a precursor to embryo maturation and germination. If this is correct, then either dry after-ripening (Turner *et al.* 2009) or warm/cold stratification (Merritt *et al.* 2007b) may improve and enhance the germination of *Cephalotus* seeds.

Our germination data imply that the recruitment of *Cephalotus* under field conditions is likely to occur only within a narrow range of thermal and moisture conditions during lengthy periods of seasonal environmental stability, although after-ripening or stratification may further widen the hydrothermal range for germination. Achenes mature and disperse in late February or March (late summer to early autumn) (Cross *et al.* 2019), when daily temperature minima and maxima are generally between 10–15 °C and 20–23 °C, respectively (Fig. 1). The achene likely enhances dispersal of seeds and precludes germination, narrowing the germination window until it is sufficiently eroded. Temperatures over the following 3–4 months of autumn and early winter appear conducive to stimulating embryo maturation and subsequent germination, as daily minimum temperatures are generally 10–15 °C and the daily maximum rarely exceeds 20 °C. Critically, rainfall during this period is usually predictable and moisture levels in the mesic habitats inhabited by *Cephalotus* are likely to remain high enough for an extended period to support gradual germination and seedling establishment. Indeed, *Cephalotus* is known to occupy a highly specific hydrological niche within occupied habitats and rarely occurs outside of drainage depressions below the seepage zone where the substrate remains moist even in summer (Sandiford and Barrett 2010; Cross *et al.* 2019). Given the long period of embryo growth required under optimal thermal and moisture conditions before germination can occur in *Cephalotus*, the dispersal of seeds into less consistently mesic habitats outside this very narrow niche is unlikely to result in successful recruitment. This may, at least in part, explain why the species typically exhibits such highly localised distribution patterns (Conran 2004; Lowrie 2014; Cross *et al.* 2018c; 2019), why there is little evidence of successful long-distance dispersal or genetic connectivity between isolated populations (N. Kalfas unpubl. obs., in Cross *et al.* 2019), and why recruitment in natural populations is observed so infrequently (Cross *et al.* 2018c; 2019).

Although the germination success of seeds from all collections on water agar was highest at 15 °C (Fig. S1), the data showed a degree of both inter-population and inter-seasonal variability in maximum germination success (0.2–0.8). Although neither population nor collection year were significant factors influencing germination success in our analyses, this variability may indicate that seed dormancy depth in *Cephalotus* is influenced by fine-scale or seasonal variability in the moisture and temperature conditions of the maternal environment as has been observed for other species exhibiting a physiological component to seed dormancy (e.g., Long *et al.* 2014; Cochrane *et al.* 2015; Cross *et al.* 2018a). Seeds that failed to germinate following incubation for 16 weeks on water agar largely remained viable, suggesting that a variable fraction of each collection was more deeply dormant at maturity. Additionally, although both the viability and germinability of seeds (of the AP1 population) declined following drying for seven days at either 50% or 20% RH, ca. 30% of the dried seeds germinated readily when incubated at 20 °C on water agar after 12 months in storage at -18 or -80 °C (Fig. 4). The decline in viability upon drying initially observed may reflect variability in the maturity within the seed populations upon collection, and a greater sensitivity to drying in less mature seeds with higher seed moisture content. Nevertheless, mortality was high, and no germination was observed in seeds stored at 23 °C for 12 months. This suggests the seeds of *Cephalotus* may possess only limited capacity for persistence through warm, dry seasonal periods or following dispersal into less mesic habitats. The efficacy of a soil seed bank in conferring population-level resilience to stochastic environmental processes such as drought or fire would depend greatly upon factors such as the capacity for seedling emergence and resilience to the heat from fire at different burial depths (e.g., Auld and Bradstock 1996; Auld and Denham 2006; Cross *et al.* 2017); the persistence of the achene and its potential role as a seed-protecting structure under field conditions (e.g., Sheldon 1974; Joley *et al.* 2003; Erickson *et al.* 2016); the spatial and temporal patterns of seedling emergence in response to recruitment-stimulating events such as flooding (e.g., Carta *et al.* 2013; Cross *et al.* 2014; 2015; Metzner *et al.* 2017); and the longevity of seeds in the soil seed bank under field conditions (Bekker *et al.* 1998; Long *et al.* 2014). These should be priority areas for future research focus.

We observed no significant germination response of *Cephalotus* seeds to the smoke derived chemical KAR_1. A germination response to KAR_1 is typically observed for many species from fire-prone habitats for which fire plays a part in recruitment from seed (Flematti *et al.* 2004; Merritt

et al. 2007a; Baskin and Baskin 2014), including numerous other carnivorous plants that occur sympatrically with, or grow in similarly mesic habitats to *Cephalotus* (Cross *et al.* 2013; Cross *et al.* 2018b). Although *Cephalotus* appears to be resilient to fire, producing vigorous vegetative regeneration from the subterranean rhizome following summer wildfires (Bradshaw *et al.* 2018; Cross *et al.* 2019), it appears unlikely that fire plays a direct role in the stimulation of seed germination. However, it is plausible that *Cephalotus* possesses an alternative fire-responsive reproductive strategy. It has been hypothesised that insect prey may be present at greater-than-average abundance following fire events (e.g., Cross *et al.* 2017) and the rapid production of insect-trapping pitchers and inflorescences observed for *Cephalotus* following fire (Conran 2004; Lowrie 2014; Cross *et al.* 2019) may represent a strategy to exploit prey availability to meet the high energetic demand of regeneration. Enhanced flowering following fire has been previously reported for species from genera such as *Xanthorrhoea* Sol. ex Sm. (Asphodelaceae), *Haemodorum* Sm. (Haemodoraceae), and *Pyrorchis* D.L.Jones & M.A.Clem. (Orchidaceae) in southwestern Australia (e.g., Lamont and Downes 2011; Miller and Dixon 2014). The late autumn achene release by *Cephalotus*, coming after the natural timing of mainly summer wildfires (Sandiford and Barrett 2010; Bradshaw *et al.* 2018), may therefore help to improve the chances of seed dispersal into environments characterised by low competition and high resource availability.

Alteration to natural hydrological regimes has been implicated directly as a causal factor in the decline of multiple *Cephalotus* populations (Lowrie 2014; Clarke *et al.* 2018; Cross *et al.* 2018c; Cross *et al.* 2019) and climate studies predict that in the near future the southwest of Australia will experience an increasingly warmer climate with markedly reduced rainfall and runoff, as well as higher rates of evaporation (e.g., Silberstein *et al.* 2012). Under such predicted future climate and land use scenarios for the Southwest region, the recruitment of *Cephalotus* from seed may become increasingly episodic and unpredictable and, in the long-term, may be largely or completely compromised. Previously the scarcity of information pertaining to the recruitment and reproduction of *Cephalotus* has limited the knowledge supporting land use decisions, for example regarding fire management in its conservation (Cross *et al.* 2019). Accordingly, we undertook this assessment of the species' germination biology to help inform future conservation and management initiatives. With recent work indicating that *Cephalotus* is becoming increasingly impacted by ongoing habitat loss, alteration to natural fire regimes and hydrological changes from

modified land uses (Jennings and Rohr 2011; Lowrie 2014; Clarke *et al.* 2018; Cross *et al.* 2019), there is a clear and urgent need for scientifically-guided conservation actions to safeguard the future of this iconic and distinctive species.

In the face of predicted ongoing declines in the extent, connectivity and quality of suitable *Cephalotus* habitat (Cross *et al.* 2019), we propose that careful fire and hydrological management of habitat remnants will be instrumental to conserving and protecting this ancient and evolutionarily isolated plant lineage. Future studies are needed to examine how far, frequently and successfully the seeds of *Cephalotus* are dispersed, as well as to determine the capacity for, and resilience of, any *in situ* seed bank through seed burial and retrieval experiments. There is also an urgent need to quantify the success and dynamics of seed regeneration under field conditions, over spatial and temporal scales and in response to environmental perturbation in the form of fire and low-rainfall periods. This will help determine to what extent *Cephalotus* is reliant upon recurrent or episodic recruitment from seeds; in the absence of this information, land managers are likely to be poorly equipped to develop meaningful long-term conservation strategies for this species.

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Conflicts of interest

The authors declare no conflicts of interest.

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Tables

Table 1. Location information and habitat descriptions of *Cephalotus follicularis* populations in the Warren region of southwestern Australia from which seeds were collected for the present study.

Population	ID	Collection date	Post-harvest handling	Population location	Habitat description
Ant Pool	AP1	January 2005	15 °C, 15% RH	*34° 41' S*	*Homalospermum firmum/Callistemon glaucus* peat thicket;
	AP2	January 2006	15 °C, 15% RH	*116° 30' E*	*C. follicularis* occurring with *Drosera hamiltonii* and *Lycopodiella serpentina* in dense seepage sedgeland of *Leptocarpus tenax*, *Gymnoschoenus anceps*, *Evandra aristata*, *Schoenus multiglumis* and *S. efoliatus* growing under *Callistemon glaucus* and *Homalospermum firmum*.
Gull Rock	GR	January 2017	Hermetically sealed	*34° 59' S* *117° 59' E*	*Homalospermum firmum/Callistemon glaucus* peat thicket; *C. follicularis* occurring with *Lycopodiella serpentina* in open seepage sedgeland of *Leptocarpus tenax*, *Gymnoschoenus anceps*, *Evandra aristata*, *Schoenus multiglumis* and *S. efoliatus* with scattered *Callistemon glaucus* and *Homalospermum firmum*.
Marbelup	MA	January 2018	Hermetically sealed	*34° 59' S* *117° 43' E*	*Homalospermum firmum/Callistemon glaucus* peat thicket; *C. follicularis* occurring in dense seepage sedgeland of *Leptocarpus tenax*, *Gymnoschoenus anceps*, *Evandra aristata*, *Schoenus multiglumis* and *S. efoliatus* with *Homalospermum firmum* and scattered *Callistemon glaucus*.

642 **Figure captions**

643

644 **Figure 1.** Localities in the southwest of Western Australia from which *Cephalotus follicularis*
645 seeds were collected for this study (A), and monthly minimum (bold) and maximum temperature
646 and rainfall ranges for the Albany region in which this species occurs (B).

647

648 **Figure 2.** Seed germination morphology in *Cephalotus follicularis*. The diaspore comprises an
649 achene (A) containing a single indehiscent seed (B) with a linear embryo (C). Germination is
650 phanerocotylar, with cotyledons (and seed coat remnant) emerging from the persistent achene (D_I–
651 D_{II}). The fully-expanded cotyledons are obovate (D_{II}), and the first leaves are alternate and non-
652 carnivorous (D_{IV}). Extracted seeds become slightly ovoid in shape once imbibed for 23 hours (E_I),
653 then, after several weeks incubation, they are observed to swell significantly with the papery testa
654 beginning to crack (E_{II}) several days prior to hypocotyl extension (E_{III}). Shortly thereafter a non-
655 vascularized parenchymatous and fusiform hypocotyl extension develops and emerges from the
656 seeds (Fig. 2E_{III}–E_V) before the emergence of the cotyledons (Fig. 2E_{VI}) followed lastly by the
657 radicle (Fig. 2E_{VII}–E_{VIII}). Scale bars = 1 mm.

658

659 **Figure 3**. Germination success (mean probability with exact binomial 95% confidence limits) of
660 freshly collected *Cephalotus follicularis* achenes and seeds extracted from achenes incubated on
661 0.7% (w/v) water agar (control), water agar containing GA_3 and water agar containing KAR_1 at
662 constant 5, 10, 15, 20 and 25 °C for 16 weeks with a 12-h photoperiod. Data are pooled germination
663 probabilities for all populations. Annotated lettering represents the results of conditional pairwise
664 comparison tests (contrasts within each treatment). Values followed by the same or no letters are
665 not significantly different among temperatures (at $P \leq 0.05$).

666

667 **Figure 4.** Germination success (mean probability with exact binomial 95% confidence limits) of
668 freshly collected (AP1) *Cephalotus follicularis* seeds, seeds equilibrated at 50% or 20% relative
669 humidity for seven days, and seeds equilibrated at 50% or 20% relative humidity for seven days
670 then stored for 12 months at 23, -18 or -80 °C , incubated on 0.7% (w/v) water agar and water agar
671 containing GA_3 at 15 °C for 16 weeks in constant darkness. Annotated lettering represents the

results of conditional pairwise comparison tests (contrasts within each treatment). Values followed by the same or no letters are not significantly different for each estimate (at $P \leq 0.05$).

Figure 5. Distribution of seed length, embryo length and E:S ratio of fresh seeds (FS) and pre-germinating seeds (PG) of *Cephalotus follicularis* from MA incubated on 0.7% (w/v) water agar at 20 °C for 16 weeks on a 12-h photoperiod.