



Effects of fire season on the reproductive success of the post-fire flowerer *Doryanthes excelsa*

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ARTICLE INFO

Keywords:

Doryanthes excelsa
Post-fire flowerer
Fire regime
Fire season
Reproduction
Seed ecology

ABSTRACT

Fire is an integral part of many ecosystems and recent record-breaking fires in natural systems around the world are indicative of changes occurring to the fire regime. Fire seasonality is one regime element that is shifting, and can impact the reproductive success of plant species, but rarely receives the spotlight when impacts of fire regimes are studied. We examined the effect of fire season on the reproductive effort and seed resourcing of the post-fire flowering *Doryanthes excelsa* from eastern Australia. We used field experiments at sites of different soil moisture levels (wet vs dry) burnt in different seasons (summer, autumn, spring) to measure the reproductive output of *D. excelsa*, including the percentage flowering and the number of seed pods. Seed vigour including seed germination and the percentages of lipids, carbohydrates, and crude proteins of seeds produced after different seasons of burn were analysed. There was a strong effect of fire season on *D. excelsa* reproduction, with percentage flowering, number of seed pods, germination and lipid levels in seeds all higher after summer compared to spring or autumn burns. Burns outside of summer also delayed flowering. The results of this study highlight the importance of fire and the season in which it occurs in the reproductive success of post-fire flowering species like *D. excelsa*, a species representative of a functional type often dominant in some fire-prone systems. Importantly, we found that the impacts of season of burn are not merely a product of differences in fire severity. Ongoing climatic changes driving shifts in the fire regime pose an imperative to consider fire season when managing to conserve this important functional group.

1. Introduction

Fire is an integral part of many ecosystems around the world (Whelan, 2006; Bowman et al., 2009; Miller and Murphy, 2017). The long history of fire as a driver of plant population dynamics has resulted in specific fire regimes (defined by the frequency, intensity and season of fires) among vegetation types (Gill et al., 1981; Pausas and Keeley, 2009). Fire has promoted the evolution of numerous plant traits that enable persistence within these regimes, often specific and complex (Keeley et al., 2011; Lamont et al., 2019; Paroissien et al., 2020). However, recent record-breaking fires around the world, including in Australia, Brazil and the United States, are indicative of changes occurring to fire regimes (Fidelis et al., 2018; Nolan et al., 2020; Higuera and Abatzoglou, 2021; Le Breton et al., 2021). Climate change can alter fire regimes, particularly by increasing fire weather risk days, increasing the severity and frequency of fire and altering the season of occurrence (Williams et al., 2001; Hughes and Alexander, 2016; Balch et al., 2017;

Nolan et al., 2020). Additionally, while humans have co-existed with and used fires for many thousands of years, current increases in ignition and alterations to management have also resulted in significant changes to fire regimes (Syphard et al., 2006). Understanding the impacts of changing fire regimes is essential to predicting plant population dynamics and species persistence. It may also inform management of ecosystems, which in the face of ongoing global change is increasingly important for maintaining biodiversity.

Two key strategies, post-fire obligate seeding and post-fire obligate resprouting (*sensu* Prior and Bowman, 2020), provide two fundamentally different mechanisms driving plant persistence in fire-prone regions (Auld and Bradstock, 2000; Whelan, 2006; Miller and Murphy, 2017). Post-fire obligate seeders are a functional group that are killed during fire and rely on seed banks for recovery and population persistence (Auld and Bradstock, 2000; Prior and Bowman, 2020). Post-fire obligate resprouters have some proportion of plants surviving fire, and regrowth then occurs from buds stored either underground, aurally or at

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<https://doi.org/10.1016/j.envexpbot.2021.104634>

Received 5 July 2021; Received in revised form 24 August 2021; Accepted 25 August 2021

Available online 28 August 2021

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the plant's base (Clarke et al., 2013; Prior and Bowman, 2020). Resprouting species, particularly facultative resprouters (also called 'facultative seeders'; Buhk et al., 2007; Pausas and Keeley, 2014) that depend on regeneration vegetatively and from seed, are less dependent than obligate seeders on seeds for post-fire recovery, but still require recruitment to maintain genetic diversity and to replace senescent individuals (Clarke et al., 2013). Some resprouting species have enhanced flowering following fire, with little or no flowering during the inter-fire period, and therefore limited opportunities to recruit in the absence of fire (Pyke, 2017). Post-fire flowering is a dominant mechanism in some flammable ecosystems such as savannas in Brazil (Massi et al., 2017; Zironi et al., 2021) and also well represented in Mediterranean-type climate regions (Borchert and Tyler, 2009; Lamont and Downes, 2011). Despite this, study of the ecology of post-fire flowering species is remarkably limited (Lamont and Downes, 2011; Pausas, 2017; Pyke, 2017).

Many studies have investigated the impacts of altered fire frequency, and this is the key fire regime element that is considered when predicting species persistence and informing management (Bradstock and Myerscough, 1981). However, other elements of the fire regime, in particular fire season, have not been as broadly studied (Palmer et al., 2018; Miller et al., 2019; Nolan et al., 2021). Up to now, empirical studies quantifying fire season impacts, particularly on key plant functional groups like pyrogenic flowerers, are relatively rare, and there is often an assumption that any effects are a result of variation in fire intensity (Miller et al., 2019). Fires occurring outside of the historical season may interfere with plant phenology and impact recruitment success (Miller et al., 2019; Ooi, 2019). The post-fire flowering functional group is one that may be particularly impacted by changes in fire seasonality (Brewer and Platt, 1994; Bowen and Pate, 2008; Borchert and Tyler, 2009; Pyke, 2017). Seeds of post-fire-flowering species lack dormancy and may only have one chance at reproduction over many decades (Denham and Auld, 2002). Timing of fire season thus has a key impact on the ability of seeds to successfully recruit (Fenner and Thompson, 2005b). For example, Bowen and Pate (2008) found that *Stirlingia latifolia* plants, when burnt in spring, only had a short recovery period before the onset of summer drought and were less likely to produce inflorescences than plants burnt in summer/autumn. As such, this limited both the total reproduction possible and narrowed the window of opportunity for post-fire recruitment.

Fires occurring in different seasons may impact post-fire flowerers through changing patterns of resource allocation or availability. For example, soil nutrients (e.g. nitrogen and phosphorous) are at their highest levels during spring or the wet season, and low nutrient availability during winter may inhibit plant uptake (Koller and Phoenix, 2017; Ellsworth and Sternberg, 2019). These seasonal fluctuations may interact with the increase in available nitrogen produced after the fire, which can be important when vegetative resprouting is driven by the timing of fire (Pereira-Silva et al., 2019). Internal allocation of resources, towards growth or reproduction, is also influenced by seasonal cycles (Miller et al., 2019), and there may be an interaction between allocation and soil nutrient availability in determining recovery (Brewer and Platt, 1994). Depending on the season in which fire occurs, the timing of flowering may also be delayed, or the time between fire and flowering reduced, an outcome that may deplete available resources for flowering and seed provisioning. For post-fire flowering species, which are restricted to reproduction *en masse*, this could be particularly detrimental, limiting reproductive and recruitment success (Brewer and Platt, 1994; Delwiche, 2010; Zhao et al., 2018). Overall, it appears that fires may be detrimental for resprouting and post-fire reproduction when they occur just before or during peak flowering (Miller et al., 2019), and this may either reduce the number of plants flowering and/or the number of seeds produced. However, this has rarely been quantified (Miller et al., 2019).

Of the few studies that have investigated the impacts of changes to historical fire season on post-fire flowerers, reduced reproductive output

when burnt outside of summer appears to be a consistent finding. When burnt in spring rather than summer, a reduction in flowering has been recorded in the Western Australian grass tree *Xanthorrhoea preissii*, while significant decreases in inflorescence development and fruit output were found for *Stirlingia latifolia* (Lamont et al., 2000; Bowen and Pate, 2008). A study in Florida also observed that season of burn could affect floral induction and seedling success (Brewer and Platt, 1994). However, fire season impacts on key seed resource traits has never previously been assessed. Both the amount and type of resources accumulated in seeds, and seed size itself, can determine germination and recruitment success, with lipids, carbohydrates and storage proteins all contributing to germination and early seedling growth (Kitajima, 2002; Soriano et al., 2011).

In this study, we hypothesise that altered fire seasonality will cause a change in multiple reproductive traits for post-fire flowerers, and that this is likely to impact plants independently of other elements of the fire regime (Miller et al., 2019). We aim to address this by investigating how changes in fire seasonality may impact the reproductive success of the post-fire-flowering species *Doryanthes excelsa*, a large herb and often dominant understorey species from south eastern Australia. In doing this, we aim to gain insights into the fire-driven dynamics of this understudied group and provide information on the broader issue of fire management for conservation. We address four key questions:

- 1 How does fire, and fire season in particular, influence reproductive effort at the population level?
- 2 Can fire season affect seed biology, including seed number and size?
- 3 Can the season of fire affect seed provisioning and nutrient storage, and subsequently germination characteristics?
- 4 What is the preferred fire season to maximise chances of recruitment of *D. excelsa*?

2. Materials and methods

2.1. Study species

Doryanthes excelsa Correa (family: Doryanthaceae), is a large perennial herb (Fig. 1), with a basal rosette of ensiform leaves up to 3 m in length and 15 cm wide (Nash, 1996; Dimech et al., 2009) (Fig. 1d). The inflorescence occurs at the end of a scape varying in length from 1.5 m to over 8 m, with many individual leaves (Nash, 1996). Inflorescences may contain up to 30 pinkish-red flowers, which occur in a compact raceme (Newman, 1928) (Fig. 1c). Fruits are loculicidal capsules which as they ripen, dry out, eventually ejecting the seeds (Nash, 1996) (Fig. 1a). Seeds are large and papery forming a wing that facilitates wind dispersal (Nash, 1996; Denham and Auld, 2002) (Fig. 1b). *Doryanthes excelsa* may also reproduce clonally (Nash, 1996).

Doryanthes excelsa is a post-fire-flowering species endemic to the state of New South Wales (NSW), Australia, and one of only two species in the genus. It has a fragmented distribution, with the majority of populations occurring between Karuah (32°38'36.64"S, 151°58'31.69"E) in the north and Mt Keira (34°24'13.2"S, 150°51'23.94"E) in the south, with three isolated disjunct populations further north, near Grafton (Nash, 1996; ALA, 2020). *Doryanthes excelsa* resprouts after fire from underground buds, a process that can be quick with large amounts of biomass produced within weeks of a fire event (see Environment Recovery Project for examples; Kirchhoff et al., 2021). Flowering post-fire may take a year and a half to occur, and release of seeds may not occur for another six months (Denham and Auld, 2002). While it is known that *D. excelsa* flowers after fire, the mechanism that causes this is unknown. It is estimated that seedlings take up to 10 years to reach maturity (Dimech et al., 2009) and attain fire resistance within seven years (Denham, 2007). It is a long-lived species, but while the precise lifespan is unknown, it is estimated to live for many decades (Dimech et al., 2009). When conditions are dry, adult plants maintain contractile roots which pull the underground stem further down to

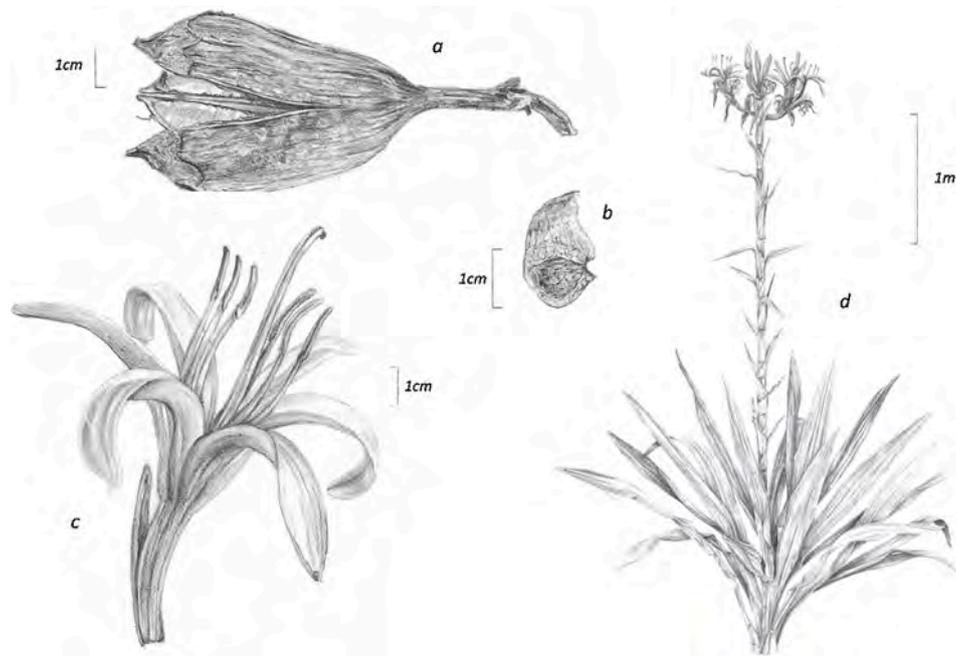


Fig. 1. *Doryanthes excelsa*, (a) seed pod, (b) seed (c) flower and (d) whole habit. Illustrations by R. Paroissien.

protect it (Nash, 1996). Due to their longevity and the fire regime of the study region, individual plants will experience many fires during their lifetime.

There is incongruity in the timing of flowering of *D. excelsa* noted between studies. While Denham and Auld (2002) suggested flowering to be late winter to spring (July to October), Nash (1996) outlined flowering to occur from July to March, with most occurring in spring to early summer (August to December). Whereas The Australian National Botanical Gardens identified flowering in Canberra to be in October–November (Rymer, 1982), and in March–April (Noltie, 2014). The lack of study of *D. excelsa* makes it difficult to determine when flowering occurs and may vary with its distribution. From our observations of *D. excelsa*, development of the flower stem appears predominantly in winter with peak flowering mid-spring (October) and development of seeds late summer/early autumn, though this may vary.

2.2. Study area and abiotic variables

We conducted field sampling within and just outside the Royal and Heathcote National Parks (34°04'30" S, 151°34'24" E, 34°07'49" S, 150°58'17" E) located on the south east coast of NSW in June 2020. We selected sites from maps supplied by the NSW Department of Planning

Industry and Environment, showing history of burnt areas in NSW. The aim of the experimental design was to enable comparison of post-fire reproductive response between burnt and unburnt sites, and between sites burnt in different seasons. Sites were selected from across multiple years to achieve sufficient replication across burn season.

Sites were chosen based on season (via month) of last burn and the presence of *D. excelsa*. The characteristics of each site was also assessed to maintain consistency by ensuring similar vegetation and abiotic features. To control for fire history, we ensured that the penultimate fire experienced at each site occurred at a similar time. This meant that prior to the burn surveyed, fires had occurred in the 2001/2002 fire season for all but one of the 15 sites (Table 1). All sites occurred on shallow infertile soils with underlying Triassic Hawkesbury Sandstone. Qualitative data including elevation, vegetation type, species present, whether *D. excelsa* occurred on ridges, slopes or in gullies, and canopy cover were recorded. Sites were primarily dry sclerophyll forest, with canopy species dominated by *Angophora costata*, *Corymbia gummifera*, *Eucalyptus sieberi* and *Eucalyptus punctata* (Nash, 1996; Tozer et al., 2017). Dominant families in the understorey included Proteaceae, Myrtaceae, Fabaceae, and Orchidaceae, and also included species that share a similar post-fire-flowering life-history such as *Telopea speciosissima* and *Xanthorrhoea australis* (Nash, 1996; Tozer et al., 2017). Climate is humid

Table 1

Sites sampled within the Royal and Heathcote National Parks. (*) indicates sites with seeds collected.

	Site	Altitude (metres)	Date of last fire event	Time since previous burn (years)	Date of sampling
Spring	Wet				
	McKell Ave, Waterfall Site 1*	164	October 2017	16	26/03/20
	McKell Ave, Waterfall Site 2*	144	October 2017	16	26/03/20
Dry	Heathcote	80	August 2015	14	03/04/20
	Werrong Point, Burning Palms Walk	390	August 2017	23	19/05/20
	Bundeena	25	August 2015	13	19/05/20
Summer	Wet				
	Sir Bertram Stevens Drive, Royal National Park Site 1*	138	January 2018	17	03/04/20
	Sir Bertram Stevens Drive, Royal National Park Site 2*	206	January 2018	17	03/04/20
	Dry				
	Hacking River, Helensburgh	206	February 2018	17	26/03/20
Autumn	Wet				
	Maddens Plains Site 1*	390	December 2015	14	16/03/20
	Maddens Plains Site 2	388	December 2015	14	16/03/20
Dry	Barden Ridge	85	April 2018	16	16/03/20
	Menai	56	April 2018	16	12/06/20
	Cawleys Rd, Helensburgh	176	June 2018	17	26/03/20
	Maianbar	53	May 2016	14	19/05/20
	Woronora Dam	207	June 2016	18	22/06/20

subtropical (using the Köppen Climate Classification Cfa). Rainfall in the region is aseasonal with maximum temperatures averaging 26 °C in February and 15.8 °C in July (Bureau of Meteorology, 2020a). Dry sclerophyll forests are fire-prone, historically experiencing fires every 10–30 years during warm months (November to March). However, prescribed fires (i.e. for management such as hazard reduction) are implemented outside of these times in the cooler months (Williams et al., 2001; Tozer et al., 2017).

Sites were split based on moisture (dry versus wet sites) using GIS layers of streams, as classified by the Strahler system (NSW Department of Industry, 2018), overlaid onto fire history maps. The closest branch of the river within the burnt site was recorded; if this stemmed off a higher river close by within the burn, the higher river was recorded. If no stream line occurred in the burn site, the nearest was recorded. This was paired with data collected from sites on the streams, soil, slope and vegetative coverage to determine sites as either 'wet' or 'dry'. Sites were dry if the closest stream was classified as "1" by the Strahler system (i.e. ephemeral streams) (NSW Department of Industry, 2018). Sites were also determined dry if mapped streams were completely dry at the time of sampling.

Sites were selected from a mixture of large and small fires, and prescribed burns and naturally occurring fires. Fires usually resulted in a mosaic of complex patterns, in which large areas may burn over multiple days and have significant variation in intensities, due to changes in weather and topography (Christensen et al., 1981; Hammill and Bradstock, 2006; Werner, 2012). Therefore, we sampled multiple sites within large burnt areas. This was not done for small burnt areas, as these burnt areas were more homogenous. Sampling was also conducted in unburnt sites adjacent to each burnt site, as a control.

Rainfall was recorded for each site, using data from the Australian Bureau of Meteorology (Bureau of Meteorology, 2020a). For each site, rainfall was recorded for the four weeks following fire, using data from the closest weather station, including Darkes Forest (Kintyre), Audley (Royal National Park) or Lucas Heights (ANSTO). To estimate fire severity at each site, an important covariate when comparing fire season, remaining stem tip thickness of burnt individuals of woody species occurring in the shrub layer (e.g. *Leptospermum* spp., *Hakea* spp., and *Lambertia* spp.) was recorded. This was based on the method outlined in Whight and Bradstock (2000), who found that remaining stem tip thickness was a good surrogate for fire intensity (Whight and Bradstock, 2000). Callipers were used to measure the diameter of five burnt tips of a woody shrub, around 1 m from ground level. This was repeated for five plants along a transect, at each site.

To compare rainfall and severity between burn seasons, we used a Generalised Linear Model (GLM) with season of burn used as the fixed factor. Rainfall during the two months following fires was not different between seasons, consistent with the area receiving aseasonal and relatively uniform rainfall throughout the year [Fig. S1 Supplementary information]. Fire severity estimates from stem tips did not differ between sites burnt in different seasons (Fig. S2).

2.3. Field measurements

Reproductive effort was estimated by counting the number of flowering stems and seed pods produced by plants at each site. It was possible to estimate flowering effort for the first season or two after fire as *D. excelsa* plants retain their flowering stems for many years, and have been noted to produce most flowering post-fire (Nash, 1996). The colour of the flowering stems also provided a clear record of age of the flowering event. Green and brown stems represented flowering in the current season (stems take approximately six to nine months to change from green to a smooth brown colour – Paroissien unpubl. data). Weathered grey stems were greater than two seasons old.

2.3.1. Field measurements 1: impacts of fire on reproductive output

We used the 15 identified burnt sites and 15 nearby unburnt sites to

investigate the effects of fire on the proportion of plants flowering and the number of seed pods produced (reproductive effort). At each site, a 50-metre transect was laid down within the *D. excelsa* population, at least 200 m in from the roadside, with the transect running perpendicular to the slope. We sampled *D. excelsa* individuals if their base occurred in a zone 5 m either side of the transect. For each transect, a minimum of 20 individuals were measured. The number of distinct whorls was recorded, and plants were considered the same individual (linked by ramets) if they were within 0.5 m of each other, to avoid sampling clonal individuals. We measured the diameter of the base and the length of the longest leaves, using a tape measure. A linear regression was used to compare plant size (leaf length, base diameter and number of whorls) between wet and dry sites and between seasons. Comparisons between flowering (flowering present/absent) and plant size (diameter at base, length of longest leaf and number of whorls) used a binomial GLM, conducted separately for wet and dry sites and a pseudo R^2 value for goodness of fit of the model was generated using Nagelkerke's R^2 ('rms' package) (Nagelkerke, 1991; Harrell, 2020). There was a relationship between the three plant size parameters measured (longest leaf, diameter of plant base and number of whorls) and likelihood of flowering [Supplementary information S3]. There was however no difference in plant size between wet and dry sites [Supplementary information S3].

An estimate of when inflorescences emerged during the flowering season was made (described below in Field measurements 2), in both the burnt and paired unburnt sites, to directly assess fire effect on recent peaks in flowering. For the older 2015/2016 burnt sites, grey stems represented the post-fire flowering peak, but may also have included inflorescences from more than one flowering season, and will therefore have overestimated flowering effort. We also recorded the number of flower stems and whether they bore an inflorescence, as well as the number of seed pods per inflorescence. Seed pods were determined as viable or unfilled based on size and shape. If they narrowed towards the base and were thin, they were deemed unfilled.

2.3.2. Field measurements 2: impacts of season of burn on reproductive output

We used the 15 burnt sites to investigate the effects of season of burn on the proportion of plants flowering and the number of seed pods produced. Sites were burnt either August to October (spring burns), December to February (summer burns) or April to June (autumn burns). Sites were classed as wet or dry, based on the stream analysis (Table 1).

We derived an estimate of when inflorescences emerged during the flowering season from the colour of the flower stem. Green stems commonly possessed inflorescences that had not yet bloomed. As sites were surveyed during June (our southern hemisphere early winter), we deemed green stems as having flowered recently (i.e. autumn or early winter, approximately April to June) compared to brown stems that had undergone weathering. Brown stems predominantly contained inflorescences with seed pods that had already opened. Stems took approximately six to nine months to turn brown (R. Paroissien, pers. obs), so brown stems were deemed as having flowered either in early or during peak flowering, the previous spring (approximately October). For the more recently burnt 2017/18 fire sites, the total number of brown and green stems were added to count as the post-fire peak flowering event. Sites that had been burnt in the 2015/16 fires had predominantly grey stems. Grey stems were estimated to have flowered more than one season ago and were an indication of post-fire peak flowering after the 2015/2016 fires. As grey stems were representative of more than one flowering season for these older burn sites (i.e. 2017 and 2018), this was an overestimation of total flowering peak that occurred post-fire.

A percentage flowering at each site was estimated using the number of stems as described above over the total number of individuals at each site. The proportion of brown stems as a fraction of recent flowering (i.e. green and brown stems combined totals) in the 2017 and 2018 fires were also analysed to assess whether there was a relationship between the

timing of peak flowering (i.e. early or late in the flowering period) and season of burn.

Of the plants that flowered, the number of seed pods was counted to estimate per plant reproductive effort, but only for sites burnt in 2017/2018. The older (burnt in 2015/16) sites contained primarily grey stems in which pods had decayed and were not easily countable, and it was therefore difficult to confidently determine seed pod numbers. Additionally, seed pod production estimates were also only done for wet sites, due to limited production in dry sites.

2.4. Seed experiments

To assess the effects of season of burn on seed and germination traits, we collected 1–3 ripe seed pods per individual from 7–10 individuals along each transect from two sites for each of two seasons (summer, spring) (Table 1). Seed pods appeared ripe if the pod had started to crack at the top. Collection was carried out using a tree pruner to cut individual pods from the inflorescence, before ballistic dispersal occurred. A minimum of 30 seeds per site was weighed and, for full seed pods, number of seeds per pod counted. Seeds were then used for either a germination experiment or for nutrient analysis.

2.4.1. Seed experiment 1: germination

A single-factor experiment was established to test the effects of season of burn on seed germination characteristics, with two levels of season burnt (summer or spring). Note that, as control sites did not contain many flowering or fruiting individuals, there were insufficient seeds to conduct germination experiments in unburnt sites. Comparisons were therefore made between the sites subjected to different burn seasons. Seeds from individual pods were pooled for each site, two representing summer and two representing spring burns. Four replicates of 20 seeds were sown onto filter paper (Whatmans No. 42) in 15 cm petri dishes for each fire season x site collection and sealed with plastic film. Dishes were watered as required to maintain a moist substrate. Germination experiments were conducted in a Memmert incubator (model: Peltier Incubator IPP110plus) set at 12/12 light/dark hour cycles, at 25 °C for day, 15 °C for night to replicate approximate temperatures experienced in sampled study sites during periods of germination (March, April). Seeds were checked every day until germination rates decreased to less than one germination per two weeks and terminated after approximately eight weeks. Germination was assessed on the presence of a radicle. Total germination was calculated based on the number of germinants divided by the number of viable seeds used. Germination rate (speed) was estimated using the time taken to reach 50 % of seeds germinated (T_{50}), calculated using the 'drc' package in R studio (RStudio Team, 2015; Ritz and Strebig, 2016).

2.4.2. Seed experiment 2: nutrient analysis

To test whether season burnt had an impact on seed nutrient levels, a single factor experiment was established using seeds from two sites burnt in summer and two burnt in spring. The compounds tested were crude protein, carbohydrates and lipids as these are the primary nutrients stored in seeds (Alencar et al., 2012). Seeds were tested for nitrogen with an Elementar varioMACRO cube (CHNS) by XRF Laboratory, Solid State and Elemental Analysis Unit at the UNSW Mark Wainwright Analytical Centre. Three seeds from five plants per site (as allocated for germination) ($n = 15$ seed per site) were analysed for nitrogen. The samples were placed into tin foil into the varioMACRO cube, combusted at 1150 °C, and the gases produced were analysed to record nitrogen (Elementar Analysensysteme GmbH, 2017). Crude protein was then calculated by multiplying nitrogen content by the assumed factor 6.25 (AOAC, 2002) for each seed. Seeds were tested for total lipid and carbohydrate content by the Faculty of Engineering UNSW. The seed coat was removed using a scalpel to provide a more accurate analysis of seed provisioning, and three lots of 2 g of seeds were weighed out for each site (as allocated for germination) for carbohydrates and total lipid.

Triplicate values were produced for Carbohydrates. Lipid was calculated using method 925.12 as outlined by AOAC International (AOAC, 2002). Total carbohydrates was measured using the Sulfuric-Acid-UV method outlined by Albalasmeh et al. (2013).

2.5. Statistical analysis

R studio version 4.0.2 was used for all data analysis (RStudio Team, 2015). We used Generalised Linear Mixed Models (GLMM) for analysis ('lme4' package), including site as a random factor for the field measurements, and replicate seed trays for the seed experiments (Bates et al., 2020). To compare percent flowering and number of pods between burnt and unburnt sites, three fixed factors – season of burn (Burn), dry or wet sites (Moisture) and severity of burn (Severity) – were used, including an interaction term between Burn and Moisture, with Severity as a covariate. The same model structure was used to compare between burn seasons but with the random factor removed (therefore reverting to a GLM), as there was only a single proportion calculated per site. To compare the number of brown stems as a proportion of total stems, season was used as a fixed factor. For number of seed pods and seed experiments two fixed factors (Season and Severity) were used. For all proportional data inclusive of the dependent variables; proportion flowered, germination and seed nutrients (protein, carbohydrates and lipids), a binomial error distribution was used, while a Gaussian distribution was used for continuous data (for dependent variables; T_{50} , size of seed pods and weight of seeds). A Poisson distribution was used for the dependent variables, number of seed pods and number of seeds. A variance inflation factor (vif) from the 'car' package, was used for each variable to test for collinearity (Fox et al., 2020). To account for significance for moisture or an interaction between moisture and season, and to assess differences within the treatments a pairwise interaction analysis was done using the 'emmeans' package (Lenth et al., 2020). A Post hoc Tukey's HSD test was used for the number of seed pods to assess differences between seasonal treatments.

3. Results

3.1. Impacts of fire on reproductive output

There was a strong effect of fire on the proportion of plants flowering, however this was conditional on whether it was a wet or dry site. This was highlighted by a strong interaction between burnt treatment and site moisture ($X^2 = 15.31$, d.f. = 1. $P < 0.001$). Post-hoc analysis showed that there was no difference between burnt and unburnt treatments for the dry sites ($P = 0.524$), but a considerable difference for the wet sites ($P < 0.001$) (Fig. 2a). Burnt wet sites had a higher percentage of plants flowering, on average 49 % ($n = 7$, s.d. = 0.18), with one site reaching 70 %; this was 42 % higher than the average at unburnt sites (7%, $n = 7$, s.d. = 0.09). There was no effect of fire severity ($P = 0.458$). There was a main effect of fire on the number of seed pods produced per flowering individual, by which burnt sites had a greater number of pods ($X^2 = 49.78$, d.f. = 1. $P < 0.001$; mean pods Burnt 4.79 ± 0.44 , Unburnt 2.52 ± 0.58), but no interaction with moisture availability. There was also no effect of severity on the number of pods ($P = 0.284$).

3.2. Impacts of season of burn on reproductive output

There was a marginally non-significant interaction between site moisture and season of burn ($X^2 = 5.15$, d.f. = 2. $P = 0.076$) and a significant main effect of site moisture ($X^2 = 42.52$, d.f. = 1. $P < 0.001$) for the percentage of flowering plants. Pairwise comparisons for season of burn for the percentage flowering for dry sites showed no differences between seasons (all $P > 0.710$). However, for the wet sites, percent flowering after spring burns were lower than summer burnt sites ($P = 0.004$) and autumn burnt sites ($P = 0.003$) (Fig. 2b). Percentage flowering in summer was on average 66 % ($n = 2$, s.d. = 0.06), autumn 54 %

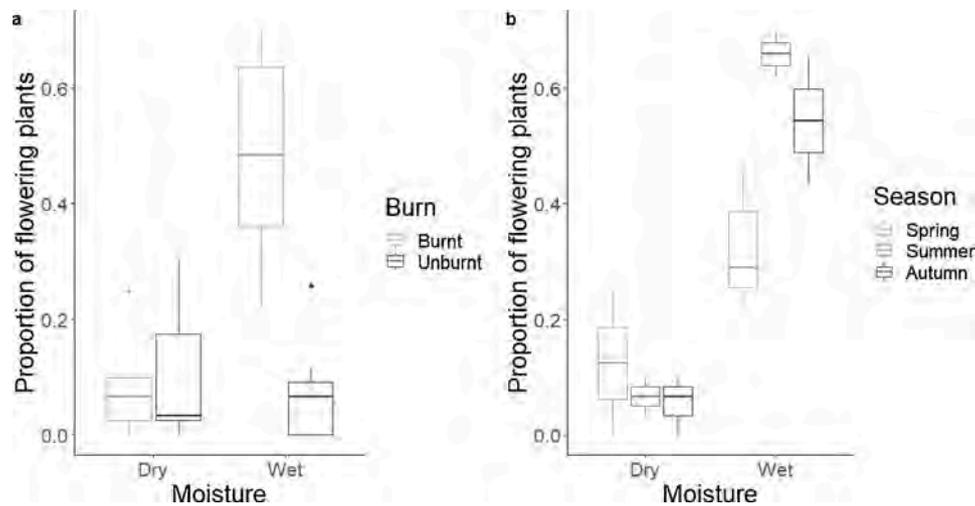


Fig. 2. Box plot showing the proportion of flowering plants during the peak of the flowering season at wet and dry sites (a) under burnt (□) and unburnt (■) conditions; and (b) burnt in spring (□), summer (■) and autumn (▒). The thick line indicates the median, with the boxes either side of the median line the lower and upper quartiles. Whiskers indicate the maximum and minimum values with outliers shown as circles.

($n = 2$, $s.d = 0.16$) and spring 33 % ($n = 3$, $s.d = 0.14$). The percentage flowering in dry sites were on average 8% ($n = 8$, $s.d = 0.08$), with the highest (25 %) occurring in spring.

For the proportion of brown stems (stems emerged during peak of flowering season), there was an effect of season ($X^2 = 16.501$, $d.f. = 2$, $P < 0.001$) at the wet sites. Spring and summer burnt sites were higher than autumn burnt sites (both $P < 0.01$) (Fig. 3) indicating a delay in the flowering time in the autumn burnt sites.

3.3. Seed Pods and season of burn

There was a considerable effect of season of burn on the number of seed pods produced per flowering plant ($X^2 = 103.68$, $d.f. = 2$, $P < 0.001$) (Fig. 4a). Pods from summer burn sites averaged 7.47 ($n = 40$, $s.d = 4.27$), approximately 0.62 higher than spring ($n = 24$, $s.d = 5.89$) and significantly higher than autumn (0.42, $n = 38$, $s.d = 1.96$). A Post hoc Tukey's HSD test revealed summer and spring were different from autumn (both $P < 0.001$), however not from each other ($P = 0.863$).

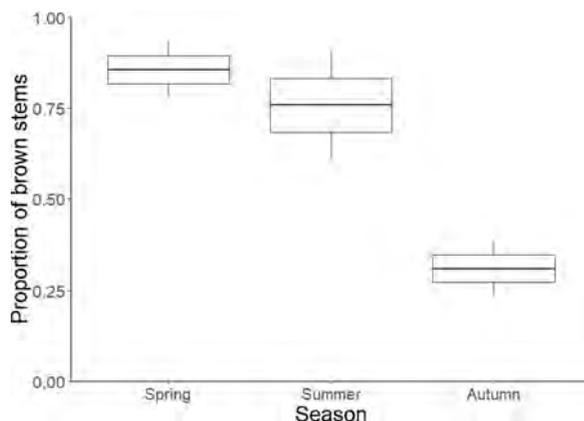


Fig. 3. Box plot showing effect of season of burn (autumn, summer, spring) on the proportion of total stems that are brown. The thick line indicates the median, with the boxes either side of the median line the lower and upper quartiles. Whiskers indicate the maximum and minimum values with outliers shown as circles.

3.4. Seed experiments

There was high variability in the levels of all seed biology parameters measured. There were no effects of season burnt for the seed weight ($X^2 = 0.68$, $d.f. = 1$, $P = 0.41$) or number of seeds ($X^2 = 0.002$, $d.f. = 1$, $P = 0.96$), or the length ($X^2 = 0.84$, $d.f. = 1$, $P = 0.36$) and weight of the pods ($X^2 = 0.99$, $d.f. = 1$, $P = 0.32$). Though increased severity did not have an effect on the weight or number of seeds (both $P > 0.746$), resulted in shorter ($P < 0.05$) and lighter seed pods ($P < 0.01$). Number of seeds varied from 30 to 300 per pod in correlation with the size of the seed pod.

3.4.1. Germination

Germination was different between seasons, on average 92 % ($n = 8$, $s.d = 0.10$) of viable seeds germinating in summer, an increase of 17 % compared to germination following spring fires (75 %, $n = 8$, $s.d = 0.24$) ($X^2 = 14.11$, $d.f. = 1$, $P < 0.001$) (Fig. 4b). Severity also impacted germination ($X^2 = 15.86$, $d.f. = 1$, $P < 0.001$). However, the effect of severity appeared only in spring, in which higher severity caused a decrease in germination. Higher severity in summer did not appear to reduce germination. The rate of germination was calculated using the time to 50 % germination, and was different between seasons, with summer taking 3.8 weeks ($n = 8$, $s.d = 0.49$) to germination and spring 2.7 weeks ($n = 8$, $s.d = 0.41$) ($X^2 = 38.04$, $d.f. = 1$, $P < 0.001$) (Fig. 4c). Severity did not affect the time to 50 % germination ($P = 0.194$).

3.4.2. Nutrient analysis

The nutrient composition of the seeds that the flowering individuals produced also differed by season of burn. Seed nutrients differed only in the level of lipids (Table 2). Summer seeds on average contained 2.4 % more lipids than spring seeds. Higher severity also decreased the lipid content, this effect was stronger in summer ($X^2 = 41.96$, $d.f. = 1$, $P < 0.001$). Crude protein and carbohydrates were however not different between season of burn (Table 2).

4. Discussion

The ability of *D. excelsa* to persist and reproduce after fire is affected by moisture availability and the season in which it burns. Our results indicate that *D. excelsa* is favoured by summer burns, and that burns occurring outside of this season reduce reproductive output. Compared to summer burns, spring burns reduced the percentage of flowering

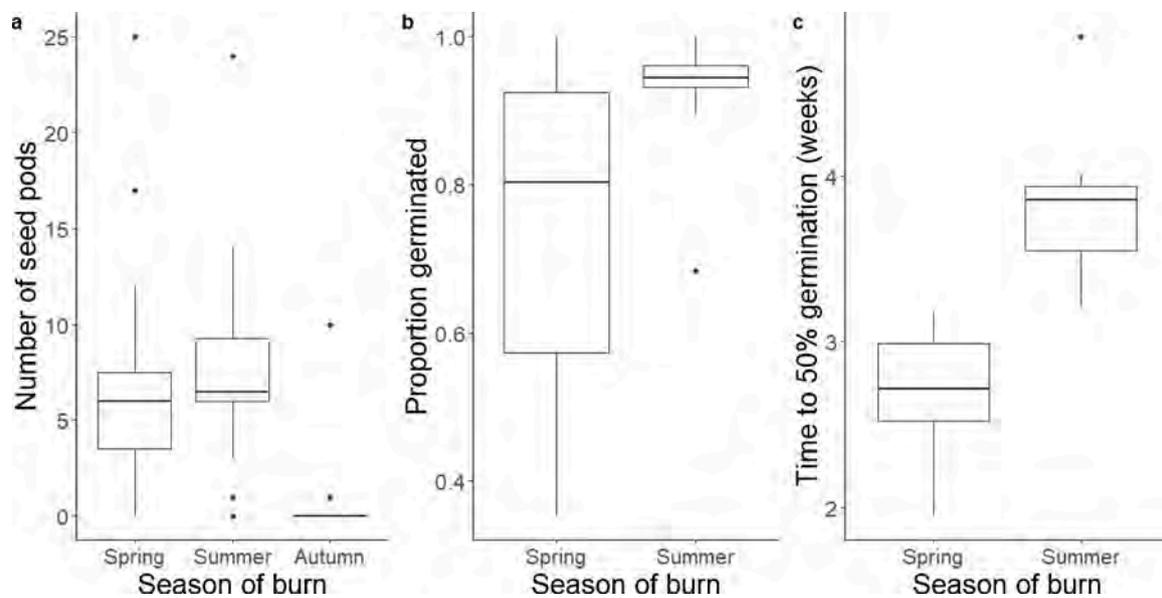


Fig. 4. Box plot showing the effect of season of burn (spring, summer, autumn) on (a) the number of seed pods produced per flowering individuals; and the season of burn (spring, summer) on (b) the final germination after 59 days, as a proportion of viable seeds; and (c) time in weeks for seeds to reach 50 % germination. The thick line indicates the median, with the boxes either side of the median line the lower and upper quartiles. Whiskers indicate the maximum and minimum values with outliers shown as circles.

Table 2

Mean \pm standard deviation of nutrients (crude protein, carbohydrate, lipid) measured as a percentage of the dry weight of seeds. Nutrient comparisons that are significant ($P < 0.05$) are bolded.

Measure	n	Summer	n	Spring	χ^2	d. f	P value
Crude Protein	29	30.84 \pm 2.51	30	31.03 \pm 2.57	1.04	1	0.307
Carbohydrate	18	17.65 \pm 2.75	18	18.54 \pm 2.89	0.91	1	0.339
Lipid	6	32.44 \pm 1.54	6	30.04 \pm 0.48	138.20	1	P < 0.001

plants, autumn burns produced shifts in the season of peak flowering, while burns in either spring or autumn caused a reduction in the number of seed pods. Reproductive output was not the only implication of altered fire season, as laboratory experiments also showed effects on resource allocation to seeds. Both germination response and seed lipid stores significantly decreased after spring burns. While these results are consistent with previous studies of post-fire flowerers, suggesting that seasonal aspects of the fire regime can impact post-fire response of plant species (Platt et al., 1988; Lamont et al., 2000; Bowen and Pate, 2008; Miller et al., 2019; Ooi, 2019; Thomsen, 2019), this work is the first, to our knowledge, to highlighting impacts on seed resourcing. Importantly, we found that seasonal fire effects occurred independently of fire severity.

Fire season may interfere with plant phenology (Miller et al., 2019) and we found this to be the case depending on moisture levels at the study sites. The wet sites had a greater percentage of flowering plants following summer and autumn burns, but considerable and significantly lower percentages in spring burnt sites. As noted by Miller et al. (2019), the impacts of fire season may be most detrimental when they occur during peak flowering and this was the case with *D. excelsa*, which has a flowering peak in spring. However, another particularly interesting result was that autumn fire delayed flowering, with the greater number of brown stems after autumn fires indicating flowering occurring later in the season (April to June). This means that fires occurring outside of the historic summer fire season, be it spring or autumn, had different, but potentially negative impacts.

There was a direct effect on fitness after spring fires, with reduced levels of flowering and therefore reduced seed output. However, while the proportion of plants flowering was not reduced at autumn burnt sites, there was a significant reduction in the number of seed pods produced per plant, resulting in a similar direct negative effect on fitness. Furthermore, autumn fires caused delayed flowering, a response that has been the more frequently observed impact. Gill and Ingwersen (1976) showed that the timing of fire at *Xanthorrhoea australis* sites in eastern Australia was able to alter timing of flowering with, for example, a fire in September (spring) causing flowering earlier. Johnson et al. (1994) noted in *Blandfordia nobilis* sites in NSW that the majority of flowering occurred in the first post-fire season after both summer and autumn fires, but that fires occurring in either winter or spring could delay flowering to the second season. Delayed flowering may impact synchronisation with pollinators, reducing pollination and thus seed output (Borchert and Tyler, 2009; Pyke, 2017), a possible mechanism for the lower number of pods recorded in this study following autumn burns. However, reduced production per plant was also recorded in post-fire flowerers in Western Australia, where Lamont et al. (2000) found sites burnt in summer produced over three times more fruit than sites burnt in autumn, and five times more than sites burnt in spring, suggesting this to be a result of resource limitation.

The potential driver of limited resources may be related to seasonal dynamics of nutrient or storage reserves held by plants (Abbott, 1985) such as non-structural carbohydrates (NSC) which are critical for resprouting after disturbance (Smith et al., 2018). A number of studies show NSC concentrations of plants cycle through the year and often peak in summer (Tolsma et al., 2010; Martínez-Vilalta et al., 2016). Burning in summer for such species would therefore be the most suitable for well-resourced recovery. However, burning when NSC levels are low, means that resprouting would further deplete this resource. When NSC resources are limited, studies have found that flowering of masting species is limited (Crone et al., 2009), providing a potential mechanism for reduced flowering after spring burns in *D. excelsa*. Limitation also means that resources are unequally provisioned for seed set (Vaughton and Ramsey, 1998; Clarke et al., 2013). While there was large variability in many aspects of the seed traits measured, there was no differences between season burnt for seed number and mass. This indicates that when resources are limiting, number of pods may be traded off to

maintain seed quality.

The store of lipids in *D. excelsa* seeds was strongly influenced by fire season; spring caused a reduction of lipid levels. More broadly, it has been suggested that the ability of *D. excelsa* to persist and establish lies in their large seed size, however until now the proportion of nutrients stored in the seeds had not been determined (Denham and Auld, 2002). While carbohydrates, proteins and lipids are all abundant in seeds, these may be traded off; lipids store significantly more energy per unit weight, resulting in a lighter seed with similar germination vigour (Fenner and Thompson, 2005a; Alencar et al., 2012; Borek et al., 2015). As *D. excelsa* is a wind distributed species, it would benefit from this trait. For example, a study of a number of native Australian *Acacia* species, a group with no wind dispersal traits, found an average lipid content of 11 % on a dry weight basis (Brown et al., 1987), three times less than *D. excelsa*. However, with regards to the differences in seed lipid content found between the spring and summer burn treatments, rates of accumulation may differ because of variation of depleted nutrients in the soil or less time to accumulate seed reserves relative to the lag between resprouting, seed production and timing of fire (Vaughton and Ramsey, 1998).

Differences in seed reserve accumulation may also result from interactions with the plants phenological cycles. Lipid levels in plants are known to vary seasonally via photoperiodic signals, with accumulation increasing with the onset of shorter days (Grimberg et al., 2018). Levels within the seed itself may differ over seasons (Tillman-Sutela et al., 2011). If plants are burnt during lipid accumulation, overall stores directed to the seed could be depleted. Spring burns produced seeds with slightly lower levels of lipids and also caused a decrease in germination. Evidence for a direct link between seed lipid levels and germination capacity is mixed. For example, Tillman-Sutela et al. (2011) found a similar result in which a greater lipid content and germination occurred in seeds measured in summer, which was attributable to higher nitrogen in the soil, whereas Gardarin et al. (2011) found that higher lipid levels caused a faster rate of germination, contrary to our results. There was also greater variability in the germination for spring burnt sites. Lipids have been linked to controlling the change from dormancy to germination, suggesting that higher lipid contents may result in less variation in germination (Zhao et al., 2018). The longer time taken could be a result of bet hedging, in which staggering the germination over weeks may increase success if environmental conditions are not suitable, especially given the lack of dormancy in the species (Letnic et al., 2000; Denham and Auld, 2002).

The significant difference in the proportion of plants flowering between burnt and unburnt sites confirms the assumption that fire promotes flowering in *D. excelsa* (Nash, 1996; Denham and Auld, 2002). We also found that the likelihood of flowering increased with plant size, potentially related to plant age. As resprouters store their buds underground and survive fire, the vegetative growth that occurs following fire could be considered to be correlated with the size of the ramets and age of the plant (Bowen and Pate, 2008). For example, Lamont et al. (2000) found for *Xanthorrhoea preissii*, that the size of the plant was shown to affect post-fire flowering, attributable to its age. Nevertheless, while we found a strong flush of flowering occurred following fire, flowering may still occur in years without fire. Denham and Auld (2002) in their seven year study witnessed each individual of *D. excelsa* to flower and fruit only once after fire. Yet we recorded flowering at sites unburnt for over 30 years, suggestive of a second flowering for at least some individuals. It can therefore be assumed that *D. excelsa* is opportunistic in the years between fire; however, what promotes this inter-fire flowering is unknown. While most studies on post-fire flowerers seem to be carried out for no more than 10 years, a longer temporal study may inform the underlying mechanisms of flowering (Johnson et al., 1994; Denham and Auld, 2002; Borchert and Tyler, 2009) [Supplementary information S4].

A number of studies suggest that the effect of season of burn is mainly due to the effect on fire intensity (e.g. Christensen et al., 1981). However, we found impacts of fire season independent of fire intensity by

including severity as a covariate. While severity did significantly affect the number of seed pods produced, the level of lipids and the germination, it did not impact the percentage flowering, or the rate of germination. Thomsen (2019) found a similar effect in which fire season and severity were able to impact resprouting plants independently of each other and in combination. It should therefore not be assumed that the effect of season of burn is due to a difference in severity. Additionally, fire season is quite often only the focus of research in areas with marked seasonal climatic differences, particularly seasonal rainfall (Lamont et al., 2000; Bowen and Pate, 2008), and plant response is usually interpreted in the context of seasonal drought. The significance of fire season in wet sites in our study region, where rainfall is aseasonal, presents a clear example of an independent fire seasonality effect; reduced reproductive effort after spring and autumn burns at sites with dependable moisture. Moisture obviously still plays an important role and our results presented a consistently low percentage flowering for all dry sites. The drought experienced through 2019 and at the beginning of 2020 may have exacerbated dry conditions, reducing flowering for sites limited in moisture (Bureau of Meteorology, 2020b).

Ongoing climatic changes driving shifts in the fire regime and drought provide an imperative to consider fire season when managing to conserve species susceptible to regime shifts (Le Breton et al., 2021). Alterations to seasonal elements of fire regimes may decrease the reproductive effort and thus the long-term persistence of species from the important post-fire flowering functional group. In the study region, prescribed burns are conducted either side of the summer period to ensure they are able to be controlled (Penman et al., 2011), and we suggest autumn burns are preferential, as spring burns appeared to have consistently negative impacts on reproductive effort. This and other recent studies (e.g. Thomsen, 2019) suggest that this may be applicable to post-fire flowerers more broadly, highlighting the importance of burns being carried out in favoured seasons for this functional group. Fire season very rarely receives the spotlight when looking at impacts of fire regimes, perhaps due to the complex interaction of processes that occurs when fire season is altered (Miller et al., 2019), particularly with severity and moisture. The results of this study highlight the importance that both fire and the season in which it occurs play in the reproductive success of *D. excelsa*.

Funding

This work was supported by the South Turrumurra Environment Protection (STEP) Inc via a John Martyn Research Grant for the Conservation of Bushland to RP and both an Australian Research Council Linkage Project grant (LP180100741) and NSW Office of Environment and Heritage Saving our Species Research Grant (DOC19/33961-1) to MKJO.

CRediT authorship contribution statement

Ruby Paroissien: Conceptualization, Formal analysis, Investigation, Writing - original draft, Visualization, Funding acquisition. **Mark K.J. Ooi:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships with other people or organizations that could inappropriately bias their work.

Acknowledgements

Seed testing was undertaken by Alia Rifai and Jayashree Arcot from the Faculty of Engineering UNSW and Irene Wainwright and the XRF Laboratory. We thank Eve Slavich from the University of New South

Wales Faculty of Science Stats Central for statistical analysis support and the NSW Department of Planning Industry and Environment (DPIE), for providing fire history records used to find suitable sites for this study. MKJO receives funds from DPIE via the NSW Bushfire Risk Management Research Hub.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.envexpbot.2021.104634>.

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