

The role of *Rhytidoponera metallica* (Hymenoptera, Formicidae) in facilitating post-fire seed germination of three ant-dispersed legume species

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Abstract The fire avoidance hypothesis proposes that a benefit of seed dispersal by ants (myrmecochory) is to protect seeds from being killed during fire and to facilitate post-fire germination of seeds that require heat shock to break their physical dormancy. The aim of this study was to quantify the effect of fire and seed burial by a predominant seed-dispersing ant, *Rhytidoponera metallica* (subfamily: Ectatomminae) on germination levels of three ant-dispersed legume species (*Pultenaea daphnoides*, *Acacia myrtifolia* and *Acacia pycnantha*). Experimental burial of seeds within aluminium cans at a site prior to being burnt and at an adjacent unburnt site showed that fire increased germination levels, particularly for seeds buried at 1- and 2-cm deep and that overall, germination levels differed among the three plant species. To quantify seed burial depths and post-fire germination levels facilitated by *R. metallica* ants, seeds were fed to colonies prior to fire at the burnt and unburnt sites. Of the seeds buried within nests that were recovered, between 45% and 75% occurred within the upper 6 cm of the soil profile, although unexpectedly, greater percentages of seeds were recovered from the upper 0–2 cm of nests in the unburnt site compared with nests in the burnt site. Germination levels of buried seeds associated with *R. metallica* nests ranged from 21.2% to 29.5% in the burnt site compared with 3.1–14.8% in the unburnt site. While increased seed germination levels were associated with *R. metallica* nests following fire, most seeds were buried at depths below those where optimal temperatures for breaking seed dormancy occurred during the fire. We suggest that *R. metallica* ants may provide fire avoidance benefits to myrmecochorous seeds by burying them at a range of depths within a potential germination zone defined by intra- and inter-fire variation in levels of soil heating.

Key words: fire, germination, mutualism, seed bank, seed dispersal.

INTRODUCTION

Myrmecochory (seed dispersal by ants) is a widespread seed dispersal syndrome that has evolved independently in at least 101 plant lineages and is employed by at least 11 000 species (Lengyel *et al.* 2010). Myrmecochorous plants are characterized by producing diaspores with ant-attractive appendages called elaiosomes. Dispersal typically involves ants carrying diaspores back to their nest where the lipid-rich elaiosomes are consumed and the unharmed seeds are then discarded within nest chambers or at locations outside the nest (Berg 1975; Beattie 1985).

The geographical and taxonomic prevalence of myrmecochory indicates a widespread capacity for this dispersal syndrome to benefit plants (Lengyel *et al.* 2010). Several benefits gained by plants have been identified; namely, directed dispersal, distance dispersal, predator avoidance and fire avoidance (Giladi 2006; Warren & Giladi 2014), although the

relative importance of these benefits varies among ecosystem types (Giladi 2006; Rico-Gray & Oliveira 2007). The directed dispersal of seeds to ant nests can be beneficial when nests are enriched with nutrients (Davidson & Morton 1981b; Beattie & Culver 1983), resulting in greater plant growth (Culver & Beattie 1980; Davidson & Morton 1981a; Berg-Binder & Suarez 2012) and survival compared with plants derived from undispersed seeds (Davidson & Morton 1981a; Hanzawa *et al.* 1988). The dissemination of seeds over distances typically averaging 1–3 m (Gomez & Espadaler 2013) from parent plants can reduce parent–offspring competition (Westoby *et al.* 1982; Boyd 2001; Leal *et al.* 2015) and competition among seedlings (Higashi *et al.* 1989; Gorb & Gorb 2003). Burial of seeds within ant nests can also provide protection against seed predation (Bond & Breytenbach 1985; Auld & Denham 1999; Ness & Morin 2008; Kwit *et al.* 2012) and from lethal temperatures that occur during fire (Hughes & Westoby 1992). Thus, plants may receive multiple benefits from myrmecochory, and it is worth noting that current ecological benefits are not necessarily the same

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as the ultimate selective pressures favouring the evolution of this interaction.

According to the 'fire avoidance' hypothesis, plants benefit because seeds retrieved by ants from the soil surface are transported to ant nests in which they are insulated from the lethal soil surface temperatures that occur during bushfires (Berg 1975). In addition to this protective outcome, the hypothesis also recognizes a direct interaction between seed burial by ants and the fire-related cues required to break seed dormancy, because the reception of such cues can be strongly dependent on seed burial depth (Berg 1975; Shea *et al.* 1979; Brits 1987; Hughes & Westoby 1992; Majer *et al.* 2011).

In Australia, myrmecochorous plants are commonly associated with fire-prone sclerophyll heath, woodland and forest vegetation (Berg 1975) where they can constitute close to one-third of the plant species richness (Rice & Westoby 1981) and can be dominant members of understory shrub communities (Berg 1975). Myrmecochory is a common means of seed dispersal for Australian species of Fabaceae (Berg 1975; Auld 1996; Lengyel *et al.* 2009), which typically produce seeds with hard, water-impermeable seed coats that impose physical dormancy (Auld & O'Connell 1991; Morrison *et al.* 1992). Soil heating as a result of fire provides a 'heat shock' that effectively breaks the physical dormancy of many buried seeds, which can result in mass post-fire germination and seedling emergence (Floyd 1976; Purdie 1977). Broadly, temperatures between 80 and 100°C produce the greatest germination response in a variety of legume species, although species can vary in the temperature threshold at which dormancy is broken or at which seeds are killed (Auld & O'Connell 1991). Seed burial depth has a strong influence on the temperatures experienced by seeds during fire because maximum temperatures reached decrease rapidly from shallow to deeper depths within the soil profile (Bradstock & Auld 1995). Fire intensity affects the degree to which heat penetrates through the soil profile and thus influences overall levels of seed mortality and post-fire seed germination (Tozer 1998; Williams *et al.* 2004; Knox & Clarke 2006). For example low-intensity prescribed fires usually limit the bulk of seed germination to the upper 3 cm of the soil profile (Bradstock & Auld 1995). In contrast, the greater temperatures reached during more intense fires (e.g. wildfires) can result in seed mortality at shallow burial depths (Ferrandis *et al.* 1999) but can also cause a larger fraction of a species' seed bank to emerge by breaking seed dormancy at deeper burial depths (Auld & O'Connell 1991). The effect of burial by ants on post-fire seed germination following a fire of a given intensity is therefore expected to depend on the range of depths over which they bury seeds.

Myrmecochory is increasingly considered to be an unevenly diffuse mutualism because myrmecochorous

plants are typically reliant on a restricted number of ant species for most dispersal services at local and broad geographical scales (Gove *et al.* 2007; Ness *et al.* 2009; Warren & Giladi 2014). In Australia, species of *Rhytidoponera* have been labelled as 'keystone' dispersers because they contribute disproportionately to the biotic transport of myrmecochorous seeds (Gove *et al.* 2007). *Rhytidoponera metallica* is a very common ant species that occurs across much of Australia in both natural and human-modified environments (Haskins & Whelden 1965). Colonies frequently relocate their nests (Hughes 1990; Thomas 2002), which are constructed in the soil profile under logs, rocks and under the litter layer more generally (Drake 1981; Thomas 2002). Along with other species of *Rhytidoponera* (Gove *et al.* 2007), *R. metallica* is often the most frequent disperser of myrmecochorous seeds in local populations (Drake 1981; Hughes & Westoby 1992; Gove *et al.* 2007; Beaumont *et al.* 2009, 2011, 2013).

Despite their predominant role in the transport of myrmecochorous seeds, seed removal and dissemination by *Rhytidoponera* ants are only the initial components of the dispersal process that they facilitate. Post-dispersal factors, such as seed burial depths, may also have a major influence on the effectiveness of dispersal services provided by ants. Seeds retrieved by *Rhytidoponera* species may remain buried within nests or be discarded outside the nests (Berg 1975; Hughes & Westoby 1992; Lubertazzi *et al.* 2010; Beaumont *et al.* 2013) where they are available to be redispersed and buried by other ant colonies (Hughes & Westoby 1992; Beaumont *et al.* 2013). While *Rhytidoponera* ants bury seeds across a range of depths within their nests, the greatest proportion of seeds usually occur in the upper parts of the nest, a zone where physical seed dormancy is more likely to be broken under a range of fire intensities (Shea *et al.* 1979; Hughes & Westoby 1992). While some previous studies have shown that seedlings emerge from nests following simulated (Majer *et al.* 2011) or prescribed fire (Hughes & Westoby 1992), indicating that at least some seeds are buried at appropriate depths for loss of physical seed dormancy and subsequent post-fire seed germination, the levels of post-fire seed germination facilitated by seed-dispersing ants have not previously been directly quantified.

The aim of this study was to quantify the effects of fire and seed burial by a predominant seed-dispersing ant, *R. metallica* (Ectatomminae), on germination levels of three ant-dispersed legume species – *Pultenaea daphnoides* J.C Wendl., *Acacia myrtifolia* (Sm.) Willd. and *A. pycnantha* Benth. This was undertaken by investigating the effects of a fire and seed burial depth on post-fire seed germination levels of the three plant species and by quantifying seed burial

depth distributions within *R. metallica* nests and the resulting levels of post-fire seed germination.

METHODS

Study sites and species

The study was conducted at two adjacent sites in Belair National Park, located in the Mount Lofty Ranges near Adelaide in South Australia. The area has a Mediterranean climate and receives an average annual rainfall of 722 mm. The sites were located within *Eucalyptus obliqua* woodland that was previously burnt in 1955. At the study sites, *P. daphnoides* was the predominant mid-story shrub and *A. pycnantha* was sparsely distributed. *Acacia myrtifolia* is common within the area but was absent from both sites. *Pultenaea daphnoides*, *A. pycnantha* and *A. myrtifolia* all produce seeds with hard coats that impose physical dormancy, but which can be ruptured by heat during fire (Auld & O'Connell 1991; Brown *et al.* 2003). *Pultenaea daphnoides* plants in the Mount Lofty Ranges have the capacity to resprout from basal buds following fire (Jianmin & Sinclair 1993), whereas both *Acacia* species are killed by fire and hence are solely dependent on post-fire recruitment from seeds for persistence.

Seed burial by *Rhytidoponera metallica* and post-fire germination

As part of the study, a prescribed burn was conducted in autumn 2008 at one of the two sites and is referred to as the burnt site. The adjacent site remained unburnt for the entirety of the study and is referred to as the unburnt site. Prior to the application of the prescribed fire, ten *R. metallica* colonies were located in each of the two sites. The average 'nearest neighbour' distance between located nests in the burnt and unburnt site was 14.0 m (min. = 8 m, max. = 30 m) and 15.5 m (min. = 8 m, max. = 19.5 m) respectively. Prior to the fire (i.e. during March 2008) colonies were offered 10 seeds of each of the three plant species daily, over a period of 15 days. Seeds of a given plant species were no longer offered once colonies had taken 50 seeds. However, not all colonies received a full complement of seeds by the end of the 15-day period (Appendix S1) due to variation in the rate at which colonies collected seeds. To assess the proportion of seeds later discarded from nests, metal enclosures (25 × 25 cm) were installed around the nest entrances. Enclosures extended to approximately 5 cm below the soil surface to 15 cm above the soil surface and a strip of Tangle-Trap gel (The Tanglefoot Co., Grand Rapids, MI, USA) was applied to the rim of the enclosures. These enclosures prevented other ants from gaining access to seeds and restricted the distance that the *R. metallica* colonies could discard seeds from their nest entrances, making it possible to collect discarded seeds from the soil surface.

Acacia pycnantha and *P. daphnoides* plants were present at the study sites and therefore the seeds of these plants were expected to be in the soil. *Acacia myrtifolia* plants were not present at the study sites and thus their seeds were

expected to be absent from the soil seed bank. This was later confirmed by soil samples excavated at locations where active ant nests were not present ('non-nest' locations). The seeds offered to *R. metallica* colonies were collected from the study region, approximately 3 months prior to offering them to colonies. Seed viabilities (based on cut tests) were 100%, 96% and 94% for *A. pycnantha*, *A. myrtifolia* and *P. daphnoides* respectively. Cut tests revealed that all non-viable seeds were distinguished by external features (soft or insect-damaged seed coats) and hence such seeds were not used. All seeds were marked with a dot of paint at the end furthest from the elaiosome. Seeds were marked with the initial aim of quantifying differences between nest and non-nest locations in the number and vertical distribution of marked *A. pycnantha* and *P. daphnoides* seeds (i.e. those buried by focal *R. metallica* colonies) and unmarked seeds (i.e. those already present in the soil at nest and non-nest locations and buried by unknown means). However, during the period of offering seeds to colonies, it was noted that a proportion of seeds that were discarded outside of the nests (as expected) had lost their markings. It was assumed that seeds within the nests may have also lost their marking and therefore, we could not confidently distinguish between *A. pycnantha* and *P. daphnoides* seeds experimentally fed to nests and those that already occurred naturally within nest chambers or within soil associated within nest samples. Despite this, accurate estimates of seed burial and germination depths facilitated by the focal *R. metallica* colonies were obtained for *A. myrtifolia*.

The prescribed burn was conducted on 19th April 2008, 4 weeks after feeding seeds to ant nests. The fire consumed 100% of leaf litter, including litter within the metal enclosures and also resulted in 100% of the canopy being scorched. One to two weeks following the prescribed burn, 25 × 25 cm quadrats centred on the focal *R. metallica* nests were excavated in 2-cm intervals to a depth of 18 cm below the soil surface. For the samples taken from the unburnt site, the leaf litter was sieved to extract any seeds discarded on the soil surface, whereas in the burnt site, such 'surface' samples were not taken as any seeds on the soil surface were expected to be burnt and unrecognizable. The excavated soil was passed through a 4.0 mm sieve to remove large debris and then through a 1.4-mm sieve that retained the seeds as well as some soil. Non-nest locations positioned 25 cm away from nest locations were also sampled; however, due to the loss of seed markings and hence the inability to reliably compare distributions of seeds at nest and non-nest locations, and due to the low numbers of seeds at non-nest locations, these data have not been included in the analyses (see Appendix S2).

To assess the germinability of seeds buried at different depths in burnt and unburnt nests, the sieved soil samples (containing seeds) from each depth category were transferred into separate plastic germination trays (16.5 × 11.5 × 7.5 cm) and placed in a glasshouse. The soil was watered automatically every 12 h for a period of 2 min and the air temperature was maintained at 18°C. Samples were systematically rotated once a week to ensure that all were sufficiently watered. The number of seedlings of the three species that emerged was recorded weekly over a period of 18 weeks, by which time seedling emergence had ceased. After 18 weeks, the watering regime was

stopped and all 'above-ground' plant material was removed. To recover any ungerminated seeds, the samples were then rinsed through a 1.4-mm sieve with water, which resulted in a further reduction in the amount of soil. The remaining soil was dried and the ungerminated seeds were extracted with the aid of a binocular microscope.

The total number of buried seeds that were recovered from each nest was calculated as the sum of the ungerminated seeds recovered from the soil plus the number of emergent seedlings. The effects of burning and seed species on the proportion of buried seeds that germinated from *R. metallica* nest locations were tested using a Generalized Linear Model fitted using a binomial error distribution and logit link function. The effect of burial depth on the proportion of seeds that germinated was not included in the analysis because samples differed greatly in the number of seeds within a given depth category. Rather, trends in the relative distribution of buried seeds within the soil profiles and trends in the percentage of germinated seeds at each burial depth are described.

Experimental seed burial and post-fire germination

A second seed burial experiment was conducted in conjunction with the one described above to further estimate the effect of fire and burial depth on seed germination rates of the three plant species. The seeds used for this experiment were sourced from the Mount Lofty region by a seed company; seed viabilities of *A. pycnantha*, *A. myrtifolia* and *P. daphnoides* were 100%, 90% and 83.3%, and seed ages were 2, 3 and 3 years old respectively. Fifteen seeds (with the elaiosomes removed using forceps) of each plant species were buried at intervals of 1-, 2-, 3-, 4-, 6- and 10-cm deep within each of 20, 375 mL aluminium drink cans. The tops of the cans were removed and seeds were buried in sieved soil that was obtained from the study site. The seeds at each depth were separated within cans by a single layer of thin, cotton mesh, which later aided in recovering seeds from each depth. The cans were buried at the study sites so that the tops were flush with the soil surface, at locations <25 cm from the edge of the metal enclosures that were installed around *R. metallica* nests ($n = 10$ cans in the burnt and unburnt sites). In addition, seeds were contained in aluminium foil packets and were positioned on the soil surface next to the buried cans. The seeds were recovered following the prescribed burn and were placed in Petri dishes on a layer of heat sterilized propagation sand. Seeds were kept at room temperature (mean min, max weekly temperatures; 15.4, 20.0°C respectively) and watered weekly. The number of seeds that germinated was recorded once a week for a period of 18 weeks.

The soil temperatures reached during the fire were recorded using non-reversible temperature labels (Non-reversible Omegalabel; Omega Engineering Inc.). The labels were buried at the same depths as the seeds, but in separate cans that were buried within 10 cm of the cans containing the seeds. The temperature labels record maximum temperatures reached along a series of discrete temperatures (10 temperatures per label) with an average interval of 5.3°C. Different labels record temperatures

along different temperature ranges, hence three labels were buried at 1- and 2-cm deep to provide a recordable temperature range of 40–193°C (30 temperature points), whereas at all other depths two temperature labels were used to provide a recordable temperature range of 40–138°C (20 temperature points).

The proportion of viable seeds experimentally buried in cans that germinated was analysed using a Generalized Linear Model with a binomial distribution and logit link function. The number of viable seeds buried at each depth was estimated by adjusting for the viabilities of each lot. Also, not all seeds were recovered from each depth (98.0%, 98.3% and 99.3% of *P. daphnoides*, *A. myrtifolia* and *A. pycnantha* seeds were recovered from the cans) and thus, the number of viable seeds were adjusted accordingly. The binomial model tested for the effects of fire, burial depth and species and the interactions between these fixed factors on the proportion of viable seeds that germinated. Seeds placed on the soil surface were not included in the analysis because the fire killed all surface seeds at the burnt site.

RESULTS

Seed burial by *Rhytidoponera metallica* and post-fire germination

The average number of seeds of a given plant species that were collected by colonies ranged from 28.2 ± 5.25 to 48.0 ± 1.11 (Appendix S1). For *A. myrtifolia*, which was not already present in the soil seed bank, it may be expected that all seeds taken by ant colonies in the unburnt site could later be accounted for, either as excavated seeds or as seedlings; however, an average of $77.5 \pm 4.70\%$ were accounted for. Of the *A. myrtifolia* seeds that were recovered from colonies in the unburnt site, an average of $45.7 \pm 7.54\%$ were located within the surface litter. For *A. pycnantha* and *P. daphnoides* $31.9 \pm 6.68\%$ and $24.3 \pm 7.14\%$ of the recovered seeds, respectively, were located within the surface litter. Interestingly, discarded seeds typically had residual elaiosomes remaining attached indicating that the colonies did not completely consume elaiosomes prior to discarding them outside of their nests.

Unexpectedly, the relative distribution of seed burial depths at nest locations (estimated from counts of ungerminated seeds and seedlings) differed between the burnt and unburnt sites (Fig. 1). For the three species, between $45.7 \pm 6.51\%$ and $52.7 \pm 12.07\%$ of buried seeds occurred in the upper 6 cm of burnt nests compared with unburnt nests where between $68.9 \pm 7.66\%$ and $75.24 \pm 6.68\%$ of buried seeds occurred in this depth zone (Fig. 1 insets). This difference was particularly due to higher seed densities in the 0–2-cm layer of unburnt nests compared with burnt nests. The relative seed densities decreased from shallow to deeper depths in unburnt nests for

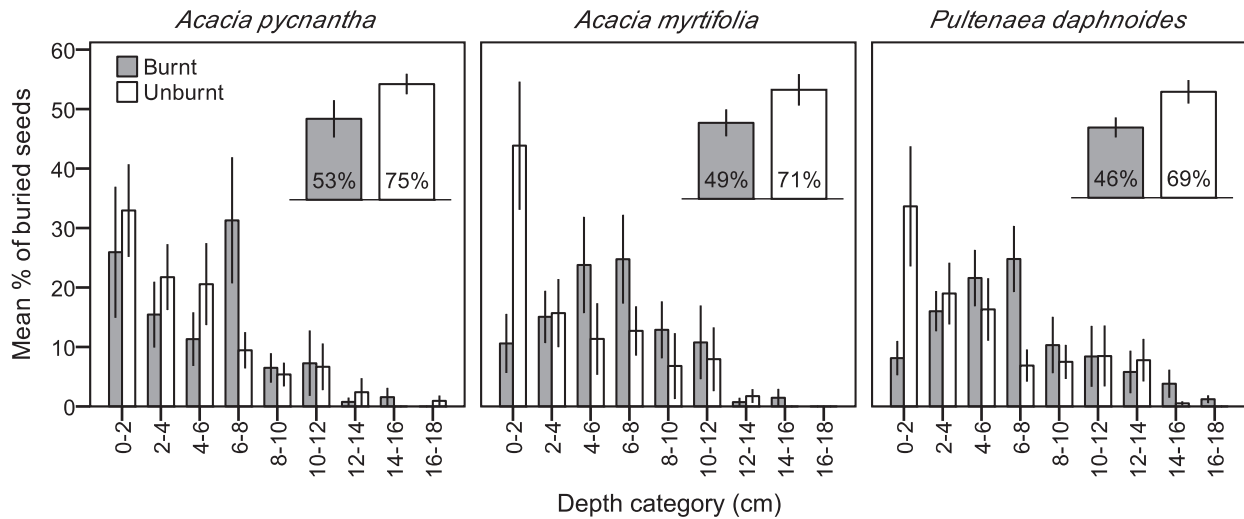


Fig. 1. Relative distributions of buried *Acacia pycnantha*, *Acacia myrtifolia* and *Pultenaea daphnoides* seeds (estimated from seeds and seedlings) within *Rhytidoponera metallica* nests located in the burnt and the unburnt site. Bars represent mean \pm SE per cent of seed per depth category. Graph insets show the percentage of seeds buried in the upper 0–6 cm of soil of burnt and unburnt nests.

all three seed species, whereas in burnt nests seed densities peaked in the 4–6 and/or 6–8-cm depth categories, although the latter pattern is somewhat less apparent for *A. pycnantha* (Fig. 1). Specifically, for *A. myrtifolia*, $43.8 \pm 10.79\%$ and $10.6 \pm 4.97\%$ of buried seeds occurred in the 0–2-cm depth category of unburnt and burnt nests respectively. *Pultenaea daphnoides* showed a similar pattern with $33.6 \pm 10.11\%$ and $8.1 \pm 2.89\%$ of buried seeds occurring in the 0–2-cm depth category of unburnt and burnt nests respectively. The difference between sites at the 0–2-cm depth category was less for *A. pycnantha* with $32.9 \pm 7.79\%$ and $25.9 \pm 11.02\%$ of buried seeds occurring in this depth category in the unburnt and burnt site respectively.

The proportion of seeds buried within *R. metallica* nests that germinated was dependent on a significant interaction between burning and seed species ($\chi^2 = 20.7$; d.f. = 2; $P < 0.001$). While average germination levels were consistently higher for burnt nests compared with unburnt nests, the germination responses differed among the plant species (Fig. 2). For *A. pycnantha*, $29.5 \pm 9.95\%$ of buried seeds germinated from burnt nests compared with $6.0 \pm 1.79\%$ from unburnt nests. Similarly, for *A. myrtifolia*, $22.1 \pm 6.55\%$ and $3.1 \pm 2.18\%$ of buried seeds germinated from burnt and unburnt nests respectively. *Pultenaea daphnoides* had similarly high levels of germination from burnt nests ($21.2 \pm 4.70\%$) although germination from unburnt nests ($14.8 \pm 5.90\%$) was relatively high compared with the two *Acacia* species (Fig. 2). Per cent seed germination levels for burnt nests peaked in the 0–2-cm depth category for all three species and decreased rapidly with increasing burial depth, whereas no

depth related pattern was evident for unburnt nests (Fig. 3). The high germination percentages of *A. pycnantha* and *P. daphnoides* seeds at 16–18-cm deep are due to sporadic germination from the few samples containing very low numbers of seeds within that depth category (Appendix S2).

Experimental seed burial and post-fire germination

Soil temperatures reached during the prescribed burn at depths of 1 and 2 cm within the soil were obtained

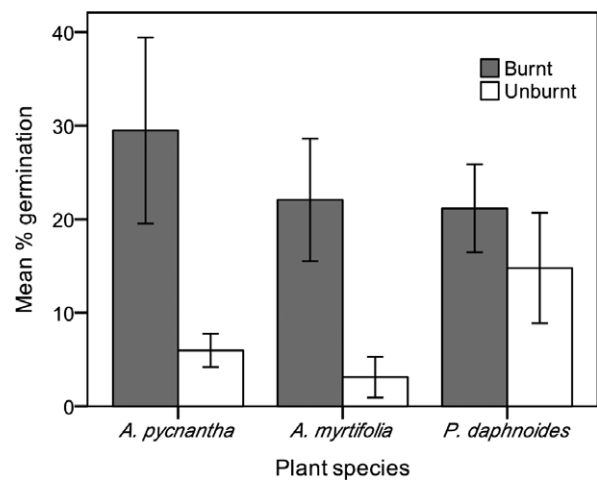


Fig. 2. Per cent of buried *Acacia pycnantha*, *Acacia myrtifolia* and *Pultenaea daphnoides* seeds that germinated from *Rhytidoponera metallica* nests located in the burnt and the unburnt site. Bars represent mean \pm SE per cent of germinants.

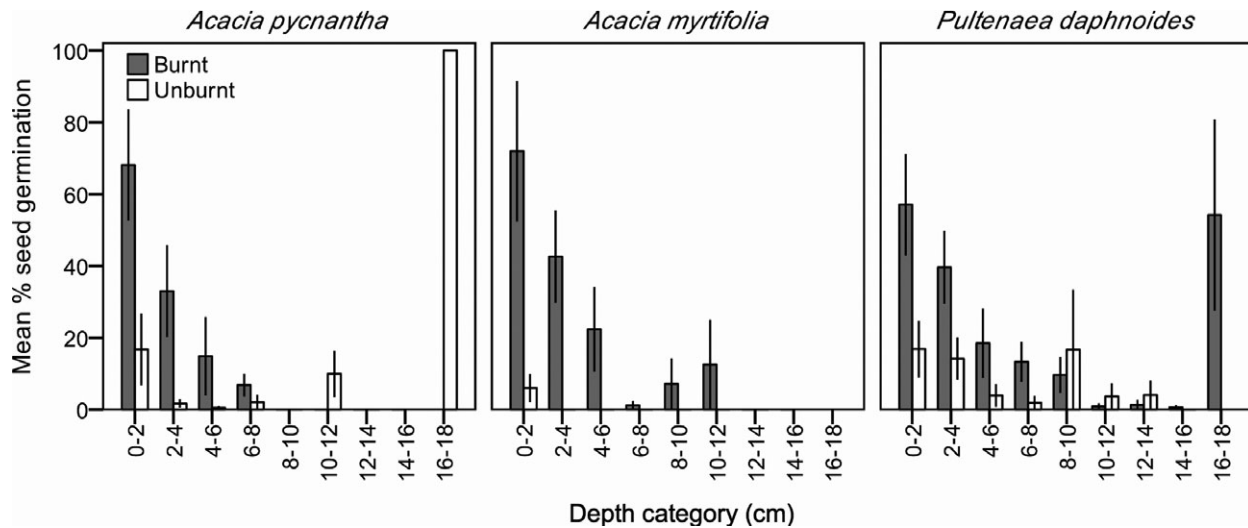


Fig. 3. Relative per cent of buried *Acacia pycnantha*, *Acacia myrtifolia* and *Pultenaea daphnoides* seeds that germinated from *Rhytidoponera metallica* nests located in the burnt and the unburnt site. Bars represent mean \pm SE per cent of germinants for each burial depth category.

from all samples and the maximum recordable temperature (193°C) was not exceeded. Soil temperature reached an average of $82.7 \pm 5.17^\circ\text{C}$ at 1-cm deep (range, 60–116°C; $n = 10$) and an average of $57.4 \pm 2.83^\circ\text{C}$ (range, 43–71°C, $n = 10$) at 2-cm deep. The minimum recordable temperature of 40°C was not recorded by any label within the unburnt site, including by those placed on the soil surface.

The proportion of viable seeds buried within cans that germinated depended on seed species ($\chi^2 = 447.0$; d.f. = 2; $P < 0.001$) and on a significant interaction between burning and burial depth ($\chi^2 = 178.5$; d.f. = 5; $P < 0.001$). For all three species, the proportions of seeds that germinated from the unburnt site were low and similar at all burial depths, whereas at the burnt site, germination levels were markedly higher in the upper 3–4 cm of the soil profile (Fig. 4). Over both sites, germination levels were highest for *P. daphnoides*, followed by *A. myrtifolia* and lowest for *A. pycnantha*, which resulted in the significant species effect.

DISCUSSION

Benefits of myrmecochory that relate to the fire avoidance hypothesis include enhanced seed survival and loss of physical seed dormancy during fire, both of which are strongly influenced by seed burial depths facilitated by ants. *Rhytidoponera* species appear effective at providing fire avoidance benefits as asserted by previous studies (Hughes & Westoby 1992; Lubertazzi *et al.* 2010; Majer *et al.* 2011), and this study provides novel evidence by specifically

linking seed burial depths facilitated by *R. metallica* with levels of post-fire seed germination. A greater fraction of the seeds buried within ant nests germinated from the burnt site compared with the unburnt site, demonstrating that *R. metallica* buried a proportion of the seeds at depths where sufficient soil heating occurred to break seed dormancy during the prescribed fire; however, a large fraction of buried seeds remained ungerminated following fire. We suggest that *R. metallica* may provide fire avoidance benefits by burying seeds at a range of depths within a potential germination zone defined by intra- and inter-fire variation in levels of soil heating.

The experimental burial of seeds within cans showed that the higher soil temperatures due to the prescribed fire were sufficient to enhance germination rates in the upper 1–3 cm of soil (burning \times depth effect; Fig. 4) and this depth related pattern is broadly similar for seeds buried within ant nests (Fig. 3). Germination levels were highest for all species at 1 cm where temperatures reached an average of 82.7°C during the fire. This is consistent with the finding that hard-seeded legumes usually require temperatures between 80 and 100°C to produce maximum levels of germination (Auld & O'Connell 1991). While no significant three-way interaction effect was found (burning \times species \times depth), germination rates in the burnt site appear to decline more rapidly with depth for *A. pycnantha* compared with *P. daphnoides*. In particular, germination of *P. daphnoides* seeds was similarly enhanced at 1- and 2-cm deep in the burnt site where temperatures at the latter depth reached an average of 57.4°C and where no temperatures exceeded 80°C. This suggests

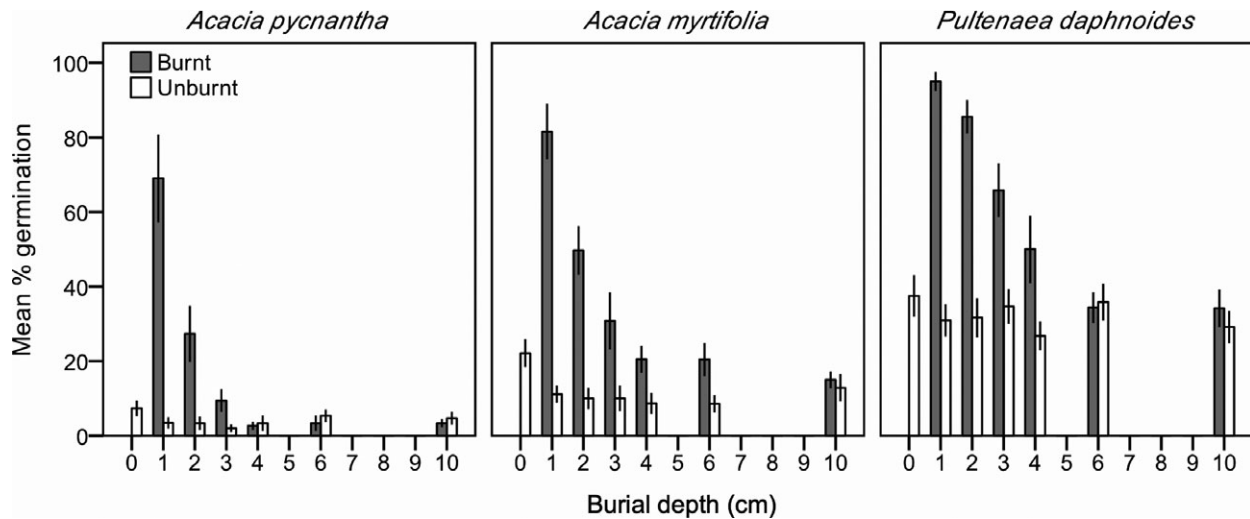


Fig. 4. Mean per cent of seeds experimentally buried within aluminium cans that germinated from each burial depth. Cans were buried in the burnt and the unburnt site and seeds at 0 cm were placed on the soil surface. Germination rates are calculated from the estimated proportion of viable seeds at each depth. Bars represent mean \pm se per cent of germinants for each depth category.

that the physical seed dormancy of *P. daphnoides* can be broken to a greater extent by lower temperatures as has been more definitively found by laboratory experiments (Auld & O'Connell 1991). Also, the experimental burial of seeds in cans showed that overall germination levels varied significantly among the plant species, which is mainly due to differences between species in the non-dormant fraction of seeds. *Pultenaea daphnoides* in particular had higher levels of germination in the absence of soil heating caused by the prescribed fire (Fig. 4). This was also reflected in the germination levels for seeds that were buried within ant nests; i.e. the significant burning \times species effect found for that experiment appears to be largely driven by higher germination rates of *P. daphnoides* seeds from unburnt nests, compared with the other two species, rather than by large differences between species in their responses to soil heating in the burnt site (Fig. 2).

Our estimates of the fraction of seeds within *R. metallica* nests that germinated following fire (21.2–29.5%) are within the wide range of previous estimates for hard-seeded legumes. For example, 45%, 65% and 77% of *A. suaveolens* seed banks at three sites were estimated to emerge as seedlings following fire (Auld & Denham 2006), whereas 6% and 12% of *A. saligna* seeds emerged after simulated soil heating that was expected to reflect those occurring during low and medium intensity fires respectively (Tozer 1998). Differences among species in their germination response to soil heating as well as the levels of soil heating likely contribute to the great variation in such estimates. Indeed, germination levels of 35 legume species with a given depth distribution of

seeds was estimated to have ranged from 0 to 24.7% and from 2.2 to 58.9% following simulated low and medium intensity fires respectively (Auld & O'Connell 1991).

The vertical distribution of buried seeds is also expected to influence the fraction of a soil seed bank that germinates following fire. In this study, the best estimate of the vertical distribution of seeds within *R. metallica* nests was obtained for *A. myrtifolia* as this estimate was not influenced by seeds already present within the soil that may not have been buried by the focal colonies. Even so, the relative distribution of *A. myrtifolia* seeds within nests at the burnt and unburnt sites closely matched the distributions of *P. daphnoides* seeds and to a lesser extent the distributions of *A. pycnantha* seeds (Fig. 1). Notwithstanding this, there was a difference in the depth distributions of seeds between burnt nests (around 6–8 cm) and unburnt nests (at 0–2 cm). Colonies in the burnt site may have redistributed some seeds to deeper depths in response to the fire event, prior to the seeds receiving sufficient heat to break seed dormancy. Alternatively, the difference may be due to seed mortality caused by lethal soil temperatures, which were likely restricted to the upper 1 cm of the soil profile given the soil temperatures that were recorded during the fire. It is possible that seeds discarded on the soil surface by *R. metallica* filtered into the very upper layer of the soil profile prior to nest excavations. Such seeds would likely be burnt during the fire but in the unburnt site they would have been recovered from the 0- to 2-cm depth category, resulting in the difference between the burnt and unburnt site in the proportion of seeds recovered from the 0-

to 2-cm depth category. It is important to note, however, that under natural circumstances (i.e. where colonies are not within enclosures), seeds discarded on the soil surface by *R. metallica* can be redispersed and actively reburied by other ant colonies, thus further reducing the chance that discarded seeds are exposed to lethal soil temperatures (Hughes & Westoby 1992; Beaumont *et al.* 2013). This process of redispersal and reburial can be enhanced by *R. metallica* because the seeds that they discarded typically had residual elaiosomes remaining attached (also see Beaumont *et al.* 2013).

Other studies of the seed banks of ant-dispersed legumes, which did not specifically target active ant nests, show that seed densities decrease with soil depth (Tozer 1998) and that most seeds occur within the top 5–6 cm (Auld 1986a; Pieterse & Cairns 1986; Holmes 2002; Auld & Denham 2006). For example 71% of *A. suaveolens* seeds were located in the top 5 cm of soil (Auld & Denham 2006) and similarly, 71.5% of *A. pulchella* (Shea *et al.* 1979) and 80% of *A. saligna* seeds (Tozer 1998) were in the top 6 cm of soil. A study of three seed-dispersing ants, including *R. metallica*, found that ants typically buried seeds within the upper 3 cm of soil (Hughes & Westoby 1992). In comparison, Shea *et al.* (1979) found that the vertical distribution of seeds within nests of a *Rhytidoponera* species extended to depths of at least 12 cm, with 36.6% occurring at 0–3 cm, 65.6% occurring in the upper 6 cm of soil and the remaining 34.4% occurring at depths greater than 6 cm (per cent values are calculated here from seed density values/nest presented in Shea *et al.* 1979). Given that an average of between 46% and 75% of seeds occurred within the upper 6 cm of *R. metallica* nests in this study, the burial depth distributions are similar to that observed by Shea *et al.* (1979).

From the perspective of the fire avoidance hypothesis, there is likely to be no single optimal depth at which ants can bury seeds that would maximize post-fire germination levels (Hughes & Westoby 1992) due to the small-scale patchiness in soil heating that occurs during fire (Hobbs & Atkins 1988; Wiggers *et al.* 2013) and due to the variable intensities that characterize natural fires (Gill & Catling 2002). Rather, ants may benefit plants by burying seeds over a range of depths within a potential germination zone defined by the range of fire intensities that occur in a given vegetation type (Hughes & Westoby 1992; Lubertazzi *et al.* 2010). For hard-seeded legumes in sclerophyll forest and woodland vegetation, it is reasonable to expect that this zone would include the upper 5–6 cm of the soil profile (Auld 1986a,b). Here, it is evident that *R. metallica* buried a large proportion of seeds at depths where they were protected from lethal temperatures but where soil

temperatures required to break seed dormancy were not reached during the prescribed fire. While low fire intensities typical of prescribed fires limit post-fire germination levels of hard-seeded legumes (Bradstock & Auld 1995), the fraction of the seed bank in the upper 6 cm of soil provides opportunity for greater levels of post-fire germination following more intense fires. For example, temperatures of 164 and 111°C were recorded at 1 and 2 cm, respectively, in jarrah forest during a fire that was considered to be more intense than the typical prescribed burns conducted in that forest type (Shea *et al.* 1979). Such temperatures would likely kill seeds at depths where optimal temperatures for breaking seed dormancy occurred during the fire studied here; however, at these temperatures, seeds at deeper depths are expected to have seed dormancy broken. Furthermore, seeds that remain dormant within a potential germination zone following fire may be important for the local persistence of plant populations, particularly for obligate seeders in instances where the return time of fires is short (Auld & Denham 2006). The seed longevity of hard-seeded legume species (Auld 1986a; Holmes & Newton 2004; Orscheg & Enright 2011) and reductions in dormancy-breaking temperature thresholds of seeds over time (Liyanage & Ooi 2017) provide further opportunities for dormant seeds to germinate following successive fire events. Notwithstanding this, *R. metallica* also buried a proportion of seeds at depths where they are unlikely to ever have seed dormancy broken by fire and/or successfully emerge as seedlings, yet the relative cost of this is unknown.

It is important to recognize that plants with a variety of dispersal modes can also form persistent seed banks and germinate after fire (Valbuena & Trabaud 2001) and that seed burial depths are not necessarily influenced by dispersal mode (Holmes 2002). Therefore, further tests of the fire avoidance hypothesis could be conducted by comparing the efficacy of burial by ants with other means of seed burial. Auld (1986a) found that a *Pheidole* species buried most seeds below the potential germination zone, whereas seeds at random locations typically occurred within the top 5 cm of soil. In this case, it was inferred that seed burial by abiotic means (or at least by means other than *Pheidole*) positioned seeds more appropriately for dormancy release during fire. The effectiveness of ants at providing fire avoidance benefits may also relate to the rate at which an appropriate depth distribution is achieved, compared with other potential mechanisms of seed burial. Presumably seed burial by abiotic means is a slow process, yet burial facilitated by ants is rapid and thus ensures that fresh seeds are placed at a variety of depths where they can be protected in the event of an imminent fire.

Consistent with the important role of *Rhytidoponera* species in myrmecochory more broadly (Gove *et al.* 2007), *R. metallica* is the major seed disperser in our study region. It disperses between 43% and 97% of the seeds (Beaumont *et al.* 2011, 2013), transports seeds greater distances than other ant species, promotes further dissemination of seeds through processes of seed redispersal (Beaumont *et al.* 2013) and buries a large fraction of seeds within a potential germination zone. This, in addition to frequent nest relocation (Hughes 1990; Thomas 2002), suggests that *R. metallica* is likely to be a major agent in determining the spatial development of myrmecochorous seed banks during inter-fire periods. The dissemination of seeds both horizontally and vertically by *R. metallica* may be beneficial by allowing a plant's seed crop to encompass small-scale patchiness in levels of intra-fire soil heating, and may allow plants to bet-hedge against the unknown intensity of the next fire event. Inherent in this process are benefits from fire avoidance, which do not necessarily negate other benefits of myrmecochory being gained. There is considerable scope to expand our understanding of post-fire seed germination and plant recruitment of hard-seeded myrmecochorous plants. This may be achieved by comparing the contributions of different ant species to post-fire seed germination with other mechanisms of seed burial and by integrating the fire avoidance hypothesis into the broader context of seed fates and seed bank dynamics in relation to fire.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Summary of the numbers of offered seeds taken into *Rhytidoponera metallica* colonies (seed intake) and the subsequent number of seed recovered from the soil surface (soil surface seeds) and from soil samples (buried seeds) at *R. metallica* nest locations. Seeds were offered to 10 colonies at

each of two sites ('burnt' and 'unburnt' sites) prior to the burnt site being treated by a prescribed fire.

Appendix S2. Mean \pm SE per cent of seeds that germinated from each depth category per ant nest and per non-nest location, in the burnt and the unburnt site.