Germination Requirements of the Lesser Known Kangaroo Paw and Catspaw Taxa

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Pictures on front cover from top left clockwise: *Anigozanthos bicolor* subsp. *bicolor*, *A. preissii*, *A. bicolor* subsp. *decrescens* and *A. humilis* subsp. *chrysanthus*

Abstract

The Kangaroo Paw, *Anigozanthos manglesii* subsp. *manglesii*, is an iconic Western Australian wildflower. However there are 20 Kangaroo Paw and Catspaw taxa in the genus *Anigozanthos*, and many of them are much less well known. The primary aim of this project was to understand the germination requirements of the lesser known Kangaroo Paw and Catspaw taxa to increase awareness of these taxa and assist in their conservation and use in horticulture and land rehabilitation. Seventeen taxa were examined in this study. The more common *Anigozanthos* taxa were included for comparative purposes. Germination tests were undertaken on freshly collected seed, after soil burial and after a period of laboratory storage. Heat treatments were also undertaken on seeds prior to imbibition. Germination of freshly collected seeds of the 16 taxa examined was negligible or low, indicating that a high proportion of seeds were dormant at maturity. Seed burial for one year, commencing in the autumn after collection, was most effective at alleviating dormancy and enabling germination stimulation in smoke water in *A. manglesii* subsp. *manglesii*. This burial treatment also resulted in some *A. flavidus* seeds germinating in nitrate or smoke water in the light. However for the remaining six taxa examined, this duration and timing of burial was not an effective means of alleviating dormancy. Likewise germination did not exceed 15% in any of the eight taxa buried at the time of seed maturation and exhumed 3 to 4 months later, except for *A. viridis* subsp. Cataby seeds treated with smoke water. Longer periods of burial, possibly extending over at least two summers, were probably required. During storage at approximately 22°C for 3 to 3.5 years, dormancy was alleviated and smoke water stimulated higher levels of germination than water in 11 of the 17 *Anigozanthos* taxa examined. In six taxa, including lesser known taxa such as *A. manglesii* var. *x angustifolius*, *A. viridis* subsp. Cataby and the conservation priority listed *A. bicolor* subsp. *exstans*, smoke-stimulated germination exceeded 49%. Heat pre-treatments at 100°C were also effective in promoting germination in most *Anigozanthos* taxa except *A. rufus*. For most taxa germination was higher following one rather than three hours at 100°C. Germination of 12 of the 17 taxa examined was >30% following the 100°C for 1 h heat treatment, and >50% in six of these taxa. These six taxa were not all the same taxa that produced the highest levels of germination following storage and smoke treatment, and included lesser known species such as *A. bicolor* subsp. *bicolor*, *A. gabrielae* and *A. preissii*. Therefore there are a number of methods that can be used to maximise the germination of these intriguing *Anigozanthos* taxa to assist in their conservation, and use in horticulture and land rehabilitation.

Introduction

Kangaroo Paws and Catspaws refer to *Anigozanthos* taxa within the Haemodoraceae. There are currently 11 recognised species and 20 taxa (including one backcrossed hybrid), and they are all endemic to southwestern Western Australia (Western Australian Herbarium, 1998-). These herbaceous plants have either strappy or subterete leaves 3 to 100 cm in length (Macfarlane *et al.*, 1987). Flowers are borne in one sided racemes on a scape that can be from 5 to 300 cm tall (Macfarlane *et al.*, 1987). The colour of the flowers is determined by the hairs on the perianth, which are red, yellow, green or orange. The most well-known Kangaroo Paw is the iconic red and green flowered *A. manglesii* subsp. *manglesii*, which occurs in Perth and is the Western Australian floral emblem.

Understanding the germination of these taxa is important for conservation, horticulture, and land rehabilitation. Although some aspects of the germination requirements of more common taxa, such as *A. manglesii* subsp. *manglesii*, have already received some attention, many taxa are still difficult to germinate or their germination requirements remain untested (Hopper, 1993; Sukhvibul and Considine, 1994; Worrall, 1996; Tieu *et al.*, 2001).

Kangaroo Paws and Catspaws are important horticulturally, the taller flowering species particularly as cut flowers and in landscaping, and the shorter species as potted plants (Macfarlane *et al.*, 1987; Goodwin, 1993; Hopper, 1993; Growns, 2015). In fact, *Anigozanthos* is among Australia's top native cut flower export lines (Foster, 2009; Gollnow, 2013). Some common species including *A. manglesii* subsp. *manglesii*, *A. flavidus*, *A. pulcherrimus* and *A. rufus*, and various hybrids are already in cultivation (Macfarlane et al., 1987). Although most *Anigozanthos* plants for large scale commercial horticultural operations are currently produced via tissue culture rather than seeds (Macfarlane *et al.*, 1987; Gollnow, 2013), an understanding of the germination requirements of these species is still important in the development of horticultural lines. For example, the ability to germinate plants is essential for phenotypic recurrent selection, whereby taxa are crossed, based on desired traits such as disease tolerance (Growns, 2015). In addition, seed is a low cost form of propagation, and in the mid-1990s some commercial plantings were still propagated this way (Worrall, 1996). Elucidating methods of germinating seeds is also useful for individual gardeners and wildflower enthusiasts, as well as groups, such as Wildflower Societies, interested in propagating these species. Hence, increasing what is known about the germination requirements of *Anigozanthos* taxa will promote their cultivation.

There are a number of lesser known *Anigozanthos* taxa that have horticultural potential, but are either difficult to germinate or their germination requirements are untested. An example of the latter is the longer-lived Catspaw, *Anigozanthos humilis* subsp. *chrysanthus* (Hopper, 1993). Not only will investigating the germination requirements of these lesser known taxa increase awareness of these taxa, it will aid in their future horticultural development.

In terms of cut flowers, most native cut flowers were initially picked from the wild (Hopper, 1993; Foster, 2009). By 1993 approximately 4% of *Anigozanthos* cut flowers in Australia were wild-sourced (Worrall, 1996). Although bush-picking of *Anigozanthos* stems for the cut flower industry is not as widespread as in the past, bush-picking of these plants continues in Western

Australia today (Alan Tinker pers. comm. 12 June 2009). Hence any information on the propagation of this group of plants will reduce pressure on wild populations.

Understanding the germination requirements of *Anigozanthos* taxa is also important in the conservation of rare or threatened taxa. Of the 20 known *Anigozanthos* taxa, two are listed as threatened (declared rare flora) and three are priority listed (Western Australian Herbarium, 1998-). Most priority listed taxa are possibly threatened but require further surveying or monitoring (Department of Parks and Wildlife 2015). The three priority listed *Anigozanthos* taxa, *A. bicolor* subsp. *exstans*, *A. humilis* subsp. Badgingarra and *A. humilis* subsp. *chrysanthus* were included in the present study. However the two threatened taxa, *A. bicolor* subsp. *minor* and *A. viridis* subsp. *terraspectans*, were not examined. Nevertheless, closely related subspecies of these taxa were investigated, and this may provide insights into the germination requirements of the rare taxa.

One method of *ex situ* conservation is storing seeds in long-term seedbanks such as the Millenium Seedbank in the United Kingdom or at the Western Australian Threatened Flora Seed Centre. For the seeds in these seedbanks to be of any conservation value, it is important that the germination requirements of the seeds are unraveled.

Seeds are an important resource for land rehabilitation (Merritt and Dixon, 2011; Kildisheva *et al.*, in press). 'Restoration ready' seeds are those in which dormancy has been alleviated to ensure maximum seed use efficiency (Merritt and Dixon, 2011; Turner *et al.*, 2013). By overcoming seed dormancy, more seeds will germinate prior to being lost through predation, decay or erosion. Understanding dormancy breaking and germination requirements will also enhance seedling establishment through, for example, spreading seeds in which dormancy has been alleviated and for which germination stimulants have been applied, in seasons when temperatures are suitable for germination and moisture is available for plant establishment.

The life history and ecology of taxa can provide insights into what cues may be important for the dormancy alleviation and germination of their seeds. Furthermore, what is already known about the germination of the more common *Anigozanthos* taxa can provide directions for research into the lesser known ones. Many *Anigozanthos* taxa are most prolific after fire (Hopper, 1993), which suggests that fire-related cues may stimulate germination. Heat treatments have enhanced germination of *A. manglesii* (Tieu *et al.*, 2001) and smoke treatments have enhanced germination of *A. bicolor*, *A. humilis*, *A. manglesii* and *A. rufus* (Dixon *et al.*, 1995; Roche *et al.*, 1997). A period of burial further enhanced germination of *A. manglesii* and *A. rufus* in response to smoke (Roche *et al.*, 1997). Nitrate has also enhanced germination in *A. manglesii*, and thus the response of other *Anigozanthos* taxa to this treatment warrants further study (Sukhvibul and Considine, 1994; Bell *et al.*, 1999; Shchori, 2000).

The primary aim of this project is to ascertain the germination requirements of the lesser known Kangaroo Paw and Catspaw taxa to increase awareness of these taxa, and to assist in their conservation and use in horticulture and land rehabilitation. The first step will be to establish whether these seeds are dormant at maturity. If so, methods of dormancy alleviation such as seed burial and laboratory storage will be tested. Following dormancy alleviation, the effect of germination stimulants such as light, smoke water (and other germination promoting chemicals

in smoke such as KAR_1 and glyceronitrile) and nitrate will be examined. The impact of germination promoting methods such as heat pre-treatments will also be investigated.

Research Methodology

Study Species

The main purpose of this study is to investigate the germination requirements of some of the lesser known Kangaroo Paw and Catspaw taxa. However more common species, including *A. flavidus*, *A. manglesii* subsp. *manglesii*, *A. humilis* subsp. *humilis* and *A. viridis* subsp. *viridis* were included for comparative purposes. Following is a short description of each *Anigozanthos* taxon grouped by species, including information such as species distribution, defining features, flower colour, plant height and rarity status. Firstly, those with unbranched scapes (subgenus Haplanthesis) will be described, followed by those with branched scapes (subgenus Anigozanthos). Information in this section is sourced from Macfarlane *et al.* (1987), Hopper (1993), the Western Australian Herbarium (1998-), and personal observation.

Subgenus Haplanthesis

Anigozanthos bicolor taxa

There are four *A. bicolor* subspecies, *bicolor*, *decrescens*, *exstans* and *minor*, and their distributions are shown in Fig. 1A. Only the first three taxa are included in the present study because *A. bicolor* subsp. *minor* is declared rare flora (Western Australian Herbarium, 1998-). Although all of these taxa have red and green flowers, they can still be differentiated using characteristics of the perianth. The perianths of *A. bicolor* subsp. *bicolor* and *A. bicolor* subsp. *exstans* are parallel to slightly constricted (Fig. 2A and 2B), whereas *A. bicolor* subsp. *decrescens* perianths are strongly constricted (Fig. 2C). In addition, *A. bicolor* subsp. *decrescens* has dark purple hairs on the ovary (Fig. 2D) and the perianths of *A. bicolor* subsp. *exstans* are relatively long compared to the size of the plants (Fig. 3). Of these three taxa, *A. bicolor* subsp. *bicolor* is the tallest, followed by *A. bicolor* subsp. *decrescens* and *A. bicolor* subsp. *exstans* (Fig. 3). *Anigozanthos bicolor* subsp. *exstans* is a Priority 3 taxa meaning that it is known from several populations and at least some of these are not immediately threatened (Department of Parks and Wildlife, 2015). *Anigozanthos bicolor* subsp. *bicolor* and *Anigozanthos bicolor* subsp. *decrescens* are not currently threatened.

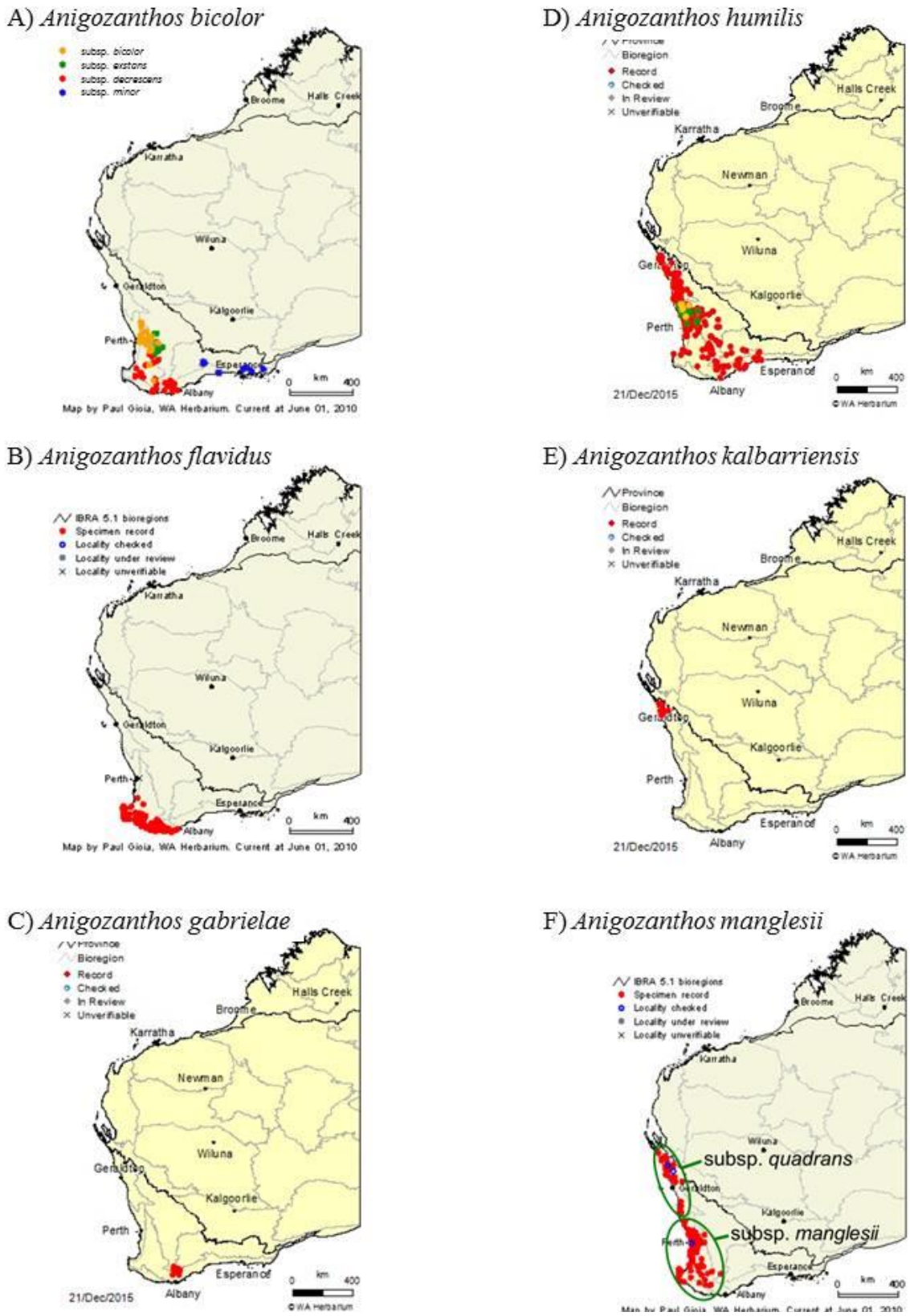

Fig. 1 Distribution maps of the different *Anigozanthos* taxa (Images used with the permission of the Western Australian Herbarium, Department of Parks and Wildlife (https//florabase.dpaw.wa.gov.au/help/copyright). Accessed 1 June 2010 and 2 December 2015).

G) *Anigozanthos onycis*

J) *Anigozanthos viridis*

H) *Anigozanthos preissii*

K) *Anigozanthos manglesii* var. *x angustifolius*

I) *Anigozanthos pulcherrimus* and *A. rufus*

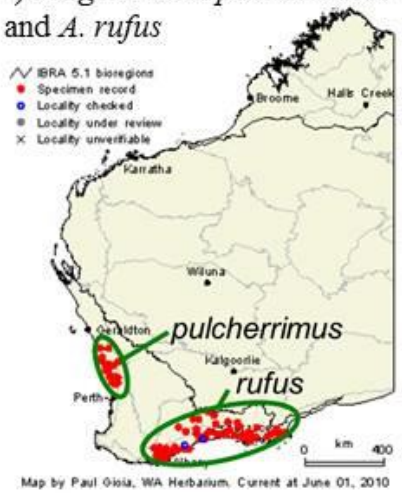

Fig. 1 Distribution maps of the different *Anigozanthos* taxa (Images used with the permission of the Western Australian Herbarium, Department of Parks and Wildlife (https//florabase.dpaw.wa.gov.au/help/copyright). Accessed 1 June 2010 and 21 December 2015).

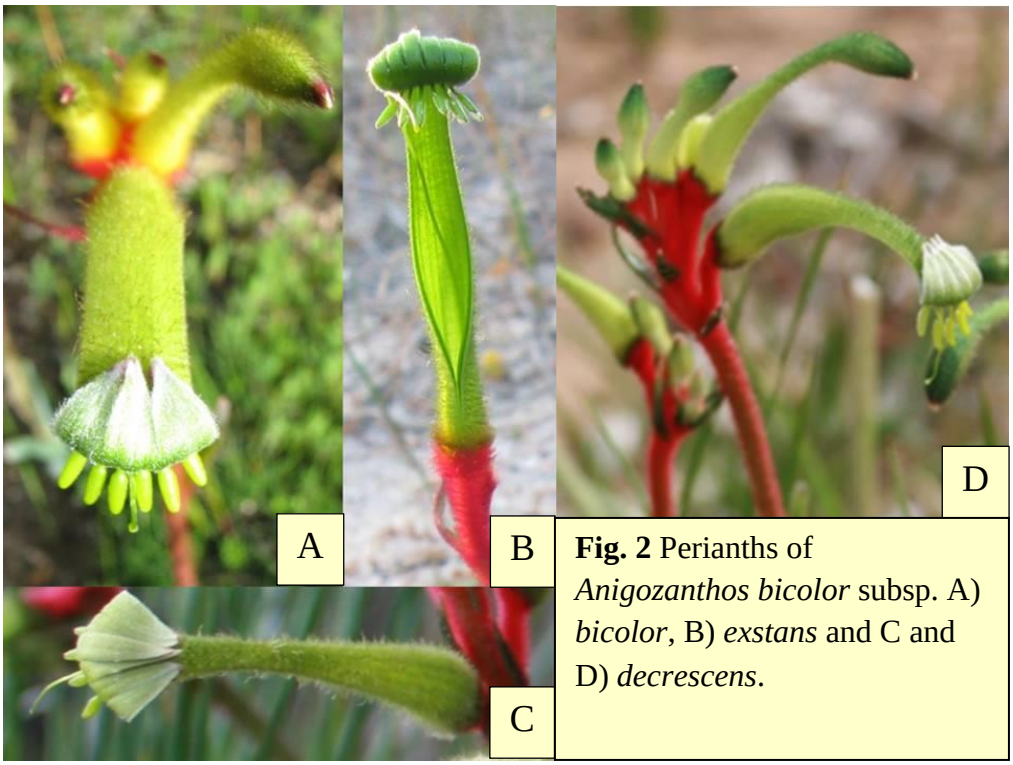

Fig. 2 Perianths of *Anigozanthos bicolor* subsp. A) *bicolor*, B) *exstans* and C and D) *decrescens*.

Fig. 3 Whole plants of three of the *Anigozanthos bicolor* subspecies, *bicolor*, *decrescens* and *exstans*, showing their heights, perianth lengths and conservation code where applicable. Note that the pictures are not to scale.

Anigozanthos gabrielae

Anigozanthos gabrielae also has red and green flowers and is the smallest of the Kangaroo Paws (Fig. 4). Each perianth is only 2 to 3 cm long and plants are 8 to 20 cm tall. It is found around the Stirling Ranges in low lying areas (Fig. 1C). This taxon is not rare or priority listed but is seldom seen because it is a fire ephemeral, meaning that it mainly only germinates after fire and is short-lived (Baker *et al.*, 2005a). Once the plant dies it exists only in the soil seedbank until a subsequent fire. An advantage of this species for horticulture is that it produces many flowers in cultivation (Hopper, 1993) and in pots in Perth it can live for longer than in its natural habitat (personal observation).

Fig. 4 *Anigozanthos gabrielae* inflorescence on the left and whole plant on the right. The inflorescence shows the progression of flowering from the base that is typical of all *Anigozanthos* taxa. Here the lowest flower has finished flowering and the ovary has started to swell with the development of seeds.

Anigozanthos humilis taxa

There are three *A. humilis* subspecies. In each subspecies the stamens are arranged in three rows (Fig. 12). *Anigozanthos humilis* subsp. *humilis*, also referred to as the Common Catspaw, is the most widely distributed *Anigozanthos* taxon (Fig. 1D). It is found from Kalbarri which is north of Geraldton, to Hopetoun which is between Esperance and Albany. In contrast, *A. humilis* subsp. *chrysanthus* and *A. humilis* subsp. Badgingarra have more restricted distributions north of Perth.

Of these three subspecies, *A. humilis* subsp. *humilis* is the shortest, growing up to 30 cm in height, *A. humilis* subsp. *chrysanthus* is intermediate in height, growing to 20 to 40 cm, while *A. humilis* subsp. Badgingarra is the tallest, with flowering scapes up to 1 m tall (Hopper, 1993; Fig. 5). Usually however, *A. humilis* subsp. Badgingarra plants are around 50 cm tall. *Anigozanthos humilis* subsp. *humilis* perianths are yellow, with varying degrees of orange and red. *Anigozanthos humilis* subsp. *chrysanthus* perianths are mainly yellow, whereas *A. humilis* subsp. Badgingarra perianths are yellow with some orange, especially at the tips of the lobes. *Anigozanthos humilis* subsp. *chrysanthus* and *A. humilis* subsp. Badgingarra are both longer-lived than *A. humilis* subsp. *humilis*. All three taxa have broad sickle-shaped leaves. They are longest in *A. humilis* subsp. Badgingarra, reaching up to 24 cm in length.

Although *A. humilis* subsp. *humilis* is fairly common, the other two subspecies are listed as Priority taxa. *Anigozanthos humilis* subsp. *chrysanthus* is a Priority 4 taxon meaning that it is rare but not currently threatened, and *A. humilis* subsp. Badgingarra is a Priority 2 species meaning that less than five populations are known but further surveying is required, and at least some of its populations are not currently threatened (Western Australian Herbarium, 1998-; Department of Parks and Wildlife, 2015).

Anigozanthos humilis subsp. *humilis* is already available in some Perth nurseries (eg Apace Revegetation Nursery, North Fremantle, Western Australia). As already mentioned, Hopper (1993) has noted that *Anigozanthos humilis* subsp. *chrysanthus* has considerable horticultural potential.

Fig. 5 Whole plants of the three *Anigozanthos humilis* subspecies, *humilis*, *chrysanthus* and Badgingarra, showing their heights, flower colour and conservation codes. Note that the pictures are not to scale. (See Western Australian Herbarium (1998-) and Department of Parks and Wildlife (2015) for more information on conservation codes).

Anigozanthos kalbarriensis

The Catspaw, *Anigozanthos kalbarriensis*, mainly occurs in winter wet depressions in the Kalbarri National Park and surrounding areas, north of Geraldton (Fig. 1E). This taxon is only seen in the first year or two after fire because it is a fire ephemeral. It is fairly small, only growing to 10 to 20 cm in height, and is similar to *A. humilis* in that the stamens are arranged in three rows. The leaves of *A. kalbarriensis* and *A. humilis* are also similar (Hopper, 1978). One of the main differences between these species is that *A. kalbarriensis* lobes are fully recurved (Fig. 6), whereas the lobes of *A. humilis* are held horizontally (Hopper, 1993). *Anigozanthos kalbarriensis* flowers are also redder in colour, particularly around the ovaries. Hopper (1993) notes that this species has interesting flower colour variations, but difficulty in germinating seed is one of the factors that restrict its cultivation.

Fig. 6 *Anigozanthos kalbarriensis* plant from the side and above. Note the recurved perianth lobes.

Anigozanthos manglesii taxa

Anigozanthos manglesii subsp. *manglesii* is perhaps the most well-known Kangaroo Paw taxon (Fig. 7A), as it occurs in Perth and is the Western Australian floral emblem. There is also a second *A. manglesii* subspecies, *A. manglesii* subsp. *quadrans* (Fig. 7B,C), which occurs further north (Fig. 1F). This taxon differs from *A. manglesii* subsp. *manglesii* in that the scape is often branched (and is therefore an exception in this subgenus of unbranched taxa), the red hairs on the ovary are more pinkish and extend further up the perianth, and the perianth is slightly constricted near the filaments (Fig. 7B,C). In *A. manglesii* subsp. *manglesii* there is no constriction of the perianth. In addition, the perianth of *A. manglesii* subsp. *manglesii* is split all the way to the

ovary, whereas in *A. manglesii* subsp. *quadrans* the perianth is only partially split at the base, and has a green flange (Fig. 7B,C).

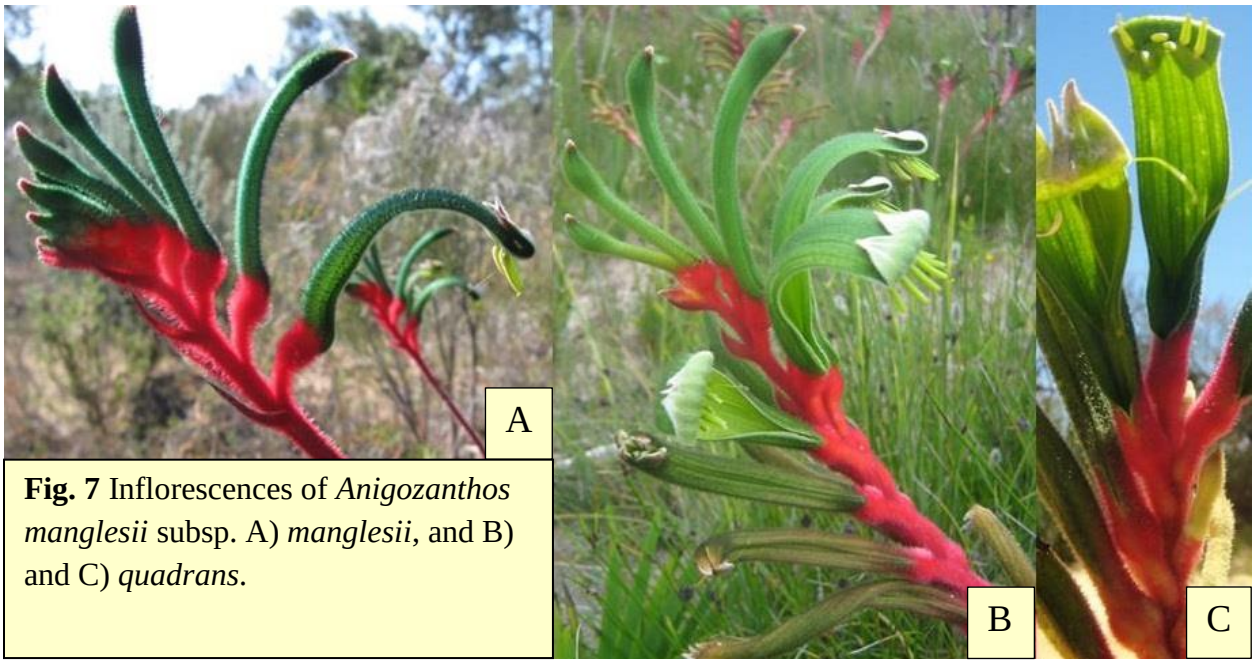

Fig. 7 Inflorescences of *Anigozanthos manglesii* subsp. A) *manglesii*, and B) and C) *quadrans*.

***Anigozanthos viridis* taxa**

The specific epithet '*viridis*' comes from the Latin word for 'green', in reference to the colour of the flowers (Fig. 8). A distinguishing feature of the leaves of these taxa is that they are subterete rather than flat, as in most other *Anigozanthos* taxa. These taxa mainly occur in winter wet depressions without a shady overstorey. Sometimes they can be seen growing in roadside gullies.

There are three *A. viridis* subspecies. The most common subspecies is *A. viridis* subsp. *viridis* which occurs along the coastal plain and adjacent scarp from Gin Gin north of Perth down to Scott River (Fig. 1J). The two other subspecies have more restricted distributions north of Perth. These subspecies are *A. viridis* subsp. Cataby, which is yet to be formally described, and *A. viridis* subsp. *terraspectans*, which is a declared rare plant and therefore not included in the present study. *Anigozanthos viridis* subspecies *viridis* is the tallest of these taxa, with flowering scapes up to 1 m tall, *A. viridis* subsp. Cataby plants are intermediate in height with scapes 15 to 30 cm tall and *A. viridis subsp. terraspectans* is the smallest subspecies, growing to only 10 to 15 cm in height (Fig. 8).

In all *Anigozanthos* taxa the flowers open over the flowering scape or stem, thus facing back toward the centre of the plant. Accordingly, the subspecies *viridis* and Cataby have flowers that open over the scape (Fig. 8). This enables birds to sit on the stem as they pollinate the flowers. This differentiates them from *A. viridis* subsp. *terraspectans* where the flowers open away from the scape rather than over the plant.

Fig. 8 Inflorescences of the three *Anigozanthos viridis* subspecies, *viridis*, Cataby and *terraspectans*, showing their heights, flower colour and conservation codes where applicable. (See Western Australian Herbarium (1998-) and Department of Parks and Wildlife (2015) for more information on conservation codes).

A. manglesii* var. *x angustifolius

This taxa is a putative backcrossed hybrid between *A. viridis* and either *A. manglesii* or *A. bicolor* (Macfarlane *et al.*, 1987; Stephen Hopper pers. comm.). This taxon can occur as whole populations rather than just isolated hybrids in populations of the parent taxa. The green flowers are similar to those of *A. viridis* but the leaves are broad and flat, rather than subterete (Fig. 9).

Fig. 9 *Anigozanthos manglesii* var. *x angustifolius* plant growing on the roadside with green flowers and flat leaves.

Subgenus Anigozanthos

Anigozanthos flavidus

Anigozanthos flavidus is a long-lived resprouter that has multi-branched scapes 1 to 3 m tall with many flowers. The evergreen leaves are up to 1 m in length. The flowers are tubular and generally yellow to green in colour, but in some populations they are slightly reddish (Fig. 10). The flowers are distinguished by orange appendages on the back of the anthers (Fig. 10). This is the only *Anigozanthos* species that extends into the wetter shaded forests of the south west (Fig. 1B). Many species are crossed with *A. flavidus* in horticulture because it is less susceptible to fungal diseases, such as ink spot, than other *Anigozanthos* taxa.

Fig. 10 Tubular flowers of *Anigozanthos flavidus*. The typical flower colour is shown on the left, while a colour variation is illustrated on the right. Note the orange appendages on the back of the anthers.

Anigozanthos onycis* and *Anigozanthos preissii

Anigozanthos onycis and *A. preissii* are also in the branched subgenus of Anigozanthos, but differ from other taxa in this group in that the scape is usually only branched once (Fig. 11A,B). Occasionally *A. onycis* has an unbranched scape (Fig. 11C). These two species, along with *A. humilis* and *A. kalbarriensis*, are referred to as Catspaws rather than Kangaroo Paws. The perianth lobes of both these taxa are spread horizontally rather than being tubular, and the ends of the lobes are flat rather than recurved (Fig. 11). One of the main differences between these two species is that *A. preissii* has a taller scape (20 to 70 cm) than *A. onycis* (15 to 30 cm). The perianths of *A. preissii* are also larger. In addition, *A. onycis* flowers are red to cream in colour, whereas *A. preissii* flowers are mostly yellow with some red at the base. Both species are fire ephemerals, meaning that they are short-lived and are mainly seen only in the year or two after fire. They are both found on the south coast of Western Australia (Fig. 1G,H). *Anigozanthos preissii* occurs in the Albany region and so is often called the Albany Catspaw, whereas *A. onycis* occurs further east. A feature of *A. preissii* is that it has the heaviest seeds of all *Anigozanthos* taxa (K. Downes unpublished data). However, they are still fairly small (approximately 1.2 to 2.2 mm long; Macfarlane *et al.*, 1987).

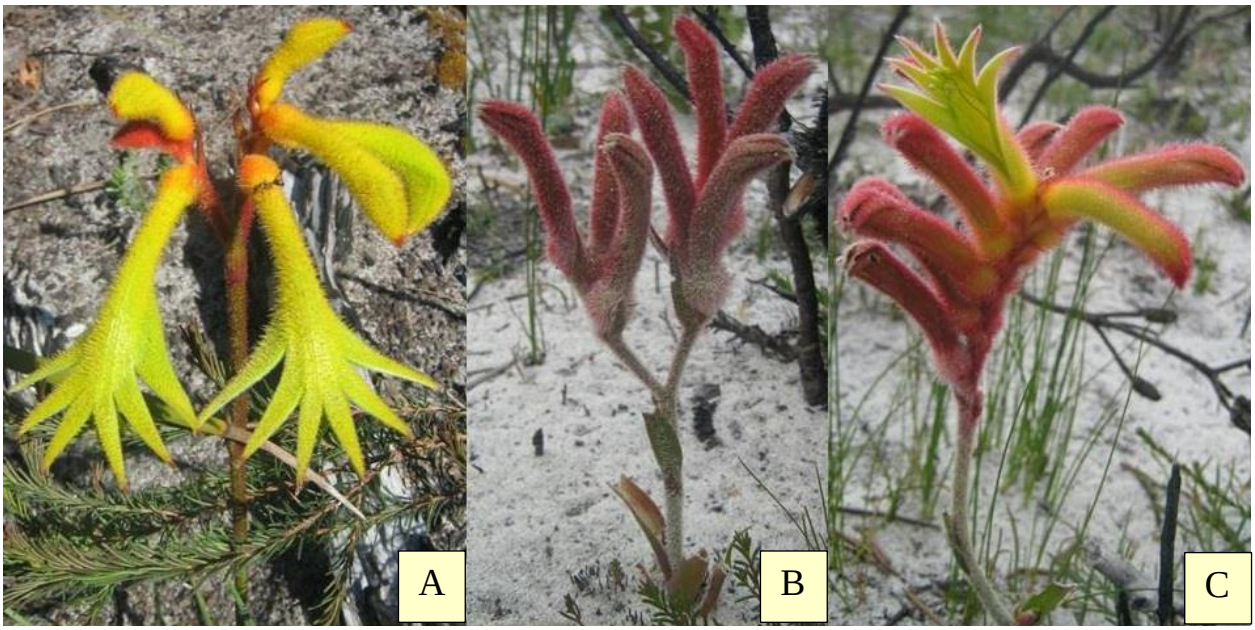

Fig. 11 Flowers of *Anigozanthos preissii* (A) and *A. onycis* (B,C). In B) the flowers of *A. onycis* have just finished and in C) an unbranched *A. onycis plant* is shown.

Sometimes *A. onycis* co-occurs with *A. humilis* subsp. *humilis*. These taxa can be differentiated by the slightly larger perianth and longer filaments of *A. onycis*, and the different stamen arrangements of the two species (Fig. 12). In *A. onycis* the four central stamens are in a row and the two outer stamens are lower with the anthers curved inward, whereas in *A. humilis* subsp. *humilis* the shorter stamens are arranged in three rows.

Fig. 12 Surface and underside of perianths of *Anigozanthos onycis* on the left and *A. humilis* subsp. *humilis* on the right.

Anigozanthos pulcherrimus* and *Anigozanthos rufus

Anigozanthos pulcherrimus and *A. rufus* are closely related species that are long-lived resprouters. They both have scapes greater than 40 cm in height that are multi-branched with many flowers (Fig. 13). The main difference between these species is the colour of their flowers. *Anigozanthos pulcherrimus* flowers are yellow whereas *A. rufus* flowers are red (Fig. 13). The specific epithet '*rufus*' is derived from the Latin, meaning 'red'. These species also have distinct geographic distributions (Fig. 1I). *Anigozanthos pulcherrimus* is found on the northern sandplain of southwestern Australia, and *A. rufus* is found on the southern sandplain. The distribution of *A. rufus* extends further east than any other *Anigozanthos* species. These two species only have two to four ovules in each segment of the ovary, which is much fewer than in other *Anigozanthos* taxa. Another characteristic of these two species that differs from all other *Anigozanthos* taxa is that the whole fruit is shed, rather than the fruit wall splitting to release the seeds when they are ripe.

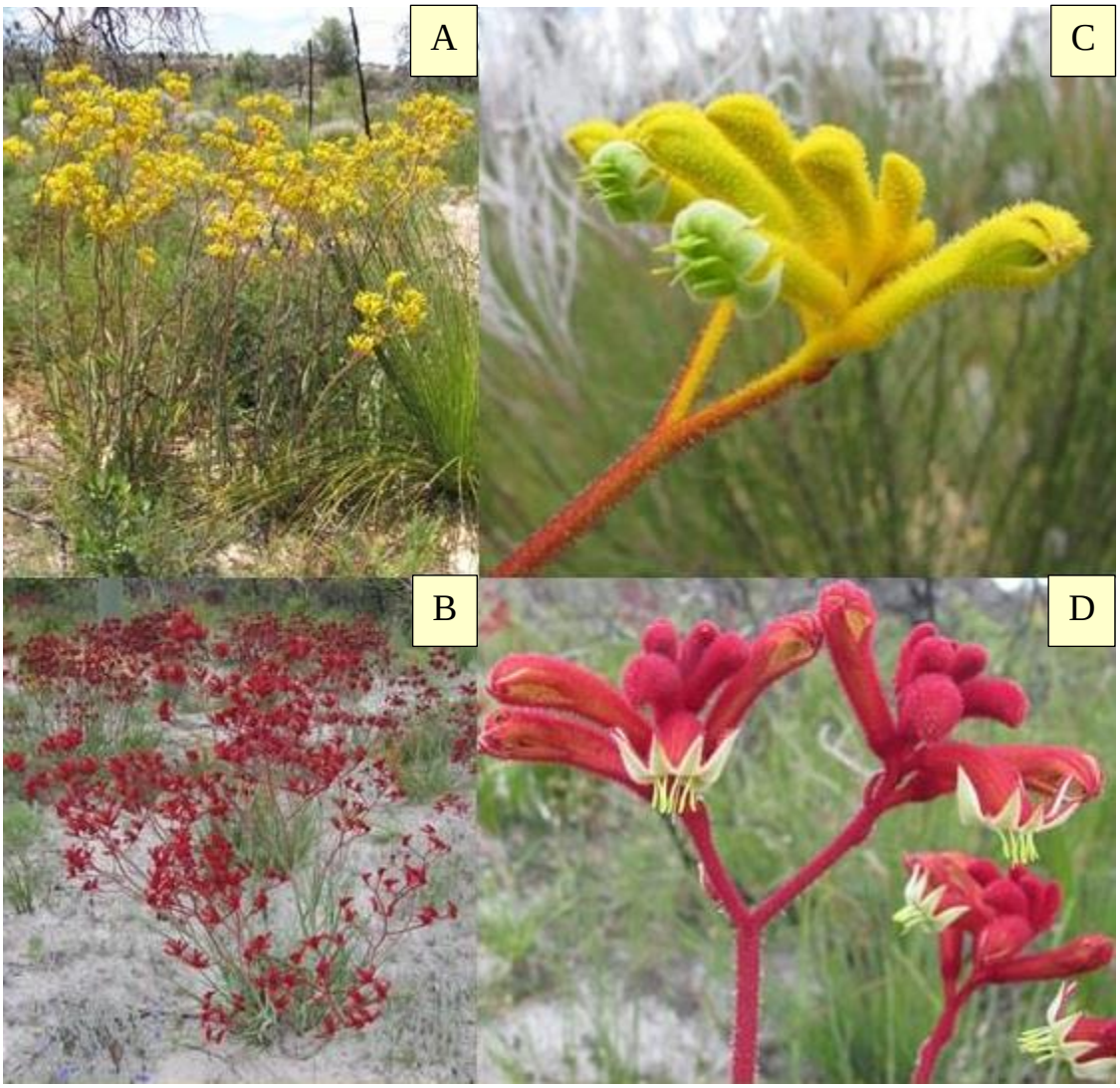

Fig. 13 Whole plants of *Anigozanthos pulcherrimus* (A) and *A. rufus* (B) and a close up of their flowers (C,D).

Pilot Study of *Anigozanthos manglesii* subsp. *manglesii*

Anigozanthos manglesii subsp. *manglesii* seeds were purchased from Tranen Revegetation Systems in Jolimont, Perth. These seeds were collected in Wanneroo, Perth (~31°45'S, 115°45'E) in January 2008. To ensure that a high proportion of the seeds used in the germination trials were filled, a Kimseed vacuum separator (Kimseed International, Osborne Park, Perth) was used to remove lighter seeds and chaff. Germination trials commenced in April 2008.

Germination Protocol

Surface Sterilisation

To minimise fungal contamination, seeds were surface-sterilised in a 2% sodium hypochlorite solution with a drop of Tween 80 (polyoxyethylene sorbitan mono-oleate) as a surfactant. This solution was placed under vacuum for 5 minutes, returned to normal atmospheric pressure for 5 minutes and then placed under vacuum for a further 5 minutes. Seeds were then rinsed at least twice with autoclaved deionised water to remove the hypochlorite.

Germination Trial

For each of the three replicates, 50 seeds were placed in 9 cm Petri dishes lined with two pieces of Whatman No. 1 filter paper over three 4 cm^2 pieces of Wettex. Ten mL of deionised water, 10 mM potassium nitrate or a 1 : 10 dilution (v/v) of 'Seed Starter' smoke water, were added to the Petri dishes, and sealed with Parafilm. Seed were incubated at 15°C, because this is the optimum temperature for germinating many southwestern Australian species (Bellairs and Bell, 1990), and exposed to either continuous light provided by cool white fluorescent tubes or continuous darkness (wrapped in aluminium foil). Germination was defined as emergence of the radicle and scored after 3 and 5 weeks of incubation.

Burial

Additional seeds were counted out, sealed in nylon mesh bags and buried ~1–2 cm beneath the soil surface at three locations (replicates) in the sandy soil of the Curtin University Field Trial Area, Bentley, Perth (32°0'39.2"S, 115°53'24.8"E) in May 2008. Seeds were exhumed in early April 2009, as a similar period of soil burial has previously reduced seed dormancy and enhanced seed receptiveness to stimulants such as smoke water in other southwestern Australian species (Baker *et al.*, 2005a, b). Seeds were also exhumed at this time of year because Hopper (1993) noted that most *Anigozanthos* germination occurs after the first autumn rains following a bushfire. Seeds were cleaned as previously described and the germination trials repeated as above. KAR_1 (0.1 µM) was also tested as it is the main germination-promoting chemical in smoke water (Flematti *et al.*, 2004; Van Staden *et al.*, 2004). Germination was scored after 4 weeks of incubation.

Germination of Freshly Collected Seeds of 16 *Anigozanthos* Taxa

To test the germination of freshly collected seeds, seeds of 16 *Anigozanthos* taxa were collected during the summers of 2008/09 and 2009/10 and cleaned (chaff and empty seeds removed) using a Kimseed vacuum separator. The 16 taxa and the seed collections used are indicated by an 'F' in the experiment column of Table 1. Initial germination studies were set up within one month of collection to ensure that the results are ecologically meaningful (Baskin *et al.*, 2006). Seeds were surface sterilised as per the pilot study, rinsed twice with autoclaved deionised water and placed in 9 cm Petri dishes on filter paper and 'Vileda' sponge. The filter paper was then moistened with 10 mL of deionised water, 10 mM nitrate, a 1:10 dilution of 'Seed Starter' smoke water or KAR_1, and sealed with Parafilm. All treatments consisted of three replicates of 50 seeds, except for those noted in Table 1 as having 25 seeds per replicate. Seeds were incubated at 15°C and were exposed to either continuous light or continuous darkness (wrapped in aluminium foil). Germination was scored after four weeks.

Germination of Seeds of Eight *Anigozanthos* Taxa Following 1 Year of Burial

Seeds of eight *Anigozanthos* taxa collected in the summer of 2008/09, *A. bicolor* subsp. *decrescens*, *A. flavidus*, *A. humilis* subsp. *chrysanthus*, *A. humilis* subsp. *humilis*, *A. manglesii* subsp. *manglesii*, *A. onycis*, *A. preissii* and *A. viridis* subsp. *viridis*, were buried in the autumn following collection and exhumed one year later as per the pilot study. The collections used in the burial trials are denoted by a 'B' in the experiment column of Table 1. In contrast to the pilot study, seeds were buried at three replicate sites in the source populations. GPS readings were taken to assist in re-locating the seeds. At the time of exhumation (between 2 and 10 April 2010) seeds were examined to check if any had started germinating. Seeds were cleaned using the Kimseed vacuum separator, surface sterilised and incubated in water, nitrate, smoke water or KAR_1 as per the fresh seeds.

Germination of Seeds of Eight *Anigozanthos* Taxa Following 3 to 4 Months of Burial

Seeds of eight different *Anigozanthos* taxa, *A. bicolor* subsp. *bicolor*, *A. bicolor* subsp. *exstans*, *A. gabrielae*, *A. humilis* subsp. Badgingarra, *A. kalbarriensis*, *A. manglesii* subsp. *quadrans*, *A. manglesii* var. *x angustifolius* and *A. viridis* subsp. Cataby were collected in the summer of 2009/10. See Table 1 for the collection details of these taxa. The collections that were employed in the burial trial are indicated by a 'B' in the experiment column. These seeds were mostly buried at the source populations within a few days of collection. A few taxa were buried slightly later, but still within a month of collection. Seeds were buried soon after collection to mimic when seeds enter the seedbank under natural conditions. Seeds were exhumed after 3 to 4 months of burial (between 2 and 10 April 2010), cleaned, surface sterilised and incubated as per the fresh seeds and seeds buried for one year.

Table 1. Collection details of the *Anigozanthos* taxa, including date of collection and latitude and longitude of the source population, examined in the fresh seed (F), burial (B), storage (S) and heat (H) experiments.

Taxa	Collection date	Latitude, longitude	Experiment
A. bicolor subsp. *bicolor*	31/12/09	31°03'45.0"S, 116°2'47.5"E	FBS^{25}H
A. bicolor subsp. *decrescens*	9/1/09	34°51'38.9"S, 117°33'23.8"E	FB
	14/1/10	34°37'38.9"S, 117°39'6.7"E	SH
A. bicolor subsp. *exstans*	27/12/09	32°14'32.4"S, 116°59'5.8"E	F^{25}BS^{25}H
A. flavidus	2/3/09	34°51'32.6"S, 117°33'14.8"E	FB
	8/3/10	34°51'30.7"S, 117°33'14.0"E	SH
A. gabrielae	14/1/10	34°18'24.6"S, 118°1'26.5"E	F^{25}BSH
A. humilis subsp. Badgingarra (S.D. Hopper 7114)	1/1/10	30°40'11.2"S, 115°33'14.0"E	FBSH
A. humilis subsp. *chrysanthus*	15/12/08	[A]	FB
	31/12/09	[A]	S^{25}H
A. humilis subsp. *humilis*	11/11/08	30°51'35.8"S, 115°36'45.4"E	FB
	26-27/11/09	30°51'35.8"S, 115°36'45.4"E	SH
A. kalbarriensis	21/12/09	27°48'13.8"S, 114°27'59.8"E	F^{25}BS^{25}
	23/12/09	27°45'23.9"S, 114°22'20.2"E	H
A. manglesii subsp. *manglesii*	11/12/08	32°01'10.4"S, 115°58'54.5"E	FB
	30/1/10	32°01'10.4"S, 115°58'54.5"E	SH
A. manglesii subsp. *quadrans*	20/12/09	27°40'56.2"S, 114°15'54.5"E	FB
	23/12/09	27°45'55.3"S, 114°22'46.3"E	SH
A. manglesii var. *x angustifolius*	9/1/10	30°42'41.2"S, 115°36'51.7"E	FBSH
A. onycis	17/12/08	34°15'8.6"S, 119°23'44.5"E	FB
	16/1/10	34°39'10.9"S, 118°20'12.6"E	SH
A. preissii	2/3/09	35°00'26.1"S, 117°58'38.8"E	FBS^{25}
	9/3/10	35°00'26.1"S, 117°58'38.8"E	H
A. rufus	9/4/10	34°39'22.3"S, 118°20'15.5"E	SH
A. viridis subsp. Cataby (S.D. Hopper 1786)	15/12/09	31°11'29.8"S, 115°46'31.4"E	F^{25}B
	5/12/10	31°11'29.8"S, 115°46'31.4"E	SH
A. viridis subsp.*viridis*	17/1/09	32°01'13.5"S, 115°58'44.3"E	FB
	30/1/10	32°01'13.5"S, 115°58'44.3"E	SH

[A] Location details are not provided for this taxon because it is listed as vulnerable by Briggs and Leigh (1996)

[25]indicates that 25 rather than 50 seeds were used per replicate for the FB and S treatments. Note that there were 25 seeds per replicate in all heat treatments.

Germination of 17 *Anigozanthos* Taxa Following 3 to 3.5 Years of Storage

Seeds of 17 *Anigozanthos* taxa were stored for 3 to 3.5 years from the time of collection at approximately 22°C (except for *A. preissii* seeds which were stored at 15°C). The taxa and seedlots employed in this study are denoted by an 'S' in the experiment column of Table 1. Following storage, seeds were surface sterilised and placed in Petri dishes as previously described with 10 mL of either deionised water, a 1:10 dilution of 'Seed Starter' smoke water, 50 µM glyceronitrile or 0.1 µM KAR_1. Glyceronitrile was included as it has recently been identified as a second germination promoting chemical in smoke water (in addition to KAR_1), and it has effectively promoted the germination of some *Anigozanthos* taxa (Flematti *et al.*, 2011; Downes *et al.*, 2013). Glyceronitrile was synthesised as a racemic mixture according to Kopecký and Šmejkal (1984). KAR_1 was synthesised from pyromeconic acid according to Flematti *et al.* (2005) and Light *et al.* (2010). For each taxon, three replicates of 50 seed were tested, except for those marked in Table 1 with a '25'. For those taxa, there were 25 seeds per replicate. Seed were incubated in the dark (wrapped in aluminium foil) at 20°C, except for *A. onycis*, *A. preissii* and *A. viridis* subsp. *viridis* which were incubated in the dark at 25°C. Germinants were counted after four weeks.

Germination of 17 *Anigozanthos* Taxa Following a 1 or 3 Hour 100°C Heat Pre-treatment

Seeds of 17 taxa were included in a heat trial. The taxa and seedlots used are indicated by an 'H' in the experiment column of Table 1. Seeds were subjected to a heat pre-treatment of 100°C for 1 or 3 hours because Tieu *et al.* (2001) reported that 3 hours at this temperature increased *A. manglesii* subsp. *manglesii* germination from negligible levels to over 80%. A 1 hour treatment was also tested to determine whether a shorter duration could produce similar results. Tieu *et al.* (2001) found that 30 minutes of heating promoted germination of *A. manglesii* subsp. *manglesii* but was less effective than 100°C for 3 hours, but 1 hour was not tested. The heat treatment was undertaken within 4 months of seed collection. For all taxa four replicates of 25 seeds were used per treatment and non-heated seeds were also set up at the same time as controls. The heat treatment was undertaken prior to surface sterilisation and each replicate was placed in the oven at separate times prior to incubation in deionised water to minimise pseudo-replication (Morrison and Morris, 2000). The ovens were pre-calibrated using data loggers placed on the top shelf (where the seeds were heated). This was because the oven heat sensor was in the roof of the oven and thus slightly overestimated the temperature on the oven shelves. Temperatures were also logged during the heating period and replicates were discarded if the temperature deviated outside 97-103°C once the temperature had returned to 100°C after opening the oven to place the seeds inside. Seeds were placed in Petri dishes as per the previous experiments, moistened with 10 mL autoclaved deionised water, sealed with Parafilm and incubated for 4 weeks at 15°C in continuous light.

For more details regarding the methods, other than the heat pre-treatments, see Downes *et al.* (2014).

Data Analysis

Prior to all statistical analyses, germination percentage data were converted to a value between zero and one, and arcsine square-root transformed.

One-way ANOVAs were conducted in Genstat 16 to test whether there were differences between treatments, and Fisher's protected LSD was run to determine where the differences, if any, arose. Treatments with zero germination in all replicates were excluded from analysis as these did not conform to ANOVA assumptions.

To determine whether there was a difference in germination levels following a 1 and 3 hour 100°C heat pre-treatment, either t-tests or Mann-Whitney Rank Sum tests (for those taxa with data that failed either a normality test (Shapiro-Wilk) or an equal variance test) were undertaken in SigmaPlot 11.

Results

Pilot Study of *Anigozanthos manglesii* subsp. *manglesii*

In the pilot study, three month old *A. manglesii* subsp. *manglesii* seeds germinated to low levels (≤8%) after 3 weeks of incubation, and there was no difference in germination between the water, nitrate and smoke water treatments (Fig. 14A, $F_{5,12}$=2.81 P=0.066). Levels of germination were not significantly different between seeds germinated in the light and the dark. Following a further two weeks of incubation in the light, no additional seeds germinated in the water (remaining at 1 ± 0.7% germination), whereas more seeds germinated in both the nitrate and smoke water treatments to 9 ± 3.5% and 16 ± 3.1%, respectively. After five weeks, germination promoted by nitrate and smoke water was significantly higher than that promoted by water alone ($F_{2,6}$ = 9.23, P = 0.015).

Following one year of burial, germination in smoke water exceeded 65%, whereas germination in the nitrate and KAR_1 treatments was between 9 and 15% and no higher than in water alone (Fig. 14b, $F_{7,16}$ = 3.08, P = 0.030). Each of the treatments exhibited high levels of variation between the three burial sites (replicates). Germination across treatments was consistently either higher or lower according to site (data not shown).

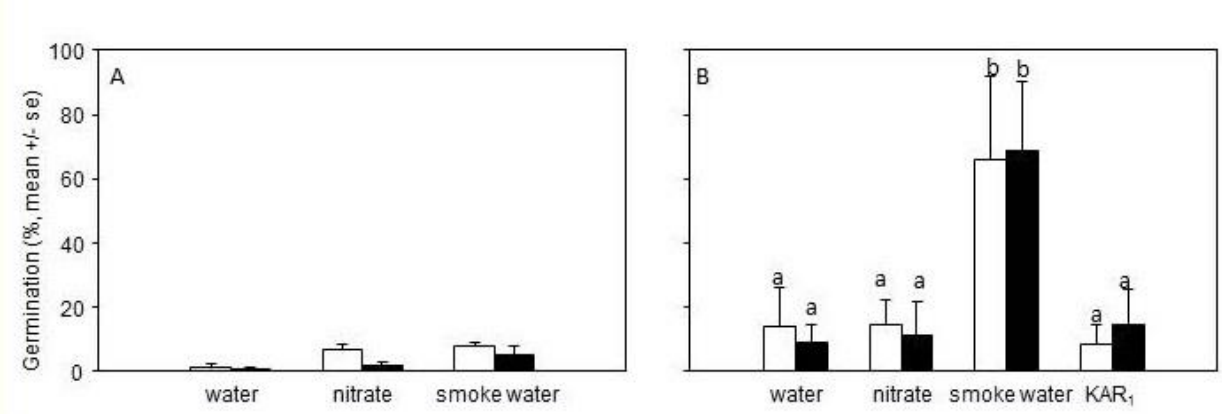

Fig. 14 Germination (%, mean ± s.e.) of *Anigozanthos manglesii* subsp. *manglesii* seeds tested A) three months after collection, and B) after one year of burial in response to water, nitrate and smoke water, in continuous light (open bars) or continuous darkness (filled bars) for three and four weeks, respectively, at 15°C. After burial, seeds were also incubated in KAR_1. Different letters above the bars indicate significant differences (P<0.05) in germination between treatments.

Germination of Freshly Collected Seeds of 16 *Anigozanthos* Taxa

Germination of freshly collected seeds of most (13 out of 16) of the *Anigozanthos* taxa tested was ≤3% across all treatments (water, nitrate, smoke water and $KAR_{1;}$ Fig. 15), indicating high levels of dormancy at 15°C. For the other three taxa, *A. manglesii* var. *x angustifolius*, *A. onycis* and *A. viridis* subsp. *viridis*, maximum germination levels of fresh seeds were ≤11%, and so dormancy levels were still fairly high. In each of these three taxa one of the highest levels of germination resulted from the dark nitrate treatment. (Note though that there was statistically no significant difference between treatments in *A. onycis*). Six per cent of *A. manglesii* var. *x angustifolius* seeds also germinated in the light nitrate treatment, and 11% of the *A. viridis* subsp. *viridis* seeds germinated in smoke water in the dark.

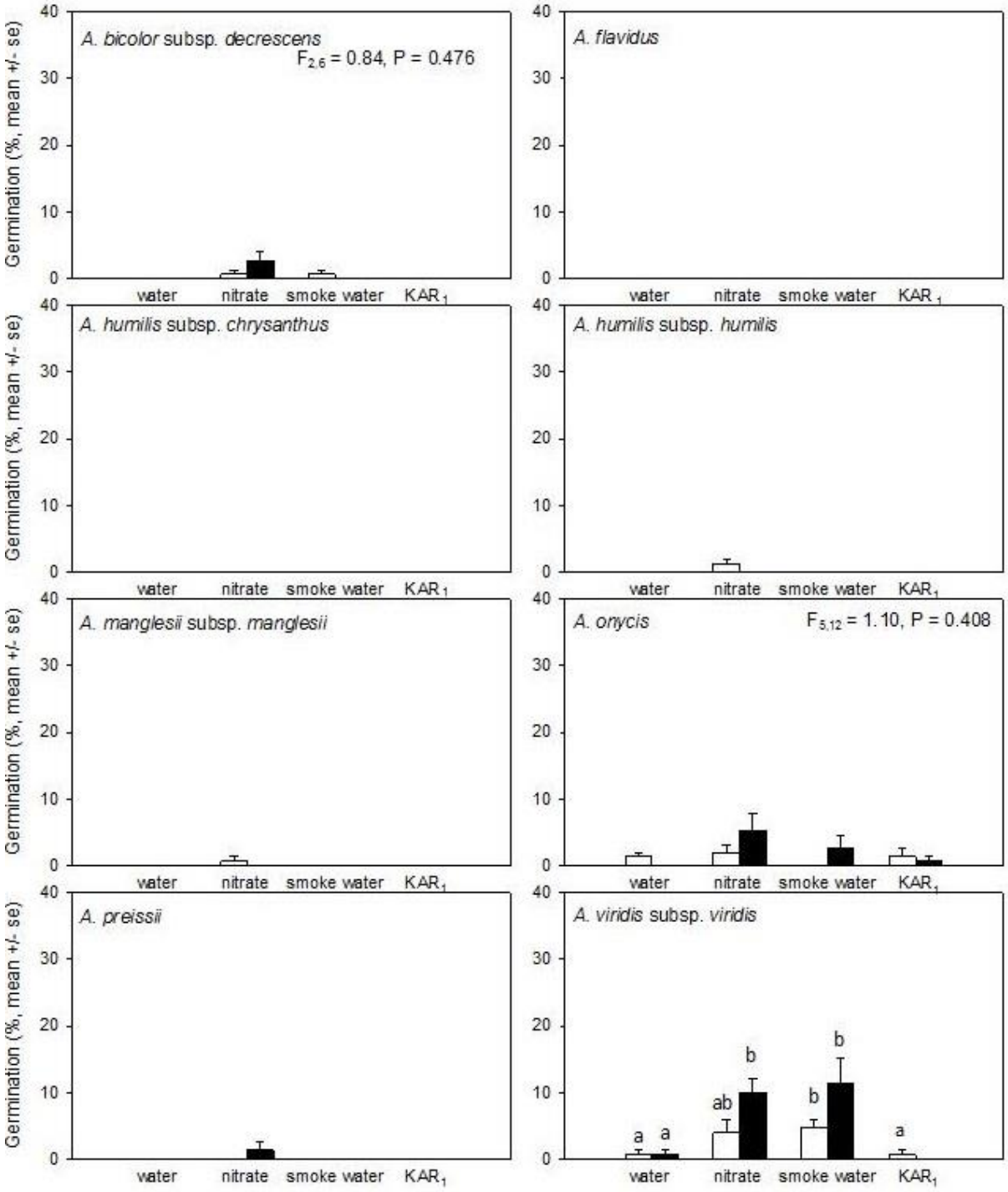

Fig. 15 Germination (%, mean ± s.e.) of 16 *Anigozanthos* taxa when freshly collected and incubated in the light or the dark, in water, nitrate, smoke water or KAR_1 for 4 weeks at 15°C. Different letters indicate significant differences between treatments (P<0.05).

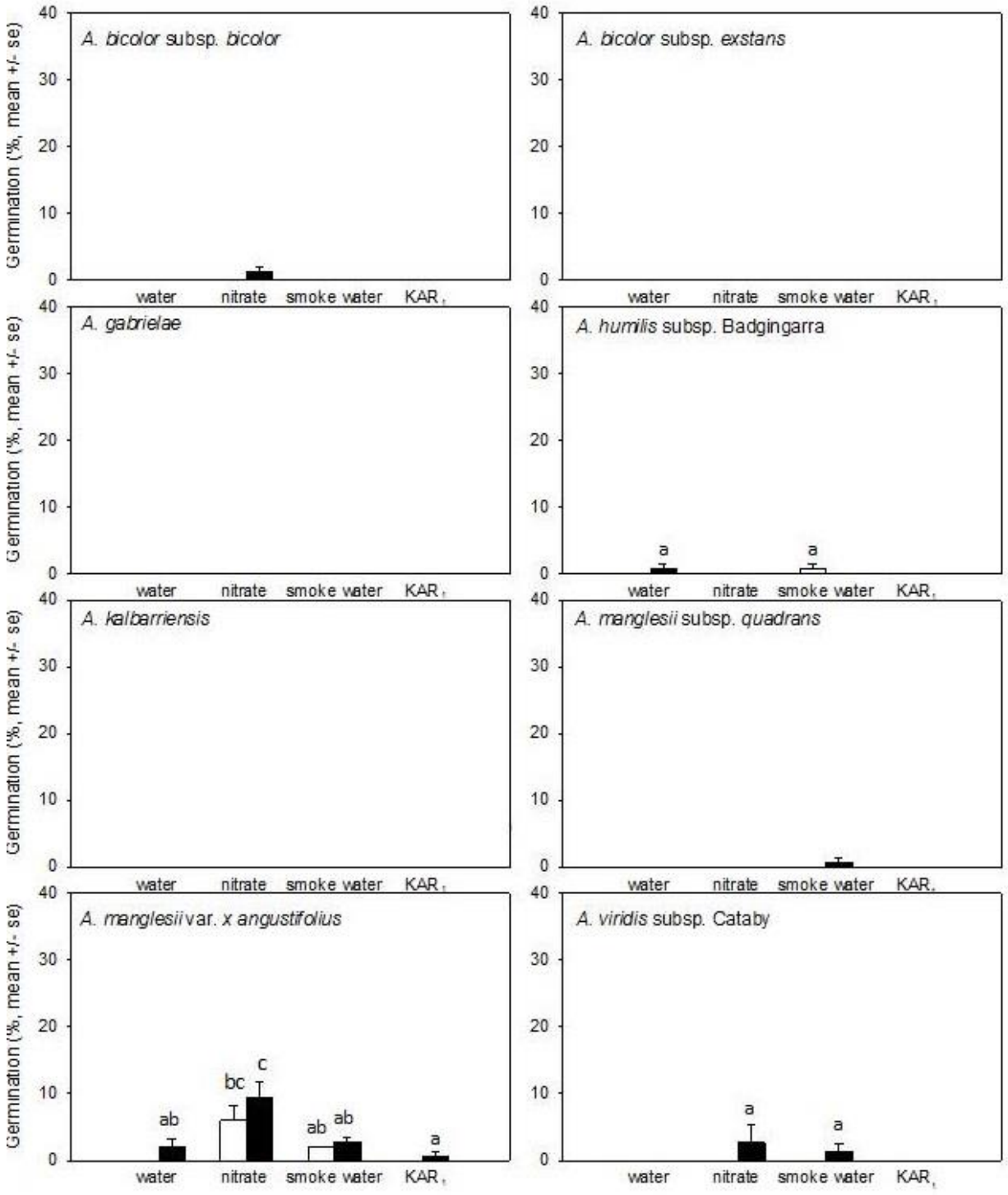

Fig. 15 (cont.) Germination (%, mean ± s.e.) of 16 *Anigozanthos* taxa when freshly collected and incubated in the light or the dark, in water, nitrate, smoke water or KAR_1 for 4 weeks at 15°C. Different letters indicate significant differences between treatments (P<0.05).

Germination of Seeds of Eight *Anigozanthos* Taxa Following 1 Year of Burial

Of the eight taxa buried for one year and exhumed in autumn, *A. manglesii* subsp. *manglesii* was the only one with >30% germination in any of the treatments (Fig. 16). Smoke water following burial resulted in >70% germination in both the light and dark treatments. Some *A. manglesii* subsp. *manglesii* seeds also germinated in water alone following burial. In addition to *A. manglesii* subsp. *manglesii*, smoke water also stimulated some *A. humilis* subsp. *humilis and A. viridis* subsp. *viridis* germination, though to lower levels. Smoke water in the dark also promoted the highest level of *A. humilis* subsp. *chrysanthus* germination (6%) after one year of burial. However, statistically, smoke stimulated germination in this taxon was no higher than that promoted by the other treatments. The taxon with the second highest level of germination after one year of burial, after *A. manglesii* subsp. *manglesii*, was *A. flavidus* (Fig. 16). This species had started germinating in the soil before seed retrieval, so germination levels of retrieved seeds was possibly an underestimate of the proportion of seeds in which dormancy was alleviated. Germination of this taxon was higher in the light than the dark, and highest in the nitrate followed by the smoke water treatments. Following burial, germination was low (<3%) in *A. onycis* and *A. preissii*, and absent in *A. bicolor* subsp. *decrescens.*

Germination of Seeds of Eight *Anigozanthos* Taxa Following 3 to 4 Months of Burial

Of the eight taxa exhumed after 3 to 4 months of burial, germination only exceeded 15% in *A. viridis* subsp. Cataby (Fig. 17). Prior to burial, germination of this taxon did not exceed 3% in any of the treatments (Fig. 15). Following burial, germination of *A. viridis* subsp. Cataby was highest in the smoke water treatments, reaching 29% in the dark and 16% in the light. Eight % of seeds also germinated in the light nitrate treatment post-burial. For *A. manglesii* subsp. *quadrans* 5% of seeds germinated after burial in the dark nitrate treatment (Fig. 17) compared to ≤1% germination across treatments in the fresh seeds (Fig. 15). Post-burial, germination of *A. manglesii* var. *x angustifolius* was 3 to 8% in the nitrate and smoke water treatments (Fig. 17). Germination following 3 to 4 months of burial was ≤3% across all of the treatments in the remaining five taxa tested. This included *A. onycis*, which had germinated to higher levels (5% in the dark nitrate treatment) when fresh (Fig. 15).

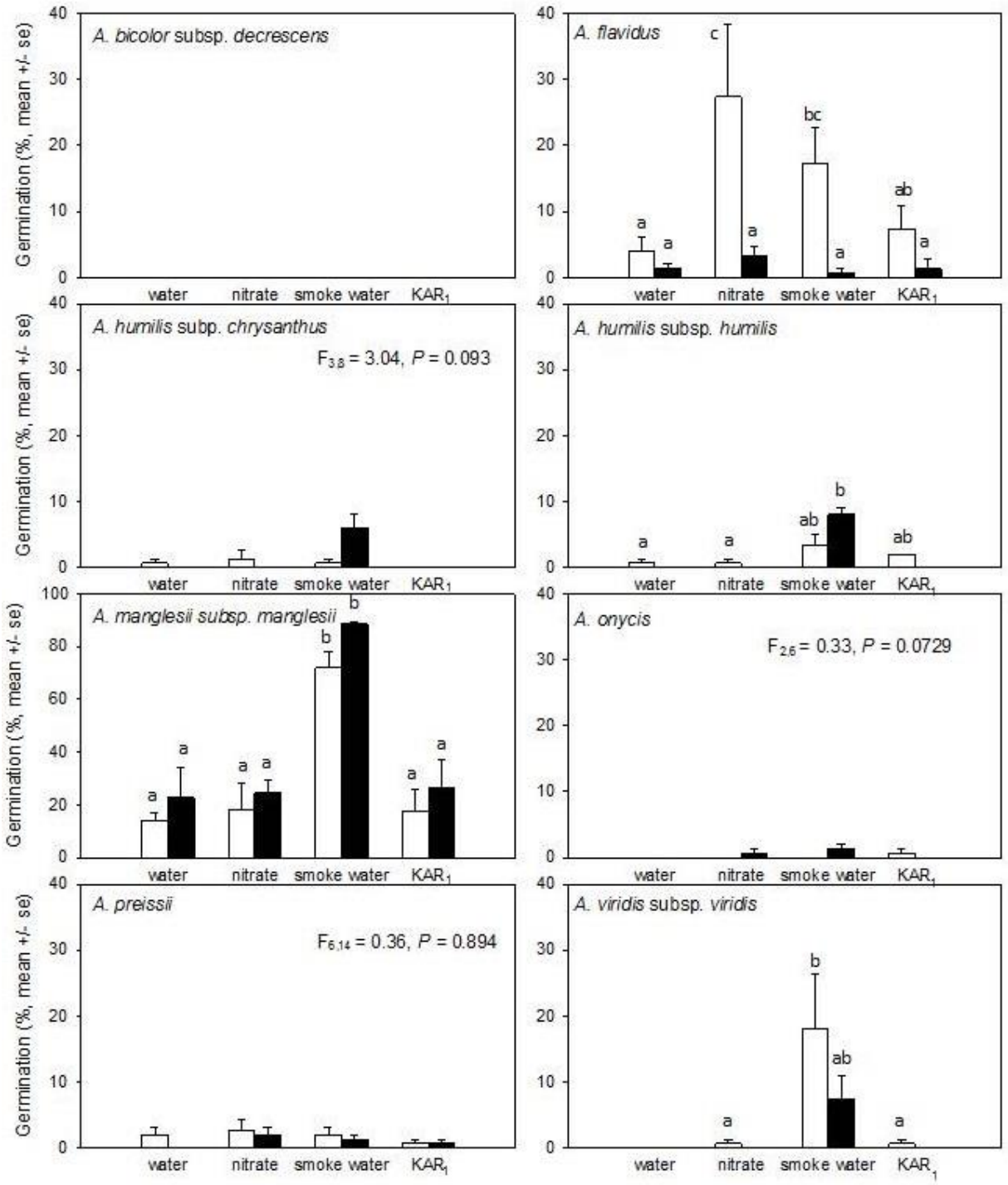

Fig. 16 Germination (%, mean ± s.e.) of eight *Anigozanthos* taxa following one year of burial commencing in the autumn following seed shed, and incubated in the light or the dark, in water, nitrate, smoke water or KAR_1 for 4 weeks at 15°C. Note that the y-axis for *A. manglesii* subsp. *manglesii* is 0 to 100, whereas for all other taxa it is 0 to 40. Different letters indicate significant differences between treatments (P<0.05).

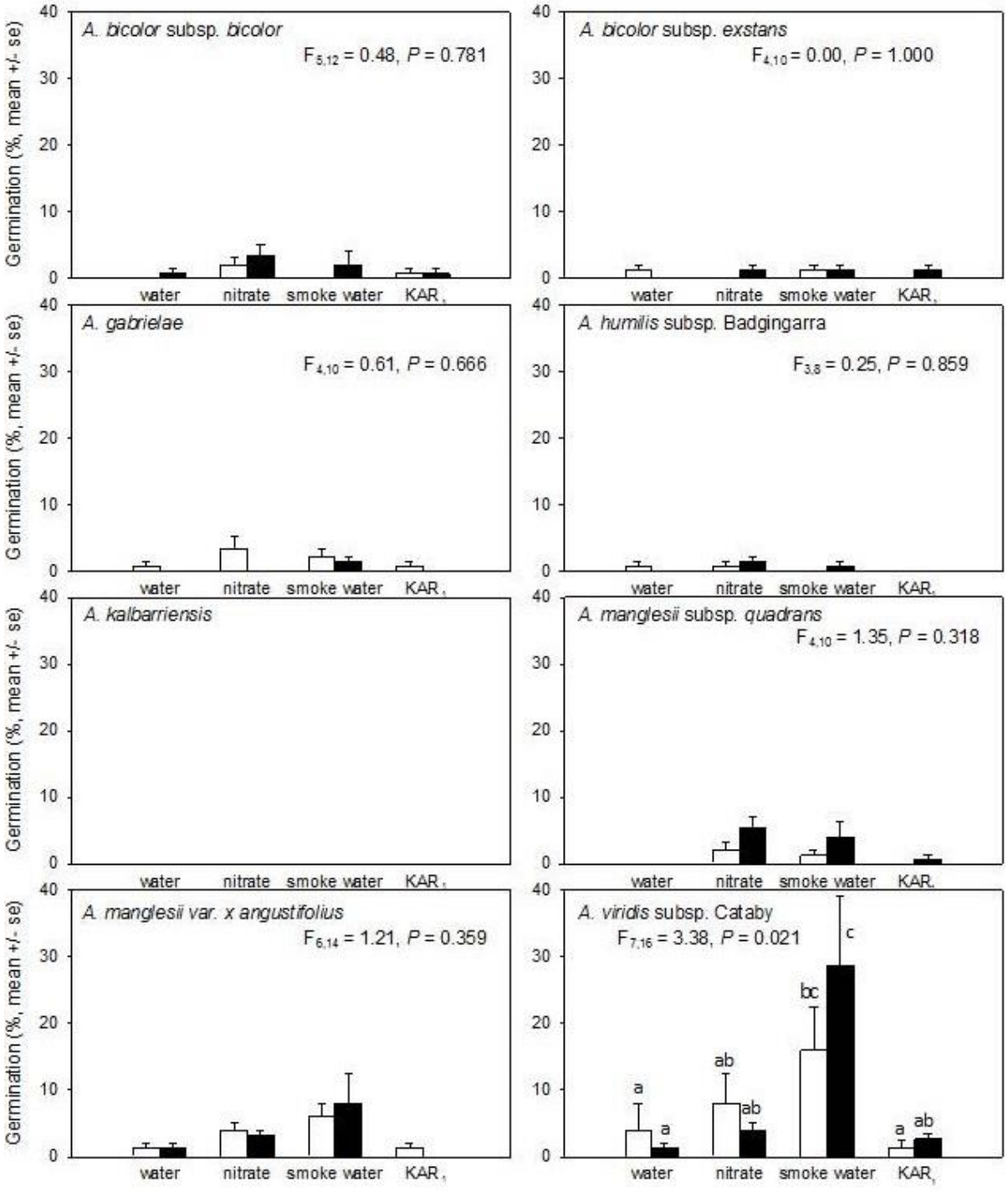

Fig. 17 Germination (%, mean ± s.e.) of eight *Anigozanthos* taxa following 3-4 months of burial from the time of seed shed, and incubated in the light or the dark, in water, nitrate, smoke water or KAR_1 for 4 weeks at 15°C. Different letters indicate significant differences between treatments (P<0.05).

Germination of 17 *Anigozanthos* Taxa Following 3 to 3.5 Years of Storage

Following 3 to 3.5 years of storage, germination in smoke water was >30% in 9 of the 17 *Anigozanthos* taxa examined, and ≥49% in 6 of these (Table 2). Germination was higher in both smoke water and the smoke-derived chemical, glyceronitrile, than water alone for 11 taxa. *Anigozanthos humilis* subsp. *humilis* and *A. rufus* germination was promoted by glyceronitrile but not smoke-water. KAR_1, another germination-promoting chemical in smoke, was ineffective at stimulating germination above control levels in any *Anigozanthos* taxa examined. *Anigozanthos kalbarriensis*, *A. manglesii* subsp. *quadrans*, *A. onycis,* and *A. preissii* were not stimulated to germinate to higher levels in smoke water, glyceronitrile or KAR_1 than in water alone. Nevertheless the germination of *A. onycis* in water alone was still moderate (12%), and *A. kalbarriensis* and *A. preissii* germination was 7% in smoke water. Treatment with smoke-related chemicals after storage was least effective at promoting *A. manglesii* subsp. *quadrans* germination under the conditions tested.

Table 2. Germination (%, mean ± s.e.) of 17 *Anigozanthos* taxa following a period of storage in response to water, smoke-water, glyceronitrile or KAR_1 after incubation for 4 weeks in the dark. Different letters indicate significant differences between treatments (P<0.05).

Taxa	Water	Smoke water	Glyceronitrile	KAR_1	F- and P-values
A. bicolor subsp. *bicolor*	5.3 ± 1.3 a	14.7 ± 1.3 b	17.3 ± 2.7 b	4.0 ± 2.3 a	$F_{3,8}$ = 6.62, P = 0.015
A. bicolor subsp. decrescens	4.7 ± 1.8 a	33.3 ± 4.7 b	36.0 ± 5.3 b	5.3 ± 1.3 a	F3,8 = 27.23, P < 0.001
A. bicolor subsp. *exstans*	13.3 ± 2.7 a	49.3 ± 5.8 b	58.7 ± 8.7 b	12.0 ± 6.1 a	$F_{3,8}$ = 9.68, P = 0.005
A. flavidus	11.3 ± 3.7 a	53.3 ± 1.8 b	55.3 ± 7.1 b	5.3 ± 1.8 a	$F_{3,8}$ = 35.44, P < 0.001
A. gabrielae	2.0 ± 0.0 a	32.7 ± 0.7 c	16.0 ± 1.2 b	4.6 ± 1.8 a	$F_{3,8}$ = 85.92, P < 0.001
A. humilis subsp. Badgingarra	0.0 ± 0.0	10.7 ± 2.4 b	18.0 ± 2.3 b	0.7 ± 0.7 a	$F_{2,6}$ = 24.94, P = 0.001
A. humilis subsp. *chrysanthus*	6.7 ± 3.5 a	40.0 ± 6.9 b	41.3 ± 2.7 b	9.3 ± 2.7 a	$F_{3,8}$ = 12.59, P = 0.002
A. humilis subsp. *humilis*	0.7 ± 0.7 a	4.7 ± 1.3 a	16.0 ± 4.6 b	0.0 ± 0.0	$F_{2,6}$ = 12.34, P = 0.007
A. kalbarriensis	1.3 ± 1.3	6.7 ± 2.7	2.7 ± 1.3	0.0 ± 0.0	$F_{2,6}$ = 2.27, P = 0.184
A. manglesii subsp. *manglesii*	2.7 ± 1.3 a	66.0 ± 0.0 b	70.7 ± 0.7 b	2.0 ± 1.2 a	$F_{3,8}$ = 118.52, P < 0.001
A. manglesii subsp. *quadrans*	0.7 ± 0.7	1.3 ± 0.7	2.7 ± 1.8	0.0 ± 0.0	$F_{2,6}$ = 0.53, P = 0.611
A. manglesii var. *x angustifolius*	5.3 ± 2.4 a	49.3 ± 6.8 b	66.7 ± 0.7 c	7.3 ± 0.7 a	$F_{3,8}$ = 69.38, P < 0.001
A. onycis	12.0 ± 3.5	17.3 ± 2.7	12.7 ± 2.7	10.7 ± 0.7	$F_{3,8}$ = 1.17, P = 0.380
A. preissii	1.3 ± 1.3	6.7 ± 3.5	6.7 ± 3.5	0.0 ± 0.0	$F_{2,6}$ = 0.76, P = 0.506
A. rufus	0.7 ± 0.7 a	2.0 ± 1.2 ab	8.7 ± 3.7 b	0.7 ± 0.7 a	$F_{3,8}$ = 4.22, P = 0.046
A. viridis subsp. Cataby	2.7 ± 0.7 b	50.0 ± 3.5 c	52.0 ± 2.0 c	0.7 ± 0.7 a	$F_{3,8}$ = 152.32, P < 0.001
A. viridis subsp. *viridis*	12.0 ± 4.2 a	57.3 ± 6.4 b	66.7 ± 4.8 b	7.3 ± 2.9 a	$F_{3,8}$ = 31.57, P < 0.001

Germination of 17 *Anigozanthos* Taxa Following a 1 or 3 Hour 100°C Heat Pre-treatment

In the absence of a 100°C heat pre-treatment, none of the *Anigozanthos* taxa germinated, except for *A. viridis* subsp. *viridis* which produced low germination (4%; Fig. 18). Germination of most *Anigozanthos* taxa was higher following the 100°C treatment for either 1 or 3 hours (compared to no heat pre-treatment), the one exception being *A. rufus* in which germination remained low (≤2%; Fig. 18). Germination exceeded 30% in at least one of the heat treatments in 12 taxa, and for six of these taxa, germination was >50%. Heating seeds at 100°C for 1 hour generally produced higher germination than heating for 3 hours. This difference was significant in 12 of the 17 *Anigozanthos* taxa examined. The statistical power of the performed test was below desired levels for *A. flavidus*, *A. humilis* subsp. Badgingarra and *A. humilis* subsp. *humilis*, meaning that a difference may have also been present in these taxa but was not detected.

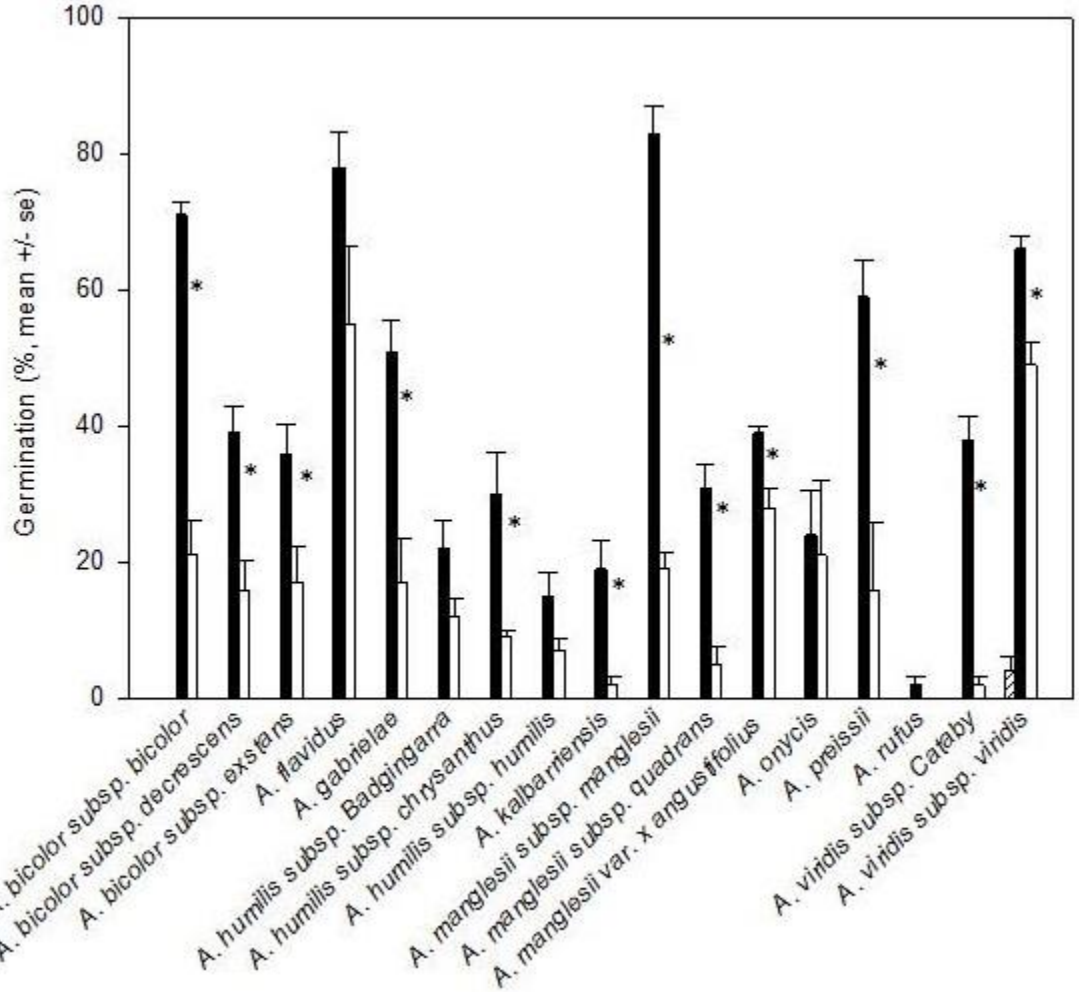

Fig. 18 Germination (%, mean ± se) of 17 *Anigozanthos* taxa following a 100°C heat pre-treatment for either 1 or 3 hours, and then incubation in deionised water in the light at 15°C for 4 weeks. The hatched bars denote germination of unheated seeds (control), the filled bars represent germination following the 1 hour 100°C treatment, and the empty bars indicate germination following 3 hours at 100°C. The asterisk denotes taxa with a significant difference (P>0.05) in germination following the 1 hour versus the 3 hour heat treatments.

Discussion

Seeds of all *Anigozanthos* taxa tested were dormant at maturity and therefore required some form of treatment to alleviate dormancy. This study highlighted various techniques that can be used to alleviate dormancy in these taxa, such as burial, storage and heat pre-treatments. Under the conditions tested, the two most effective methods of promoting germination of most *Anigozanthos* taxa were the storage treatment combined with incubating seeds in smoke water, and the heat pre-treatment. Germination following storage of fresh seeds at approximately 22°C for 3 to 3.5 years resulted in over 30% of seeds germinating in smoke water in 9 of the 17 *Anigozanthos* taxa examined. This treatment was effective in promoting germination of approximately 50% of seeds or more of *A. bicolor* subsp. *exstans, A. flavidus, A. manglesii subsp. manglesii, A. manglesii* var. *x angustifolius*, *A. viridis* subsp. Cataby, and *A. viridis* subsp. *viridis.* Glyceronitrile also promoted the germination of stored seeds, but because it is not readily available commercially, the use of smoke water is recommended.

Despite the effectiveness of storage for alleviating dormancy, storing seeds for increasingly longer durations of time does not necessarily result in increasingly higher germination levels. This is because seeds lose viability over time. Generally seeds lose viability faster when stored at higher temperatures and moisture contents (Bewley and Black, 1982). Thus there is a trade-off over time between dormancy alleviation and seed viability. This highlights the importance of knowing the age and storage conditions (temperature and humidity) that seeds have been exposed to, for example, when deciding whether to purchase seeds. The storage history of the seeds to be germinated can also influence what germination treatment is most appropriate. For example, if seeds are a few years old they could be incubated directly in smoke water, whereas a heat pre-treatment at 100°C would be more suitable for fresh seeds.

The heat pre-treatment improved germination of all 17 *Anigozanthos* taxa examined except *A. rufus.* Germination levels were promoted to over 30% in 12 of the 17 taxa examined. Heating seeds at 100°C for one hour was more effective at promoting germination than heating them for three hours. The one hour heat pre-treatment promoted at least 50% germination in *A. bicolor* subsp. *bicolor, A. flavidus, A. gabrielae, A. manglesii* subsp. *manglesii, A. preissii and A. viridis* subsp. *viridis*. An advantage of the 100°C heat pulse method of germinating *Anigozanthos* seeds is that germinants can be produced relatively quickly without requiring long periods of seed burial or storage. Also the promotive effect of a heat pulse has been shown to be retained in *Anigozanthos manglesii* subsp. *manglesii* seeds for at least three years of subsequent storage (Turner *et al.*, 2013).

Although burial from autumn one year until autumn the next was highly effective in alleviating dormancy in *A. manglesii* subsp. *manglesii*, and rendering seeds responsive to smoke water, it was less effective in the seven other taxa tested. This is probably because the duration and timing of burial and exhumation, and/or the incubation temperatures, were not optimal for most of the taxa. For example, seeds of most taxa are shed in summer (Table 1) and so under natural conditions seeds would enter the soil seedbank in summer rather than autumn. This is the reason why the seeds collected in the second year (2009/10) were buried as soon as possible after collection. However germination levels of these taxa were also low when tested following exhumation three to four months later. Future tests could examine whether seeds of some of

these taxa require longer burial extending over at least two summers before dormancy is alleviated. Exhuming seeds in different seasons and testing a range of incubation temperatures could also be examined. Determining optimum incubation temperatures would also enhance germination levels further following the storage and heat treatments.

Therefore this study highlights that *Anigozanthos* seeds are dormant at maturity and that germination can be promoted in many taxa by either a dry heat pre-treatment of 100°C for one hour, or storage for 3 to 3.5 years at room temperature followed by incubation in smoke water. Such methods can be used to enhance the propagation of these fascinating lesser known Kangaroo Paw and Catspaw taxa, for conservation, horticultural and land rehabilitation purposes.

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A range of *Anigozanthos* taxa, hybrids and colour forms.

Top row: *A. bicolor* subsp. *exstans*, *A. bicolor* subsp. *bicolor*, *A. humilis* subsp. *humilis* and *A. humilis* subsp. *chrysanthus*

Middle row: *A. manglesii* subsp. *manglesii*, *A. humilis* subsp. *humilis x gabrielae*, *A. preissii*, *A. kalbarriensis*, *A. bicolor* subsp. *decrescens*

Bottom row: *A. manglesii* var. *x angustifolius*, *A. manglesii* subsp. *manglesii x humilis* subsp. *humilis*, three *A. manglesii* subsp. *manglesii* flower colour variants