Germination of the rarely seen Pink Flannel Flower (*Actinotus forsythii*; Apiaceae)

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Background

Fire is an important driver shaping the composition of plant communities in Australia. Fire ephemerals are a unique element of the fire-vegetation relationship, emerging in the first few months after fire and persisting only for one to several years. These species have a strong dependence on fire to germinate and, as ephemerals only live a short time in their plant phase, spend the majority of their life cycle as seeds in the soil. Consequently, it is likely that they have developed relatively complex and specific requirements to overcome dormancy and promote germination.

Fire ephemerals make up a significant component of the floras in Mediterranean-type rainfall climates, including Western Australia, California and South Africa, however, they are much less common in aseasonal rainfall climates like in south-eastern NSW. With support from the Menai Wildflower Group, we have been studying *Actinotus forsythii* (Pink Flannel Flower; Figures 1 and 2), one of the few fire ephemerals from the greater Sydney region in NSW, at the Centre for Ecosystem Science at the University of New South Wales.

Studying seeds and their germination cues may provide insight into how a species persists (Baskin and Baskin, 2014). Because of the dependence of fire ephemerals on very long-lived soil seed banks, it is highly likely that seeds of the Pink Flannel Flower would have deep dormancy. The type of dormancy species within the family Apiaceae have, either physiological or morphophysiological (Baskin and Baskin 2014), also indicates that seeds are likely to have specific germination requirements. This is similar to other physiologically dormant species from the region, including many Rutaceae, where dormancy is overcome with combinations of seasonal temperatures and germination responding to fire (Mackenzie *et al.* 2016; Collette and Ooi 2017).

Studies of fire ephemerals carried out in Western Australia have highlighted a significant increase in germination in response to smoke and heat (Baker *et al.* 2005). A seed burial experiment also suggested a strong dependence on seasonality as germination increased to 95% after a summer compared to a winter burial (Baker *et al.* 2005). Studies have also been carried out in the Sydney region, focusing on the more common Flannel Flower *Actinotus helianthi,* a closely related fire ephemeral, and have also demonstrated a complex dynamic of germination cues (Emery *et al.* 2011). We aimed to understand the dormancy and germination cues of the Pink Flannel Flower to give insights both for improving its use in cultivation and its ecology in the wild.

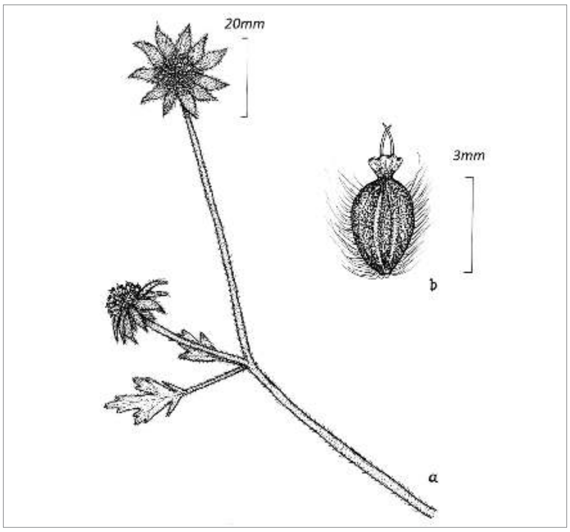

Figure 1. ***Actinotus forsythii*** **a - flower stem and b – seed. Illustration: Ruby Paroissien**

Figure 2. ***Actinotus forsythii*** **in flower. Photo: Lloyd Hedges**

Methods

We established an experiment to assess the germination requirements of *Actinotus forsythii*. Our main aims were to understand:

- Whether this species had specific seasonal temperature requirements for germination.
- The relative importance of the different fire cues, smoke and heat, for germination.
- If seeds could germinate in both light and dark conditions.

To do this, seeds were collected in March 2017 from plants cultivated by Lloyd Hedges at Menai Wildflower Group nursery. Parent seeds for cultivated plants were collected from near Lithgow, NSW. We first assessed viability of the seed lot by cutting three replicates of 20 seeds in half and observing if the endosperm was healthy (Ooi *et al.* 2004). An experiment was then implemented to test:

1. Seasonal temperatures: incubators were used to mimic seasonal day/night temperatures of 11/3ºC, 18/6ºC, or 25/11ºC approximating winter, spring or autumn, and summer conditions respectively, for the study region.
2. Fire cues: smoke water was generated through burning leaf litter from the local area and pumping the resulting smoke through distilled water for 3 hours.
3. Light and dark: incubators had light/dark conditions on a 12/12 hour cycle. Constant dark was implemented by covering trays in two layers of aluminium foil.

Four replicates of 25 seeds were sown on water agar (7 g/L) in 9cm petri dishes for each treatment. Treatments included the control, smoke, dark and smoke plus dark. A heat and smoke plus heat treatment were also carried out at the 25/11°C incubation temperature only. The smoke treatment was applied using smoke water at a concentration of 2%, with 1ml spread onto the top of the agar before seeds were sown. Heat was applied to heat treatment seeds for 10 minutes at 80°C in an oven. Dishes were incubated at the three different seasonal temperatures on 12 h/12 h light/dark and maximum/minimum temperature cycles. Petri dishes were checked once a week for 7 weeks except for the dark and dark plus smoke treatments, which were checked just once after 50 days. Final germination was calculated based on number of seeds germinated divided by the number of viable seeds in each dish. Time to reach 50% of total seeds germinated (t_{50}) was calculated to assess the rate of germination by fitting a linear model for the percentage of seeds germinated in each replicate against time and solving for y = 50. Germination data were analysed using two-factor Generalised Linear Models and T_{50} with ANOVA. Comparisons between treatment levels were made using Tukey's tests.

Results

After 50 days, seeds in the 11/3ºC temperature had not germinated. However, at 18/6ºC, germination of untreated seeds reached approximately 20% and smoke significantly increased this ($P = 0.006$). At 25/11ºC, untreated seeds germinated to even greater levels (ca. 35%) and the slight increase with smoke was not significant. The dark treatments significantly reduced germination at both temperatures to negligible levels (Figure 3). The heat shock experiment indicated that fire-related temperatures did not increase germination (Figure 4). The rate of germination differed between seasonal temperatures. On average, T_{50} was 1.89 weeks (untreated seeds) and 1.84 weeks (smoke) at 25/11ºC, and 3.45 weeks (untreated) and 3.02 weeks (smoke) at 18/6ºC. Germination was significantly faster at 25/11ºC ($F = 82.7$, df = 1, $P < 0.001$).

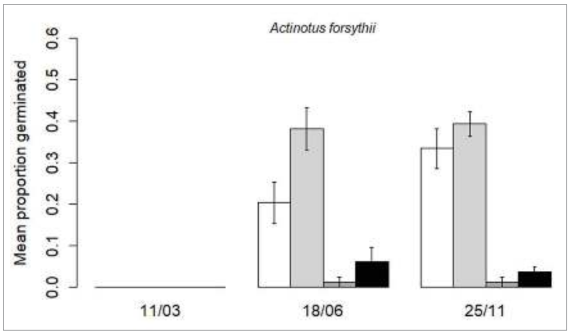

Figure 3. Final *Actinotus forsythii* seed germination after 50 days at the three different seasonal temperatures. Treatments: Control (□), Smoke ( ), Dark ( ), Dark and Smoke (■). Error bars are standard errors.

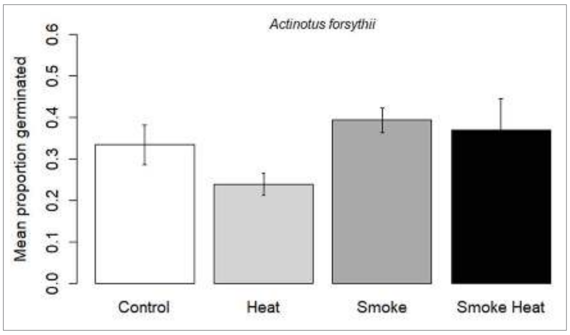

Figure 4. Final *Actinotus forsythii* seed germination after 50 days at temperature 25/11ºC with factorial applications of heat (80°C) and smoke.

Conclusion

Specific seasonal temperatures are required for the Pink Flannel Flower, with germination strongly correlated with warmer seasons. Similar germination in spring/autumn and summer temperature regimes indicated that the species may have adapted to avoid emergence during very cold winter temperatures typical of higher elevations. *Actinotus forsythii* had a strong germination requirement for smoke, indicating that it would emerge after fire in the field, but no response to heat shock. Dark treatments also had a very strong inhibiting effect on germination, suggesting that seeds need to be near the surface where light can reach to ensure germination. This also has implications for cultivation of the species, in which germination in light conditions is recommended before sowing seeds. An unexpected result was that a relatively high overall proportion of seeds germinated, which may reflect that seeds were sourced from regularly watered cultivated plants, as seeds produced in harsher conditions in the wild tend to have deeper dormancy (Paroissien, Porter & Ooi, unpublished data).

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Rapid assessment of future habitat suitability: a case-study of the Snowy Mountains endemic alpine flora using the Biodiversity and Climate Change Virtual Library (BCCVL)

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Big ecological data and climate change

Recent years have seen an exponential increase in the amount and quality of ecological and climatic data freely available online. Ongoing digitisation of herbarium specimens provides a rich source of vouchered and verified botanical information spanning hundreds of years. Similarly, citizen science platforms such as iNaturalist and the Atlas of Living Australia (ALA) allow users to upload species occurrences in real-time via a smartphone app. This source of information is more prone to error but orders of magnitude greater in volume, with tens of millions of occurrence records generated in the past few years alone. Big data from these complementary sources can be used to understand complex ecological processes, including species range shifts, biotic interactions and responses to the changing abiotic environment. In response to the availability of ecological data, the tools to understand these processes are becoming increasingly sophisticated and user-friendly.

The cloud-based Biodiversity and Climate Change Virtual Library (BCCVL) is part of the ALA network that allows users of all skill levels to conduct sophisticated species distribution modelling and secondary experiments such as climate change projections within short timeframes (Hallgren *et al.* 2015). Species occurrences can be imported directly from the ALA data repository or cleaned within the ALA spatial portal and exported into the BCCVL. Educational tools are provided such that users can make informed decisions about algorithm selection and