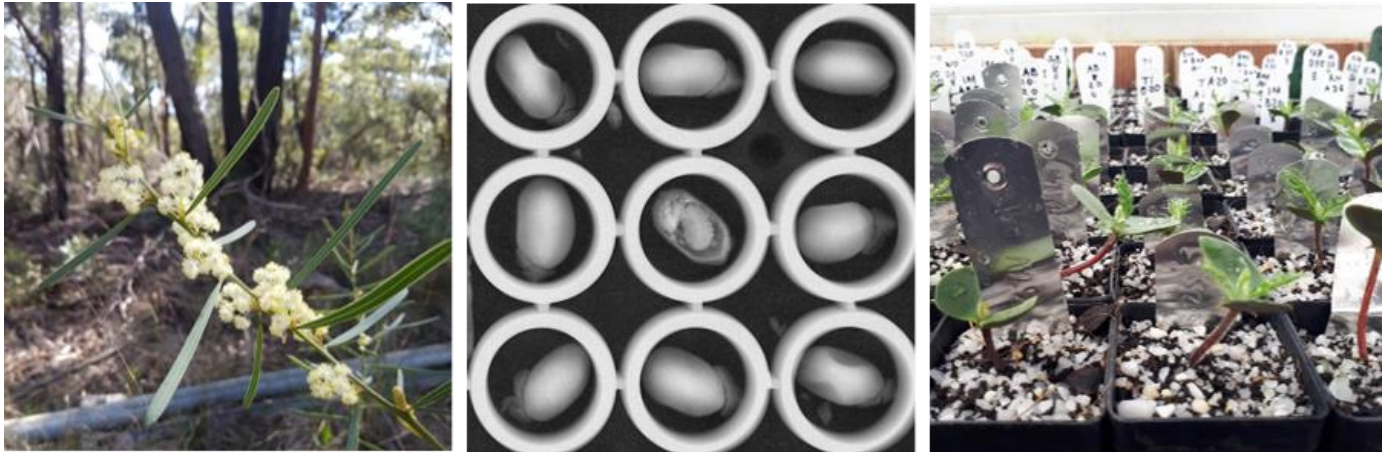# Local adaptation to climate in Sydney sandstone plant species

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This thesis was submitted in partial fulfilment of the requirements for the degree of Masters of Research

Submission date: 15th October 2018

DECLARATION

I confirm that this thesis entitled *Local adaptation to climate in Sydney sandstone plant species* was carried out by me for the Degree of Master of Research under the supervision of Dr Rachael Gallagher, Department of Biological Sciences, Macquarie University. I certify that the material of this thesis has not been previously submitted as part of the requirements for a higher degree to any other university or institution.

With the exception of Figure 9 which was used with permission from the author, this thesis contains no material previously published or written by any other person. I certify that all information sources and literature used are indicated in the thesis.

All other research described in this report is my own original work.

Thomas Pyne

Note to examiners

This thesis is written in the form of a journal article for *Oecologia*. The majority of the author guidelines have been followed, except for minor deviations detailed here and where the guidelines clash with Macquarie University thesis formatting requirements. For example, extending the page limit as per Macquarie preparation guidelines. All figures and tables have been presented at the appropriate places in the text to enhance readability.

ACKNOWLEDGMENTS

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GENERAL ABSTRACT

Determining the extent and effect of local adaptation on survival is central to understanding plant responses to the abiotic environment. Understanding species capacity to respond to rapid, human-driven changes to climate is essential for conservation planning and ecological restoration. For instance, the presence of local adaptation also influences decisions about the sourcing of seed for revegetation programs. Here, I examine whether germination and establishment of two plant species (*Acacia suaveolens* and *Banksia serrata*) show evidence of local adaptation to temperature using germination trials and common garden experiments. Experimental populations were sourced from cold and warm margins of a temperature gradient between the Blue Mountains and Central Coast, New South Wales. We hypothesise higher fitness for individuals grown in 'home' conditions relative to 'foreign' (e.g higher seedling growth and survival). We found contrasting evidence of local adaptation to local temperature across the range of traits measured in each species. Growth-chamber trials showed *B. serrata* radicle growth and time till germination were higher at temperatures closer to those experienced by populations in the field, whereas common garden trials at both ends of the temperature gradient showed little to no evidence of local adaptation in the early establishment phase of either species. We discuss the implications of these results for climate change adaptation and ecological restoration projects.

INTRODUCTION

At broad geographic scales, climate is a key determinant of species distributions which influences the establishment, growth and reproduction of plants (Woodward and Williams 1987). Adaptation to local conditions (precipitation, temperature) has the potential to shape the fitness of plant populations and, ultimately, limit species ranges (Hargreaves et al. 2015; Kreyling et al. 2014; Moran et al. 2017). However, climatic conditions – in particular temperature – are undergoing substantial directional changes as a result of human interventions in the biosphere with important consequences for biological systems (Chen et al. 2011; Parmesan 2006; Parmesan and Hanley 2015). For instance, in the context of localised population adaptation to temperature, climate change may shift 'home' environments toward more 'foreign' conditions and impact on population viability and migration (Valladares et al. 2014). Populations on the leading edge of the migration front are predicted to persist if they can disperse at the same rate as climate change (Gibson et al. 2009). By contrast, trailing edge populations have been argued to be more at risk from extinction due to habitat deterioration especially if they are unable to adapt (Aitken et al. 2008; Parmesan et al. 1999; Sheth and Angert 2016). Understanding the impact of temperature change on populations distributed across strong climatic gradients provides important context for the impact of global change on natural systems and informs applied ecological activities, such as conservation management and restoration (e.g. selecting between local vs non-local seed for restoration).

Plants are sessile organisms during their growth phase, but have mobile dispersal stages which facilitate the establishment of populations distant from mother-plants (Pakeman 2001). Dispersal distances, however, may not be sufficiently large to allow plants to track suitable conditions under future climates. Plants must rely on dispersal and germination of seed at an equal or greater pace than climate change to maintain viable populations (Renton et al. 2013). For some species, suitable climate conditions may not exist under future climates which may limit their response (Wiens 2016). Global average temperatures have increased by 1°C since pre-industrial periods (1851-1880) (Jianjun et al. 2018) and are projected to continue to increase between 1.4 – 5.8 °C by 2100 (IPCC 2014). In Australia, average air surface temperature has increased by 0.9 °C since 1910 and modelled projections of temperature increase for 2100 of between 0.6 –

54 1.7 °C for conservative scenarios (RCP2.6) and 2.8 – 5.1 °C for extreme though likely

55 scenarios (RCP8.5). Understanding how increases in temperature may affect the

56 recruitment phase of plant life cycles (i.e. germination and establishment) will increase

57 our capacity to predict demographic responses to climate change.

58 In this study, we explore the effects of temperature on the establishment phase of

59 two widespread Sydney sandstone species: *Acacia suaveolens* (Sm. Willd.) (family:

60 Fabaceae) and *Banksia serrata* (L.f) (family: Proteaceae). We approach this question by

61 comparing the performance of multiple populations of these species sourced from two

62 contrasting climatic regimes – cold conditions in the Blue Mountains west of Sydney,

63 New South Wales (NSW) and warm conditions on the coastal fringe to their east. We

64 test hypotheses about local adaptation to climate conditions and restoration potential

65 using a mixture of experimental techniques. These techniques include germination

66 temperature assays to determine optimum germination conditions in contrasting

67 provenances and common garden plantings to explore the effect of 'home' and 'foreign'

68 environments on warm- and cold-sourced provenances. The intention of the thesis is to

69 explore the effect of a key driver of global vegetation change – temperature warming – on

70 the establishment of common species in the flora of NSW.

71

Adaptation of populations to local climate conditions

73 Local adaptation may have important implications for the ability of plant species to

74 adapt to rapid anthropogenic climate change (Franks et al. 2014). Heterogeneous

75 environments can produce adaptations in organisms which can enhance, or hinder,

76 success in changing environments (Cook and Johnson 1968; Oyama et al. 2018). For

77 instance, Etterson (2004) and Kim and Donohue (2013) show evidence that fitness

78 declines in locally adapted populations exposed to changed aridity and temperature

79 conditions. Plant species exhibit signals of local adaptation in their morphology,

80 phenology and physiology which aid in their successful establishment, growth and

81 reproduction. The term local adaptation refers to both the processes involved in

82 adaptation to conditions (e.g. the acquisition and maintenance of advantageous alleles)

83 as well as the observed patterns which result (e.g. increased average levels of fitness in

84 'home' conditions compared to 'foreign') (Whitlock 2015).

An important mechanism for local adaptation is the increase of beneficial alleles, or the purging of maladapted ones (Bourne et al. 2014). Gene flow of these alleles between populations plays a complex role in shaping local adaptation. For example, local adaptation can be assisted in a population with restricted gene-flow (e.g. via population isolation-by-distance). Low gene flow allows for selective pressures to fortify beneficial allele frequency, aiding in local adaptation (Günther and Coop 2013). However, restricted gene flow can also result in inbreeding depression which reduces levels of local adaptation (Leimu and Fischer 2008). Unrestricted gene flow can limit population differentiation retarding local adaptation (Brancalion et al. 2018). While gene flow of negative traits can result in maladaptation where incoming deleterious alleles are not purged by selection (Moran et al. 2017) it has been argued that intermediate levels of gene flow can result in maximal local adaptation (Blanquart and Gandon 2011; Gandon 2002).

Local adaptation is often measured via transplant and common garden experiments involving populations sourced across strong environmental gradients. The first formal transplants of this kind were carried out in the 1920s (Turesson 1922). Transplant experiments typically involve growing individuals in plots where conditions are similar ('home') or dissimilar ('foreign') to their collection location. Populations are considered locally adapted where home populations have higher average fitness than do foreign populations (Kawecki and Ebert 2004) or when fitness differences occur in populations planted in home conditions compared to away conditions (Blanquart et al. 2013). For instance, a reciprocal transplant study of local adaptation (Joshi et al. 2001), found differential plant performance of germinated seeds of a forb and two grass species in home and foreign sites. Seeds germinated at home locations had, on average, a 34% increase in tillering, 20% more inflorescences, and were 44% larger compared to foreign sites (Joshi et al. 2001). This local advantage underpinned significantly higher survival rates (+9.3%) of home populations; consistent with other studies indicating evidence of local adaptation from reciprocal transplant studies (Brancalion et al. 2018; McDonough MacKenzie et al. 2018; Schmidt et al. 2018) but see (Pahl et al. 2013; VanWallendael et al. 2018) for studies showing no evidence of local adaptation in plants. The presence of a home-site advantage in these studies may reflect population level genetic differentiation caused by local adaptation.

Local adaptation plays an important role in regulating successful recruitment and maintenance of plant populations (Blanquart et al. 2013; Levene 1953). Practices for the regeneration of degraded habitat are grounded in ideas around the prevalence and importance of local adaptation. For instance in the adherence to the 'local is best' paradigm – the practice of using highly localised seed banks to revegetate degraded sites (Giencke et al. ; Török et al. 2018). However, the long-term success of regeneration projects is contingent on the impact of changing climates on population viability (Cochrane et al. 2014; Hancock and Hughes 2014). Experimental evidence about the importance of using non-local provenances to restore populations shows that there may be a case for selecting seed from non-local sources, multiple sources, from populations with anticipated future suitable conditions, or based on the abiotic conditions at the restoration site (Bischoff et al. 2006; Kramer and Havens 2009; O'Brien and Krauss 2010; Whalley et al. 2013). For instance, Broadhurst et al. (2008) advocate using non-local seed to reduce overharvesting while Hoffmann et al. (2017) suggests non-local seed to maximise genetic diversity. The use of non-local provenances in revegetation may be viewed as a modest form of assisted migration at the genetic level as it may be possible to future-proof habitats against climate warming when seed is sourced from warmer areas of a species distribution (Castellanos-Acuña et al. 2015; Gallagher et al. 2015). Although, there are arguments against the risk:reward ratio of assisted migration, including the potential for invasiveness of translocated species (Reichard et al. 2012) and disturbances to locally adapted genotypes (Weeks et al. 2011).

Plant establishment: a key phase in determining species response to climate change

A seed consists of an embryonic plant, supplied with energy from the endosperm (Shearer et al. 2004) and a protective outer shell (testa), often with appendages to aid dispersal. For example, the protein and lipid-rich elaiosome attached to some *Acacia* seeds promotes ant dispersal (myrmecochory; Beaumont et al. 2018; Edwards et al. 2006), while winged structures on some proteaceous seeds, like those in the genus *Banksia*, can assist with wind dispersal (Fitzpatrick et al. 2008). These structures assist in dispersal of an embryo in the period between seed maturation and seedling establishment (Koornneef et al. 2002). Germination may also only occur under specific environmental conditions and disturbances (Moreira and Pausas 2012). For example, some *Acacia* and

Banksia species are adapted to germinate post-fire and this type of physical dormancy is common in habitats where fire recurs cyclically (e.g. Mediterranean ecosystems (Moreira and Pausas 2012; Ooi et al. 2014).

Plants employ a wide range of strategies to mitigate against establishment failure, which are often cued by climatic or edaphic signals. For instance, temperature is a significant factor determining the rates of seed germination (Cochrane et al. 2014) and this factor also varies along environmental gradients. Seed dormancy increases seedling survival by offsetting germination to avoid unfavourable climate conditions, competition or predation (Tozer and Ooi 2014). Specific seed germination requirements and dormancy act as critical influences during the establishment phase of new generations (Willis et al. 2014). Seed dormancy is influenced by surrounding climate as well as physical conditions such as light levels, temperature and water availability (Koornneef et al. 2002). It is therefore possible that seed dormancy in natural populations will be affected by warming due to anthropogenic climate change (Ooi 2012; Walck et al. 2011).

The optimum temperature for seed germination varies between species and is described by the temperature associated with the highest successful germination (Cochrane et al. 2014; Roberts 1988; Roché et al. 1997). Shifts away from this temperature optima can result in decreases in germination rate and the steepness of this slope may indicate species susceptibility to temperature shifts under climate change (Cochrane et al. 2014; Currey et al. 2015; Ellis et al. 1986; Geo 1921; Giménez-Benavides et al. 2005). Likewise, temperatures moving away from optimum can fail to break seed dormancy (Ooi et al. 2014; Thompson and Ooi 2010). Determining how species and populations respond to changing conditions away from their temperature optimum is an important step in determining their future persistence. For instance, populations of species spread across strong environmental gradients of temperature may exhibit differences in their germination optima, as has been previously seen in *Alliaria petiolata* (Blossey et al. 2017), *Cardamine hirsuta* (Kudoh et al. 2007) and *Ambrosia artemisiifolia* (Leiblein-Wild et al. 2014). By contrast, Alba et al. (2016) showed that local populations of *Verbascum thapsus* from warm regions germinated at a higher rate under cold conditions relative to those collected from cold regions. This counterintuitive result may arise from a cold avoidance strategy present only in cold populations. A 'cautious' germination strategy of this kind can arrest germination, potentially to reduce the danger

181   of frost damage to young emergent seedlings (Erfmeier and Bruelheide 2005; Hierro et

182   al. 2009).

183   **Study aims**

184   The two specific objectives of this study are to:

185

186   (1)   Characterise the germination niche of *Acacia suaveolens* and *Banksia*

187   *serrata* seed sourced from populations occupying contrasting temperatures

188   (cold/mountain and warm/coast). We test the hypothesis that seed

189   germination behaviour (germination rate, radicle growth, time to germination)

190   will differ between cold and warm adapted populations, showing evidence for

191   local adaptation to temperature.

192   (2)   Quantify survival and growth of seedlings from contrasting temperature

193   environments in common garden field trials in 'home' and 'foreign'

194   environments. We test the idea that seedling performance (plant height,

195   survivorship) will be higher where populations are grown in 'home' relative to

196   'foreign' environments. That is, populations sourced from warm

197   environments will outperform those from cold environments when grown in

198   common gardens in coast/warm locations (Centennial Parklands), with a

199   similar pattern for cold populations relative to warm in cold/mountain

200   locations (Mount Tomah).

201

202   With the evidence generated we seek to improve the efficacy of revegetation by showing

203   the effect of provenance in seed-sourcing to restore plant communities (i.e., local vs

204   foreign seed source paradigm) under future climates. To our knowledge, limited

205   information on the effect of local adaptation of populations exists for our two target

206   species.

MATERIALS AND METHODS

Study species

We targeted two species with contrasting life-history characteristics which are common and abundant elements of the Sydney sandstone flora: *Banksia serrata* and *Acacia suaveolens*.

Banksia serrata is a small, single-stemmed tree that can reach a height of 10 m in favourable conditions (Bradstock 1990). Individuals are typically long-lived (~100 years) and can resprout after fire from lignotubers or through epicormic shooting following less intense burns (Benson and McDougall 2000). *Banksia serrata* is serotinous – retaining seeds until fire – making seeds of this species easy to harvest year-round (Midgley 2000) and seeds do not require pre-treatment to induce germination, however they are typically only released from follicles in response to fires or heat (Huss et al. 2018). Seeds are dispersed from opened follicles via gravity or by wind and optimal germination temperature has previously been documented to fall within the range 18-24°C (Sonia and Heslehurst 1978). Fire is only likely to be beneficial for adult individuals with seed ready for dispersal; the juvenile phase of *B. serrata*'s life cycle are sensitive to fire due to deficient lignotuber development (Bradstock and Myerscough 1988). Fire tolerance typically occurs after 5 to 7 years of growth, when lignotuber diameter reaches sufficient size (i.e. 4- 5 cm) to survive high intensity fire (Bradstock and Myerscough 1988).

Banksia serrata is native to the coast regions in the east of Australia, with the species range extending from Maryborough, Queensland in the north to Wilsons Promontory, Victoria in the south, with populations also occurring in Tasmania (Fig. 1). Natural populations exist mostly within altitudes of 0-1000 m with annual rainfall of 800-1400 mm (Benson and McDougall 2000). The species is common and locally abundant in vegetation within the Sydney bioregion. Across its range, the area of occupancy of *B. serrata* (area covered by the number of 2 x 2 km grid cells occupied by records in the *Atlas of Living Australia;* www.ala.org.au) is 11,540 km^2. The area of a minimum convex polygon enclosing all occurrences (Extent of Occurrence; EOO) is 1,381,130 km^2. *Banksia serrata* is a scrubland and low woodland species primarily adapted for growth in sand-rich soils but can also be found in a stunted form in coastal heathlands or shallow rocky soils (Bradstock 1990). *Banksia serrata* is a characteristic

species of Eastern Suburbs Banksia Scrub (ESBS) - a Critically Endangered Ecological

Community under the NSW *Biodiversity Conservation Act 2016*.

Figure 1. Distribution of *Banksia serrata* in Australia. Black circles indicate the location of occurrence records associated with preserved specimens digitised for the *Australian Virtual Herbarium* http://avh.chah.org.au/

Acacia suaveolens is a short-lived, nitrogen fixing shrub with individuals rarely living longer than 15 years (Benson and McDougall 1996). The species reaches a maximum height of 3 m and reproductive maturity is often reached within 2-3 years (Benson and McDougall 1996). *Acacia suaveolens* is easily identified in the field from its mucronate phyllodes which are sometimes covered in a fine white powder. The species is heterophyllous, where bipinnately compound 'true leaves' are replaced by phyllodes.

Flowering in *A. suaveolens* occurs from April-September (Plantnet; www.plantnet.rbgsyd.nsw.gov.au; though can be noted at other times in response to interannual variability in climate and local conditions), and, unlike *B. serrata*, plants do not retain their seed within the pod (Auld 1986). Seed is typically mature in early summer and is dropped from the pod where it enters the soil seed bank awaiting fire to cue germination (Auld and Myerscough 1986). Previous work by Auld and Myerscough (1986) identified optimal temperature to break *A. suaveolens* seed dormancy at between 60-80 °C with low intensity and low duration fires resulting in reduced seed germination. Acacia seeds often have physical dormancy that needs to be treated to initiate germination (Palmer et al. 2018).

262   Recruitment of seedlings occurs from the seed bank following fire and

263   germination usually occurs 1-2 months post-fire (Auld and Myerscough 1986). *Acacia*

264   *suaveolens* is fire-sensitive (i.e. killed by fire) resulting in cohorts of similar age forming

265   populations (Warton and Wardle 2003). This is in direct contrast to *B. serrata* where in

266   some cases adults can survive fire whilst also releasing seed. Low intensity fires can

267   cause serotinous seed to be released and germinate such that both juvenile and adult

268   plants can be found coexisting in the same population (Bradstock 1990).

269   *Acacia suaveolens* is common and locally abundant in the Sydney Basin Bioregion,

270   but populations are predominately located in coast districts (Plantnet;

271   www.plantnet.rbgsyd.nsw.gov.au) . The species range extends from Bundaberg,

272   Queensland in the north to Wilsons Promontory Victoria in the south, with populations

273   also occurring in Tasmania (Fig. 2). It occurs within altitudes of 0-1000 m with an

274   annual rainfall of 700-1800 mm (Benson and McDougall 1996). The species favours well

275   drained, coastal soils such as those associated with Hawkesbury Sandstone substrates

276   and sand dunes which are low in nutrients, but can persist in heavier soils (Benson and

277   McDougall 1996). *Acacia suaveolens* is mildly tolerant of salt and has been documented

278   surviving frost conditions down to -7 °C. Across its range, the area of occupancy of *A.*

279   *suaveolens* (area covered by the number of 2 x 2 km grid cells occupied by records in the

280   *Atlas of Living Australia,* www.ala.org.au) is 12,096 km^2. The area of a minimum convex

281   polygon enclosing all occurrences (Extent of Occurrence; EOO) is 1,656,410 km^2.

282   *Acacia suaveolens* seeds provide a food source for a range of invertebrate larvae,

283   including *Megastigmus* sp. and *Melanterious corosus* (Coleoptera: Curculionidae) where

284   eggs are laid inside seeds, as well as from post-dispersal seed grazers (Auld and

285   Myerscough 1986). Biotic interactions of this kind result in significant losses in seed

286   production. Myrmecochory in *A. suaveolens* increases the distance of dispersal in which

287   ants can manipulate the seed via the elaiosome (aril) and move it away from the mother

288   plant (Auld and Myerscough 1986). Ants in the genus *Pheidole* (Hymenoptera:

289   Formicidae) carry dropped seed short distances to underground nests but only the aril is

290   consumed, aiding in the dispersal of seeds (Auld and Myerscough 1986). Flowers

291   provide a source of pollen for native and exotic bees and a source of nectar for birds

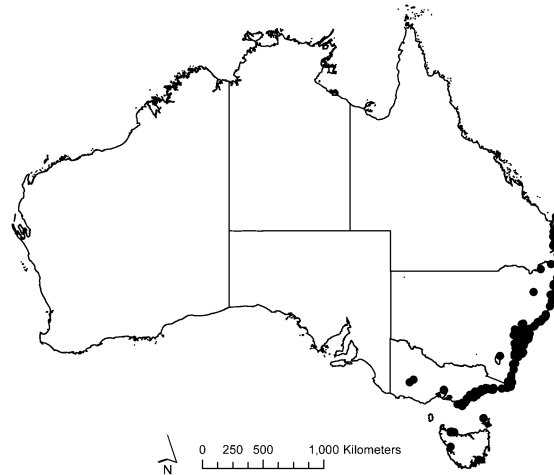292   (Benson and McDougall 1996).

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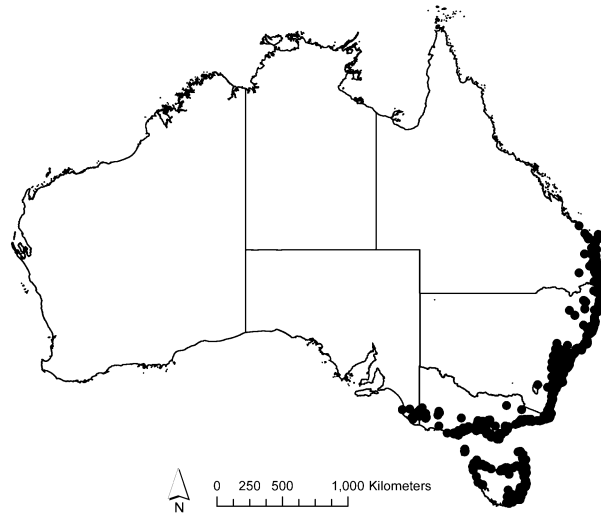
Figure 2. Distribution of *Acacia suaveolens* in Australia. Black circles indicate the location
of occurrence records associated with preserved specimens digitised for the *Australian*
Virtual Herbarium http://avh.chah.org.au/

297

Sampling of seed populations

Seeds for all experimental work were sourced from natural populations in two distinct
climatic and altitudinal zones in NSW: Coast (Central Coast/Royal National Park) and
mountain (Blue Mountains National Park) (Table 1; Fig. 3). These zones were chosen to
maximise variation in the long-term average temperature conditions to which species
may be adapted. For example, across zones, mean annual temperature and frost
exposure declines in a westerly direction from 13 °C/102 days per year at Mount Wilson
in the Blue Mountains to 17.7 °C/0 days per year at Patonga on the coast, largely due to
increasing altitude (Table 1; Bureau of Meteorology (BOM) (www.bom.gov.au). Soil
type and texture was broadly consistent between collection locations (quartz-rich
sedimentary derived from Hawkesbury sandstone) and mean annual precipitation ranges
between 1072-1218 mm. *Banksia serrata* seed was collected from Newnes Plateau (Table
1) however this population failed to germinate so was replaced with seed from Mount
Hay at a later date.

Seed collection sites are part of a long-term transect study – the Biological
Adaptation Transect Study (BATS) – which is part of the Australian Transect Network
coordinated by the Terrestrial Ecosystem Research Network (TERN). The BATS is a
collection of 36 permanent 50 m × 50 m plots managed by the National Herbarium of
NSW. Both *A. suaveolens* and *B. serrata* are common and abundant across the BATS and

16

317 the research presented in this thesis complements a range of other genetic and ecological

318 studies being undertaken on common species on the BATS.

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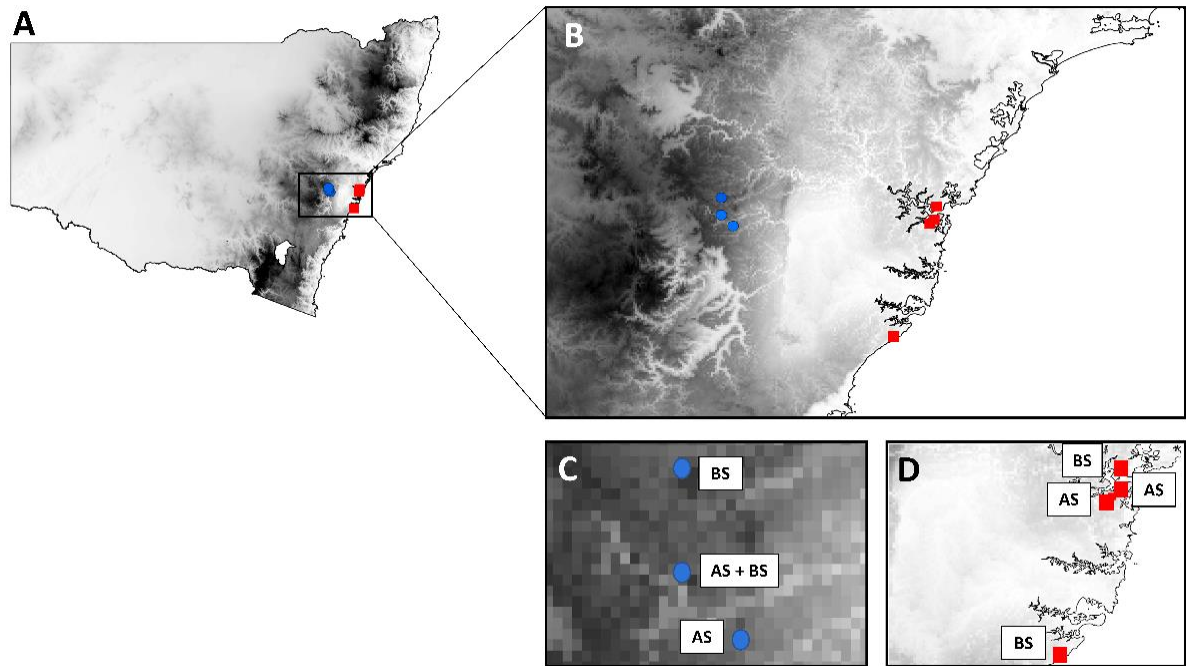
322 **Figure 3.** Seed collection sites in NSW, Australia for populations of *Acacia suaveolens* and
323 *Banksia serrata* used in experimental treatments in NSW (A; B) showing cold (C) and
324 warm (D) sampling sites. Seeds were collected for each species from two populations in
325 each of the two climatic zones: mountain (blue circles) and coast (red squares).
326 Mountain populations were collected at Mount Wilson, Mount Banks and Mount Hay
327 (top to bottom circles in C). Coast populations were collected at Patonga, West Head
328 and Ku-ring-gai (top to bottom circles in D). AS =*Acacia suaveolens* populations; BS =
329 *Banksia serrata* populations. Climatic and altitudinal differences between coast and
330 mountain zones are detailed in Table 1.

331   **Table 1. Seed collection sites for populations of *Acacia suaveolens* and *Banksia serrata*† used in experimental treatments.**

Site	Species collected	Zone	Location (latitude, longitude)	Altitude (m)	Mean annual temperature (°C)	Mean annual rainfall (mm)	Mean minimum temperature coldest month (°C)	Frost exposure (mean days/year)
Wattamolla, Royal National Park	*Banksia*	Coast	-34.138, 151.113	34	17.7	1086	7.7	0.1
Patonga, Brisbane Water National Park	*Banksia*	Coast	-33.538, 151.283	162	17.6	1185	6.7	0.6
Ku-Ring Gai Chase National Park	*Acacia*	Coast	-33.621, 151.250	157	17.5	1198	6.9	0.6
West Head, Ku-Ring Gai Chase National Park	*Acacia*	Coast	-33.600, 151.274	139	17.6	1199	7.0	0.6
Mount Hay, Blue Mountains National Park	*Banksia*	Mountain	-33.627, 150.409	727	13.5	1218	2.5	47
Mount Banks, Blue Mountains National Park	*Banksia, Acacia*	Mountain	-33.579, 150.365	838	13.6	1075	2.4	49
Mount Wilson, Blue Mountains National Park	*Acacia*	Mountain	-33.498, 150.364	904	13.0	1072	2.0	52
Newnes Plateau, Wollemi National Park	*Banksia*	Mountain	-33.43887 150.23003	1131	11.1	1054	-1.1	86

332   †Seeds for each species were collected from two natural populations of each species in each climatic cline (coast, mountain). Altitude was
333   derived from the Shuttle Radar Topography Mission (SRTM) 1km product (https://lta.cr.usgs.gov/SRTM1Arc) and all climate averages
334   were calculated from daily climate data in the CSIRO Australian Water Availability Project dataset (http://www.csiro.au/awap/) for
335   reference period 1990-2009. Frost exposure was derived from the BOM *Annual and monthly potential frost days* gridded dataset
336   (http://www.bom.gov.au/jsp/ncc/climate_averages/frost/index.jsp?period=an&thold=lt2deg#maps). Herbarium specimens
337   accompanying collections are lodged with the *National Herbarium of NSW*.

338 Seed was only sampled from individuals that were visually assessed as healthy and free
339 from disease and no more than 10% of seed was taken from a single individual. *Banksia*
340 *serrata* seed was collected over a two-month period between July and August 2017 by
341 staff at the National Herbarium of NSW. The serotinous nature of *B. serrata* allows for
342 seed collecting at any time of the year. This feature was important for the experimental
343 design, because a population originally collected from Newnes Plateau failed to
344 germinate (see **Results**) and was easily replaced with seed from populations at Mount
345 Hay. Banksia seed was collected from 8-10 individuals with 3 infructescences being
346 sampled at each. Infructescences were selected randomly from healthy ones one trees.
347 This means that seed may have been from a range of ages and maternal conditions.

348   *Acacia suaveolens* seeds were collected between December 2017 and January 2018
349 by staff at the National Herbarium of NSW. Seed from *A. suaveolens* was sampled from at
350 least ten individuals at each site. Infructescences were placed into drying rooms at the
351 Australian Botanic Gardens, Sydney at 40 °C for ten days to reduce the spread of
352 pathogens and invertebrates.

353

354 **Germination experiment**

355 *Seed preparation and seed mass measurements*

356 We opened *Banksia serrata* follicles by placing infructescences in a convection oven at 160
357 °C for 30 minutes (Ecocell LSIS-B2V / EC 55) and seeds were removed with forceps.
358 Undeveloped seeds were discarded. Seeds were counted and weighed and the average
359 seed weight was calculated per individual infructescence. *Acacia suaveolens* seed from
360 each individual was removed from pods and separated from other plant material. Ten
361 seeds from each location were randomly selected and weighed. For both species, only
362 seeds which were within 1.5 mg of the interquartile range of its population weight were
363 retained for the germination experiments. This reduced the influence of differences in
364 maternal provisioning on the outcomes of germination trials.

365

366 *Seed viability via predation assessment using X-ray analysis*

367   *Acacia* seed infested with invertebrates would result in an incorrect assessment of

368   germination rate as predated seed would not germinate. X-ray radiographs of seed can

identify infested and non-infested seed, as well as any other defects that would make seed unviable. *Banksia* seed morphology meant that an infestation assessment by eye could be achieved and X-raying was not required.

X-ray radiographs of *A. suaveolens* seed were acquired using a Faxitron X-ray MX-20 Specimen Radiography System. The Faxitron system was attached to an integrated built in workstation that captured, digitized and produced real time X-ray images. 42 seeds per image were placed into 10 mm wells and positioned in X-ray cabinet and 30 cm from the emission source. X-ray images were developed using a 10-35 kV potential at a 300 µA current. DX 1.0 Software with ImageAssist and Automatic Exposure Control automatically selected the correct kV potential and exposure time as well as capturing and producing the radiographs. An inbuilt 19" workstation monitor was used to compare infested to non-infested seed (Fig. 4).

We used an established method of measuring infestation of wheat seeds (Haff and Slaughter 2004) as our basis for quantifying infestation levels in *Acacia* seeds. We used two distinctions of seed condition: predated or un-infested. Predated was any of the conditions described in Fig. 5 apart from un-infested. Any seeds identified to be in an infested condition were discarded. Measurements of embryo size can be used to identify germination vigour, with smaller visibly damaged embryos having lower chance of germination (Sawarkar 2017). Seeds with highly reduced embryos were also discarded.
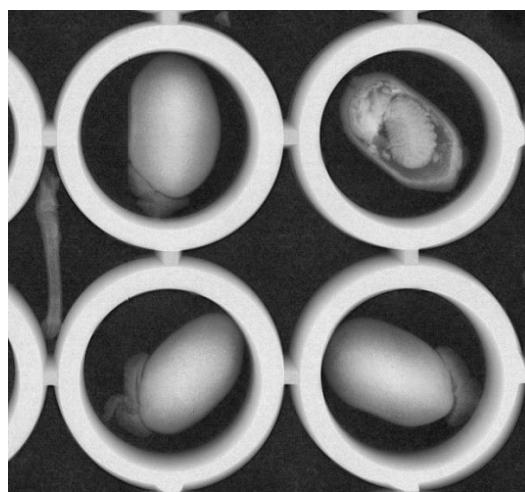
Figure 4. Photographic X-ray image of *Acacia suaveolens* seeds showing three un-infested seeds (upper left and bottom left and right) and one infested seed (top right). The invertebrate (possibly *Megastigmus* sp.) appears to be in a pupa-adult stage.

| Egg | Instar 1 | Instar 2 | Instar 3 | Instar 4 | PrePupa | Pupa | Adult | Vacant | Uninfested |
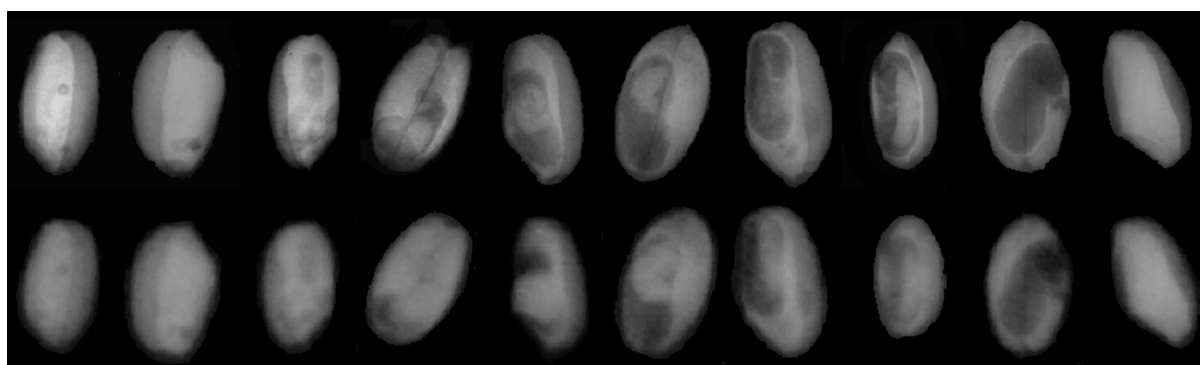
Figure 5. Standard predation of seeds used by Haff and Slaughter (2004) showing different levels of invertebrate stage in host seed. Image used with permission from (Haff and Slaughter 2004).

Treatments of seed prior to germination

Banksia serrata seeds were surface-sterilized using a modified method from Doan and Davis (2015). Seeds were placed inside tea-strainers, submerged and agitated in 1% bleach for two and a half minutes before being washed in reverse osmosis water and a final wash in autoclaved water. *Acacia suaveolens* was treated with boiling water to initiate germination. The boiling treatment consisted of placing the seeds in a 250 ml sealed glass jar with boiled water for 12 hours.

Germination trials

Potato dextrose agar (PDA) was used as a medium to germinate seeds of both species. Seed from both species was germinated in autoclaved 90 x 15 mm petri dishes filled with 25 ml of medium. PDA was prepared by suspending agar at 8 g per 1 litre of reverse osmosis water spun and heated at 100 °C.

All seeds were germinated across six set temperatures (10, 15, 20, 25, 30, 35 °C) in growth chambers located at the NSW Seed Bank at the Australian Botanic Garden, Mount Annan, New South Wales. Seeds were germinated in two walk-in-growth chambers (set at 20 and 25 °C respectively) and four smaller Thermoline Scientific growth chambers for the remaining four temperatures (10, 15, 30, 35 °C). All growth chambers used had consistent humidity and were kept at a 12-hour day/night photoperiod using a Labec Temperature Cycling Chamber. Although growth chambers

419   are designed to produce consistent conditions plates were randomly shuffled once a week

420   to prevent any blocking effects.

421   Four seed plates were prepared for each combination of germination temperature

422   and species (n = 48 seed plates). To test our hypothesis about local adaptation in

423   germination behaviour in relation to temperature, we randomly assigned an equal

424   number of seeds from cold and warm populations of both species (see Fig. 3) to seed

425   plates being germinated across the range of six temperatures (Fig. 6). Seed plates were

426   split into ten segments using a marker pen, and each position marked according to the

427   population it contained in order to track the fate of seeds during germination trials.

428   Using this technique, seeds at all positions could be traced back to their source

429   population and individual mother plant from field collections. Once seeds were

430   distributed to dextrose plates, lids were sealed using Parafilm (Bacto Laboratories,

431   Sydney) and placed in growth chambers. Petri dishes were rotated and their position

432   swapped in the growth chamber during measurements to minimize shelf effects.

433
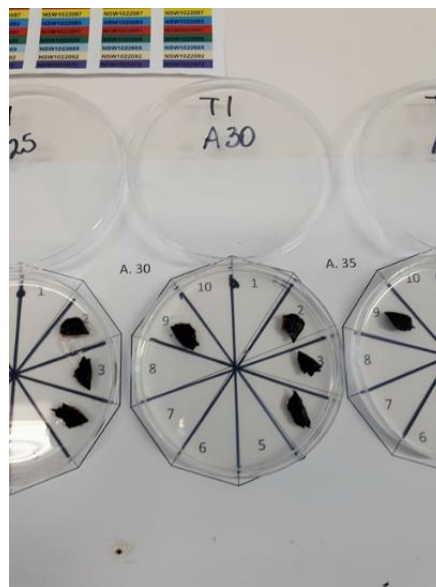
434   **Figure 6.** *Banksia serrata* seeds being prepared for use in germination experiments. Seeds
435   were randomly assigned a position between 1 -10 in segmented petri dishes to track their
436   germination response under six different temperature treatments. Note the empty spaces
437   in plates, as all seed was yet to be placed in the seed plate.

438

439   *Assessing germination behaviour*

440   Germination was scored daily for a period of 37 days for *A. suaveolens* and 42 days for *B.*

441   *serrata* and intermittently (every 4 days) once most seeds had germinated. Successful

germination was defined in this study as the appearance of the radicle from the embryo as suggested by (Müller et al. 2013). Time till germination (days) was calculated for each seed and percent germination (%) was based on the total amount of seeds germinated and un-germinated on the final scoring day of the experiments.

After 28 and 41 days of germinating the *A. suaveolens* and *B. serrata* seeds in plates respectively, germinated seeds were transferred to 50 x 50 x 120 mm forestry tubestocks to be grown before being planted in common gardens. Native plant mix made by the Botanic Garden Nursery team was used in the tubestock. Whilst transferring the seedlings, radicle length was measured. Radicle length (mm) was determined by the root tip to the root collar, indicated by a change from white to green colour. Attention was given as not to include any part of the shoot into the measurement. All seeds were moved to tubestock on the same day to standardize age and therefore radicle length growth period.

Common garden field experiments

Study sites

Seedlings of *B. serrata* and *A. suaveolens* from all mountain and coast populations were transferred to two common garden planting sites across the Sydney region: the Blue Mountains Botanic Gardens at Mount Tomah and Centennial Parklands in Kensington (Table 2).

Mount Tomah is in the upper Blue Mountains, 105 km north of the Sydney CBD at an altitude of 1001m. The vegetation type is Moist Basalt Cap Forest, dominated by *Eucalyptus viminalis, E. blaxlandii* and *E. radiata*. The associated vegetation also includes *E. cypellocarpa, E. oreades, E. fastigata*. Understory species include *Acacia melanoxylon, A. penninervis, Polyscias sambucifolia, Astrotricha floccosa, Daviesia ulicifolia, Leucopogon lanceolatus* and *Cyathea australis*. The soils in this region are part of the Hawkesbury Sandstone soil landscape and border the Mount Tomah soil grouping. Hawkesbury Sandstone soils are generally described as shallow, with high erosion potential and low soil fertility which are highly permeable soils (Office of Environment and Heritage, 2018).

472   **Table 2.** Climate and soil characteristics of plots. Climate data was collated from nearest weather stations. Phytophthora refers to the
473   presence of *Phytophthora cinnamomi* detected by Royal Botanic Gardens Sydney[†]. Soil characteristics analysed at Southern Cross
474   University, Lismore, NSW[‡]. MAT = mean annual temperature, Light density was calculated by measuring nearest area of direct sunlight
475   to sunlight within the plots.

Site	Coordinates (°S,°E)	MAT (°C)	Rainfall (mm)	Frost risk days	Light density (% µmol s-1 m-2)	Spherical Densitometer reading	Phytophthora present	Soluble phosphorus (mg/kg)	Nitrate Nitrogen (mg/kg N)
Mount Tomah Watershed	-33.534979, 150.421485	13.1	1122	49	42.8	43.3	Negative	0.4	0.9
Mount Tomah Spurside	-33.533668, 150.421209	13.1	1122	49	39.6	53.8	Negative	0.4	0.9
Centennial Parklands Eva	-33.904584, 151.235516	17.9	1194	1	92.4	0	Negative	4.2	1.6
Centennial Parklands Martin	-33.902973, 151.238366	17.9	1194	1	98.3	0	Negative	1.7	6.4

476   † See Supplementary Material Appendix 1 for Phytophthora results.
477   ‡ See Supplementary Material Appendix 2 for Soil results.

478   Centennial Parklands are a 189 ha multi-purpose greenspace in eastern Sydney.
479 The soil is composite of the traditional Aeolian sand dunes at a range of depths
480 intermixed with bedded clays and peats (NSW Department of Environment and
481 Conservation, 2004). The original Quaternary Hawkesbury sandstone has been
482 significantly modified starting from the 1800's. Over 115 tree species exist within the
483 park including in areas of remnant native vegetation. For instance, remnants of the
484 endangered Eastern Suburbs Banksia Scrub (ESBS) community can be found in
485 Centennial Parklands (Clews 2011). Both *A. suaveolens* and *B. serrata* are listed as
486 characteristic species in ESBS.

487   The ESBS community is a sclerophyllous heath and scrub association which has
488 been reduced in extent from 5,300 to 143 hectares since European colonisation of
489 Australia (NSW). ESBS exists on disjunct Aeolian dune sand in nutrient poor soil, for
490 this reason, a major threat to the ESBS is alteration to nutrient levels. Furthermore,
491 ESBS is likely to be susceptible to *Phytophthora cinnamomi* which has also been described
492 as a Key Threatening Process in NSW. Thus, the remaining fractions of ESBS are of
493 high value to protect, and restoration programs are underway to improve remnants. Low
494 soil phosphate is a determinant of many heath communities, and alterations to nutrient
495 flows has been described as threat to ESBS communities (NSW Department of
496 Environment and Conservation, 2004).

497 *Temperature characteristics of sites*

498 Daily climate data for each common garden was collated from the nearest Bureau of
499 Meteorology (BOM) weather station (Observatory Hill (33.8607 °S, 151.2050 °E) for
500 Centennial Parklands; Katoomba (33.7122°S, 150.3087 °E) for Mount Tomah. We
501 compared daily minimum temperatures encountered by plants across the duration of the
502 common garden trials (20/4/2018 – 29/8/2018) to daily temperatures in the preceding
503 17 years during the same time period (Fig. 7). Sub-zero temperatures were experienced
504 for 5 days in Katoomba and there were 32 days below 2°C – a commonly used threshold
505 for frost formation in the presence of clear sky (Frost Potential; www.bom.gov.au)
506 Observatory Hill had no days with minimum temperatures below 4 °C (Fig. 7B). July 6,
507 2018 (day 80; see Fig.7 asterisk) was the coldest temperature recorded at the Katoomba
508 weather station in the previous 15 years.

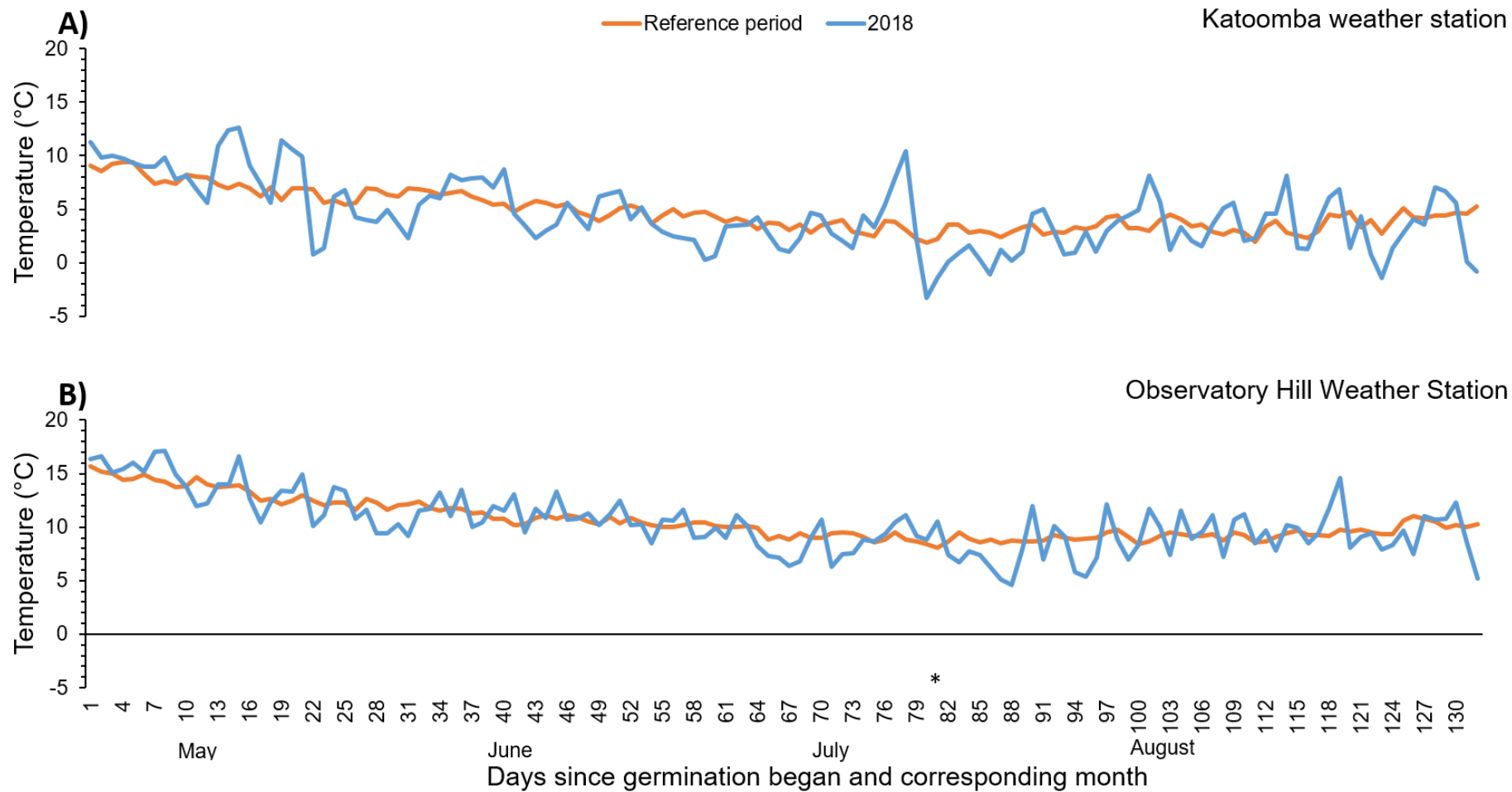**Figure 7.** Minimum temperature data during experimental transplants at two common garden planting sites. Climate data are sourced from daily records at the nearest weather stations to each site: (A) Katoomba = Mount Tomah, (B) Observatory Hill = Centennial Parklands. Orange lines are the minimum temperature recorded during the growth experiment, while blue is the averaged minimum temperature from 2000-2017.

515   *Plot preparation*

516   We prepared two replicate 4 m x 5 m plots at both sites. Plots were positioned

517   approximately 50-100 m apart and a fenced to a height of 1.2 m to exclude mammalian

518   herbivores (e.g. deer, kangaroos, rabbits). At Mount Tomah, three rings of high-tension

519   wire were wound laterally across the top half of the fence to deter kangaroos from

520   jumping into plots. To discourage rabbits from entering the plots at Centennial

521   Parklands, chicken wire was attached to the fence and the base of the fence was bent

522   outward forming an L-shape to prevent digging under the fence. Where necessary, plots

523   were cleared of weeds using a fixed flail mulcher, brush cutter, hand mower, and hand

524   weeding.

525   We measured canopy light penetration in each plot using a LI-COR LI-250A

526   Light Meter. Six measurements were taken in both the fully open canopy in surrounding

527   vegetation and in each plot. Each measurement was taken using the 15-second averaging

528   mode and a 30 second wait between measurements. Measurements were averaged and

529   full canopy light was divided by plot light levels to give a proportion of light penetration

530   in plots (Table 2). Overstory density was measured using a Forestry Suppliers Spherical

531   Crown Densiometer, Convex Model A. Six individual measurements were taken in a

532   grid pattern across each plot and averaged into a single metric of canopy cover.

533   To characterize difference of local soil conditions between replicate plots at each

534   site, a full soil analysis was performed (Environmental Analysis Laboratory, Southern

535   Cross University, Lismore, NSW). Ten 2.5 cm diameter soil cores were taken from a

536   depth of 10-15 cm within each plot using a grid pattern. The top organic layer and coarse

537   debris were removed before sampling. The following variables were returned form soil

538   analysis: pH and electrical conductivity (1:5 water); Available Ammonium, Nitrate,

539   Sulphur; Exchangeable Sodium, Potassium, Calcium, Magnesium, Hydrogen,

540   Aluminium, Hydrogen, Cation Exchange Capacity; Bray I and II Phosphorus; Available

541   Micronutrients Zinc, Manganese, Iron, Copper, Boron, Silicon; Basic Colour, Basic

542   Texture (See Supplementary Material 1).

543   We tested for the presence of *Phytophthora cinnamomi* at both sites using

544   diagnostic tests performed by the Plant Pathology Laboratory at the Royal Botanic

545   Gardens, Sydney. *Phytophthora* cinnamomi is an oomycete (water mould) and is a

546   known risk to *B. serrata (Tynan et al. 2002)* and could reduce seedling survival where *A.*

547   *suaveolens* is considered tolerant (Weste 1981). Soil samples were collected between April

548   and May with same day drop-off to the pathology lab to ensure viable samples. Ten

549   samples were randomly chosen at each plot. The top organic matter was removed before

550   collecting a sample to 10-15 cm deep and all soil was mixed into a composite sample.

551   Tools were disinfected with 70% methylated spirits before each new sample (see

552   Supplementary Matirial 2).

Planting design in plots

554   Five replicate seedlings from each combination of species and population were planted

555   into each of the two plots at Mount Tomah and Centennial Parklands in April 2018.

556   Seedlings of each species from different populations were planted randomly in a grid

557   pattern in each plot, ensuring equal spacing for each replicate. An auger and mattock

558   were used to dig holes 20 cm deep and wide. Plants were carefully removed from the

559   forestry tubes to maintain soil around the roots, planted and watered immediately (2 L

560   per plant). Watering was continued at a rate of 1.5 L per plant every four days for two

561   weeks after planting due to anomalously dry conditions during the planting period

562   relative to long-term rainfall averages for April.

Measuring plant growth rate and performance

564   We used measurements of seedling growth to assess differences in performance between

565   coast and mountain populations of *A. suaveolens* and *B. serrata*. Given the short timeframe

566   available for the study we were not able to assess lifetime fitness in these species (i.e.

567   overall fecundity) though these measurements will be made by collaborators at the Royal

568   Botanic Gardens in coming years. We measured three different aspects of growth: plant

569   height (cm), seedling survival (count) and leaf damage (% damage).

570   Plant height was recorded as the distance from cotyledon to highest terminal

571   shoot, which involved measuring the full length of the stem. We use the term growth rate

572   to refer to differences between plant height between the first and last recording date.

573   These differences were divided by the number of days plants were grown in the common

574   garden experiment to provide an overall growth rate. We measured growth four times

575   across the duration of the experiment but used differences between the initial and final

576   plant height as a measure of growth rate.

577

578 *Quantifying leaf damage*

579 On the last measurement day of each plot, leaf damage was visually estimated to the

580 nearest 5% of leaf damage. This was to see if seed origin had any significant difference

581 on leaf damage. Leaf damage was assessed by estimating the total amount of leaf

582 discolouration (yellowing, rusting or browning) compared to healthy leaves. *Acacia*

583 leaves and phyllodes were both measured.

584

585 **Statistical analyses**

586 We used a combination of *t*-tests, analysis of variance (ANOVA) and generalized linear

587 models (GLM) to assess differences in germination and growth behaviour between

588 treatments. Comparisons were made between two regions (coast and mountain) with

589 two populations sampled in each area, however *A. suaveolens* and *B. serrata* were

590 examined separately in all analyses. Data that did not meet the assumptions of

591 parametric tests were $\log_{10}$ transformed. All analyses were performed in R (R Core

592 Team, 2017) using the packages *multcomp (Hothorn* et *al. 2017)* and *mvtnorm* (Genz et al.

593 2018) and were considered significant at α = 0.05.

594 *Analysis of seed germination experiments*

595 Prior to the start of germination experiments the size (mass; mg) and viability (%

596 undamaged *A. suaveolens* seeds) of seeds sampled from all populations was compared

597 using students *t*-test for seed mass and a Test of Equal or Given Proportions to compare

598 viability. This test was used to assess differences in seed condition between populations

599 and regions which may affect the interpretation of experimental results (e.g. maternal

600 provisioning may differ between populations).

601 Type-II two-way factorial ANOVA was used to compare germination behaviour

602 (time to germination (days) and radicle length (mm) in six germination temperatures (10,

603 15, 20, 25, 30, and 35 °C) between coast and mountain regions. A post-hoc Tukey's test

604 was used to determine the nature of group differences between significant terms in the

605 ANOVA. Significant interaction terms were retained in the model and were considered

606 to evidence of local adaptation to germination temperature.

607 A binomial generalized linear (GLM) model was used to determine if region

608 (coast, mountains) and germination temperature interacted during seed germination

609 across the six different temperatures. This was measured by the observed and expected

610 levels of percent germination. This is where the *multcomp* function *glht* (Hothorn et al.

611 2017) was used to compare the group means using a Tukey post-hoc test. An exact

612 binomial test was used to determine the 95% confidence intervals.

613   Note that one mountain population of *B. serrata* (Mount Hay) was delayed due to

614 later sampling. This was the result of an original population having zero germination.

615 Therefore, combining populations would result in lower growth rate in the mountains

616 population, so these were analysed as individual populations. *A. suaveolens* seed from all

617 sampling populations were combined into either mountain or coast.

Analysis of common garden field experiment

619 We compared growth and survival metrics in coast and mountains plots for seedlings

620 translocated from plots of warm or cold origin. Plant height was analysed using a two-

621 way factorial ANOVA. Plant height change was measured by comparing the initial

622 height at the start of the growth period and height at the end. The interaction model

623 analysed if plant height had significantly changed during the growth period; and, if this

624 rate significantly differed in coast or mountain collected seedlings. Plant height increase

625 was tested using two factors: (1) sample day which is either the first or last plant height

626 measurement and (2) seed origin, either mountain or coast. Minimal intra-population

627 variation was found within regions so were pooled as either 'coast' or 'mountain'.

628 Differences in seedling survivorship were analysed using a binomial GLM on the

629 observed and expected proportions of living and dead individuals from each region. We

630 then compared between regions to determine if seedling origin had an effect on

631 survivorship. A Student's *t*-test was used to compare leaf damage means between

632 mountain and coast collected individuals within plots.

633

RESULTS

Pre-experimental assessment of seed quality

Seed mass

637 Seed mass ranged from 4.2-43.4 mg for *A. suaveolens* and 35.5-104.6 mg for *B. serrata*

638 across all sampled populations (Table 3). No significant differences were found between

639   *A. suaveolens* seed mass between regions ($t_{308} = 0.07$, $p = 0.94$; Fig. 8A, Table 3), however

640   post-hoc analysis indicated that populations of *A. suaveolens* had significantly different

641   mean seed mass within regions (mountain: $F_{1,189} = 133$, $p < 0.001$; coast: $F_{1,134} = 6.37$, $p =$

642   0.01; Fig. 8B). There were significant differences in seed mass between mountain and

643   coast populations of *B. serrata* ($T_{74} = 5.02$, $p < 0.001$; Table 3, Fig. 8C; coast: 71.94 ± 2.59

644   mg, mountains: 53.3 ± 1.56 mg). Similarly, the two coast populations significantly

645   varied in mean mass ($F_{1,48} = 13.15$, $p < 0.001$; Fig. 8D). A lack of data for Mount Hay

646   means a within region comparison could not be performed for *B. serrata* in the mountain

647   region.

648
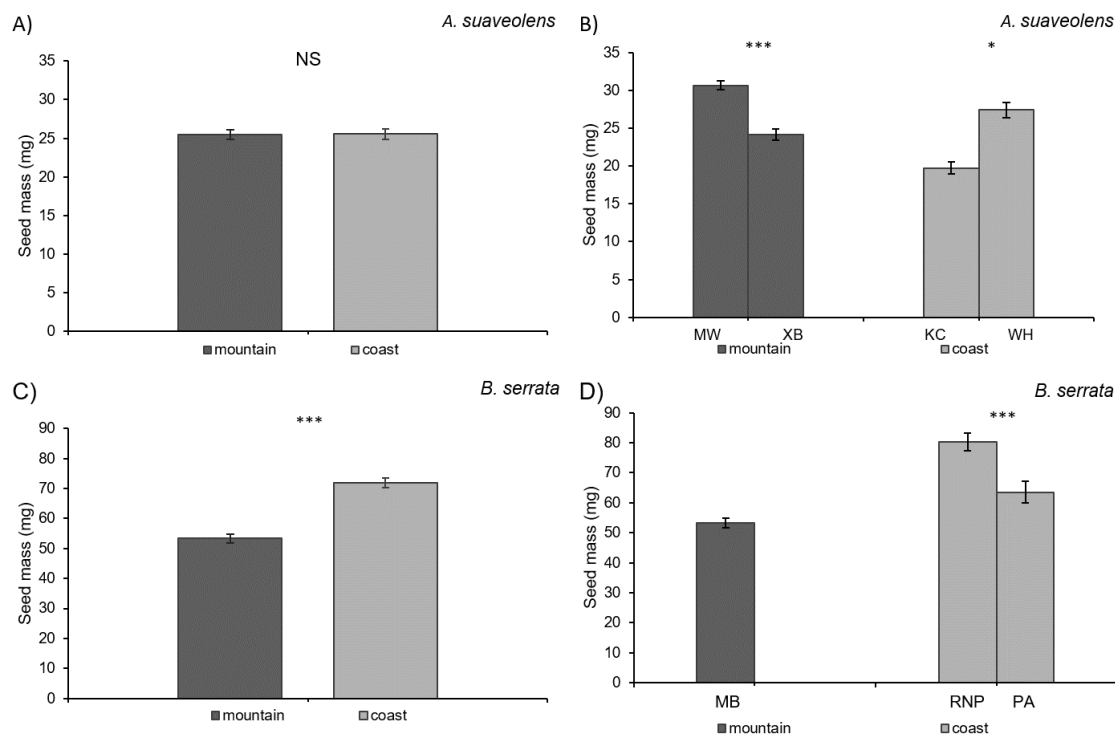
649   **Figure 8.** Comparisons of average seed mass (mg) between populations of *Acacia suaveolens* and
650   *Banksia serrata* sourced from coast and mountain regions in the Sydney area, New South Wales.
651   (A) and (C) depict differences in average seed mass across all sampled populations, and (B) and
652   (D) depict seed mass variation in individual populations collected from cold, mountain (dark grey)
653   and warm, coast (light grey) climates. The significance of differences between the seed rate for
654   intraregional populations was determined by a series of ANOVAs: '***' $p < 0.001$ '**' $p < 0.01$ '*' p
655   >0.05.

Table 3. Seed and germination profile for data collected from mountain (M) and coast (C) populations of two species from New South Wales, Australia. Germination at the centre temperature optimum (20°C) is shown, as well as total germination behaviour across all growth chamber temperatures (10-35°C). 20°C was chosen as a comparison temperature as it is the closest to Sydney's mean annual temperature. This data is a summation of data that is further explored in following results section. Numbers shown in bold are data that are a significant difference between both mountain and coast regions.

Species	Region †	Seed mass (mg; mean ± SE)	Midpoint (20 °C)			All temperatures (10 – 35 °C)		
			Mean time to germination (days)	Germination (%)	Radicle growth rate (mm; ± SE per day)	Mean time to germination (days)	Germination (%)	Radicle growth rate (mm; ± SE per day)
Acacia suaveolens	M	25.47 ± 0.62	**8.29 ± 0.71**	68.75	**1.98 ± 0.3**	**9.08 ± .036**	51.13	2.1 ± 0.19
	C	25.54 ± 0.65	**5.68 ± 0.41**	64.06	**3.75 ± 0.49**	**6.27 ± 0.24**	50	3.35 ± 0.27
Banksia serrata	M	**53.23 ± 1.53**	8.37 ± 0.31	77.55	**3.92 ± 0.41**	13.06 ± 0.71	63.88	3.09 ± 0.18
	C	**71.94 ± 2.59**	8.62 ± 0.32	75.32	**2.27 ± 2.01**	12.7 ± 0.65	52.52	2.34 ± 0.14

† Seed of both species were collected from either end of their climate gradients across New South Wales. Populations were either mountain (M) or coast (C) collected.

33

663 *Seed viability and predation*

664 Significantly higher levels of inviable seed were found in seeds from mountain

665 populations relative to those collected from coast populations ($\chi^2_1 = 68.68$, $p < 0.001$;

666 Fig.9).

A. suaveolens

667
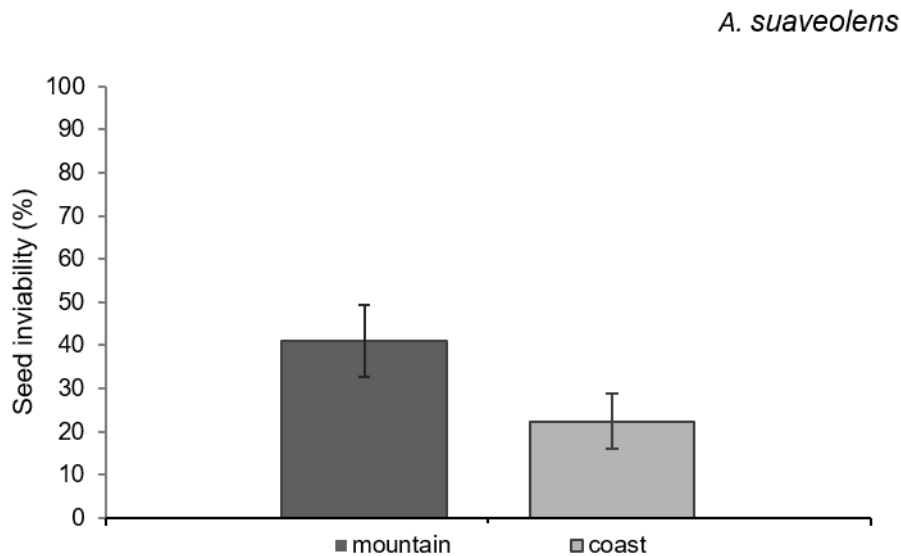
668 **Figure 9.** Mean ($\pm$SE, $n = 2218$) of mountains (dark) and coast (light) collected seed inviability in
669 *Acacia suaveolens.* Seed unviability was measured using a Faxitron X-ray machine.
670

Seed germination experiment

672 Seed germination behaviour (% germination, time to germination (days) and radicle

673 growth rate (cm)) was compared between regions (coast and mountains) across six

674 temperatures (10, 15, 20, 25, 30, or 35 °C). In all tests, *A. suaveolens* and *B. serrata* were

675 analysed separately.

676 *Percent germination*

677 Percent germination of *A. suaveolens* seeds collected at both coast and mountain

678 responded similarly across the six germination temperatures. Percent germination in *A.*

679 *suaveolens* was not significantly different in a binomial GLM model which included an

680 interaction between seed region and germination temperature ($\chi^2_5 = 9.58$, $p = 0.09$; Fig.

681 10). This result did not support our hypothesis of local adaptation to temperature in the

682 germination behaviour of *A. suaveolens.* There was, however, a significant relationship

683 between germination temperature and % germination across both regions ($\chi^2_5 = 131.57$, p

= 0.01). That is, germination percentage was highest (optimal) between temperatures 15-30 °C (Fig. 10A).

Analysis of *B. serrata* germination showed a significant interaction between region and germination temperature (χ^2_5=11.52, p = 0.04; Fig. 10B). This result indicates that populations from the two different regions differ in their germination response to the six experimental temperatures. Post-hoc tests showed significant difference in % germination between regions only at 35°C (z = 3.1, p = 0.01) however there was also a trend towards convergence in % germination at 10°C and 30°C (Fig. 10B).
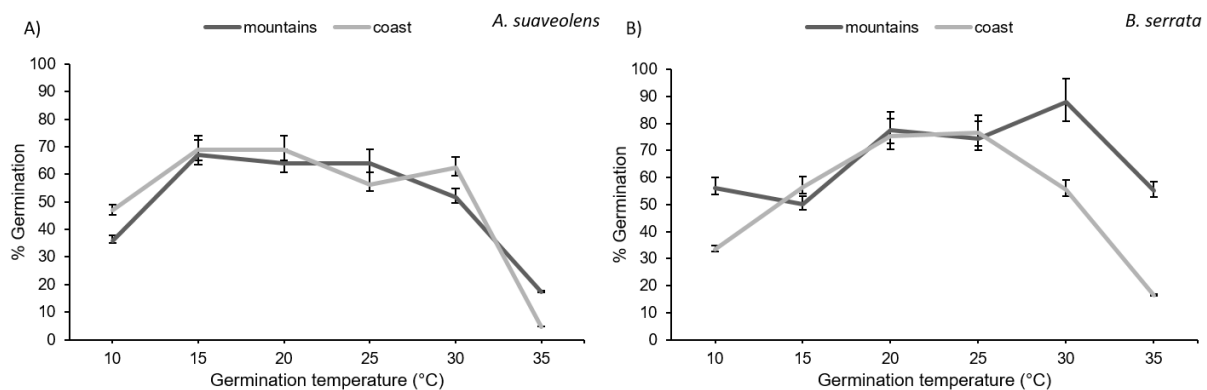
Figure 10. Percent germination in Acacia suaveolens (A) (± 95-1% confidence interval; n = 764 and Banksia serrata (B) (± 95-1% confidence interval; n = 1339) seed sourced from coast and mountain regions in the Sydney area, New South Wales. Seed were germinated on agar plates in growth chambers at six temperatures (10, 15, 20, 25, 30, or 35 °C) for 20 days. A GLM test was used to determine differences in the levels of expected vs observed seed germination percent and if any interaction exists between germination temperature and seed source. And finally, an exact binomial test was used to determine the 95% confidence intervals.

Time (days) to germination

A two-way factorial ANOVA showed significant differences in days to germination (days to radicle emergence) between coast and mountain regions for *A. suaveolens* across all six experimental temperatures ($F_{1,5}$ = 2.34, p <0.001; Fig. 11A). That is, seed from the coast region germinated faster than did seed sourced from the mountain region across all temperatures tested. Across both regions, germination appeared to be fastest between 15 – 30 °C. There was no significant interaction between region and germination temperature ($F_{1,5}$ = 0.94, p = 0.45; Fig. 11A).

Time until germination of *B. serrata* seeds showed significant interaction between region and germination temperature using a two-way ANOVA ($F_{1,5} = 7.49$, $p < 0.001$, Fig. 11B). A post-hoc Tukey test indicated significant differences in germination between regions at 10°C. That is, cold-adapted mountain populations germinated faster at colder temperatures as predicted in our hypothesis of local adaptation in germination behaviour. Similarly, there was a (non-significant) trend toward faster germination at 30°C and 35°C in populations from the warmer coast region, as predicted. Like *A. suaveolens*, germination in *B. serrata* was fastest between 15 – 30°C (Fig.11B) in both coast and mountain regions.
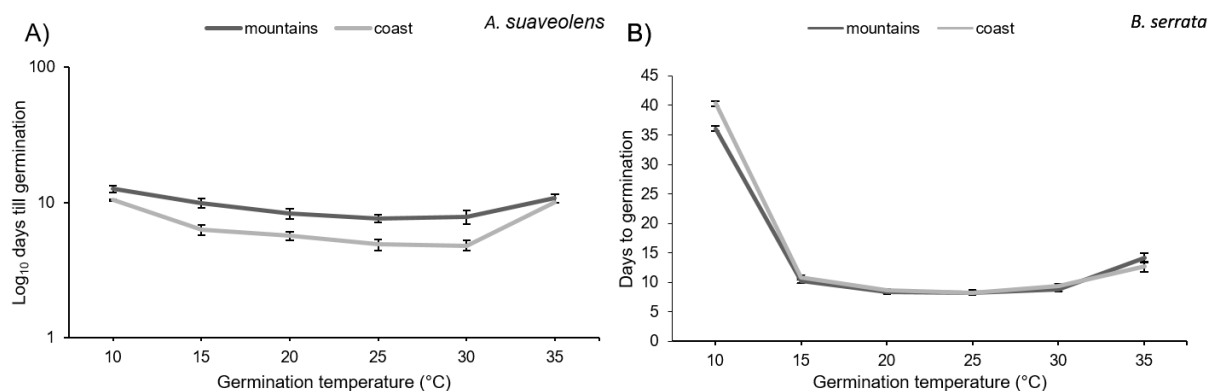
Figure 11. Mean days until germination in (A) *Acacia suaveolens* ($\log_{10}$, ± SE, $n = 385$) and (B) *Banksia serrata* (± SE, $n = 866$) seed sourced from coast (light grey) and mountain (dark grey) regions in the Sydney area, New South Wales. Seeds were germinated on agar plates in growth chambers at six temperatures (10, 15, 20, 25, 30, or 35 °C) for 20 days. A Type-II two-way factorial ANOVA was used to test for interactive effects between germination temperature and region.

Radicle growth rate

For *A. suaveolens* both germination temperature and region had a significant effect on radicle growth rate (two-way ANOVA - $F_5 = 10.97$, $p < 0.001$; $F_1 = 17.46$, $p < 0.001$, respectively; Fig. 12A) and no significant interaction was found between these terms ($F_5 = 0.70$, $p = 0.62$; Fig. 12A).

For *B. serrata*, radicle growth rate in response to temperature exhibited a unimodal distribution in both regions. Peaks in radicle growth rate were seen at 20°C (and to a lesser extent 30°C) for the mountain region and at 25°C coast regions (Fig.

12B). A significant interaction effect on radicle growth rate was found between germination temperature and region ($F_4 = 2.76$, $p = 0.02$; Fig. 12B).
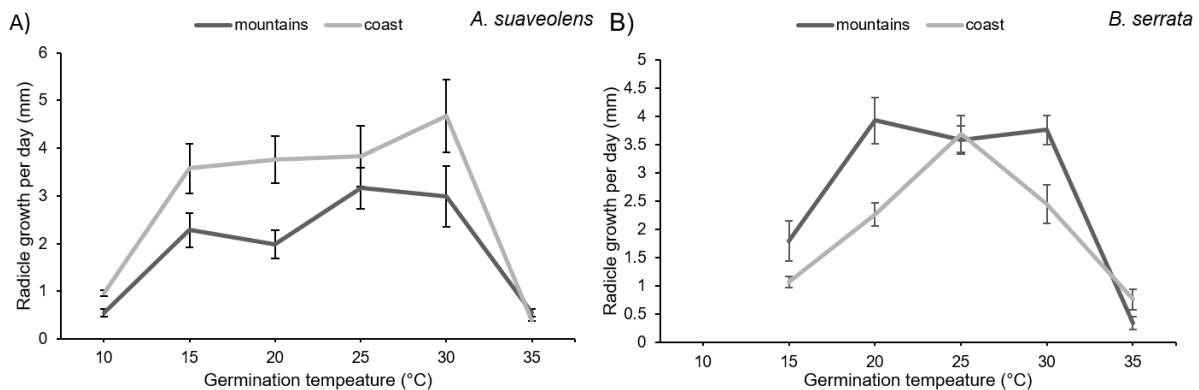
Figure 12. Mean radicle growth per day in (A) *Acacia suaveolens* ($\pm$ SE, $n = 207$) and (B) *Banksia serrata* ($\pm$ SE, $n = 323$) seed sourced from coast (light grey) and mountain (dark grey) regions in the Sydney area, New South Wales. Seed were germinated on agar plates in growth chambers at six temperatures (10, 15, 20, 25, 30, or 35 °C) for 20 days. A Type-II two-way factorial ANOVA was used to test for interactive effects between germination temperature and region. Note the different scales in the *y*-axes.

Common garden experiment

Seedling growth and survivorship was compared between coast and mountain regions in two common gardens (coast – Centennial Parklands; mountain – Mount Tomah Botanic Gardens). Due to differences in soil nutrient conditions (see **Methods –** *Study sites*) and vegetation characteristics between Centennial Parklands and Mount Tomah Botanic Gardens growth response of seedlings in each common garden was analysed separately.

Plant height (cm)

For *A. suaveolens*, we found no evidence of an interaction between plant growth (height increase) and region in either the mountain common garden (Mount Tomah; $F_1 = 0.72$, $p = 0.39$; Fig. 13A) or coast common garden (Centennial Parklands; $F_1 = 0.02$, $p = 0.88$; Fig. 13B). That is, we found no support for our prediction of local adaptation (species growing faster in 'home' conditions relative to 'foreign') in height increase as a measure of seedling growth. In the mountain common garden, we found no significant differences in height increase between replicate seedlings from coast and mountain regions ($F_1 = 0.45$, $p = 0.50$; Fig. 13A). However, plant height was significantly higher in coast

762   replicates than in mountain replicates in the coast common garden ($F_1 = 6.83$, $p = 0.01$;

763   Fig.13B).

764   We found no evidence of an interaction between plant height increase and region

765   in either the mountain common garden (Mount Tomah; $F_2=0.12$, $p=0.86$; Fig. 13D) or

766   coast common garden (Centennial Parklands; $F_2=1.75$, $p=0.18$; Fig. 13C) for *B. serrata*.

767   That is, we found no evidence to support our prediction that a signal of local adaptation

768   can be found in plant height as a measure of *B. serrata* plant performance. A small

769   increase in *B. serrata* plant height was recorded using a type-II ANOVA from the initial

770   to final measurement at cold ($F_1 = 0.14$, $p = 0.91$; Fig. 13C) but no significant change at

771   the warm ($F_1 = 7.3$, $p = 0.02$; Fig. 13D) plots.
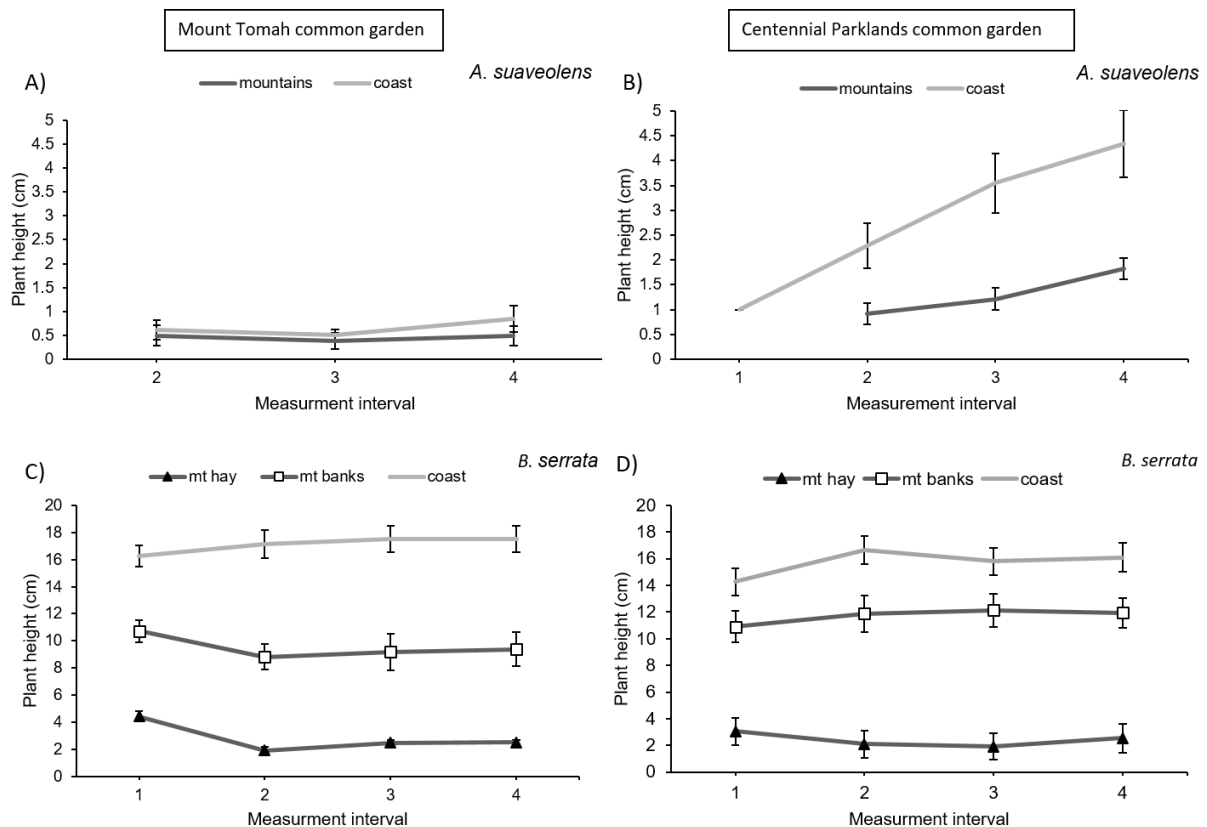
772   **Figure 13.** Mean plant height for *Acacia suaveolens* ($\pm$ SE, $n = 42$) over growth period in (A) Mount
773   Tomah and (B) Centennial Parklands common garden and for *Banksia serrata* ($\pm$ SE, $n = 42$)
774   growth in grown in common gardens in (C) Mount Tomah and (D) Centennial Parklands.
775   Seedlings were planted at common gardens at coast or mountain areas with a measured growth
776   period from 20-4-18 to 29-8-18. Note the different scales in the *y*-axes.
777

778   ***Seedling survivorship***

779   Survivorship was compared between seed region and between plot type (warm or cold)

780   using a binomial GLM. There was no interaction between the observed and expected

numbers of surviving seedlings and plot type for *A. suaveolens* or *B. serrata* ($\chi^2_2 = 0.1$, $p =$ 0.76 and $\chi^2_1 = 3.5$, $p = 0.19$ respectively; Fig. 15A, Fig. 14B). Tests on individual regression parameters for *A. suaveolens* found that seedling survival was not significantly influenced by seedling origin ($\chi^2_1 = 0.31$, $p = 0.65$) or plot location ($\chi^2_1 = 1.49$, $p = 0.24$). Exploring the results from the *B. serrata* showed no significant changes in survival between seedling populations ($\chi^2_1 = 27.75$, $p = 0.96$), but significantly higher mortality at the Mount Tomah plots than the Centennial Parklands plots ($\chi^2_2 = 0.09$, $p < 0.001$).
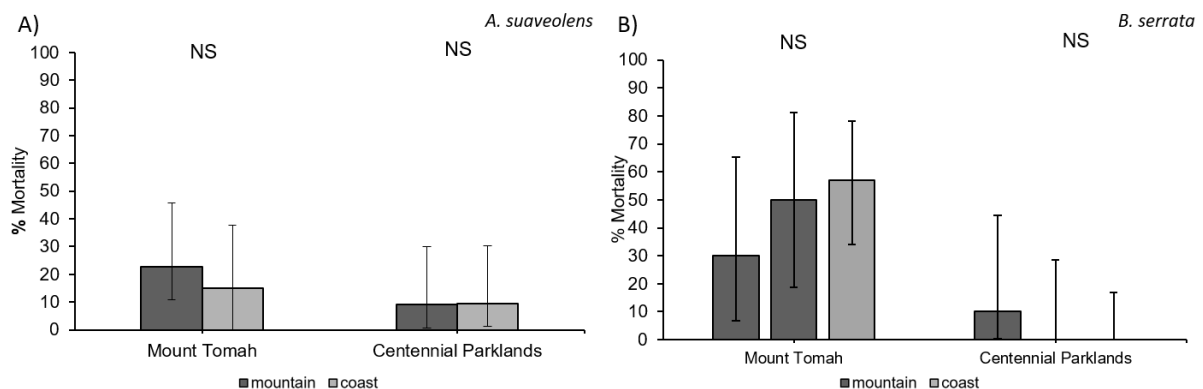
Figure 14: Mean survival of (A) *Acacia suaveolens* (±95% CI, *n* = 42) and (B) *Banksia serrata* (±95% CI, *n* = 42) sourced from mountain (dark grey) and coast (light grey) regions in the Sydney area, New South Wales. Seedlings were grown in plots for 132 days between 20-April-18 and 28-Aug-18. Binomial generalized linear model was used to test for an interaction between common garden location (mountain vs coast) and seedling region``x.

Leaf damage

For *A. suaveolens,* we found no evidence of differences in the percentage of leaf damage between seedlings from warm or cold regions when grown at coast ($t_{41} = 0.83$, $p = 0.41$) or at the mountain site ($t_{32} = 1.12$, $p = 0.28$; Fig. 15A). For *B. serrata*, no significant difference in leaf damage was found at Centennial Parklands ($t_{39} = 0.09$, $p = 0.93$). At Mount Tomah, warm region seedlings were found to have significantly higher leaf damage than those from the cold region ($t_{20} = 4.22$, $p = <0.001$; Fig. 15B).
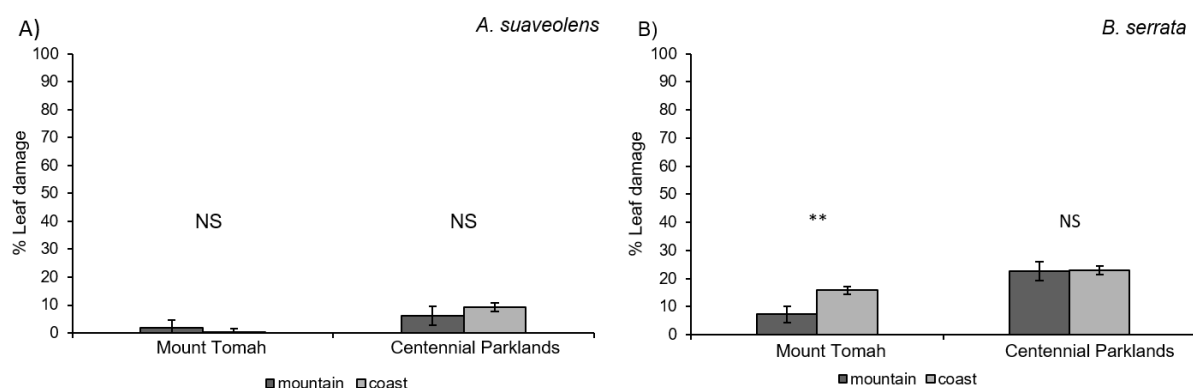
803

804

Figure 15: Mean percent leaf damage of (A) *Acacia suaveolens* (±SE, *n* = 42) and (B) *Banksia serrata* (±SE, *n* = 42) *sourced* from mountain (dark grey) and coast (light grey) regions in the Sydney area, New South Wales. Seedlings were grown in plots for 132 days between 20th April 2018 and 28th August 2018. A comparison within plots for leaf damage between coast and mountain collected seedlings was achieved using a Student's *t*-test. The significance of differences between the seed rate for intraregional populations was determined by a series of *t*-tests: '***' *p* <0.001 '**' *p* <0.01 '*' *p* >0.05.

DISCUSSION

This study illustrates the benefits of combining laboratory and field experiments to examine the presence of local adaptation in germination behaviour and establishment. Results from both our seed germination and common garden experiments showed mixed evidence for adaptation to local climate conditions in the establishment phase of the two plant species examined. For instance, percent germination was higher at cold and warm germination temperatures in cold, mountain and warm, coast seedlings respectively for *B. serrata*. Similar, cold-adapted mountain populations germinated faster at colder temperatures as predicted in our hypothesis of local adaptation in germination behaviour. However, there was no evidence of an interaction between seed origin and germination temperature for *A. suaveolens* and we also found no evidence of differences in growth and survival of seedlings of either species sourced from cold and warm regions when grown in home or foreign common gardens in the field. Given this low level of support for our hypotheses about local adaptation during seedling establishment we conclude that growth and survival may not be increased by sourcing seed from local climate regions when conducting revegetation in the Sydney region with *A. suaveolens* and *B. serrata*. Our findings have implications for the approaches used to restore Eastern

830   Suburbs Banksia Scrub – a Critically Endangered Ecological Community where these

831   two species are commonly found.

Evidence of local adaptation in germination behaviour

833   We compared the germination niche of seeds collected from warm and cold regions

834   across the east-west distribution of two Sydney sandstone species. Our primary aim was

835   to germinate seeds across a range of temperatures to test for evidence of local adaptation

836   to temperature in percentage germination, time until germination and radicle length. We

837   defined local adaptation as evidence for performance increases in temperatures more

838   closely approximating 'home' conditions relative to 'foreign' conditions (Kawecki and

839   Ebert 2004). In support of our first hypothesis, our results indicate that germination

840   behaviour in *B. serrata* differed between home and foreign temperatures which may

841   provide evidence of local adaptation. For instance, we found evidence that temperature

842   was associated with all three metrics of seed behaviour measured in this species (Fig.

843   10B, Fig. 11B, Fig. 12B). By contrast, we found no evidence of local adaptation in *A.*

844   *suaveolens* germination behaviour for any factor tested. There was evidence of a local

845   based adaptation for *A. suaveolens* in germination rate only (Fig. 10A) but not in time till

846   germination (Fig. 11A) or radicle growth (Fig. 12A).

847   Interestingly, *A. suaveolens* seed had greater rate of successful germination at the

848   coldest temperature for warm collected individuals and higher at the warmest

849   temperatures from cold collected individuals (Fig. 10A). This result could emerge from

850   the presence of maladaptation in some populations tested. Maladaptation occurs due to

851   a reduction of fitness at local sites when compared to foreign (Crespi 2000; Gellie et al.

852   2016), which can be caused by biotic interactions (Biere and Verhoeven 2008; Bucharova

853   et al. 2016), changed mating system (Breed et al. 2013; Lowe et al. 2015) or a legacy of

854   founder effects (Gellie et al. 2016; Leimu and Fischer 2008; Travisano et al. 1995).

855   Although maladaptation may affect the germination behaviour of *A. suaveolens*, resulting

856   in lower germination success at conditions more similar to home, the likelihood of

857   detecting this effect is reduced when growth chambers are used. Controlled growth

858   conditions largely eliminate confounding edaphic and biological factors which may

859   affect germination behaviour.

860   Faster rates of germination may not always equate to higher seedling fitness in

861   populations. For instance, similarly counterintuitive results about germination in local

862 conditions shown in our study were found by Marcora et al. (2017) who showed that

863 high altitude, cold-adapted seeds of *Maytenus boaria* and *Escallonia cordbensis* had higher

864 germination rates at warm temperatures relative to low, and *vice versa*. This relationship

865 may result from cold-adapted populations avoiding germination during cold conditions

866 which can be physiologically challenging for emergent seedlings (Baskin and Baskin

867 2001; Giménez-Benavides and Milla 2012; Moles et al. 2007). Equally, reductions in

868 germination at warmer temperatures in seeds from warm-adapted source populations

869 may be a drought or extreme heat avoidance mechanism to reduce risk of seedling

870 establishment (Engelbrecht et al. 2007; Fenner 2006). Reduced germination at warmer

871 temperatures may be a cue to prevent germination during drought conditions (Levine et

872 al. 2008), which is only present in the warmer populations.

873

Measuring local adaptation using common garden experiments

875 Common garden experiments are a powerful method for examining the performance of

876 individuals sourced from different conditions or regions in a shared ecological setting

877 (Broggi et al. 2005; Ebeling et al. 2011; Gao et al. 2018; Lepais and Bacles 2014;

878 McDonough MacKenzie et al. 2018; von Wettberg et al. 2016). In our common garden

879 trials, we grew seedlings from populations at both ends of a strong temperature gradient

880 across the Sydney region - from the Blue Mountains (cold) and Central Coast (warm).

881 Seedlings for both regions were grown in two common gardens which reciprocally

882 emulated home and foreign temperature conditions at Mount Tomah (cold) and

883 Centennial Parklands (warm) (temperature data given in Fig.7). Due to confounding

884 abiotic factors (e.g. soil nutrient differences, strength of competitive interactions), growth

885 and survival could not be directly compared between common gardens and were

886 restricted to comparison between regions in each garden separately. We did not find any

887 evidence of local adaptation in *A. suaveolens* or *B. serrata* in any of the growth traits we

888 measured in either of the common gardens: plant height (Fig.13), seedling survivorship

889 (Fig.14), or leaf damage (Fig.15).

890 Our lack of evidence for local adaptation in growth and survival in common

891 gardens are not atypical. Previous studies have also found little or no evidence for

892 adaptation to local climates (see studies who did not find any evidence of local

893 adaptation (Pahl et al. 2013; Tíscar et al. 2018). An analysis of pair-wise local adaptation

from 32 published studies was performed using the strict definition of local adaptation (pair-wise increase of fitness at both sites). The meta-analysis found that in 1032 compared population pairs, only 45.3% found evidence of local adaptation (Leimu and Fischer 2008).

Contrary to our hypothesis, neither *A. suaveolens* nor *B. serrata* showed any evidence for local adaptation when grown in common gardens. Signals of local adaptation were found in seed germination behaviour for *B. serrata*, however were absent in the early establishment phase in common garden trials. Both these key life stages (germination and establishment) have been shown to be under strong selection in previous studies (Blossey et al. 2017; Donohue 2009; Postma and Ågren 2016), although local adaptation in early life history traits can be difficult to quantify (Garrido et al. 2012; Raabová et al. 2011; Tíscar et al. 2018).

Potential explanations for the lack of local adaptation identified in our study include maternal effects on seed establishment (Galloway 2005; Imbert et al. 1999), low genetic diversity/small population size (England et al. 2003; Stöcklin et al. 2009) or the relatively short duration of experimental treatments (Volis et al. 2015). Transgenerational plasticity can occur because seed is produced on the mother plant and will experience similar conditions during development (Volis et al. 2015). For instance, maternal plants grown in reduced soil-nutrient availability resulted in progeny with higher root growth allocation in genetically similar offspring of *Polygonum persicaria* (Sultan 1996). In *Campanulastrum americanum*, maternal light levels significantly affected progeny fitness for those grown under the same light regime (Galloway and Etterson 2007). Parental effects can arise from either parent but are less common from the paternal line. This may be due to pre-zygotic effects being overpowered by post-zygotic ones (Lacey 1996; Luzuriaga et al. 2006). Post-fertilization seed development occurs on the mother plant and can expose the developing seed to similar conditions of the mother plant. Seed mass, dormancy and germination rate are adaptive traits that have strong implications for seed fitness (Luzuriaga et al. 2006; Singh et al. 2017). These traits vary with maternal temperature (Lacey 1996), photoperiod (Munir et al. 2001), soil nutrient availability (Sultan 1996) or soil moisture conditions (Riginos et al. 2007). Although we standardised seed by mass (Table 3, Fig. 8), this measure may not have adequately corrected for differences in maternal influences on germination rate or dormancy. Maternal effects on seeds can be reduced by using F_1 seeds from plants reared in

927 greenhouse conditions, however the lengthy generation time of our two perennial study
928 species meant this was not an option.

929   Small populations may exhibit lower levels of local adaptation than do larger
930 populations (e.g. >1000 flowering individuals (Leimu and Fischer 2008)). The benefits of
931 local adaptation may be overridden by genetic phenomenon like inbreeding depression
932 (Leimu and Fischer 2008; Menges 2008) as detected in other Australian native plant
933 populations (Hufford et al. 2012; Pickup et al. 2012; Seed et al. 2006). Larger
934 populations may harbour higher levels of genetic variation and accumulate a higher
935 proportion of beneficial mutations (Hill 1982; Weber and Diggins 1990). Local
936 adaptation can be reduced when adapted alleles are not preserved due to gene flow and
937 genetic drift (Kawecki and Ebert 2004). High gene flow can also restrict local adaptation
938 if a genotype with high fitness swamps the entire population (Akerman and Bürger
939 2014). The benefits of local adaptation can also be hindered by too little gene flow.
940 Where the benefits of adaptation are outweighed by the negatives of isolation (e.g.
941 inbreeding depression) (Verhoeven et al. 2011). Further genetic testing would be required
942 to quantify gene flow between populations.

943   Importantly, we may have seen greater evidence for local adaptation in a longer-
944 term common garden experiment. For example, local adaptation may only be detected
945 over multiple generations within populations; Voiles et al. (2015) only found evidence of
946 local adaptation in reciprocal transplanting of *Triticum dicoccoides* after three years of
947 monitoring (Volis et al. 2015).This may be because maternal phenotype effects can
948 persist for multiple generations (Miao et al. 1991). Temporally rare events, such as frost
949 or drought may be required for the expression of local adaptation. Selective conditions
950 that drive local adaptations from stochastic events even be completely absent from some
951 generations. Meaning in may take many years before a selective agent results in
952 improved fitness from local adaptation (Geber et al. 2003; Rice and Mack 1991;
953 Thompson et al. 2007), or reduced fitness from maladaptation (Miller and Libby 1989),
954 may not become apparent after many years. It is also possible that the temperature
955 gradient across which the populations were collected may not have been steep enough to
956 drive selection for differences in germination, though local adaptation has previously
957 been found in studies conducted over small spatial scales (Bastida et al. 2015; Byars et al.
958 2007).

959 *Local adaptation paradigms in plant species restoration*

960 The original extent of ESBS – the Critically endangered plant community where *A.*
961 *suaveolens* and *B. serrata* are characteristic species – has been reduced to around 97%, with
962 much of the remaining community being highly fragmented (NSW Department of
963 Environment and Conservation, 2004). Habitat fragmentation can result in reduced
964 population fitness due to random genetic drift, increased inbreeding (Bartlewicz et al.
965 2015), and reduced genetic diversity (Tumas et al. 2018). Although gene flow will be
966 occurring from *A. suaveolens* and *B. serrata* populations into ESBS, barriers may still be
967 increasing inbreeding levels. Furthermore, as climate changes strong emphasis on local
968 provenance may become increasingly difficult to justify (Wilkinson 2001).

969 We found mixed evidence of local adaptation in our study species which has
970 implications for the ecological basis of restoration and vegetation management strategies
971 in ESBS. Previous evidence shows that sourcing individuals from a population which
972 has become locally adapted and establishing these in dissimilar climates can reduce
973 transplant survival (Galloway and Fenster 2000; Helenurm 1998; Hufford and Mazer
974 2003; Montalvo and Ellstrand 2000; Pickup et al. 2012; Wilkinson 2001). As the cost of
975 restoration projects is often high, and financial resources scarce, it is important to
976 maximise the chance that plants will survive and reproduce (Adame et al. 2015; Menz et
977 al. 2013; Possingham et al. 2015). One widely accepted paradigm in ecological
978 restoration is importance of using seed sourced locally (Broadhurst et al. 2008; Callaham
979 1963; Hamilton 2001; Hancock and Hughes 2014); although see studies questioning the
980 importance of local seed (Broadhurst et al. 2008; Byrne et al. 2011; Jones 2013; Sgrò et
981 al. 2011; Weeks et al. 2011; Wilkinson 2001). Seed sourcing should be adaptive to the
982 goals of the project. If there is a serious concern about outbreeding and loss of local
983 adaptation, than local seed should be used. Alternatively, when inbreeding is a serious
984 concern and no local adaptation is found non-local seed may be favourable.

985 We found no evidence of local adaptation in the survival and growth of seedlings
986 in their home compared to foreign temperatures in this experiment. This finding implies
987 that, when considering climate alone, using local seed to restore ESBS at Centennial
988 Parklands (or other locations in the Sydney region) may not be important. However,
989 there are a range of other genetic factors which also need to be explored, but are beyond
990 the scope of this study. In many restoration contexts using seed from local populations

991 may not be a viable (or desirable) option. For instance, the identification of high levels of

992 inbreeding depression, or small effective population size in source populations may

993 hinder restoration success (Marsden et al. 2013; Weeks et al. 2011) or reduce the amount

994 of viable available seed for harvest (Krauss and Koch 2004; Meissen et al. 2017;

995 Mortlock 2000; Rowe 2010; Walker et al. 2015).

996   Maximising fitness of the population is not only important for the current

997 generation but also for subsequent recruitment. Invasion of alleles may create hybrid

998 progeny with lower fitness than parental populations due to dilution of associated genes

999 to local adaptation (Fenster and Galloway 2000). Conversely, genetic invasiveness can

1000 result in intraspecific hybrids having higher fitness than the parents via population

1001 admixture (Ellstrand and Schierenbeck 2000; Escobar et al. 2008). In this lens, lack of

1002 any visible local adaptation between ESBS local and non-local progeny may be a

1003 promising sign for revegetating the area. Heterosis (hybrids out-competing local) can

1004 occur when deleterious mutations, which are partially or completely recessive, become

1005 fixed within populations via genetic drift (Oakley et al. 2015), making the F1 hybrid

1006 superior in some measure of parental performance (Hahn and Rieseberg 2017; Hei et al.

1007 2016). Population admixture can result in a fitness cost via the dilution of locally

1008 adapted genomes (Hufford and Mazer 2003; Shi et al. 2018). This can have negative

1009 effects if these local genotypes are adaptations to stochastic events. Mixing of non-local

1010 progeny can reduce the fitness of the population via outbreeding depression (Fenster and

1011 Galloway 2000). In which local × introduced hybrids have some measure of lower trait

1012 fitness (Goto et al. 2011; Pantoja et al. 2018). Outbreeding depression and other genetic

1013 risks which are associated with transplanting have been reasons for consideration for

1014 moving non-local progeny into genetically different populations within a species range

1015 (Burbidge et al. 2011; Severns 2013; Sletvold et al. 2012)

1016

CONCLUDING REMARKS

1017

1018 We found mixed evidence to support the hypothesis that the germination niche and

1019 establishment in populations of *A. suaveolens* and *B. serrata* are adapted to local

1020 temperature conditions. That is, although we did find some evidence from germination

1021 experiments of local adaptation, no significant patterns were detected in the early

1022 establishment phase of the plants when using a common garden experiment. This study's

result of no signals of local adaptation in *A. suaveolens* and *B. serrata* may provide positive implications for restoration efforts. Despite the large climate gradient across the coast and mountains study sites, we found no evidence that these species were adapted to their local temperatures. This means that germplasm for restoration can be collected from across the gradient since genotypes from each area are equally likely to have similar plant height, leaf damage and survival in any habitat. However, studies across both species' entire distribution are recommended as to identify potential adaptations over larger geographic patterns. Furthermore, sampling from just one year may fail to provide a clear picture of adaptations. Germination traits can be driven by spatio-temporal heterogeneity (Norden et al. 2007), meaning the results gathered in this experiment only provide a snapshot of the adaptation potential of these populations. Initially this study aimed to compare the mountain and coast site, however difference in edaphic conditions meant this was not feasible. Future studies would benefit from characterizing soil conditions at sampling sites and incorporating it into the study.

While this study did not extend to measuring fitness over the entire growth period of the plant, future studies could return to the plots and identify any possible signs of local adaptation during the reproductive stage. Furthermore, other potential issues may occur when using a non-local seed source for restoration which need to be understood more thoroughly. These factors include competition of local progeny by non-local (Oakley et al. 2015), introduction of pathogens (Ricciardi and Simberloff 2009), or founder effects (Alpert 2006). If we were without limitations, it would be best that studies on seed source analyse both local adaptation and genetic variation (Wheeler et al. 2003); though this information is rarely available (Hancock and Hughes 2012). The present study provides evidence in the form of identifying locally adapted traits. However, local adaptation is just one component of a complicated question of local vs non-local seed. Specifically, if the goal is to maximise genetic diversity, then seed from a wide mosaic of populations would be recommended; or if local adaptation is present, then local seed is more appropriate.

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SUPPLEMENTARY MATERIAL

There are two appendices to this thesis:

Appendix 1: Results of pathology tests for the presence of *Phytophthora* in experimental plots.

Appendix 2: Results of soil analyses from common garden experimental plots

Appendix 1: Results of pathology tests for the presence of *Phytophthora* in experimental plots.

PDDU Reference Number:	I18/115
Date/Time of Sample Receipt:	9.3.18
Test Week:	18W20
Invoicee (Person or Company/Organisation paying the Invoice): SAP:	Thomas Pyne
ABN (Commercial or Organisation) or Date of Birth (Non-Commercial):	13/02/1992
Purchase Order Number (for organisations that require Purchase Orders):	
Purchase Order Upload:	
Primary Contact(s). Please note that results will only be sent to the Primary Contact(s):	Thomas Pyne
Email:	thomas.pyne@hdr.mq.edu.au
Phone numbers:	0408 646 714
Street Address:	8 Canberra Crescent East Lindfield, NSW 2070
Description of Problem (s):	No observed issues.

	Testing for phytophthora within research plot.
Description of Sample(s):	The samples are a composite of 7 soil cores from two plots. The plots are 50m apart. Surrounding vegetation are Brachychiton paradoxus and Ficus marophylla.
Test Required:	Sample 4 = PHYTO Sample 5 = PHYTO
Fee Rate:	BGCP-Internal
Do you agree to our service charges?:	Yes
PHYTO-Results:	S4 = Negative S5 = Negative
Repeat DNA Extraction & Sequencing:	No
Lab Work Complete?:	Yes
SC-AS6 sent to OEH?:	

Appendix 2: Results of soil analyses from common garden experimental plots

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