The role of plant provenance in restoration ecology under climate change

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Abstract

The rapidly changing climate poses a challenge for many land management and conservation activities. In particular, the need to ensure future sustainability of revegetated communities has focused attention on the critical decision as to where to source seed (and other propagules). Traditionally, industry 'best' practice has adhered to the principle of sourcing propagation material locally. This practice has been based on a perception that locally-sourced material is adapted to local conditions and will therefore confer superior plant performance. Additionally, the use of local provenance is often considered desirable as a means of 'preserving' the genetic integrity of local populations, reducing risks of outbreeding depression. In situations where source populations are small and inbred and where the environment is rapidly changing (a situation we are currently facing), this 'local is best' practice needs to be challenged. Moreover, sourcing seed from genetically-impoverished populations with little adaptive potential is increasingly being viewed as more detrimental to restoration success than potential outbreeding depression. Improved seed-sourcing guidelines, developed in the context of changing environmental conditions and based on empirical evidence, are urgently needed to support restoration projects that are sustainable in the long term.

This thesis explores the 'local is best' paradigm using field and glasshouse experiments. In Chapter 2, I describe a common garden experiment comparing the establishment success of different provenances (one local vs four non-local provenances) of six widespread species (*Acacia falcata, Bursaria spinosa* ssp. *spinosa, Eucalyptus crebra, E. tereticornis, Hardenbergia violacea* and *Themeda australis*), all community dominants and / or widely used in restoration projects on the Cumberland Plain, western Sydney. In Chapter 3, I describe an experiment designed to test the establishment success of four provenances each of *E. tereticornis* and *T. australis* under both current and simulated future temperature conditions for 2050 in western Sydney. In Chapter 4, I describe a glasshouse experiment comparing the survival and early growth rates of three provenances each of *Acacia falcata* and *Eucalyptus crebra* under ambient and

elevated CO_2. In Chapter 5, I describe the results of a survey (conducted in New South Wales) investigating understanding of local provenance issues among restoration practitioners. The overall results and conclusions of the research are summarized in the final Chapter.

Little evidence was found that local provenance plants had superior establishment success in the field studies. In the glasshouse experiment, intraspecific variation was found between the provenances for both species, regardless of the CO_2 treatment. The results of the survey identified several inconsistencies of practice and belief within the restoration industry and that the definition of 'local provenance' is very flexible. The majority of respondents are in favour of a review of seed-sourcing policy/guidelines to allow for the inclusion of non-local provenance material. Overall, this research provides empirical support to challenge the validity of the strict adherence to the 'local is best' paradigm in general, but particularly on the Cumberland Plain, and underpins the need for improved seed sourcing guidelines.

Certificate of candidate

I certify that the work in this thesis ‘The role of plant provenance in restoration ecology under climate change’ is an original piece of research and has been written by myself. The contributions of others are listed below (Statement of contribution) and assistance by volunteers has been acknowledged either at the end of each chapter or in the Acknowledgement section. All sources of information and literature used are indicated in the thesis. This thesis has not previously been submitted in any form for a higher degree at any other university or institution.

Ethics Committee approval (Ref number: 5201200068 dated effective 24th February 2012) was obtained for the survey (Chapter 5).

Nola Hancock
October 2012

Statement of contribution

I, Nola Hancock, declare that the research contained in this thesis entitled 'The role of plant provenance in restoration ecology under climate change' is my own work. The contribution of co-authors and other sources are indicated below.

Chapter 2

Testing the 'local provenance' paradigm: a common garden experiment in Cumberland Plain Woodland, Sydney, Australia.

Lesley Hughes and Michelle Leishman were involved in concept development, experimental design and manuscript preparation:

Concept & development: NH 50%

Data collection: NH 100 %

Data analysis: NH 85%

Writing: NH 80%

Chapter 3

What role does 'home-site' advantage play in restoration ecology under heatwave conditions?

Lesley Hughes was involved in concept development, experimental design and manuscript preparation:

Concept & development: NH 70%

Data collection: NH 100 %

Data analysis: NH 90%

Writing: NH 90%

Chapter 4

Intraspecific responsiveness to elevated CO_2 of two widespread native Australian species, *Acacia falcata* and *Eucalyptus crebra*

Lesley Hughes was involved in concept development, experimental design and manuscript preparation:

Concept & development: NH 70%

Data collection: NH 100 %

Data analysis: NH 75%

Writing: NH 90%

Chapter 5

How far is it to your local? A survey on local provenance use in New South Wales

The concept for this chapter initially came from Lesley Hughes and Jessica Gough

Concept & development: NH 80%

Data collection: NH 100 %

Data analysis: NH 100%

Writing: NH 95%

Chapters 1 & 6

The Introduction and Discussion and conclusion chapters are my own work, with valuable feedback provided by Lesley Hughes.

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CHAPTER 1

Introduction

Climate and restoration ecology

Over the past 25 years, atmospheric temperatures have increased at a rate of 0.19°C per decade (Allison et al. 2009), substantially more than the trend of 0.13°C per decade for the last 50 years and double that of the past 100 years (IPCC 2007). Carbon dioxide (CO_2) emissions, a major contributor to increasing global temperatures, continue on a high growth trajectory (Peters et al. 2012 and references therein). Under the IPCC's high emission scenario (A1FI), it is likely that by the end of the present century, global average temperatures will have increased by 2.4 – 6.4°C compared to the 1980 – 1999 baseline (IPCC 2007). This increase is considered to be beyond a safe operating threshold for many species and systems (Steffen et al. 2009). Increases in atmospheric temperatures may not drop significantly for at least 1000 years after emissions of CO_2 cease (Solomon et al. 2009). A concomitant increase in the frequency and intensity of extreme weather events is also expected (Allison et al. 2009). Extreme weather events are considered to have a greater impact on biological systems than average trends and to play a disproportionate role in the evolution of organisms (Gutschick & BassiriRad 2003; Jump & Peñuelas 2005).

Climate is the main factor driving the distribution of major vegetation types (Woodward 1987). Concurrently, species interactions and distributions are being modified by other drivers of global environmental change (e.g., land use practices, CO_2 enrichment, nitrogen deposition, biotic invasions) (Tylianakis et al. 2008 and references therein). In addition, as a result of human-induced changes, natural landscapes are becoming increasingly fragmented. The consequences of fragmentation are complex, but generally it is considered that it will impede geographical range shifts of species and will create small, genetically-impoverished populations that no longer have sufficient genetic diversity to adapt to rapidly changing environments (Aguilar et al. 2008 and references therein) (but see Young et al. 1996; Hobbs & Yates 2003).

Whilst the scale and magnitude of environmental changes are predicted to vary and will be context-dependent (Tylianakis et al. 2008), there is mounting evidence that climate change will alter the distribution and phenology of species and disrupt ecosystem processes (Walther et al. 2002; Root et al. 2003; Cleland et al. 2006; Chen et al. 2011). There is growing concern within the scientific community that rapidly changing environments will create ‘no-analog’ communities (communities that have not previously existed) (Hobbs et al. 2009). Restoration projects are increasingly being implemented as a means to rehabilitate natural landscapes disturbed by human activity and to reduce rates of biodiversity loss (Benayas et al. 2009). However, a rapidly changing climate means that restoration practices that seek to return landscapes to historical form and function will be increasingly challenged (Hobbs et al. 2009).

Local adaptation

Inherent in every restoration project (that involves revegetation) is the decision as to where to source seed and other propagules. Traditionally, it has been regarded as desirable to source propagation material locally. This practice has been underpinned by two main assumptions. The first assumption is that locally-sourced material is better adapted to local conditions than propagules of the same species sourced from elsewhere in their range. Implicit in this assumption is that locally-adapted propagules will have a greater probability of successful establishment and sustainability of the restoration project. Since local adaptation in plants was first noted (Turesson 1922), its importance has been the subject of much debate (Sackville Hamilton 2001; Wilkinson 2001) and the focus of many reviews, meta-analyses, and surveys (Knapp & Rice 1994; Linhart & Grant 1996; McKay et al. 2005; Broadhurst et al. 2008; Leimu & Fischer 2008; Hereford 2009; Vander Mijnsbrugge et al. 2010). Whilst local adaptation has been demonstrated in some reciprocal transplant and common garden field studies, the scale and magnitude of local advantage varies considerably and is unpredictable (Gordon & Rice 1998; Montalvo & Ellstrand 2000; Joshi et al. 2001; Leimu & Fischer 2008; Hereford 2009).

The second assumption is that using locally sourced material will retain the ‘genetic integrity’ of the site. Of particular concern is that interpopulation crosses of transplanted genotypes with local plants may increase the probability of outbreeding depression (reduced fitness in the offspring compared to the parental populations). In a review by Hufford and Mazer (2003), the majority of studies comparing offspring fitness between local and transplanted genotypes, found reduced fitness in second and third generations. However, concerns about outbreeding depression are now thought to be ‘excessive’, and the risk of outbreeding depression can broadly be predicted (Frankham et al. 2011).

A further concern is that the introduction of non-local genotypes may invade nearby remnant populations of the species. An invasion of non-local genotypes of *Phragmites australis* occurred in North America, where the local genotype was outcompeted by an introduced genotype, resulting in the loss of genetic diversity (Saltonstall 2002). In Western Australia, remnant roadside patches of *Acacia saligna* ssp. *lindleyi* were ‘genetically contaminated’ by pollen dispersed from a planted stand of *A. saligna* ssp. *saligna* (Millar et al. 2012). However, the likelihood of negative consequences arising from the introduction of non-local provenance material can be reduced with the application of risk management tools. For example, a ‘risk assessment protocol’ that evaluates the likelihood of adverse genetic change from revegetation sites on surrounding plant populations has recently been published (Byrne et al. 2011). In addition to concerns about plant performance and genetic integrity, the disruption of trophic interactions has also been demonstrated as a mechanism by which the introduction of non-local propagules may have negative consequences (Vander Mijnsbrugge et al. 2010).

Despite the potential negative outcomes resulting from the introduction of non-local propagules, there can be advantages associated with the introduction of propagules from elsewhere within the species range. These advantages include an increase in the amount of genetic variation within the source population, the formation of new combinations of traits through the creation of new genotypes (and increasing adaptive potential) and the masking of deleterious mutations (Verhoeven et al. 2011 and references therein). An improved performance of offspring, especially in novel environments can result from successful introductions (Jones & Johnson 1998; Fenster & Galloway 2000).

While it may be useful to consider the traditional reasons for using locally-sourced propagules under stable environmental conditions, the fact that the climate is changing rapidly means that new policies are required. In particular, adherence to a policy that restricts seed collection to a specific geographical distance from the proposed planting site may restrict the adaptive potential of vegetation at restoration sites and reduce long-term sustainability of restoration projects. Indeed, there are good arguments that seed-sourcing strategies should aim to maximize genetic variation and thus increase adaptive potential (Sgrò et al. 2011). Further, various studies have suggested that guidelines should consider: the environmental conditions of the donor and recipient sites (Hereford 2009); the size of the source population (to maximize genetic diversity) (Broadhurst & Young 2006); the degree and size of the disturbance at the proposed revegetation site (Lesica & Allendorf 1999); and the future climatic conditions of the recipient site (Jones & Monaco 2009). A mix of local and non-local seed sources that consider the above factors are increasingly being recommended as an insurance policy for revegetation success (Lesica & Allendorf 1999; SER Science and Policy Working Group 2004; Broadhurst et al. 2008; Sgrò et al. 2011). The decision as to which non-local provenances to include in seed mixes is complex. In some cases, recommendations need to be made on a population by population basis (particularly for populations of species with different breeding systems and cytology and where taxon lineages differ (Coates 1988; Murray & Young 2001; Holmes et al. 2008; Stöcklin et al. 2009; Millar et al. 2012). Where the long-term persistence of these populations would benefit by increasing effective population size, it has been suggested that the introduction of non-local genotypes be conducted as controlled scientific experiments so that the effects of the introduction can be monitored (Guerrant Jr & Kaye 2007; Frankham et al. 2011). To assist with the development of these introductions, guidance is available for management protocols for the 'genetic rescue' of threatened populations and for ecological restoration (Weeks et al. 2011). Guidelines suggesting strategies that provide a range of outcomes such as providing evolutionary resilience and biodiversity conservation are also available (Sgrò et al. 2011; Weeks et al. 2011).

Climate and restoration ecology in Australia

In Australia, average daily mean land surface temperatures have increased by 0.9°C since 1910, with most of the warming occurring since 1950 (CSIRO and Bureau of Meteorology 2010). An increase in temperatures of 0.6 – 1.5°C is expected by 2030 and a range of + 1 - 5°C by 2070 (compared with the climate of 1980 – 1999). These projections are based on global greenhouse gas emissions remaining within the range of IPCC projected future emission scenarios (CSIRO and Bureau of Meteorology 2010). Rainfall projections are less certain than those of temperature but climate models suggest that rainfall patterns will change, with droughts expected to become more frequent in southern Australia (CSIRO and Bureau of Meteorology 2010). More recent modelling, however, suggests that within decades, most environments within Australia will be substantially different from those currently experienced by biodiversity (Dunlop et al. 2012).

Improved seed-sourcing guidelines for restoration projects, developed in the context of changing environmental conditions and based on empirical evidence, are urgently needed in Australia to support restoration projects that are persistent in the long term. Reciprocal transplants or common garden field experiments that investigate local adaptation within Australia (and thereby underpin seed-sourcing guidelines) are scant. Most studies are performed under current conditions rather than being future-focused and have produced equivocal evidence of local provenance superiority. Generalities between studies are difficult to make because local adaptation appears to depend on the landscape and the species. For tree species, in a 15-year trial, the Western Australian species, *Eucalyptus marginata* was found to be locally adapted for survival and growth when northern and southern regional provenances were compared, but not at a smaller provenance scale (O'Brien et al. 2007). In a provenance trial for three south-western Australian forest trees, after two years, home-site advantage was shown for one species for survival and for one species for growth (O'Brien & Krauss 2010). In Tasmania, considerable local adaptation was found between populations of the *E. gunnii-archeri* complex (Potts 1985). For shrubs, local adaptation was not found for any fitness traits in a 23-month study of *Leptospermum scoparium* in central Victoria *(Price & Morgan 2006)*. Research has provided equivocal results for herbs and forbs. No local adaptation was found in terms of survival or leaf morphology for *Craspedia lamicola* during a 18-month trial in the alpine area of Victoria, but some home-site advantage was found for leaf number (Byars & Hoffmann 2009). In a 12-month study, local genotypes of *Rutidosis leptorrhynchoides* displayed a small but significant advantage over non-local genotypes for survival but not for emergence or phenology (Pickup et al. in press). The results are also mixed for grass species. *Poa hiemata* demonstrated home-site advantage in terms of survival in an 18-month study in the alpine region of Victoria (Byars et al. 2007). However, in a large study of 29 grasses in central New South Wales, home-site advantage for survival rates was mixed (Waters et al. 2005).

Studies that delineate genetic diversity among populations are common and are useful tools for the development of seed-sourcing guidelines. Eucalypts tend to dominate the species investigated in these studies (possibly due to their commercial value) and significant levels of genetic diversity between populations have often been documented (Potts 1990 and references therein). However, these studies do not identify if the significant intraspecific diversity found amongst populations translate into superior performance of the locally sourced plants at the local site. Studies that investigate the relationship between population size and plant performance are also important for seed-sourcing guidelines. Many of these studies have found that the performance of plants whose seeds were sourced from small populations, and those with limited outcrossing potential, is significantly reduced (Buza et al. 2000; Broadhurst & Young 2006; Heliyanto et al. 2006; but see Yates et al. 2007).

In summary, these studies provide equivocal empirical evidence in support of the ‘local is best’ paradigm and demonstrate that significant genetic diversity occurs among populations of many Australian plant species. Strong evidence exists that population size influences plant performance (Broadhurst & Young 2006; Pickup et al. 2012), mirroring the global experience (Leimu & Fischer 2008 and references therein), and thus, questions the validity of the current adherence to seed-sourcing guidelines that limit collection to a geographical distance from the planting site (see Montalvo & Ellstrand 2000).

Despite strong empirical evidence supporting an inverse relationship between population size and plant performance, legislation, policies or contractual obligations that stipulate the use of local provenance are inconsistent among and within the States and Territories of Australia. An informal internet search (and discussions with relevant Government personnel) revealed that only three States (New South Wales, South Australia and Western Australia) have either legislation or formal policies that stipulate the use of local provenance (Table 1). However, the use of local provenance can be requested as part of funding agreements by government departments within those States and Territories that have no formal policies. Inconsistencies in the conditions under which the use of local provenance is required exists between departments and even among personnel within the same department.

Table 1. Summary of Australian State and Territory's practices regarding the use of local provenance. Information was obtained by searching government department web sites and by conducting informal discussions with relevant government personnel. Where the appropriate government contact could not be identified, information was obtained from participants within the restoration industry (e.g. Greening Australia) in the relevant State or Territory.

State/ Territory	**Responsible Department**	**Relevant government department and comments on local provenance use**
Australian Capital Territory	Environment and Sustainable Development Directorate	A draft policy was developed many years ago specifying the use of local provenance but is not enforced due to more recent information from CSIRO that the negative effects of inbreeding depression outweigh that of outbreeding depression. The use of local provenance can still be required, depending on the situation, but the decision is often left to the seed collectors (Senior Vegetation Ecologist, Environment and Sustainable Development Directorate, July 2012, personal communication).
New South Wales	N.S.W. Office of Environment & Heritage	The use of local provenance genetic material is a requirement for the attainment of a Section 132C licence to bring in new plant material &/or to collect seed for revegetation in Threatened Ecological Communities (TECs) and for threatened species in NSW. The use of local provenance is also a recommendation or specification of many restoration projects and grant applications (Scientific Licensing Officer, Wildlife Licensing and Management Unit, Office of Environment and Heritage, January 2012,

		personal communication).
Northern Territory	Natural Resources, Environment, the Arts and Sport	1. There are no requirements to use local provenance in restoration projects but some individuals may prefer its use (Program Manager, Natural Resources, Environment, the Arts and Sport, August 2012**,** personal communication). 2. There are no specific requirements for provenance selection but direction may come from either the Parks and Wildlife Service or the Department of Resources. Some individuals within the Parks division may prefer local provenance material. There is very little stipulation within the Resources Department, because revegetation success is difficult, especially if replanting tailings and often the only concern is that the stock is a species found in the area and that the provenance is from the wider area, i.e. the tropical savannah (CEO, Greening Australia N.T., July 2012, personal communication).
Queensland	Department of Environment and Heritage Protection	Using local genetic stock is no longer ‘flavour of the month’ and State Government guidelines that once stipulated a preference for locally-collected seed are no longer relevant (e.g. http://www.derm.qld.gov.au/environmental_management/land/mining/pdf/tech-guidelines-env-management-mining-d-8.pdf, 1995 and http://www.derm.qld.gov.au/wildlife-ecosystems/nature_refuges/pdf/koala-

		reveg-guideline.pdf, 2001). However, some guidelines still state a preference for local provenance but these are not mandatory. For example, the Department states in its guidelines for revegetation of post-mining sites '*Seed should preferably be collected from the site to ensure it is genetically adapted to local conditions*' (last modified 14 Oct 2009) (Senior Project Officer, Greening Australia, July, 2012, personal communication).
South Australia	Department of Environment and Natural Resources	1. The *Planting Indigenous Species Policy*, 2003, states '*Locally indigenous seed and plants are to be used whenever possible'* (Principal Advisor –Landscape Management, Department of Environment and Natural Resources, July 2012, personal communication).
Tasmania	Department of Primary Industries Parks Water and Environment	No policies exist concerning provenance in restoration. The Forest Practices Authority, an independent statutory body that administers the Tasmanian forest practices system on both public and private land, states in the Forestry Practices Code '*Seed to be sown should be collected from the stand to be felled or from the nearest similar ecological zone'.* This is interpreted to mean that local provenance is preferred but not mandatory: http://www.fpa.tas.gov.au/__data/assets/pdf_file/0020/58115/Forest_Practices_Code_2000.pdf. (Forest Practices Officer, SFM Forest Products, July 2012, personal communication).

Victoria	Department of Sustainability and Environment	No legislative requirement but the use of local provenance material can be required to receive government funding for revegetation contracts &/or planning applications. These are made on a case by case basis but if stipulated, it becomes a legal requirement. (Environment Research Co-ordinator, Department of Sustainability and Environment, July 2012, personal communication).
Western Australia	Department of Environment and Conservation	Focus is more on preventing the actions of removal and clearing of native vegetation rather than its rehabilitation or revegetation. The relevant Act (the Wildlife Conservation Act, 1950) needs updating. Using local provenance is considered 'best practice' and is sometimes a condition of approval for certain projects. (Botanist, Department of Environment and Conservation, July 2012, personal communication). For example, the current seed collection policy in State Forests is to collect locally (anecdotally, seed collection zones are within 15 kms from the proposed revegetation site) but a recent report by DEC recommends 'an eco-geographic' approach to widen seed collection zones (Millar et al. 2007).

Aims of thesis

The research described in this thesis investigated the ‘local is best’ paradigm using species from an assemblage known as the Cumberland Plain Woodland (CPW), from western Sydney, NSW. The CPW and Cumberland Plain Shale Woodlands are listed as Critically Endangered Ecological Communities under the *NSW Threatened Species Conservation Act* (1995) (N.S.W. Government Office of Environment & Heritage 2009) and under the federal *Environment Protection and Biodiversity Conservation Act 1999* (Australian Government Department of Sustainability 2009) respectively. The Cumberland Plain’s native vegetation is distributed in highly fragmented landscapes, with only 13% remaining, compared to pre-European times (N.S.W. Department of Environment Climate Change and Water 2010). This thesis provides empirical support to underpin seed-sourcing guidelines in general, but particularly for the CPW. It is the first study to investigate the role of local provenance for multiple species within a whole vegetation community and the first to select species commonly found in western Sydney. The study species are representative of each vegetation layer present in the CPW (trees, shrubs and ground covers) (Table 2), are either community dominants and/or are widely used in CPW restoration projects, and are widely distributed throughout eastern Australia.

Thesis scope and structure

This thesis comprises a series of papers investigating the role of local provenance in restoration ecology, in the context of future climate change. A ‘thesis by publication’ format has been applied to all data chapters (Chapters 2-5 inclusive,) but not including the introduction (this chapter) or the conclusion (Chapter 6). Currently, Chapters 2 and 5 have been accepted for publication and it is my intent to also submit Chapters 3 and 4 to peer-reviewed journals.

Table 2. Nomenclature and life history details of study species

	Family	Life form	Longevity
Acacia falcata *Willd*	Fabaceae: Mimosoideae	Shrub	5–20 years
Bursaria spinosa **ssp *spinosa*** *Cav*	Pittosporaceae	Shrub	≤ 60 years
Eucalyptus crebra F. Muell	Myrtaceae	Tree	100-200 years
E. tereticornis Sm	Myrtaceae	Tree	≤ 200 years
Hardenbergia **violacea** (Schneev.) Stearn	Fabaceae: Faboideae	Vine	5–25 years
Themeda australis (R.Br.) Stapf	Poaceae	Grass	Indefinite

Chapter 2

Testing the 'local provenance' paradigm: a common garden experiment in Cumberland Plain Woodland, Sydney, Australia

The 'local is best' paradigm was investigated by comparing the performance of plants grown from locally-sourced seeds with those from non-local seed sources (provenances) within a common garden experiment. Six species were selected that represent a range of life histories (*Acacia falcata, Bursaria spinosa* ssp. *spinosa, Eucalyptus crebra, E. tereticornis, Hardenbergia violacea* and *Themeda australis)* from an assemblage known as the Cumberland Plain Woodland, a threatened community in western Sydney. Multiple provenances were collected from within the range of each species and grown at two field sites on the Cumberland Plain. Growing time varied between species and ranged from seven months to two years. Survival, growth, leaf morphology, herbivory and where appropriate, flowering traits were measured. This chapter has been accepted for publication in *Restoration Ecology* (August 2012).

Chapter 3

What role does 'home-site' advantage play in restoration ecology under heatwave conditions?

The 'local is best' paradigm was investigated under predicted summer temperatures for 2050 in western Sydney by comparing the establishment success of *E. tereticornis* and *T. australis* seedlings grown from local vs non-local seed sources. Four provenances (including one local) were collected from within the range of each species and grown at a field site on the Cumberland Plain. Open top chambers were used to simulate the future temperature conditions. The seedlings were grown under the temperature treatment for approximately 17 weeks. Survival, growth (non-reproductive and where appropriate, reproductive) for both species and leaf SLA and herbivory for *E. tereticornis* were measured. These species were chosen to complement the results for two of the species investigated in Chapter 2 and because they are both relatively quick growing. *E. tereticornis* was also chosen due to its low mortality rate, regardless of provenance, as demonstrated in Chapter 2. *T. australis* was one of the two species to demonstrate some home-site advantage in Chapter 2 and further investigation is warranted. This paper is intended for submission to *Austral Ecology.*

Chapter 4

Intraspecific responsiveness to elevated CO_2 of two widespread native Australian species, *Acacia falcata* and *Eucalyptus crebra*

Intraspecific differences in responsiveness to elevated CO_2 in survival and establishment were investigated in *A. falcata* and *E. crebra.* Three provenances (including one local) were collected from within the range of each species and grown in glasshouses under ambient and *c.* 550 ppm CO_2 to reflect projected atmospheric CO_2 levels in 2050. Plants were subjected to the CO_2 treatment for an average of 71 days for *A. falcata* and 54 days for *E. crebra***.** Survival, growth, leaf SLA and C: N ratio for both species and nodulation traits for *A. falcata* were measured. These species were chosen to complement the results for two of the species investigated in Chapter 2 but also because they are both C_3 plants (to allow response comparisons between two species). In addition, *A. falcata* was chosen to investigate the response to elevated CO_2 of a nitrogen-fixing legume. *E. crebra* was chosen because it is a relatively slow growing plant, thereby reducing potential confounding caused by root effects. This paper is intended for submission to the *Australian Journal of Botany.*

Chapter 5

How far is it to your local? A survey on local provenance use in New South Wales

Understanding the current usage of local provenance is pivotal to discussions on its appropriateness under a rapidly changing environment. The results of an on-line survey of restoration participants in New South Wales on attitudes and practices in relation to the use of local provenance are presented. Implications of the survey for potential changes to guidelines to better prepare for anticipated changing conditions are discussed. This chapter was published in *Ecological Management and Restoration* (September 2012).

Chapter 6

Summary discussion and conclusions

This chapter includes a general discussion and synthesis of the main findings of each chapter, in the context of the published literature, and suggests potential research directions for the future.

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CHAPTER 2

Testing the "local provenance" paradigm: a common garden experiment in Cumberland Plain Woodland, Sydney, Australia

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Abstract

Seed for restoration projects has traditionally been sourced locally to "preserve" the genetic integrity of the replanted site. Plants grown from locally-sourced seeds are perceived to have the advantage of being adapted to local conditions, and the use of local provenance is a requirement of many restoration projects. However, the processes of climate change and habitat fragmentation, with the subsequent development of novel environments, are forcing us to reconsider this basic tenet of restoration ecology. We tested the 'local provenance is best' paradigm, by comparing the performance of plants grown from local with non-local seed sources within a common garden experiment. We selected six species representing a range of growth forms (*Acacia falcata, Bursaria spinosa* ssp. *spinosa, Eucalyptus crebra, E. tereticornis, Hardenbergia violacea* and *Themeda australis*) from an assemblage known as the Cumberland Plain Woodland, a threatened community in western Sydney. Multiple provenances were collected from within the range of each species and grown at two field sites on the Cumberland Plain. Growing time varied between species and ranged from seven months to two years. With the exception of *B. spinosa,* and to a lesser extent *T. australis,* we found little evidence that local provenance plants were superior to distant provenances in terms of survival and establishment.

Keywords

Adaptive potential, home-site advantage, local adaptation, local superiority, restoration, seed source

Introduction

The changing environment poses many challenges for restoration ecology. Small populations in fragmented landscapes will be particularly vulnerable to rapid change in the future. Genetic diversity within these populations is a key consideration for climate change adaptation strategies but many seed-sourcing guidelines used by restoration practitioners do not allow for the incorporation of a broad range of genotypes in restoration projects (N.S.W. Department of Environment and Conservation 2005; State of Minnesota 2010).

Traditionally, it has been considered desirable to use seeds collected within a defined radius of the restoration site to "preserve" the genetic integrity of the replanted site. Plants sourced from local seed (hereafter referred to as 'local provenance') are generally assumed to be better adapted to local conditions, with superior survival and faster growth rates conferring a greater probability of restoration success. In addition, the use of non-local provenance is considered to increase the potential for the negative effects of outbreeding depression (Edmands 2007) and to initiate unplanned gene flow into neighboring populations either by hybridization between sub-species (Sampson & Byrne 2008; Millar et al. 2012), or by 'cryptic' invasions (Hufford & Mazer 2003 and references therein). This may result in maladapted offspring and altered trophic interactions with associated organisms (Vander Mijnsbrugge et al. 2010 and references therein). Increasingly, these potential negative impacts are being weighed against the positive effects of avoiding inbreeding depression (Broadhurst et al. 2008; Lopez et al. 2009) and a recent review concluded that current concerns about outbreeding depression are excessive (Frankham et al. 2011). Furthermore, there is a growing recognition that the exclusive use of local material may hinder adaptive potential in the face of a rapidly changing climate (Weeks et al. 2011 and references therein). A broader approach to seed sourcing has been adopted by some restoration practitioners (e.g. Corangamite Seed Supply & Revegetation Network 2007; Native Seed Network 2011) but empirical evidence is needed to underpin improved guidelines.

Local adaptation of plants has previously been demonstrated over strong environmental gradients: altitudinal (Gimenez-Benavides et al. 2007) and latitudinal (Davis & Shaw 2001 and references therein), and in novel environments such as polluted soils (Antonovics & Bradshaw 1970). A recent meta-analysis found that local adaptation is common, but if large environmental gradients between sites are used, the frequency and magnitude of local adaptation may be overestimated due to a sampling bias caused by a priori expectations (Hereford 2009).

The few empirical studies of local adaptation undertaken in Australia have generally produced equivocal results, ranging from weak or no evidence of home-site advantage (Price & Morgan 2006; Byars & Hoffmann 2009) to evidence of selective local adaptation (Byars et al. 2007; O'Brien & Krauss 2010; Waters et al. 2011). Forestry provenance trials on Australian eucalypts have confirmed significant intraspecific variation for some traits and species (Duncan et al. 2000) and have also found that for breeding purposes, the local seed source may not be the best performer at its home site (Raymond & Namkoong 1990).

We compared survivorship and early growth of plants grown from local vs non-local seed sources using six species typical of the Cumberland Plain Woodland community in western Sydney, New South Wales. The species selected (*Acacia falcata* (Hickory Wattle), *Bursaria spinosa* ssp. *spinosa* (Blackthorn) (hereafter referred to as *B. spinosa*), *Eucalyptus crebra* (Narrow-leaved Ironbark), *E. tereticornis* (Forest Red Gum), *Hardenbergia violacea* (False Sarsparilla) and *Themeda australis* (Kangaroo Grass)) are either community dominants and/or are commonly used in Cumberland Plain Woodland revegetation programs and represent a range of different life history traits (Table 1 & Appendix Table 1). All six species have a wide geographic distribution along the east coast of Australia; across seasonal and biotic boundaries (Appendix Figure 1). Their ranges include tropical to temperate and mesic to dry conditions and therefore, would be expected to express clinal patterns in phenotype that may be due to genetic differences and possibly local adaptation. *B. spinosa* and *H. violacea* extend into South Australia and Tasmania, although discontinuously for the latter. A prostrate and a climbing form of *H. violacea* are known (Harden 1991). *Themeda australis* is found throughout the continent, is a polyploid complex (Hayman 1960) and different nomenclature is used in different States.

The Cumberland Plain is Australia's fastest growing and most populous region. Only 13% of its native vegetation remains, distributed in highly fragmented landscapes. This vegetation has attracted unprecedented investment in recovery efforts (N.S.W. Department of Environment Climate Change and Water 2010). As part of the Cumberland Plain Recovery Plan, 2011, a research priority is to investigate the benefits, or otherwise, of introducing new genetic material into the fragmented remnants through restoration (N.S.W. Department of Environment Climate Change and Water 2010). This study aims to test the "local is best" paradigm and to contribute to the identification of seed collection areas suitable for revegetation efforts.

Table 1. Nomenclature and life form details for six perennial species used in the common garden experiment (*T. australis* is a synonym of *T. triandra. T. australis* is commonly used in NSW and is used in this paper).

Species	Family	Life Form
Acacia falcata Willd	Fabaceae: Mimosoideae	Shrub
Bursaria spinosa ssp. *spinosa* Cav	Pittosporaceae	Shrub
Eucalyptus crebra F. Muell	Myrtaceae	Tree
E. tereticornis Sm	Myrtaceae	Tree
Hardenbergia violacea (Schneev.) Stearn	Fabaceae: Faboideae	Vine
Themeda australis (R.Br.) Stapf	Poaceae	Grass

Methods

Study sites and species

The Cumberland Plain, western Sydney (33° 30 - 34° 30 S and 150° 30 - 151° 30 E) is an undulating landscape, ranging in elevation from just above sea level to ~350 m (N.S.W. Government Office of Environment & Heritage 2009). The deep clay soils are derived from the Wianamatta Group of shales and alluviums that retain moisture and have higher nutrient levels than the surrounding landforms (Benson & Howell 1990). Average annual rainfall is 700–900 mm, most of which falls during summer. Maximum daily temperatures of 44.8 °C and minima of -1 °C have been recorded in western Sydney (Bureau of Meteorology 2011).

Cumberland Plain Woodland (CPW) typically consists of an open tree canopy of large, mostly *Eucalyptus* species (often dominated by *E. crebra, E. tereticornis* and *E. moluccana)*, a diverse grassy groundcover (often dominated by *Themeda australis* and *Microlaena stipoides)* and depending on the local fire regime, a shrub layer (often dominated by *Bursaria spinosa)* (Benson & Howell 1990). The CPW and Cumberland Plain Shale Woodlands are listed as Critically Endangered Ecological Communities under the *NSW Threatened Species Conservation Act* (1995) (N.S.W. Government Office of Environment & Heritage 2009) and under the federal *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) (Australian Government Department of Sustainability 2009) respectively, which provide some legislated protection for its conservation.

Common garden experiments were established at two field sites on the Cumberland Plain, approximately 20 km apart: the Australian Botanic Garden, Mount Annan (MtA) (34° 04′ S, 150° 46′ E) and at Western Sydney Parklands, Cecil Hills (CH) (33° 53′ S, 150° 49′ E). The soils on both sites are derived from Bringelly shale of the Wianamatta Group (Clark & Jones 1991) and are broadly similar (analysis performed at Sydney Environmental & Soil Laboratory, Sydney, data not shown). Seeds of each species were obtained commercially from five different geographical locations (provenances); one from the Cumberland Plain (hereafter referred to as the local provenance) and the rest from widely distributed locations within each species' geographic range (Appendices Figure 1 & Table 2). Information regarding seed collection procedures i.e. size of population, number of mother plants and the exact location of the original seed collection sites was not available from all of the commercial suppliers. Provenances were largely selected due to availability but they are generally representative of the lower temperature and rainfall areas of the core climatic envelopes that these widespread species occupy, especially for *E. crebra* and *T. australis* (Appendix Figure 2).

For all species, multiple seeds were germinated and plants were established from the largest and most-closely timed early germinants at the Macquarie University glasshouses. The method used during the processes from germination (including potting into 50 x 125 mm pots) until field transplantation, was uniform for each species and was broadly in line with that used by commercial native plant nurseries. Only three of the four non-local provenances of *B. spinosa* germinated successfully. The sites were prepared by slashing the existing weed cover and spraying with Roundup herbicide (Monsanto Company, St. Louis, M0, USA), according to the manufacturer's instructions. Pre-swollen water crystals and a slow release fertilizer (Osmocote Plus Native Gardens, NPK: 17:1.6:9.7, Scotts Australia P/L, Sydney, NSW, Australia) were placed in the bottom of the hole before planting. Planting occurred in April and May 2009, at an average age of three months. Each species was planted as a group, with provenances randomly distributed within the group, approximately 1m apart. Minor flooding occurred at the CH site and necessitated the replanting of *A. falcata*, *B. spinosa* and *H. violacea*, completed by late August 2009. Plants that died within one month of planting were replaced. All plants were watered at the time of planting with minimal supplementary watering. Both sites were mulched and occasionally hand weeded during the first 12 months of the experiment. Unless otherwise specified, 100 replicates of each species (20 per provenance) were planted (Appendix Table 2). Experiment duration varied between species, ranging from 7-24 months, to avoid shading or in the case of the vine, *H. violacea,* from intermingling with other plants (Appendix Table 3).

Data collection

<u>Survivorship</u>: Plants were assessed as 'alive' (green leaves &/or stem) or 'dead' (no green anywhere on the plant or plant missing). Plants were scored on a weekly basis during 2009, monthly during 2010 and quarterly during 2011. Survivorship was the only measurement taken for the following provenances: Illabo (*B. spinosa)* at CH, Denver (*H. violacea)* and Manilla *(T. australis)* at CH due to high mortality.

<u>Growth Traits</u>: *Stem height*: Plants were measured *in situ* from ground to apical meristem. Leaf length was measured for *T. australis* by averaging the five longest leaves (non-culms), measured from base to the tip. *Stem diameter*: Stems were cut at ground level and two measurements were taken at right angles and averaged. The *in situ* basal circumference of the clump was measured for *T. australis*. *Aboveground total biomass*: After harvest, plant material was dried for at least two days at 70° C then weighed.

<u>Phenology</u>: *Percentage of flowering plants***:** At harvest, plants were scored as "flowered" if inflorescences, capsules or pods were visible. This was cross-referenced with data collected periodically for 'time to flowering'. *Time to flowering:* Plants were scored on a weekly basis from June to July 2010 for

A. falcata when stamens were visible and from September 2009 to May 2010 for *T. australis* when seed heads were visible.

Morphology: *Specific Leaf Area (SLA).* Fresh leaves were collected as per Cornelissen et al. (2003), scanned and their area measured using ImageJ software (http://rsb.info.nih.gov/ij/download.html) before being oven-dried and weighed. SLA was calculated as leaf area (mm^2) per unit biomass (mg). *Leaf width: length ratio***:** Lamina length along the midvein and lamina width at the widest point was measured with ImageJ using the same leaves as for SLA. *Lignotuber***:** Plants were scored as having a lignotuber present if the lignotuber was visible above ground or could be felt at the base of the stem. *Branching:* The number of branches arising from the main stem that were greater than 10cm in length were counted. If the main branch split into two, only the branches from a predetermined stem were counted.

Herbivory: The total amount of defoliation and leaf necrosis per plant was visually assessed at the time of harvest and scored: 0-1%, 1-5%, 5-25%, 25-50%, 50-75% and >75% for all plants except for *H. violacea*. Due to the prostrate habit of *H. violacea,* the data presented here is from a random sample of ten mature leaves per plant, visually assessed for the percentage of area lost due to chewing and sucking herbivores.

Details of measurements taken for each species are shown in Appendix Table 3.

Statistical Analyses

The local population was defined as being superior to or having better performance than non-local provenances if there was a significant difference between the provenances such that the local provenance had the highest survival or growth compared to non-local provenances. This definition was also used to calculate the frequency of local superiority relative to the number of measurements taken. For example, the frequency of local superiority for survival = N/T, where N is the number of times local provenance significantly survived the longest and T is the total number of survival measurements.

Differences in performance between provenances were compared using a general linear model with provenance as a fixed and site as a random factor, with their interaction included in the model. A Dunnett's *post hoc* test was used to compare means where the provenance effect was significant. Data were transformed where necessary to meet the assumptions of normality and homoscedasticity. When this was not achievable, sites were analyzed separately using a Kruskal-Wallis test. There were only two provenance comparisons for *B. spinosa* at the CH site, so each site was analyzed separately for this species. Survival and Time to Flowering distributions were compared using Kaplan-Meier estimates (Log Rank Chi-Square test). Chi-Square tests were used to test for differences in herbivory, presence of lignotuber and percentage of flowering plants. For all analyses, significance was determined at $p<0.05$ for provenance and site was removed as a factor when $p>0.25$.

Results

Survivorship

There were no significant differences in survivorship between provenances of *A. falcata, E. crebra* and *E. tereticornis*: all *E. tereticornis* and 97% of *E. crebra* plants survived until harvest. This contrasted with only 2% survival of *A. falcata* plants, possibly due to root rot caused by water-logging (P. Cuneo, 2010, The Australian Botanic Garden, personal communication). Whilst there were significant differences between the provenances for *H. violacea*, (Figure 1a & 1b), the differential mortality was due to plants sourced from one non-local provenance dying significantly earlier at both sites, compared to the other four provenances. The local provenance was equal longest survivor at the Cecil Hills (CH) site and second longest survivor at the Mount Annan (MtA) site. For the remaining two species, *B.a spinosa* and *T. australis*, plants sourced from local provenances survived significantly longer than those from non-local provenances at both sites (Figure 1c & 1d and 1e & 1f). Overall, the frequency of local superiority was 0.33 i.e. the local provenance survived significantly longer only four times out of a possible 12 counts.

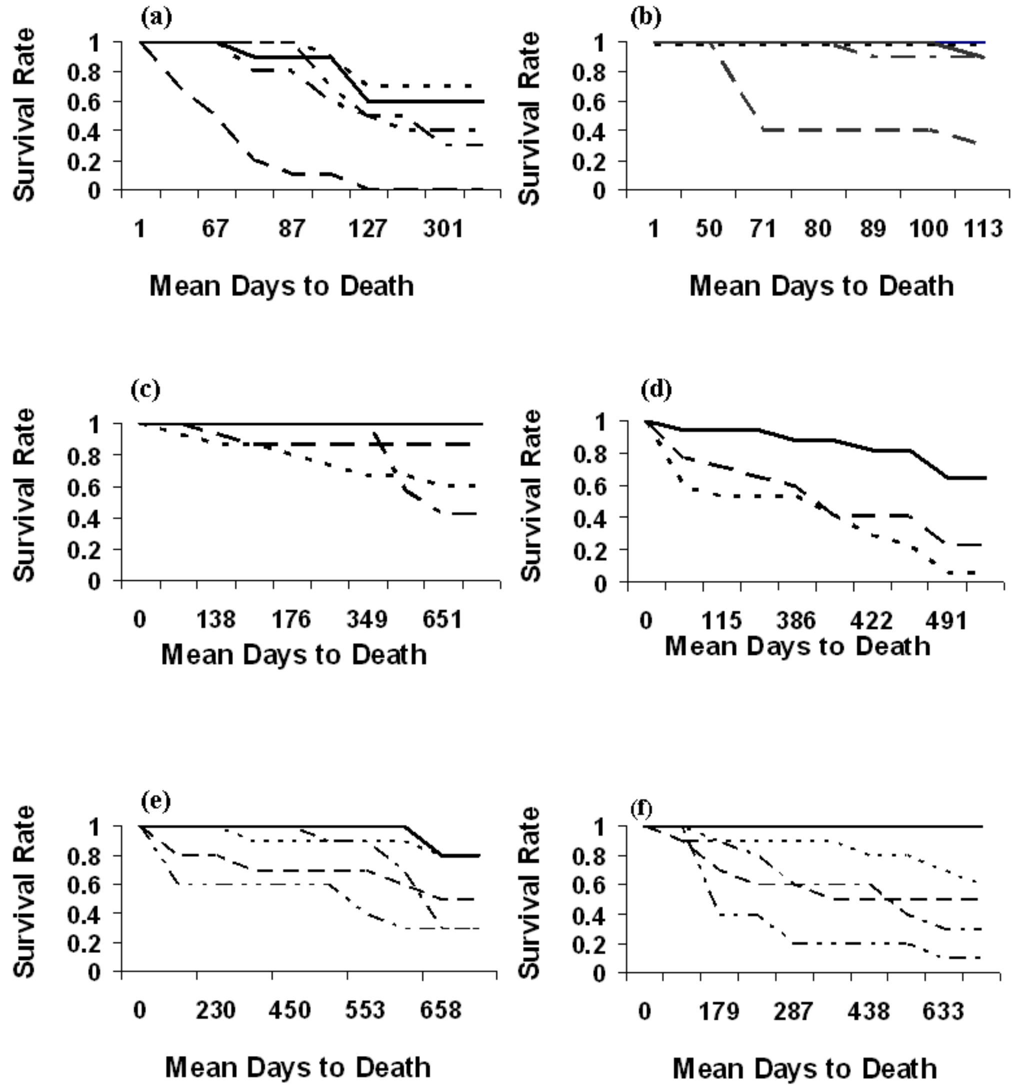

Figure 1. Proportion of surviving plants (Mean Days to Death) at the Mount Annan (MtA) & Cecil Hills (CH) sites respectively for provenances of: *Hardenbergia violacea:,* (a) $p<0.001$ and (b) $p<0.001$. ▬▬ Local, __ __ Denver,__ - __ Bathurst, ------- Nabiac, __ - - __ Manilla.(N.B. Nabiac & Local both had 100% survivorship at CH). *Bursaria spinosa:* (c) $p<0.012$ and (d) $p<0.001$: : ▬▬ Local, __ __ Port Campbell, ------- Illabo, __ - __ Jerilderie. *Themeda australis,* (e) $p<0.014$ and (f) $p<0.001$ ▬▬ Local, __ __ Gower, ------- Bethungra, __ - __ Fern Bay, __ - - __ Manilla. *p* value = log rank chi-square test statistic.
(Data not shown for non-significant differences between provenances of *Acacia falcata, Eucalyptus crebra* & *E. tereticornis*)

Table 2. Growth data by species. Provenance mean is underlined if significant differences were found between local and non-local provenances. Sites (MtA & CH) were analyzed separately for *Bursaria spinosa* due to only two provenances surviving at the CH site and for *Themeda australis* when the data could not be transformed for ANOVA analysis and Kruskal-Wallis analysis was used. Only one plant from the Manilla provenance of *T. australis* survived and this was removed from the growth analysis. Measurement units: stem height, stem diameter, leaf length & clump circumference (cm) and above-ground total biomass (biomass) (g).

	Provenance mean (±SE)					***d.f.***	***F/H* value**	***P*-value**
			Acacia falcata					
Stem Height	Broke	**Local**	Sth NSW	Grafton	Nanango	4,4	2.96	0.159
	174 (6)	162.17 (7)	148.2 (8)	137.44 (9)	133 (9)			
			Bursaria spinosa					
Stem Height	**Local**	Illabo	PCampbell	Jerilderee				
Mt A	**176.1 (9.0)**	162.9 (14.1)	104.7 (8.61)	92.7 (5.2)		3,36	13.04	0.001
C Hills	**130.2 (7.9)**		87.8 (7.4)			1,13	9.07	0.010
Stem Diam	Illabo	**Local**	Jerilderee	PCampbell				
Mt A	**19.4 (2.2)**	18.3 (1.3)	10.3 (1.2)	10.0 (0.8)		3,36	12.63	0.001
C Hills		**12.7 (0.9**)		8.3 (0.4)		1,13	8.47	0.012
Biomass	Illabo	**Local**	Jerilderee	PCampbell				
Mt A	**249.8 (46.4)**	211.2 (28.3)	66.5 (17.6)	63.5 (11.8)		3,36	13.50	0.001
C Hills		**75.2 (11.0)**		30.23 (6.9)		1,13	6.20	0.027
			Eucalyptus crebra					
Stem Height	Halcomb H	Ashford	**Local**	Gilgandra	Manilla	4,4	8.76	0.029
	95.15 (6)	93.83 (6)	80.88 (7)	70.81 (5)	60 (9)			
Stem Diam	Halcomb H	**Local**	Gilgandra	Ashford	Manilla	4,4	19.364	0.007
	13.56 (.7)	12.15 (.7)	10 (.5)	9.9 (.7)	8.72 (.7)			
Biomass	Halcomb H	**Local**	Gilgandra	Ashford	Manilla	4,86	1.32	0.268
	58.3 (7)	54.45 (7.3)	53.47 (5)	48.5 (8)	41.9 (7)			

Table 2 Cont.

	Provenance mean (±SE)					*d.f.*	*F/H* value	*P*-value
			E. tereticornis					
Stem Height	Selection Fl	Dungog	Sth NSW	**Local**	Tenterfield	4,4	2.18	0.235
	144.97 (8.1)	132.3 (4.16)	131.95 (5.5)	131.25 (7.0)	117.8 (6.51)			
Stem Diam	Sth NSW	Selection Fl	**Local**	Tenterfield	Dungog	4,4	6.09	0.054
	27.33 (1.58)	27.13 (1.94)	26.68 (1.52)	22.29 (1.19)	24.36 (1.27)			
Biomass	Sth NSW	**Local**	Dungog	Tenterfield	Selection Fl	4,4	2.50	0.198
	242.2 (30.8)	210.3 (26.6)	187.8 (21.7)	180.3 (22.8)	162.3 (23.8)			
			Hardenbergia violacea					
Biomass	Nabiac	**Local**	Manilla	Bathurst		3,3	35.83	0.008
	122.7 (24.5)	107.1 (20.7)	71.9 (19.7)	42.3 (10.6)				
			Themeda australis					
Leaf length	Gowar	Bethungra	Fern Bay	**Local**		3,3	1.128	0.462
	64.22 (6)	57.71 (5)	55.2 (6)	54.28 (2)				
Clump circum	Gowar	**Local**	Bethungra	Fern Bay	Manilla			
Mt A	**70.1 (8)**	42.5 (1.7)	37.6 (3)	37.0 (6)	36.0 (4)	4,22	8.36	0.000
C Hills	38.3 (10)	**38.9 (3)**	31.8 (2)	16.3 (3)		3	8.33	0.04
Biomass	Gowar	**Local**	Manilla	Bethungra	Fern Bay			
Mt A	**318.7 (86)**	134.4 (14)	114.8 (8)	108.0 (4)	85.6 (11)	4	8.47	0.076
C Hills	**111.7 (64)**	83.5 (23)		55.84 (9)	6.67 (2)	3	7.19	0.066

Growth: Stem height, stem diameter and total aboveground biomass

Local provenance plants exhibited consistently superior growth in only one of the six species (Table 2). Locally-sourced *B. spinosa* plants showed superior performance for all three growth measurements at the CH site but this comparison was made against only one non-local provenance. At the MtA site, where the local provenance was compared to three non-local provenances, the local was the largest only for mean stem height. The local provenance of *T. australis* had the largest mean basal circumference at one site only, with a non-local provenance having superior growth for all other measurements. Significant differences between the provenances were recorded for *E.s crebra* and *H. violacea* but the local provenance did not display the greatest growth in either case. For *H. violacea*, only biomass was measured due to its prostrate growth habit. Overall, the frequency of local superiority was calculated at 0.26: i.e. the local provenance grew significantly larger only five times out of a possible 19 counts.

Phenology

<u>Percentage of Flowering Plants</u>

Three of the species flowered during the course of the experiment but only one demonstrated local superiority. On average, the local provenance plants of *B. spinosa* had significantly more flowering plants than two non-local provenances at the MtA site and one non-local at the CH site (Figure 2a). There were significant differences between the provenances for *A. falcata* but the local provenance, on average, did not have the most flowering plants (Figure 2b). There were no significant differences between the provenances of *T. australis* ($\chi^2_{(4)}$=6.577, *p*=0.1600) with plants from two provenances, including the local, achieving 100% flowering at both sites. Overall, the frequency of local superiority was calculated at 0.40.

<u>Time to Flowering</u>

Time to flowering was recorded only for *A. falcata* (Figure 3a) and *T. australis* (Figure 3b). For *A. falcata* at the MtA site, the southerly provenances flowered, on average, significantly earlier than the northerly provenances with the local provenance plants the earliest to flower. Only the southerly provenances flowered at the CH site and in contrast to the MtA site, there were no significant differences between the provenances and the local provenance plants were not the earliest to flower. For *T. australis* at the MtA site, the local provenance plants flowered significantly earlier than all of the non-local provenance plants. At the CH site, only three provenances flowered and whilst the local provenance plants flowered the earliest, in contrast to the MtA site, it was only significantly earlier than one non-local provenance.

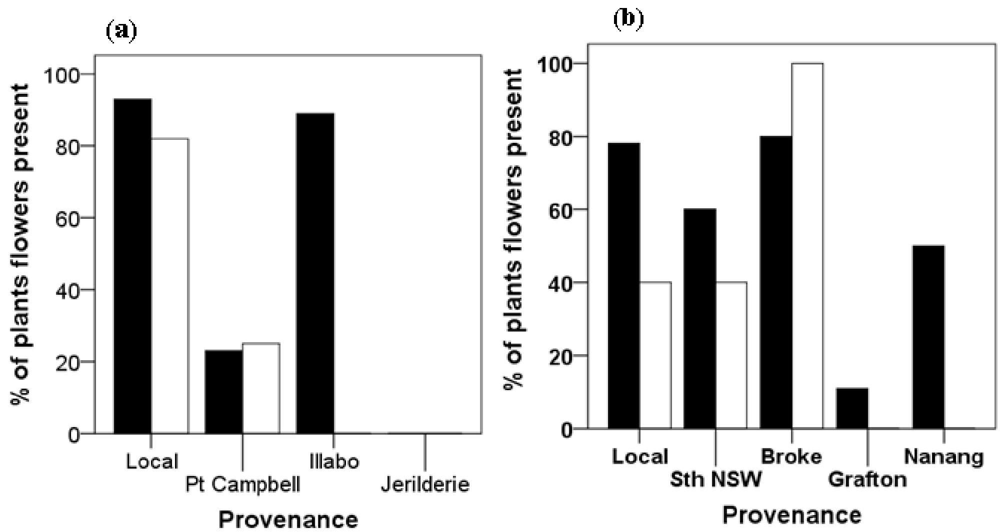

Figure 2. Percentage of plants of the different provenances that flowered (flowers present or absent) at Mount Annan (MtA) (black bars) and Cecil Hills (CH) (white bars) for (a) *Bursaria spinosa* (MtA $\chi^2_{(3)}$=22.38, $p \leq 0.001$: CH ($\chi^2_{(1)}$= 4.261, p=0.039) and (b) *Acacia falcata* (MtA $\chi^2_{(4)}$=11.64, p=0.0203: CH $\chi^2_{(4)}$=25.85, $p \leq 0.001$). No plants from the Jerilderie provenance of *B. spinosa* were planted at the CH site.

Morphological Traits

Significant differences were recorded between the provenances for many morphological traits but, with the exception of *B. spinosa,* the local provenances were always within the range of variation. The local plants and those from one non-local provenance had significantly smaller leaf width: length ratios than plants from two non-local provenances (Table 3).

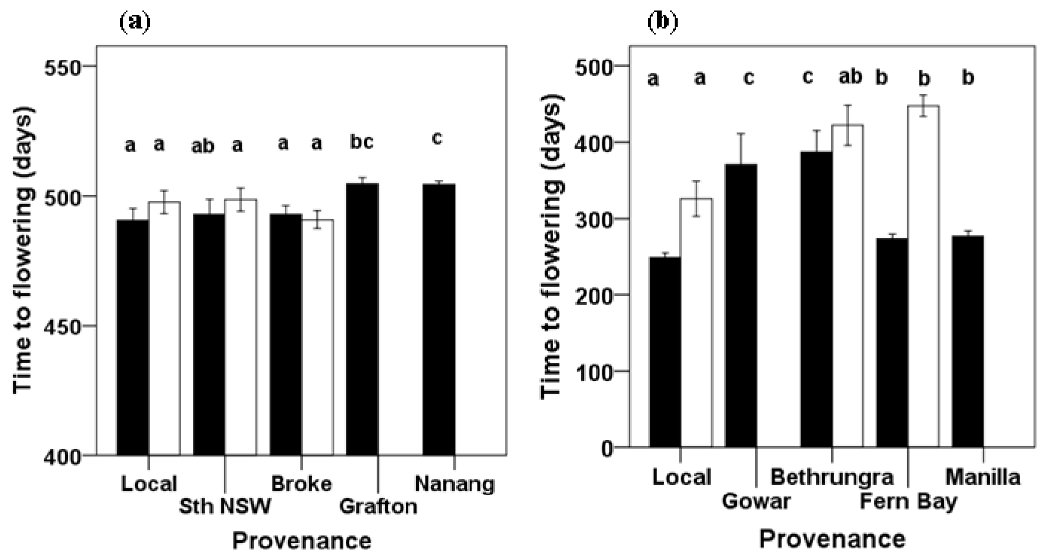

Figure 3. Mean (± SE) time to flowering for (a) *Acacia falcata* (MtA $\chi^2_{(4)}$=12.501, *p*=0.014: CH $\chi^2_{(4)}$=21.63, *p*<0.001) and *(b) Themeda australis* (MtA $\chi^2_{(4)}$=29.15 *p*≤0.001: CH $\chi^2_{(4)}$=19.54, *p*≤ 0.001) provenances grown at Mount Annan (MtA) (black bars) and Cecil Hills (CH) (white bars). Provenances sharing the same lower-case letters are not significantly different as indicated by the overlap of 95% confidence intervals. There were no flowering plants at the CH site for *A. falcata* provenances Grafton and Nanango and for *T. australis* provenances Gowar and Manilla. Note the truncated y-axis in (a).

Herbivory

A significant difference in herbivory between provenances was found only for *E. crebra* ($\chi^2_{(16)}$=28.46, *p*=0.028) (data not shown). Leaves from plants sourced from the non-local provenance with the smallest leaf width: length ratio had the least amount of herbivory (~5%) and plants from the slowest growing non-local provenance suffered the most damage (15-20%).

Site

There were significant differences between the two sites in almost all cases (*p*<0.25). Generally, survival and growth performances were lower at the CH site, most likely due to occasional water-logged conditions.

Table 3. Variation in morphological traits: Leaf width: length ratio (W:L), Specific Leaf Area (SLA), Lignotuber present or absent and number of main branches for *Bursaria spinosa, Eucalyptus crebra, E. tereticornis* and *Hardenbergia violacea.* Significant differences between local and non-local provenances indicated in bold (*p*<0.05). Sites (MtA & CH) were analyzed separately for *Bursaria spinosa* due to only two provenances surviving at the CH site and for *Eucalyptus crebra* when sites were analyzed separately using a chi-square test. ^{1}, ^{2}:represent the Mount Annan and Cecil Hills sites respectively.

	W: L	***SLA***	***Lignotuber (p/a)***	**Number of Branches**
Bursaria spinosa	$^{1}F_{(3,36)}=29.035$, $\boldsymbol{p<0.001}$ $^{2}F_{(1,13)}=2.22$, $\boldsymbol{p<0.001}$	$^{1}F_{(3,35)}=1.36$, $p=0.272$ $^{2}F_{(1,13)}=0.11$, $p=0.751$	n.a	n.a
Eucalyptus crebra	$F_{(4,82)}=14.41$ $p<0.001$	$F_{(4,4)}=8.17$ $p=0.061$	$^{1}\chi^2_{(4)}=3.89$, $p=0.422$: $^{2}\chi^2_{(4)}=3.48$, $p=0.481$	$F_{(4,4)}=8.33$, $p=0.032$
E. tereticornis	$F_{(4,89)}=33.78$, $p<0.001$	$F_{(4,89)}=5.39$, $p=0.001$	$\chi^2_{(4)}=12.22$, $p=0.016$	$F_{(4,94)}=3.47$, $p\leq0.011$
Hardenbergia violacea	$F_{(3,45)}=26.42$ $p<0.001$	$F_{(3,45)}=2.38$, $p=0.082$	n.a	n.a

Discussion

This study found equivocal evidence for superiority of local provenance plants during the establishment phase. Whilst the local provenance plants were sometimes equal top performers (and therefore not superior), we found that with the exception of *B. spinosa,* and to a lesser extent *T. australis,* the local provenance plants rarely demonstrated superiority overall. The frequency of local superiority was 0.33 for survivorship, 0.26 for growth and 0.40 for percentage of flowering plants (although not all species flowered). With the exception of leaf W:L ratio in *B. spinosa,* the local provenance plants were always within the range of variation for morphological traits and herbivory.

Intraspecific variation was evident within each species, with significant differences between the provenances demonstrated for many traits. The only published provenance trials conducted in Australia for any of the species investigated are for *T. australis*. Waters et al. (2005) found mixed home-site advantage for survival, and Groves (1975) found few significant differences between provenances in the number of tillers between five populations spread across a 14° longitudinal band in south-eastern Australia. Differences between provenances for flowering time in this widespread species are well known (Evans & Knox 1969; Groves 1975) and this was confirmed in our study. The local provenance plants of *T. australis* flowered significantly earlier than all non-local provenance plants at the MtA site. Whilst earlier flowering time does not necessarily confer a fitness advantage, it may have implications for trophic interactions (Vander Mijnsbrugge et al. 2010 and references therein).

The probability of detecting local superiority in our study may have been influenced by the timing of the census. The two species in which the local provenance demonstrated superior survival to non-local provenances (*B. spinosa* and *T. australis*) were also grown for the longest period (approximately two years). The results for *E. crebra* and *E. tereticornis* can be compared to longer-term forestry trials. Our findings are consistent with nine forestry trials spanning 3.3–13 years for five eucalypt species in which none of the local provenance trees had higher survival than those sourced from non-local provenances when grown at a common site (Johnson & Stanton 1993). However, a comparison of survival rates in two *E. pilularis* trials (for 38 and 104-months) found that although the local provenances did not survive the longest, over time, their performance improved (Raymond 2010), a trend which was not detected in our relatively short term study.

The two species that demonstrated local superiority, *B. spinosa,* and *T. australis,* were also the species with a history of taxonomic uncertainty and/or different ploidy levels. This raises the possibility that our comparisons were between subspecies or varieties within these species, rather than between provenances. This has implications for seed-sourcing zones and restoration success as gene flow between populations of species with ploidy differences and taxonomic uncertainly increases the likelihood of outbreeding depression and hybridization (Byrne et al. 2011; Frankham et al. 2011). For *T. australis*, it is beyond the scope of this paper to expand on its polyploid status but it has been established that some Sydney populations are tetraploid whilst many other populations in Australia are diploid (Hayman 1960). It is highly likely that provenances with different ploidy numbers were used in our study. *Bursaria spinosa* has long been the subject of taxonomic debate and its taxonomic status, based on morphology, was reviewed in 1999, which resulted in two subspecies currently being recognized (Cayzer et al. 1999). We obtained confirmation that all of the provenances used were of the same subspecies, *spinosa* (B. Wiecek, 2011, National Herbarium of New South Wales, personal communication) but we found that most of the traits we measured separated into two fairly distinct groups. In addition, differences in unrecorded traits (germination requirements, stem flexibility and habit differentials), also applied to this grouping. Whilst the local provenance of *H. violacea* did not show clear superiority, there is also some taxonomic uncertainty about this species; a prostrate and a climbing form are known (Harden 1991). From the provenances we studied, there appeared to be three morphological forms: prostrate round-leaved, climbing long-linear leaved and climbing round-leaved. These three forms were significantly differentiated, suggesting different ploidy levels may also exist within this species (L. Broadhurst, 2010, CSIRO, personal communication). Further investigation into taxonomic uncertainties and potential differences in ploidy levels and reproductive methods that may create problems in subsequent generations is warranted.

Several other factors may also explain our findings of equivocal evidence for superiority of local provenance plants. Firstly, there was a lack of statistical power for some analyses, including survivorship, and these results therefore need to be interpreted with caution. This includes the comparisons of *B. spinosa* plants at the CH site where the local provenance plants were only compared to one non-local provenance. Secondly, the suboptimal performance of some provenances may have been due to seeds being sourced from small populations. Information on population size was not available from all of the commercial seed suppliers and in some instances, only large regional areas could be given as the seed source location. This is a problem for practitioners who need this level of detail to enable informed decisions about appropriate seed sourcing. Thirdly, environmental maternal effects may have influenced the results of all species, particularly *H. violacea* because this species had the shortest growing period (eight months) (Roach & Wulff 1987). Lastly, it is possible that the results were affected by differences in seed age between provenances. However, germination was not assessed in our study and we should note that seed age varied most for Myrtaceae and Fabaceae species, two families in which seeds remain viable for longer than for many other families (Martyn et al. 2009).

The long term sustainability of restoration projects is under threat from a rapidly changing climate. This will present new challenges for restoration practitioners already faced with dwindling seed supplies, increasing fragmentation and the associated risks of sourcing seed from small populations. These challenges raise the need for a different approach to the traditional practice of the exclusive use of genetic material that has been collected within a defined radius of the restoration site. We found little evidence that restricting provenance choice to locally-collected seed will be detrimental to the establishment success for several Cumberland Plain Woodland (CPW) species. Longer-term field trials that investigate the transition from establishment to persistence will progress our findings. The inclusion of non-local stock (from populations with the same ploidy levels and taxon lineage) may provide potential for evolutionary adaptation in the highly fragmented CPW landscape in the face of climate change.

Implications for practice

- The common garden experiment did not detect strong evidence of local superiority for establishment success. This implies that the inclusion of non-local provenance material could be considered as an adaptation strategy to mitigate the effects of a changing environment by increasing evolutionary potential for the Cumberland Plain Woodland.
- A lack of seed collection detail will hinder informed decision making on suitable non-local seed sources. For example, details of population size and environmental conditions of the seed source and correct identification to subspecies and variety level will assist in the maximization of restoration success.
- Careful consideration should be given to seed sourcing in species known to have differing ploidy levels.

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Appendix

Appendix Table 1. Life history details for six perennial species used in the common garden experiment. Information accessed from: Benson & von Richter(undated)and general information on the genus obtained from Gibson et al (2011)[1] and Mortlock (2000)[2].

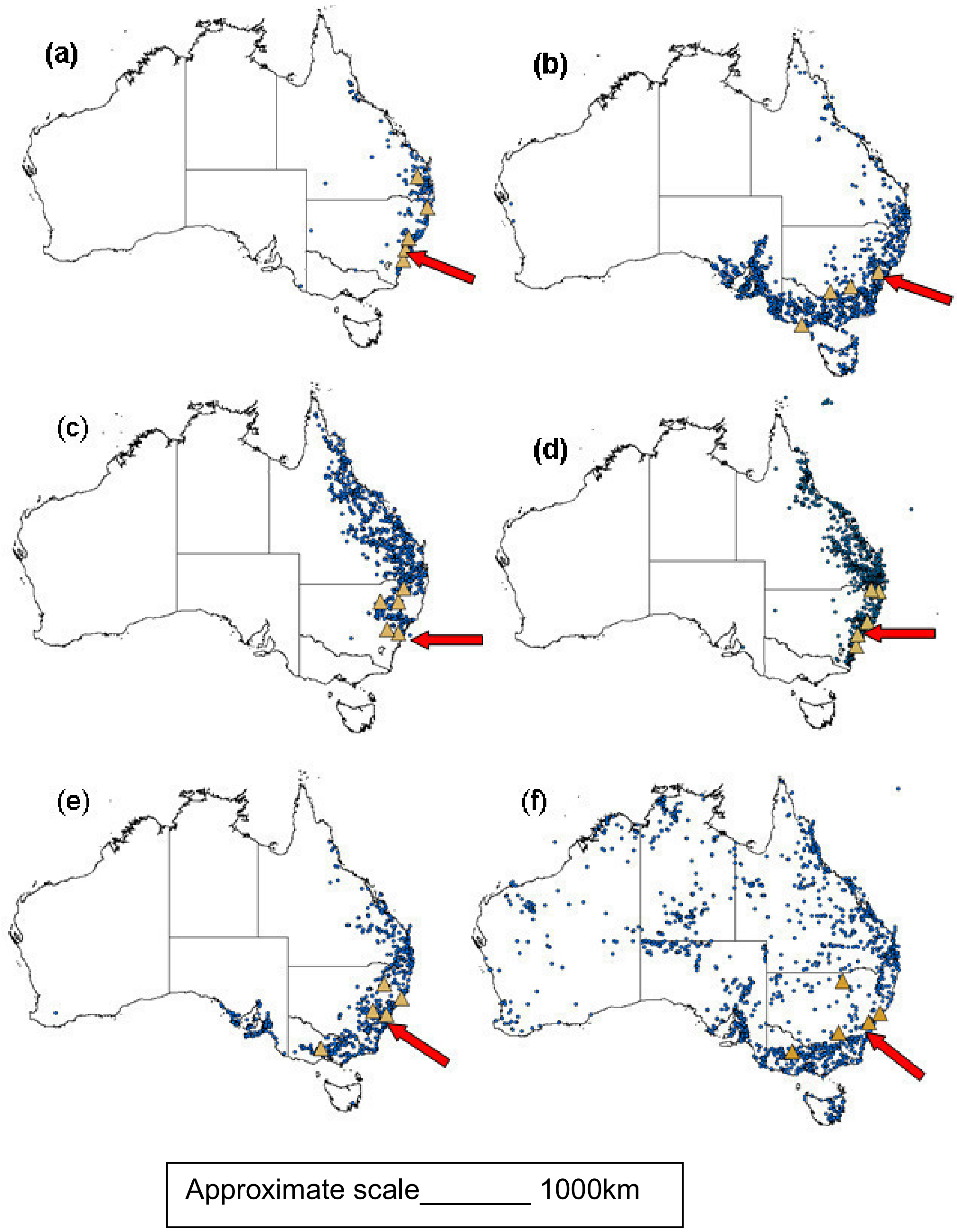
	Acacia falcata	***Bursaria spinosa* ssp. *spinosa***	***Eucalyptus crebra & E. tereticornis***	***Hardenbergia violacea***	***Themeda australis***
Pollination	Insects (bees & wasps) & birds[1]	Native bee *Chalicodoma derelicta*	Insect & vertebrates i.e. *Pteropus poliocephalus (E.t)*	Self, insects, birds[2]	Wind
Seed dispersal	Ants, gravity[1]	Wind	Wind /gravity	Ants, gravity[2]	Gravity/ possibly by adhesion
Longevity	5–20 yrs	≤ 60 years	100-200 years	5–25 yrs	Indefinite

Appendix Table 2. Location (lat/long) and climatic conditions of field collections of seed sources (provenances). Numbers in brackets represent the total number planted at both sites. Insufficient germinants of the Jerilderie provenance of *Bursaria spinosa* meant that replacement at the Cecil Hills (CH) site could not occur, so additional seedlings from the other provenances were planted. The exact location of the original seed collection sites was not available from all of the commercial suppliers. *Approximate location; where specific GPS readings not available, elevation and rainfall are estimates only.

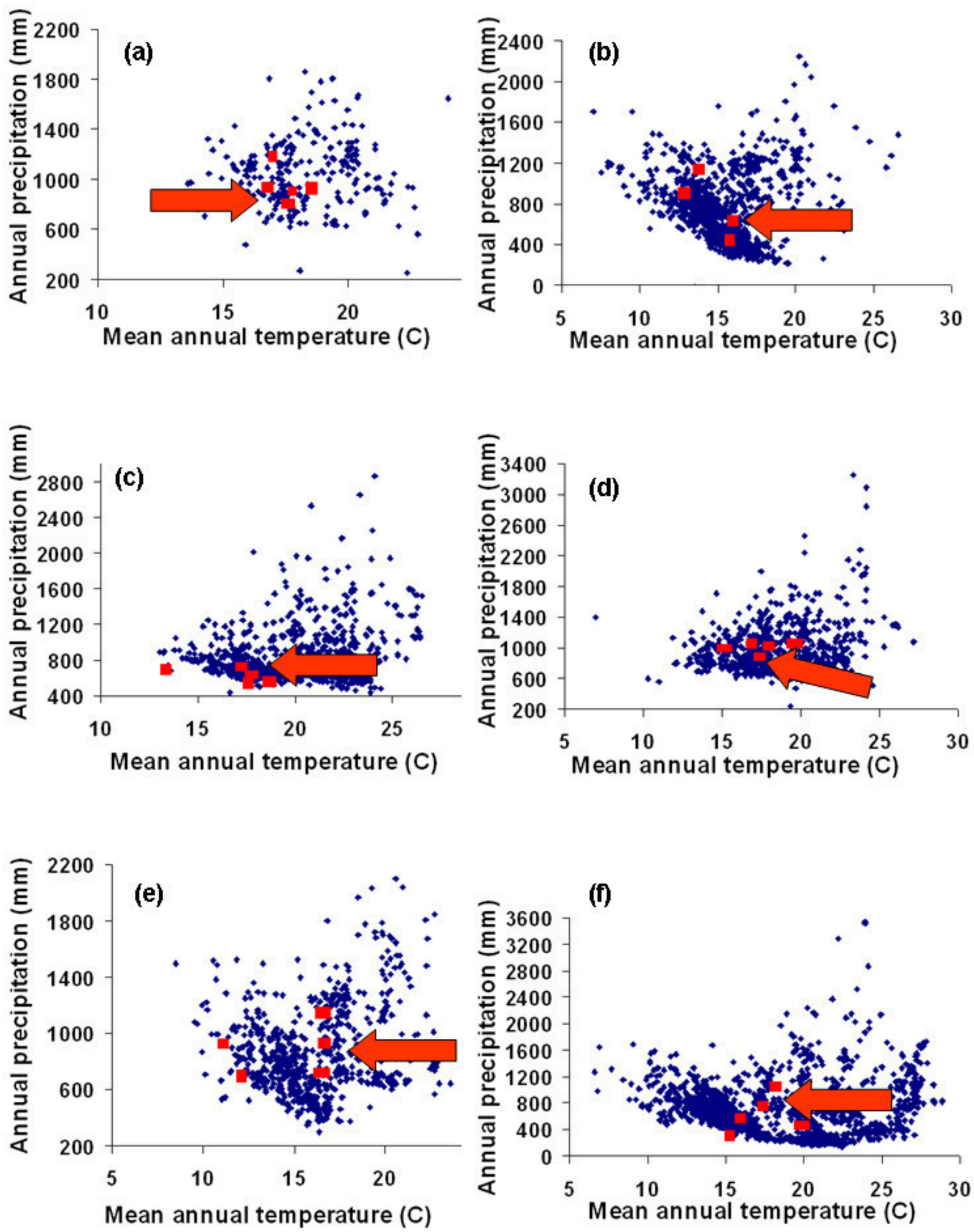
Provenance	Latitude S	Longitude E	Altitude (m)	Rainfall p.a.	Mean annual max/min temp C	Date seed collected
Acacia falcata (100)						
South Coast NSW(20)	*34 52	*150 36		*1240	*23/16	1/12/2006
Greendale, (Local) (20)	33 55 40	150 39 50	80	800	23.5/10.5	21/12/1998
Broke (20)	*32 44	*151 06	*115	650	25/11	1/11/1998
Grafton (20)	29 45 51	152 55 58	83	980	26.5/13	28/11/2004
Nanango (20)	26 46	151 54	500	785	25.5/10.5	5/12/1984
Bursaria spinosa (97)						
Port Campbell (30)	38 36 59	142 59 59	19	742	18/9.5	4/4/2008
Jerilderie (7)	*35 21	*145 44	109	390	23.5/10	4/2/2005
Illabo (30)	*34 49	*147 45	271	625	22/8	24/4/2005
Werrington, (Local) (30)	33 45 3	150 45 43	17	800	23.5/10.5	30/4/2007
Eucalyptus crebra (94)						
Mount Annan, (Local) (20)	34 4 9	150 46 4	133	800	23.5/10.5	13/2/2004
Gilgandra (20)	31 52	148 45	350	560	25/10	9/1/1996
Manilla (20)	30 44	150 52		620	24.5/9.5	12/2001
Halcomb Hill (20)	*32 09	*151 4	174	595	25/10	2/2/2006
Ashford (14)	29 23 0	151 02 0	566	685	26/8	2/11/1983
E. tereticornis (100)						
South Coast (20)	*34 52	*150 36		*1240	*23/16	1/3/2006
Mount Annan, (Local) (20)	34 4 9	150 46 4	133	800	23.5/10.5	14/6/2005
Dungog (20)	32 22 20	151 49 30	241	1145	23/10	14/9/1995
Selection Flat (20)	29 10	152 58	40	1095	27/13	1/1/1993
Tenterfield (20)	28 58 45	152 10 15	868	850	21.5/8	7/12/2006
Hardenbergia violacea (100)						
Denver (20)	37 16 35	144 18 1	588	755	18.5/5.5	1/2/08
Mount Annan, (Local) (20)	*34 4	*150 46	133	800	23.5/10.5	1/12/1993
Bathurst (20)	*33 25	*149 31	*731	567	20/7	1/1/2003
Nabiac (20)	32 7 48	152 24 9	35	1179	24.5/12	18/10/93
Manilla (20)	30 33	150 34	790	620	24.5/9.5	11/1999
Themeda australis (100)						
Gowar (20)	36 32 52	143 23 50	163	460	22.5/8.5	1/12/2005
Bethungra (20)	*34 45	*147 51	305	625	22/8	9/12/2005
Mount Annan, (Local) (20)	34 4 25	150 45 32	391	800	23.5/10.5	14/12/2005
Fern Bay (20)	*32 52	151 47	8	1139	22/14	16/11/2006
Manilla (20)	*30 43	*150 44	427	620	24.5/9.5	12/2005

Appendix Table 3. Measurements recorded for each species. Numbers in columns represent days in the field (planting date to measurement date): Mt Annan & Cecil Hills sites respectively. *Mean Days to Death [1]Observations were made throughout the period and at the time of final measurement. [2]The time period during which observations were made.

	Acacia falcata	***Bursaria spinosa***	***Eucalyptus crebra***	***E. tereticornis***	***Hardenbergia violacea***	***Themeda australis***
Survivorship (MDTD)*	751,392	756, 678	435, 416	460, 454	332, 202	734, 731
Stem height (cm)	371,260	756, 678	435, 416	460, 454		734, 731
Stem diameter (cm)		756, 678	435, 416	460, 454		734, 731
Aboveground total biomass (gm)		756, 678	435, 416	460, 454	332, 202	734, 731
[1]Percentage of flowering plants (%)	751,392	756, 678				734, 731
[2]Time to flowering	6 – 7/ 2010					9/ 2009 – 5/ 2010
Specific Leaf Area (mm^2 mg^{-1})		756,678	435, 416	460, 454	332, 202	
Leaf Width: length (cm)		756,678	435, 416	460, 454	332, 202	
No. of leaves measured/ plant		4	2	2	5	
Lignotuber (p/a)			435, 416	460, 454		
Branching (No.)			435, 416	460, 454		
Herbivory (%)	371,260		435, 416	460, 454	332, 202	



Appendix Figure 1. Distribution (dots), provenances (triangles) and location of common garden sites (and local provenance) (arrows) for: (a) *Acacia falcata*, (b) *Bursaria spinosa* ssp. *spinosa*, (c) *Eucalyptus crebra*, (d) *E. tereticornis*, (e) *Hardenbergia violacea* & (f) *Themeda australis.* (*T. australis* is a synonym of *T. triandra. T. australis* is commonly used in NSW and is used in this paper. The data supplied is for *T. triandra).* *http://chah.gov.au/avh/public_query.jsp* (Maps and distribution data sourced from Australia's Virtual Herbarium: http://www.rbg.vic.gov.au/cgi-bin/avhpublic/avh.cgi, accessed October 2008).



Appendix Figure 2. Climatic envelope (mean annual temperature (C) and annual precipitation (mm)) (blue dots) of provenances selected (red squares) for: (a) *Acacia falcata*, (b) *Bursaria spinosa* ssp. *spinosa*, (c) *Eucalyptus crebra*, (d) *E. tereticornis*, (e) *Hardenbergia violacea* & (f) *Themeda australis.* Arrows indicate location of common garden site (and local provenance). Note that the axes have different scales.

CHAPTER 3

What role does ‘home-site’ advantage play in restoration ecology under heatwave conditions?

This chapter is intended for submission to *Austral Ecology*

Abstract

A key aspect of successful restoration projects is the sourcing of propagation material suited to the environmental and biotic conditions of the proposed planting site. Traditionally, the use of propagules collected locally has been advocated for revegetation on the assumption that this material is better adapted to local conditions. A rapidly changing climate, however, is challenging the assumption that the use of local genetic stock will provide the best restoration outcome in the long term. We tested the ‘local is best’ paradigm using open top chambers to simulate the predicted summer temperatures for 2050 in western Sydney. We compared the establishment success of *Eucalyptus tereticornis* and *Themeda australis*, dominant species in Cumberland Plain Woodland, grown from local vs non-local seed. All plants survived an exceptional summer heatwave and few differences between temperature treatments were found. No evidence of local superiority was found for survival or growth of non-reproductive tissues of either species. However, local provenance plants of *E. tereticornis* had significantly more herbivory in the ambient temperature treatment than one non-local provenance, and local provenance plants of *T. australis* demonstrated significant superiority to non-local provenances in all categories of reproductive growth.

Keywords

Climate change, ecotype, extreme weather, intraspecific variation, local adaptation, temperature

Introduction

Global mean temperatures are projected to rise beyond 2°C (compared with pre-industrial levels) by the middle of this century, a level considered to be beyond a safe operating threshold for many species and systems (Steffen et al. 2009). Increases in the frequency and intensity of extreme weather events for some regions are also expected (Meehl et al. 2007). It is generally considered that natural systems will be strongly influenced by extreme climatic events such as heatwaves, and that in the past, gradual long-term biotic changes may have been influenced by brief extreme events (Easterling et al. 2000). Extreme events are considered to be a driver of strong directional selection (Gutschick & BassiriRad 2003; Jump & Peñuelas 2005) and to have a greater impact on biological systems than trends in mean temperatures (e.g. Groom et al. 2004). Despite this, most studies explore changes in average trends rather than the array of responses that extraordinary climatic events will bring (Smith 2011). Studies on plant responses to extreme temperatures have focused mainly on growth responses to frost tolerance rather than higher-than-average temperatures (Saxe et al. 2001). Temperature is an important factor in the development of plants, from germination through to flowering, and responses to lethal high temperatures and plant acclimation rates vary widely among-(Cunningham & Read 2006; Sage & Kubien 2007; Gunderson et al. 2010) and within-species (Slayter 1977; Saxe et al. 2001).

Climate change is already having a significant impact on biological and physical systems (e.g. Rosenzweig et al. 2008; Chen et al. 2011) and it is generally considered that the projected pace of climate change will be faster than most species will be able to track by shifting their geographic ranges. The pace at which climate is shifting has been calculated at 17.6 km/decade over land in the southern hemisphere (Burrows et al. 2011) but for terrestrial species, average range shifts have been reported at only 6.1km/decade (Parmesan & Yohe 2003 and references therein; but see Chen et al., 2011). The inability of species to track climate change by shifting ranges, and the ensuing biotic uncertainties, pose many challenges for management in general, and for restoration practices in particular.

An integral component of any restoration project is the decision where to source seed, or other propagation material. It has been traditional practice to source propagules locally, for two principal reasons. Firstly, it is usually assumed that locally-sourced material (hereafter referred to as local provenance) is better adapted to local conditions than propagules of the same species sourced from elsewhere in their distribution. A corollary of the assumption is that the use of local provenance material provides the greatest probability of successful establishment and growth. Secondly, it has also been commonly assumed that the use of local provenance material is desirable from a genetic point of view. It is assumed that the use of local provenance will ‘preserve the genetic integrity of the planting site’, decrease the chances of outbreeding depression and unwanted hybridization, prevent the ‘cryptic’ invasion of local plants, and avoid disruption of trophic interactions (Hufford & Mazer 2003; McKay et al. 2005; Vander Mijnsbrugge et al. 2010).

There are a number of lines of evidence that challenge the ‘local is best’ paradigm. Not all plants are locally-adapted (Gordon & Rice 1998), and both the frequency and magnitude of any local adaptation varies (Hereford 2009). Further, a recent review concluded that the concerns about outbreeding depression in recently fragmented populations are probably overstated (Frankham et al. 2011). ‘Decision trees’ that assess the likelihood of adverse genetic change from revegetation sites on surrounding plant populations and that predict the probability of outbreeding depression have recently been published (Byrne et al. 2011; Frankham et al. 2011). By predicting potential negative outcomes caused by the introduction of non-local propagules, the risks of outbreeding depression and adverse genetic change from hybridization can be minimized.

Even if the ‘local is best’ paradigm can be supported under current environmental conditions, plant populations are being increasingly subjected to factors altering their local environment. A changing climate, fragmentation, altered land use practices, and the introduction of exotic species, mean that the traditional assumptions of the benefits of using local provenance plants need to be reconsidered. Species once 'adapted' to local conditions may become increasingly poorly adapted as conditions change rapidly. In particular, small, genetically impoverished populations in vegetation fragments may increasingly lose their adaptive capacity where inbreeding depression (through genetic drift and stochastic events) results in a loss of genetic diversity (Bradshaw & McNeilly 1991 and references therein; Leimu & Fischer 2008). Studies examining genetic diversity (intraspecific variation) under future climate scenarios such as altered precipitation and temperature (Gimeno et al. 2009; Beierkuhnlein et al. 2011; Hartman et al. 2012) and elevated CO_2 (Mycroft et al. 2009), have found significant within-species variation in responses.

The aim of this study was to test the 'local is best' paradigm under both current and simulated future temperature conditions for 2050 for two dominant species from the Cumberland Plain in western Sydney, Australia. The average daily maximum temperature for summer in western Sydney is projected to increase 1.5 - 2°C above current temperatures by 2050 (Department of Environment and Climate Change NSW 2010). Mean minimum temperatures are predicted to rise between 1 - 3°C across all seasons (Department of Environment and Climate Change NSW 2008). An increase during the colder months implies that the likelihood of frosts will lessen. More frequent extreme events such as heatwaves are also predicted (Department of Environment and Climate Change NSW 2008). However, current understanding of how climate change may influence major drivers of climate variability such as the ENSO phenomenon is still uncertain (Department of Environment and Climate Change NSW 2010).

We compared the survival and growth rates of plants grown from local provenance sources with those from non-local sources in a common garden experiment, under ambient and at 1.5 - 2°C above ambient temperatures over a hot summer period. We hypothesized that the survival and establishment of plants from provenances from lower latitudes (and thus hotter climates) would perform equally or better than local provenance plants.

We selected two Cumberland Plain Woodland (CPW) species that are community dominants and commonly used in CPW revegetation programs: *Eucalyptus tereticornis* Sm (Forest Red Gum) (Myrtaceae) and *Themeda australis* (R.Br.) Stapf (Kangaroo Grass) (Poaceae)*. Themeda australis* is a known polyploid complex (Hayman 1960) and different nomenclature is used in different states. *Themeda australis* is a synonym of *T. triandra* that is commonly used in NSW and is hereafter used in this paper. Local adaptation has not been investigated under high temperature treatments in field conditions for either of these species. Provenance trials for either species under current climatic conditions, where plants are grown at local sites, are limited (Chapter 2; Waters et al. 2005).

Methods

Study site and species

The common garden experiment was established at the Australian Botanic Garden, Mount Annan, Campbelltown (34˚ 04′ S, 150˚ 46′ E), on the Cumberland Plain in western Sydney. The Cumberland Plain, (33˚ 30′ - 34˚ 30′ S and 150˚ 30′ - 151˚ 30′ E) is an undulating landscape, ranging in elevation from just above sea level in the north to approximately 350 m in the south (Tozer 2003). The soils are deep clay and derived from the Wianamatta Group of shales and alluviums that retain moisture and have higher nutrient levels than the surrounding landforms (Benson & Howell 1990). At the site, the soil is derived from Bringelly shale of the Wianamatta Group (Clark & Jones 1991), and is acidic with a very low electrical conductivity (analysis performed at Sydney Environmental & Soil Laboratory, Sydney, data not shown). Average annual rainfall is 700–900 mm, most of which falls during summer (Benson & Howell 1990). In the last three decades (1980 – 2009), maximum summer temperatures have averaged slightly above 37˚C with minima ~ 21˚C. Over this period, the maxima and minima have increased by ~ 0.8 and ~ 0.65˚C respectively (data not shown) (Bureau of Meteorology 2012a). In the last decade, a maximum daily temperature of 46˚C and a minimum of -3˚C have been recorded in western Sydney (Department of Environment and Climate Change NSW 2010).

Cumberland Plain Woodland (CPW) typically consists of an open tree canopy of large, mostly eucalyptus species (of which *E. tereticornis* is one of three main species) and a diverse grassy groundcover, often dominated by *T. australis* and *Microlaena stipoides.* A shrub layer may also be present, depending on the local fire regime (Benson & Howell 1990). *Eucalyptus tereticornis* is widely distributed on the east coast of Australia, from southern New South Wales to northern Queensland, spanning temperate to tropical climatic zones (Figure 1a). *Themeda australis* is widely distributed throughout Australia, covering arid to tropical climatic zones (Figure 1b). Life history traits for the two species are summarized in Table 1.

Table 1. Life history details of *Eucalyptus tereticornis* and *Themeda australis.* General information accessed from: Benson & von Richter (undated) and specific information obtained from [1]Grattapaglia et al (2012), [2]Evans & Knox (1969) and [3]Hayman (1960).

	Eucalyptus tereticornis	***Themeda australis***
Life Form	Tree	Grass
Mating system	[1]Mixed: mostly outcross but can self	[2]Apomictic &/or sexual
Cytogenetics	[1]Diploid	[3]Polyploid
Pollination	Insects & vertebrates	Wind
Seed dispersal	Wind /gravity	Gravity/ possibly by adhesion
Longevity	100-200 years	Perennial

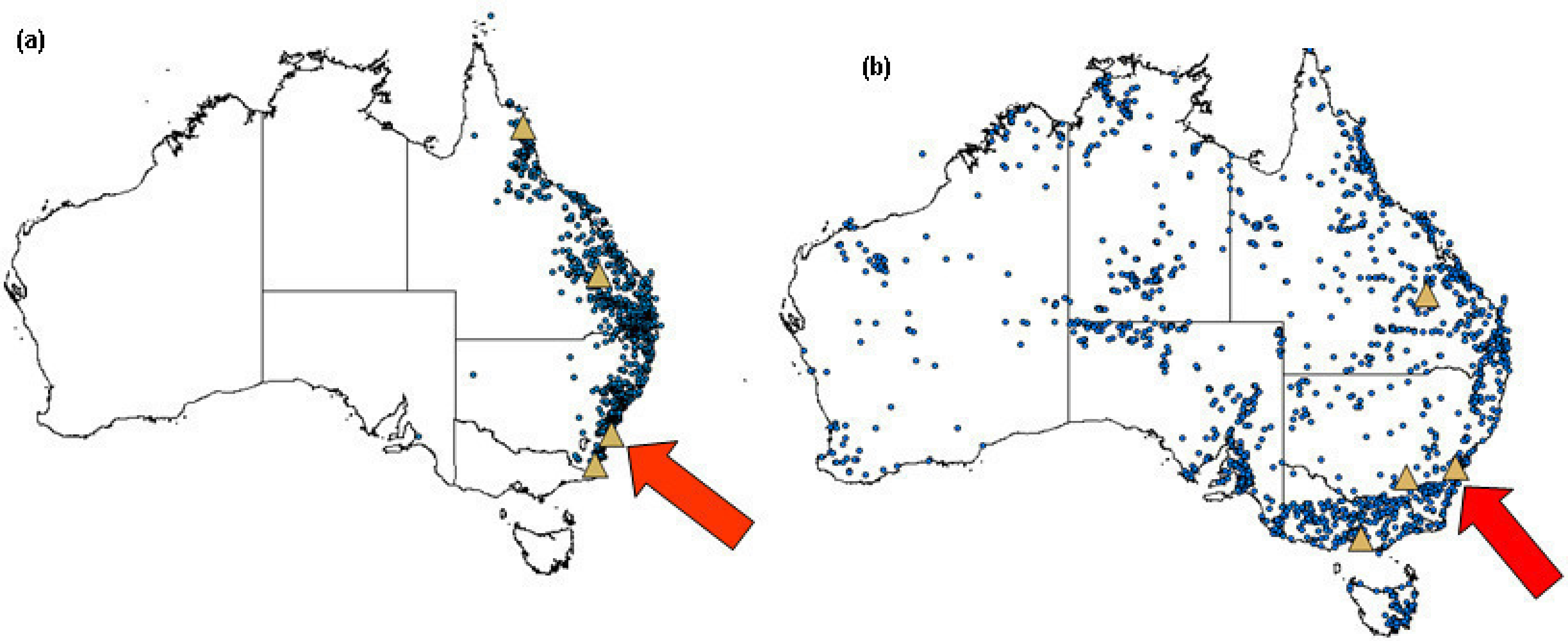


Figure 1. Distribution (dots) and provenances selected (triangles) for: (a) *Eucalyptus tereticornis*, & (b) *Themeda australis*. Arrows represent the site of the common garden experiment in the Cumberland Plain, western Sydney. (Maps and distribution data sourced from Australia's Virtual Herbarium: http://www.rbg.vic.gov.au/cgi-bin/avhpublic/avh.cgi, accessed October 2008).

Plant material and growth conditions

Seeds from four provenances of each species (one from the Cumberland Plain, hereafter referred to as the local provenance, and three non-local provenances) were obtained from commercial suppliers. The provenances selected for *E. tereticornis* are representative of most of its climatic envelope along the east coast of Australia (Figure 1a; Appendix Table 1 and Appendix Figure 1a). The provenances selected for *T. australis* are representative of the range of mean annual temperature and the lower precipitation regions within its geographic range (Figure 1b and Appendices Table 1 and Figure 1b). Mean annual temperatures of the sites from which the provenances were sourced range from approximately 13 - 24°C, with the local provenances at the lower end of this range. Collection details such as exact location, population size and number of parent plants were not always available from the commercial suppliers and are therefore cannot be included in the data analysis. Only limited supplies of *T. australis* seed were available and whilst the majority of the seed was purchased just before sowing, the Bethungra provenance seed was purchased approximately two years prior to sowing and was stored at Macquarie University under dry, stable temperatures (Appendix Table 1).

Seeds were germinated and grown in a glasshouse at Macquarie University from June to October 2010. Seedlings were raised in seedling trays filled with Debco seed raising mix and Osmocote® Plus Native Plant slow release fertilizer. *Eucalyptus tereticornis* seeds were mixed with a small quantity of fine sand and then lightly sprinkled over the mix, with a fine layer of vermiculite on top. *Themeda australis* seeds were soaked in 0.5% bleach and a drop of surfactant for 5 minutes, triple rinsed in de-ionised water, scattered on top of soil mix and covered with a thin layer of vermiculite ten minutes after a light watering (Ralph 1997; and R. Rapmund, Hornsby Shire Council Community Nursery, 2010, personal communication). Seeds were placed in a growth cabinet set at 27°C/20°C (day/night), and 14 hrs daylight until germinated and then transplanted into 250 ml tubes filled with Debco Plugger Starter Plus potting mix and Osmocote® slow release fertilizer (rate according to manufacturer's instructions).

Site preparation and experimental design

The planting site was prepared by slashing the existing herbaceous weed cover and spraying with Scotts Roundup® weed killer. The site was fenced to exclude vertebrate herbivores and divided into 10 equal plots, 3 x 3m, with 2m spacing between plots. Wooden stakes were positioned at the perimeter of 3m diameter circles and five open top chambers (OTCs) were constructed by wrapping 9.45 m of 1.2 m high natural Solarshield® reinforced greenhouse film around the stakes (Figure 2). The greenhouse film allows approximately 88% light transmission. Control plots and OTCs were alternatively placed in two rows of five plots each.

Planting holes were dug within the circular plots with approximately 75 – 80 cm spacing between *E. tereticornis* plants and 50 cm for *T. australis.* One level teaspoon of Osmocote® was added to the bottom of the hole for each *Eucalyptus* plant. Each plot contained 16 plants (eight plants of each species, with two plants from each of the four provenances), randomly positioned to the extent that no plants from the same provenance were next to each other. One hundred and sixty of the most healthy and evenly-sized plants were transplanted in October 2010. Heavy rain occurred before planting and apart from the day of transplanting, no further hand watering was performed. Stem height for *E. tereticornis* plants, and leaf length and clump circumference for *T. australis* plants, were measured approximately 10 days after transplanting.

The OTCs were erected approximately six weeks after transplanting. Air temperature and relative humidity (RH) was recorded every 15 minutes in the centre of two separate OTCs and in two separate non-OTC plots using Thermochron® iButton Data loggers (#1923), placed inside solar radiation shields, approximately 30 cm above the ground. Soil temperature was recorded every 60 minutes in the centre of the same plots using Thermochron® iButton Data loggers (#1921) placed inside petri dishes, sealed with paraffin wax, approximately 5 cm below ground level. Soil moisture in each plot was measured monthly using a moisture probe meter (MPM-160 ICT International Pty Ltd). Three readings were taken within each plot and averaged.

Measurements

The temperature treatment ceased in March 2011, approximately 17 weeks after the start of the experiment, and all plant material was measured and harvested within a week. Survivorship was assessed as 'alive' (green leaves &/or stem) or 'dead' (no green anywhere on the plant). Measurements taken for other performance-related traits are detailed in Table 2. Herbivory was measured for *E. tereticornis* because stressed trees (e.g. as a result of climate change) are thought to be more susceptible to insect attack, and the CPW has been highlighted as an area of concern (Marsh & Adams 1995; Department of Environment and Climate Change NSW 2010). Furthermore, *E. tereticornis* belongs to the subgenus *Symphyomyrtus,* which is known to commonly suffer substantial insect herbivory (Noble 1989; Marsh & Adams 1995). For *T. australis*: culms (aerial stem bearing an inflorescence) were removed from the plant during the experiment and growth measurements were divided into (1) non-reproductive traits and (2) reproductive traits.

Figure 2. Open Top Chambers (OTCs) at the field site, western Sydney.

Table 2. Measurements for *Eucalyptus tereticornis* and *Themeda australis*

Trait measured	**Measurement method**
Eucalyptus tereticornis	
Stem height	Extended length from ground level to terminal apex
Stem diameter	Stem was cut above the lignotuber and two measurements were taken at right angles and averaged
Total above-ground biomass	Plant material was dried at 80°C for 48 hours before weighing
Specific Leaf Area (SLA)	Fresh leaves were collected in accordance with Cornelissen et al. (2003), scanned and their area measured using ImageJ software (http://rsb.info.nih.gov/ij/download.html) before being oven-dried and weighed. SLA was calculated as leaf area (mm^2) per unit biomass (mg)
Herbivory	The total amount of defoliation and leaf necrosis per plant was visually assessed at the time of harvest and scored in the following categories: 0-1%, 1-5%, 5-25%, 25-50%, 50-75% and >75%
***Themeda australis*: Non-reproductive traits**	
Clump circumference	Plant was clumped together and the circumference of the base measured *in situ*
Leaf length	Extended length of the longest leaf, ground level to tip
Total above-ground biomass without culms	Plant material less culms was dried at 60°C for 72 hours and then weighed
***Themeda australis*: Reproductive traits**	
Total above-ground biomass with culms Percentage dry weight of culms/biomass Mean culm weight	All plant material was dried at 60°C for 72 hours and then weighed
Mean number of culms per plant	Individual culms counted per plant and then averaged
Percentage of flowering plants	Plants were scored as “flowered” if seed heads were visible
Mean time to flower (MTTF)	Time from sowing to first flowering was recorded weekly from 25/11/2010 – 17/2/2011 for each plant

Data analysis

Local provenance plants were defined as being superior to, or having better performance than non-local provenance plants, if they had a significantly higher survival rate or growth compared to non-local provenances. Differences in performance between provenances were compared using a general linear model. Provenance and temperature were treated as fixed factors while plot (nested in temperature treatment) was treated as a random factor, with interactions included in the model. Data were transformed as necessary to meet the assumptions of normality and homoscedasticity. When performance between provenance responses was significantly different, a Bonferroni *post hoc* test was used to compare means. An α level of 0.05 was used to determine significance in all tests. Plots and interactions were removed when $p>0.25$ and the tests were re-run. Where these assumptions were not met, each factor was analyzed separately using a Kruskal-Wallis test, and where significant, a Mann-Whitney *post hoc* test was used to compare the means. Mean time to flowering between the provenances was compared using Kaplan-Meier estimates (Log Rank Chi-Square test). The percentage of flowering plants was analyzed by using chi-square tests. *Themeda australis* plants that did not flower were removed from the analysis for mean culm weight.

To account for potential size differences between the provenances at the start of the treatment, that may influence the experiment outcomes, we tested for correlations between the pre-treatment and harvest growth measurements. When the correlation was found to be significant and positive ($p<0.05$), the residuals from these analyses were used in subsequent tests of performance. Significant and positive correlations between the pre-treatment and harvest measurements were found for *T. australis* for clump circumferences and for leaf length. We also found a significant positive correlation between clump circumference and total above-ground total biomass (without culms) for this species. Consequently we analysed the residuals of the regression between the two traits. For *E. tereticornis*, only stem height was measured pre-treatment and a significant and positive correlation was found between pre-treatment and harvest measurements. The harvest data of stem diameter and total above-ground total biomass did not correlate with the pre-treatment stem height value, so the harvest data rather than the residuals were analyzed for these two traits.

Results

Environmental conditions

Temperature: Between the end of January and the first week of February 2011, western Sydney experienced an 'exceptional summer heatwave': a record hot spell of six consecutive days of maximum temperatures above 35°C (Bureau of Meteorology New South Wales Climate Services Centre 2011). At the field site, maximum temperatures were above 35°C for seven consecutive days, with a high of approximately 47°C recorded inside one of the OTCs (Figure 3). During the heatwave, temperatures within the OTCs averaged ambient plus ~ 1.5°C. Outside the heatwave period, the measured average air temperature within the OTCS measured 0.7°C higher than the control plots, with the exception of the period between 6/1/2011 and 27/1/2011 when the data loggers failed to operate. The 3 pm air temperatures logged at the site consistently tracked the official 3 pm air temperature recorded by the Bureau of Meteorology (Bureau of Meteorology 2011) (Appendix Figure 2). One of the data loggers in a control plot gave spurious results on an *ad hoc* basis so these data were discarded. The average soil temperature inside the OTCs was consistently 0.75°C higher than the control plots for the entire period (data not shown).

Relative Humidity (RH): On average, the OTCs did not change the RH beyond the ± 5% variation within the chambers compared to the control plots (Appendix Figure 3). The average RH data at 3pm at the control plots compared to the official Bureau of Meteorology (BOM) data was within the ± 5% accuracy range of the data loggers (see exception in Temperature).

Soil moisture*:* Soil moisture levels inside the OTCs and the control plots were similar for the duration of the experiment (average 0.5% lower inside the OTCs) (Appendix Figure 4). The lowest average monthly soil moisture level of 4.36% occurred inside the OTCs during February; the driest February in Sydney for 30 years (Bureau of Meteorology 2012b).

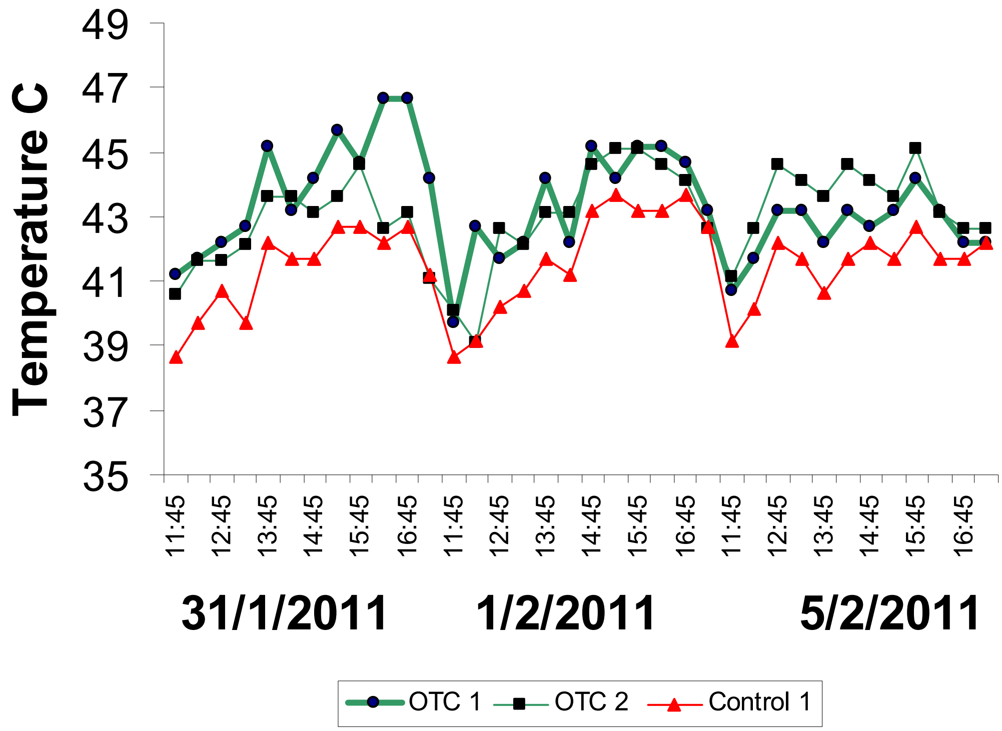


Figure 3. Diurnal air temperatures for the hottest periods during three of the hottest days during the record hot spell: 31/1/2011, 1/2/2011 & 5/2/2011 inside OTCs and control.

Survivorship and growth of non-reproductive tissue

All individual plants of both species survived the duration of the experiment. For plant growth, there were significant differences between the provenances, but the local provenance plants did not demonstrate local superiority. There were no significant differences between temperature treatments or interactions between provenance and temperature, or provenance and plot, for either species. Plots, however, were a significant factor for all growth traits. The significance was inconsistent between traits and species but for *E. tereticornis*, the plot affect was most pronounced for above-ground biomass ($F_{(8,67)}$=7.238, p=0.0258) and for leaf length in *T. australis* ($F_{(8,43)}$=4.181, p=0.0009).

For *E. tereticornis*, there were significant differences among the provenances for stem height ($F_{(3,67)}$=3.110, $p \leq 0.0321$) and stem diameter ($F_{(3,24)}$=3.690, p=0.0258) (Figure 4a & b) but not for total above-ground biomass (Figure 4c). On average, local provenance plants had the smallest (or second smallest) stem diameter and total above-ground biomass (Figure 4b & c). The ranking of the provenances for height remained constant throughout the experiment. Accordingly, we conclude that the *E. tereticornis* local provenance plants did not grow the fastest and therefore did not exhibit local superiority. For *T. australis*, the local provenance plants had the largest circumference but this was not significantly different from the non-local provenances ($F_{(3,67)}$=0.90, p=0.448) (Figure 4e). Significant differences were found among the provenances for leaf length ($F_{(3,24)}$=12.329, $p \leq 0.0001$) (Figure 4d) and total above ground biomass without culms ($F_{(3,75)}$=6.2537, $p \leq 0.0008$) (Figure 4f) but it was the most northerly non-local provenance that demonstrated superiority for these growth traits.

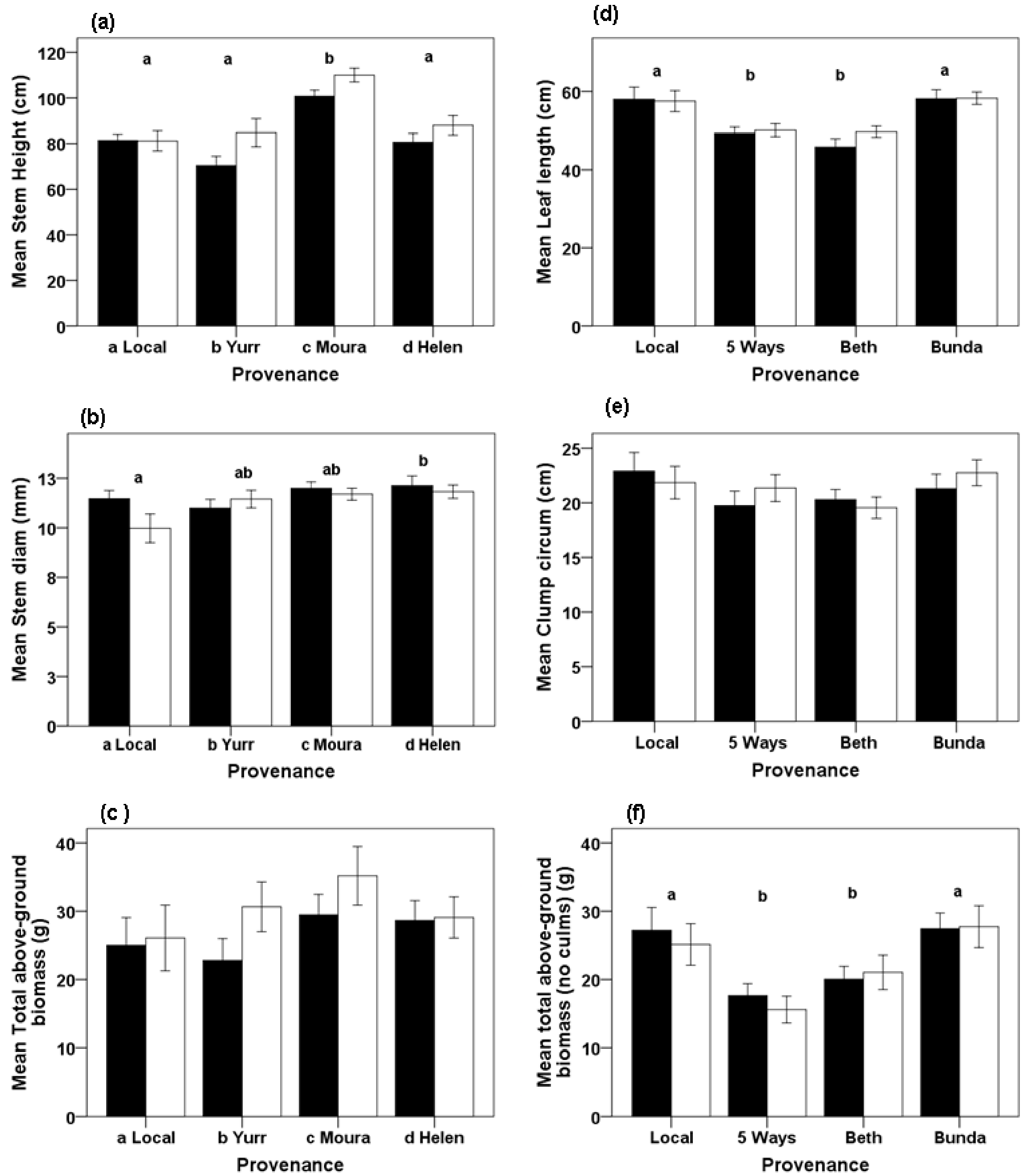


Figure 4. Mean (±SE) of non-reproductive growth data at harvest for *Eucalyptus tereticornis*: (a) stem height, (b) stem diameter (c) total above-ground biomass and for *Themeda australis*: (d) leaf length, (e) clump circumference and (f) above-ground total biomass (without culms). Ambient (black bars) and high temperature (white bars). Provenances sharing the same lower-case letters are not significantly different.

Table 3. Reproductive growth mean (±1 SE) and results of the General Linear Model, chi-square test and Kruskal-Wallis analysis for *Themeda australis***.** Figures in bold indicate where the local provenance plants were, on average, significantly superior to non-local provenances.

Provenance	Total above-ground biomass with culms	% weight of culms/biomass	Mean culm weight (g)	Flowering plants (%)	Mean no. of culms per plant
Local	**32.63 (2.7)**	**23.85 (3.35)**	**8.06 (1.7)**	**80**	**3.5 (0.7)**
Five Ways	16.91 (1.3)	6.25 (1.61)	1.13 (0.4)	25	0.4 (0.2)
Bethungra	20.89 (1.5)	6.72 (2.15)	1.37 (0.4)	25	0.35 (0.2)
Bundaberg	30.53 (1.9)	15.75 (2.65)	4.52 (0.8)	65	1.45 (0.4)
	$F_{(3,67)}$=13.789 **p≤0.0001**	$F_{(3,34)}$=6.37 **p≤0.0015**	$F_{(3,34)}$=11.781 **p≤0.0001**	$\chi^2_{(3)}$=18.96 **p=0.0003**	$H_{(3)}$=25.18 **p≤0.001**

Growth of reproductive tissues

Only *T. australis* plants flowered during the course of the study (eucalypts take several years to flower). The local provenance plants exhibited superiority in all five categories for reproductive growth: total above-ground biomass with culms ($F_{(3,67)}$=13.789, p≤0.0001), percentage weight of culms/biomass ($F_{(3,34)}$=6.37, p≤ 0.0015), mean culm weight ($F_{(3,34)}$=11.781, p≤0.0001), the percentage of flowering plants ($\chi^2_{(3)}$=18.96, p=0.0003) and mean number of culms per plant ($H_{(3)}$=25.18, p≤0.001) (Table 3). Where *post hoc* tests were conducted, the local provenance and the most northerly provenance were significantly superior to the southern provenances in all cases, except for the number of culms per plant, where the local plants were significantly superior to all other provenances. There were significant differences among the provenances for mean time to flowering (MTTF) ($\chi^2_{(3)}$=37.34, p=0.001). On average, local provenance plants flowered earliest, and significantly earlier than two non-local (southern) provenances (Figure 5).

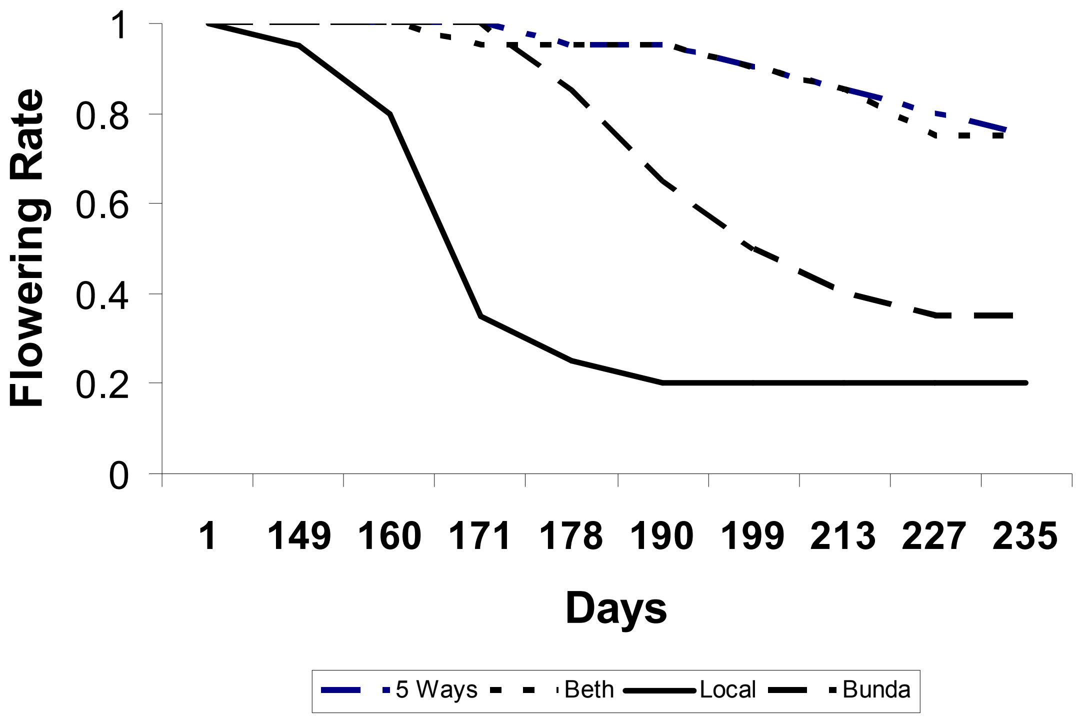


Figure 5. Mean time to flowering for four provenances of *Themeda australis* (unbroken line represents the local provenance). Each curve ends when the last plant has flowered.

Leaf morphology and herbivory

Leaf morphology and herbivory were only measured for *E. tereticornis* (Table 4). Herbivory levels were significantly different between the provenances but only under ambient conditions ($H_{(3)}$=9.04, p=0.029). Herbivory on the local provenance plants (under ambient conditions) was significantly greater than that of the most northerly provenance plants. Temperature was a significant factor for herbivory, with herbivory levels lower under high temperature compared to ambient ($H_{(1)}$=6.59, p=0.010). Significant differences were recorded between the provenances for Specific Leaf Area (SLA). Only the high temperature treatment for provenance was analyzed for SLA because only one sample from the local provenance plants was taken from the control plots (ambient) due to the high incidence of herbivore damage. The two northern provenances had a lower SLA than the two southern provenances. Temperature was a significant factor for

SLA with the median leaf size larger under the high temperature treatment compared to ambient ($H_{(1)}$=5.83 p=0.016).

Table 4. Leaf morphology and herbivory mean (±1 SE) and results of ANOVA and Kruskal-Wallis analysis for provenances of *Eucalyptus tereticornis***:** Figures in bold indicate that the local provenance plants were significantly different from non-local.

Provenance	Specific leaf area (SLA) High temp only	% Herbivory In Ambient Temperature	% Herbivory In High temperature
Local	4.14 (0.3)	**29.8 (5.2)**	12.45 (3.4)
Yurramie	4.54 (0.3)	29.4 (10.1)	9.0 (2.0)
Moura	3.50 (0.1)	19.9 (7.7)	12.75 (4.6)
Helenvale	3.84 (0.1)	9.0 (2.0)	11.25 (3.5)
	$H_{(3)}$=11.24 p=0.011	**$H_{(3)}$=9.04 p=0.029**	$H_{(3)}$=0.57 p =0.903

Discussion

No evidence of local superiority was found for survival or growth of non-reproductive phases of *E. tereticornis* or *T. australis.* All plants survived an exceptional summer heatwave and few differences between temperature treatments were found. However, local provenance plants of *T. australis* demonstrated significant superiority to non-local provenances in all categories of reproductive growth and local provenance plants of *E. tereticornis* suffered significantly more herbivore damage in the ambient temperature treatment than one non-local provenance.

Eucalyptus tereticornis

For *E. tereticornis*, our results are consistent with a 12-month provenance trial that found no superiority in survival or growth for local provenance plants grown on the Cumberland Plain under ambient conditions (Chapter 2). To the best of our knowledge, local adaptation within this taxa has not previously been investigated under high temperature conditions. However, we did find intra-specific differentiation within *E. tereticornis* for growth traits consistent with other studies conducted under both current (Otegbeye 1990; Duncan et al. 2000) and high temperature conditions (Paton 1980). The level of genetic variation for *E. tereticornis* within the Cumberland Plain Woodland (CPW) is unknown. Information on the amount of genetic diversity among and within populations is required to enable assessment of potential adaptive capacity in the face of disturbances such as climate change. The CPW is a highly fragmented landscape, which may, over time, hinder migration potential and reduce reproductive population sizes of species within the community (Hoffmann & Sgró 2011). The findings of this study suggest that revegetation strategies that include non-local provenances of this species will not be detrimental to the establishment success of the project. A seed-sourcing strategy that maintains sufficient genetic diversity within populations will maximize evolutionary potential (Weeks et al. 2011). This is especially important for plant species with long generation times such as *E. tereticornis*. It is generally assumed that species with long generation times will face greater difficulty in keeping pace with the rate of climatic change than those with shorter generation times (Jump & Peñuelas 2005 and references therein). Furthermore, such a strategy may help to avoid the possible negative affects of fragmentation such as interspecific hybridization (Broadhurst & Young 2007) or selfing (Grattapaglia et al. 2012), that are known to be common in eucalypts.

Leaf morphology plays an important role in a plant’s ability to avoid heat damage (Groom et al. 2004). Intraspecific variation in eucalypt leaf shape and size is common and can be influenced by temperature (Scurfield 1961; Shepherd et al. 1976). Our study confirms that intraspecific variability among *E. tereticornis* provenances exists for specific leaf area (SLA). In our study, local provenance plants of *E. tereticornis* suffered the most herbivore damage, significantly more than the most northerly provenance plants, but only under ambient conditions. The high herbivory rates of the local provenance plants may explain its relatively poor ranking for growth. The current study is consistent with the finding that local provenance plants of a closely related eucalypt, *E. camaldulensis,* suffered up to four times more herbivory than non-local plants (Floyd et al. 1994). Increased herbivory rates have been linked to high temperature stress (Allen et al. 2010) but this was not demonstrated in the current study.

Themeda australis

The lack of local superiority for non-reproductive growth in *T. australis* concurs with that found in a similar provenance trial on the Cumberland Plain but the survival results of the two studies differ (Chapter 2). In the current study, all plants survived, whereas in the previous study, non-local plants suffered a significant increase in mortality (Chapter 2). A difference in duration between the two studies may explain the contrasting results because local superiority for survival may become more prevalent as the plants age. However, the only similar study to compare survival rates of different provenances of *T. australis* did not support this idea (Waters et al. 2005). *Themeda australis* is a known polyploid complex but the cytologies of the different provenances used in the current study are unknown. It is possible that if the cytologies were different, levels of fitness or competitive advantage may have differed among the provenances (Prentis et al. 2008). Another unknown factor is the mating system of the different provenances; reproduction within the taxon can be either apomictic or sexual (Groves & Whalley 2002). Apomictic reproduction can limit gene flow within populations and consequently lead to high levels of local adaptation (Groves & Whalley 2002 and references therein). Whilst this may be advantageous in stable environments, in a rapidly changing environment, a lack of evolutionary potential may compromise the species' long-term persistence.

In this study, seeds were not counted, so the results for reproductive growth are not a true measure of fitness. However, these results do suggest that the local plants allocate more resources to reproductive growth earlier than non-local provenances. Whilst early allocation to reproduction may confer competitive advantages, a changing climate may disrupt traditional germination and seed-ripening conditions. Under a changing climate scenario, a successful strategy may be to prolong the seed production period. The early allocation for reproductive growth traits is consistent with local adaptation studies on other grass species (Bischoff et al. 2006; Rice & Knapp 2008) but not for *T. australis* (Chapter 2). For mean time to flowering (MTTF), significant differences between provenances have previously been demonstrated for this species (Groves 1975; Chapter 2), and were confirmed in the current study.

To the best of the authors' knowledge, local adaptation within *T. australis* has not previously been investigated under high temperature conditions. However, under lower ambient temperatures than those used in the current study, +2°C warming did not affect the survival rate of this species (Hovenden et al. 2006; Williams et al. 2007). It was surprising that temperature did not significantly affect mean time to flowering in our study, as phenology is generally thought to be very responsive to temperature (Walther 2003). In contrast to our study, Hovenden et al (2008) found that a +2°C warming substantially reduced the time to first day of flowering for *T. australis*. However, this did depend on the year and temperatures were generally lower than those used in the current study.

Other factors

The ~1.5°C increase in ambient temperatures achieved by the OTCs had little impact on any of the response parameters measured, indicating that within this range, these widespread species have relatively high heat tolerance. A high thermo-tolerance has been previously demonstrated for *E. tereticornis* (Paton 1980) with pot-grown seedlings shown to have heat resistance of up to 48°C (Kreeb, K, 1965 cited in Karschon & Pinchas 1971). *Themeda australis* is a C_4 plant, a group that is adapted to high temperatures and is considered to have originated from a tropical climate (Hayman 1960; Raven et al. 2003). A glasshouse study demonstrated that growth (tiller number) for *T. australis* plants will increase with temperatures up to at least 33/28°C (day/night temperature) (Groves 1975). In the present study, we found no significant interactions between provenance and temperature treatments. Interactions of provenance and climatic factors are more frequent when larger differences in temperature treatments or when several treatment levels are applied (compared to those used in our study) (Groves 1975; Shepherd et al. 1976; Joshi et al. 2001). However, it was not possible to test all interactions because some data could not be transformed to fit the assumptions for General Linear Model analysis and factors were analyzed separately. Plot was a significant factor for all non-reproductive growth variables, possibly reflecting the fine scale heterogeneity of the environmental conditions at the site (i.e. micro-site effects). There was no consistency in the relationship of the plot to the growth results for *T. australis* plants because the largest plants grew in different plots for each of the three non-reproductive growth measurements. However, for *E. tereticornis,* plants in one of the plots, on average, were often larger for all three growth measurements compared to the other plots. Soil moisture content in that particular plot, a possible environmental factor causing the growth differences, was not substantially different to the other plots. However, herbivory levels were lowest within that plot which may explain some of the variance.

Generally, the impacts of the open top chambers (OTCs) on the environmental conditions, other than ambient temperature, were minimal i.e. changes to sunshine hours, amount of precipitation and soil moisture (De Boeck et al. 2010). In addition, the plants were exposed to *in situ* inter-specific competition and 'hidden players' such as microbes, fungi and soil invertebrates (Jentsch et al. 2007). However, the OTCs may have mitigated the adverse effects of wind exposure, known to be a significant factor in Myrtaceae for leaf damage (Groom et al. 2004). Whilst this would not have affected the comparison between local and non-local plants it may have reduced any negative effects of the high temperature treatment. *Eucalyptus tereticornis* plants grown within the OTCs incurred approximately half of the herbivory damage compared to those outside. It is possible that the chambers created a barrier to certain insects, resulting in less herbivory. The plants in this study were not subjected to the projected increase in atmospheric CO_2. Generally, elevated CO_2 positively affects water use efficiency and growth, which may ameliorate heat stress, and it would appear that this outcome is likely for the two species investigated in the current study (see review in Hovenden & Williams 2010). However, elevated CO_2 was found to have a negative affect on net photosynthesis for C_4 species when grown under high temperature stress (albeit the high temperature treatment was lower than that used in the current study) (Wang et al. 2012). Our study may have benefitted from a longer tenure to capture any long-term effects of the heatwave (Gutschick & BassiriRad 2003). However, it was not possible to extend the timing of the experiment using *E. tereticornis* plants without raising the height of the OTCs, thereby potentially changing the effects of wind, shading, humidity and herbivory.

Overall, the results may have been influenced by the effects of the maternal environment, the population size of the seed-sources and age of seeds, factors that were not controlled for in this study. Germination results were not included in this short-term study, and as we selected only the largest and healthiest germinates for the treatment, we assumed minimal influence of the aforementioned seed properties on the results.

Conclusion

The 100% survival of *E. tereticornis* and *T. australis* seedlings during an exceptional summer heatwave is extremely encouraging because these species are both community dominants and their persistence will have a major effect on the structure of the Cumberland Plain Woodland (CPW) ecosystem. Little supporting evidence for the 'local is best' paradigm, together with the higher herbivory rates suffered by the local provenance *E. tereticornis* plants, suggests that maximizing genetic diversity by introducing different seed sources (provenances) of *E. tereticornis* or *T. australis* may not be detrimental to the establishment success of restoration projects undertaken on the Cumberland Plain.

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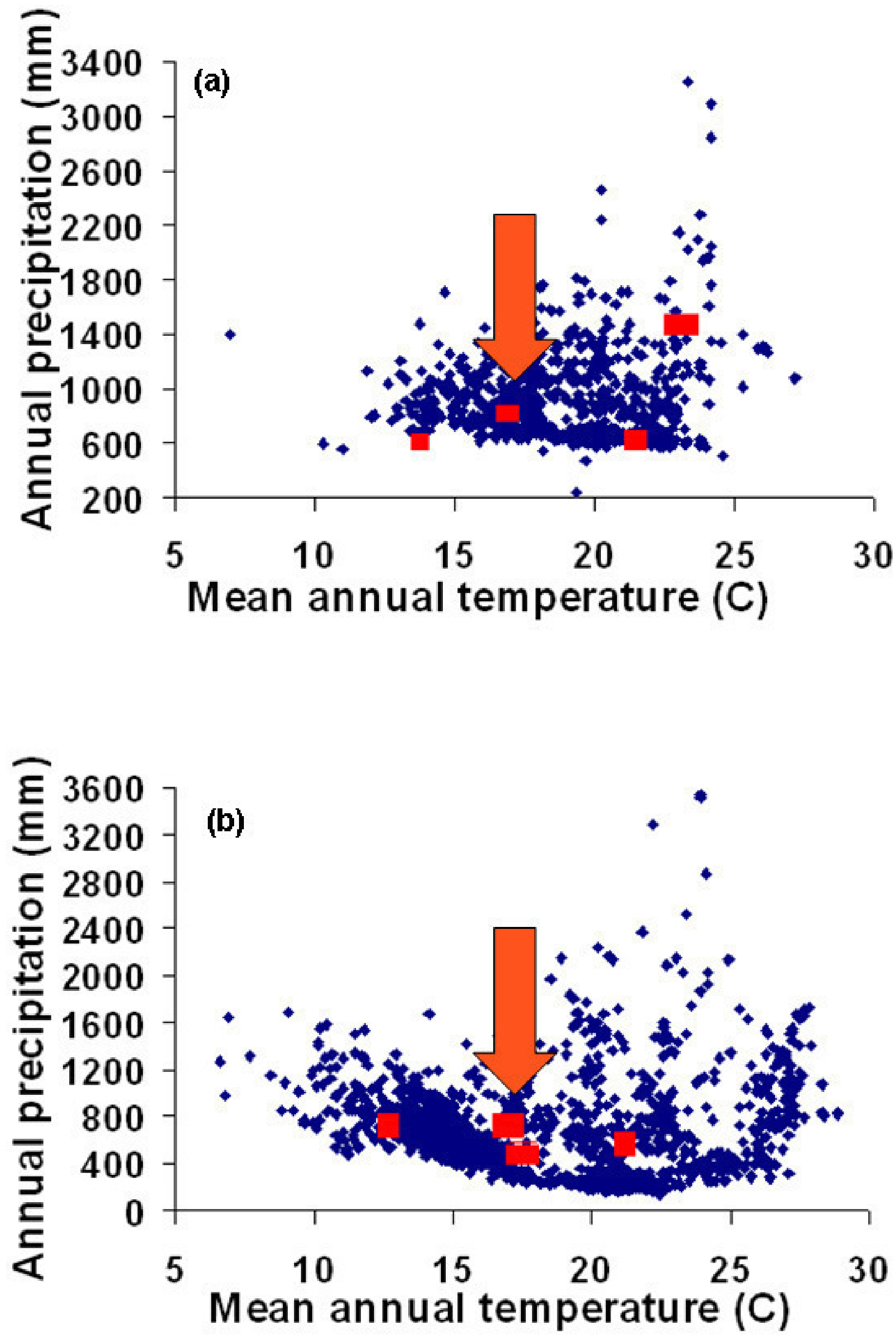
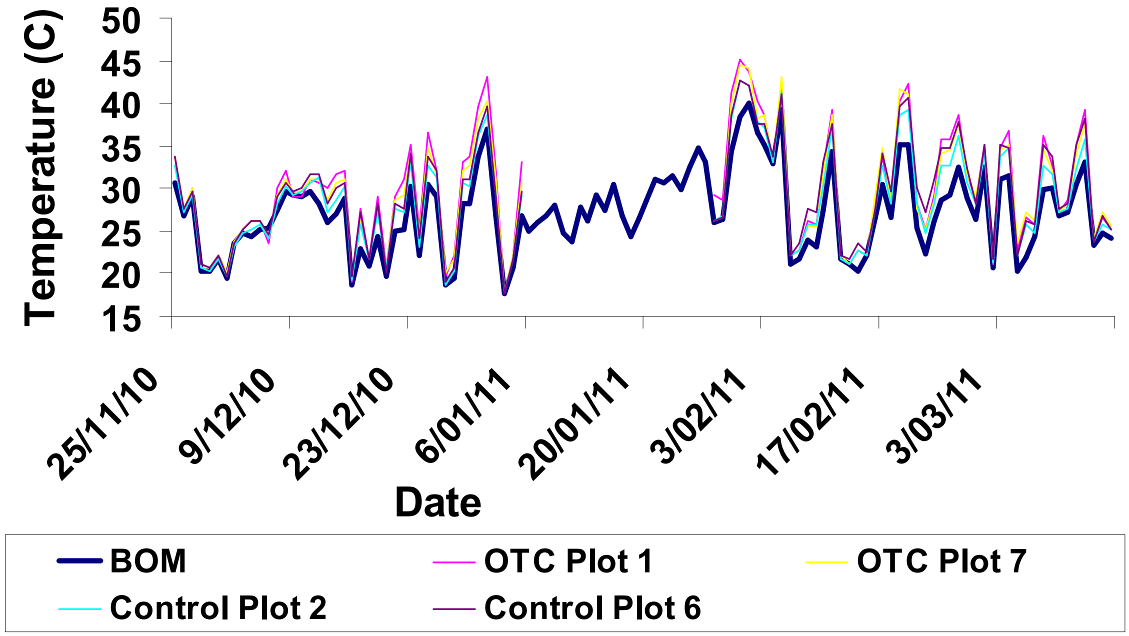
Appendix

Appendix Table 1. Provenances (source of seed), approximate latitude and longitude of seed source, approximate climate details of nearest weather station, date seeds collected and seed suppliers for *Eucalyptus tereticornis* and *Themeda australis.*[1] Approximate only. [2] 10 year data only available.

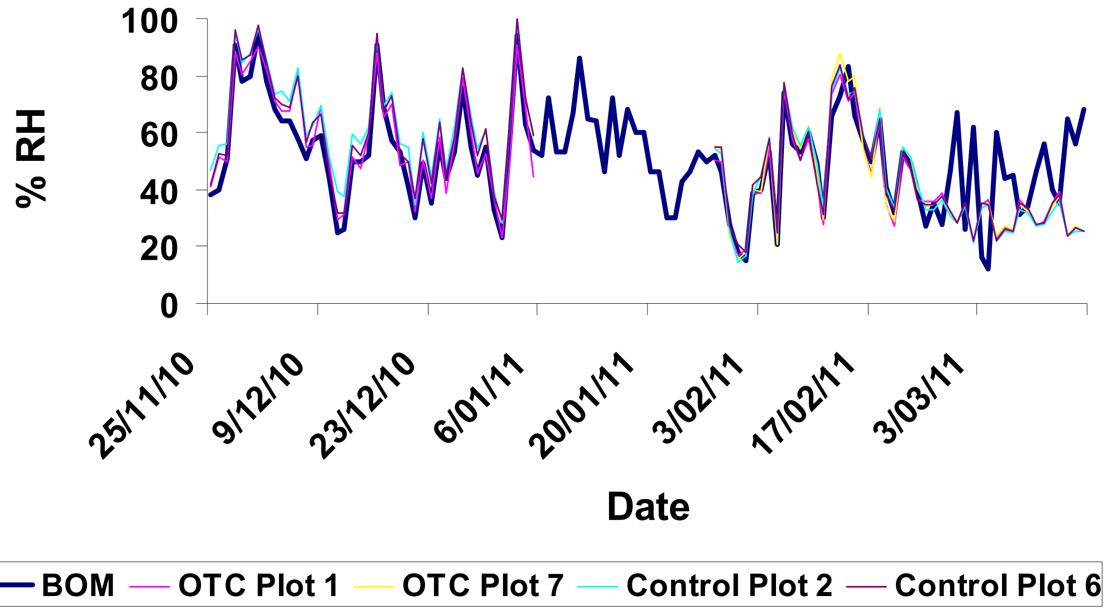
Species	***Provenance***	***Lat/Long (approx) S/E***	***Mean annual max/min temp °C***	***Highest temperature °C***	***Rainfall p.a.***	***Date seed collected***	***Seed supplier***
Eucalyptus tereticornis	[1]Yurammie, South Coast (NSW)	36.49/149.45	22/9	44.4	600	7/4/1990	Australian Tree Seed Centre
	Local. Mount Annan, Sydney (NSW)	34.04/ 150.46	24/10	45	790	14/6/2005	Botanic Gardens Trust
	Moura (Queensland)	25.00/ 150.00	29/13	43.1	670	1/1/1988	Australian Tree Seed Centre
	[2] Helenvale, (Queensland)	15.46/ 145.14	30/22	41.4	1500	1/12/1983	Australian Tree Seed Centre
Themeda australis	Five Ways (Victoria)	38.10/ 145.19	19/10	46	830	2004	Greening Australia
	Local. Picton, Sydney (NSW)	34.10/ 150.36	24/10	45	790	12/2009	Cumberland Plain Seeds
	Bethungra (NSW)	34.45/ 147.51	22/9	45.2	570	9/12/2005	Greening Australia
	Bundaberg/Moura (Queensland)	25.00/ 150.00	29/13	43.1	670	2/2009	Origin Seed



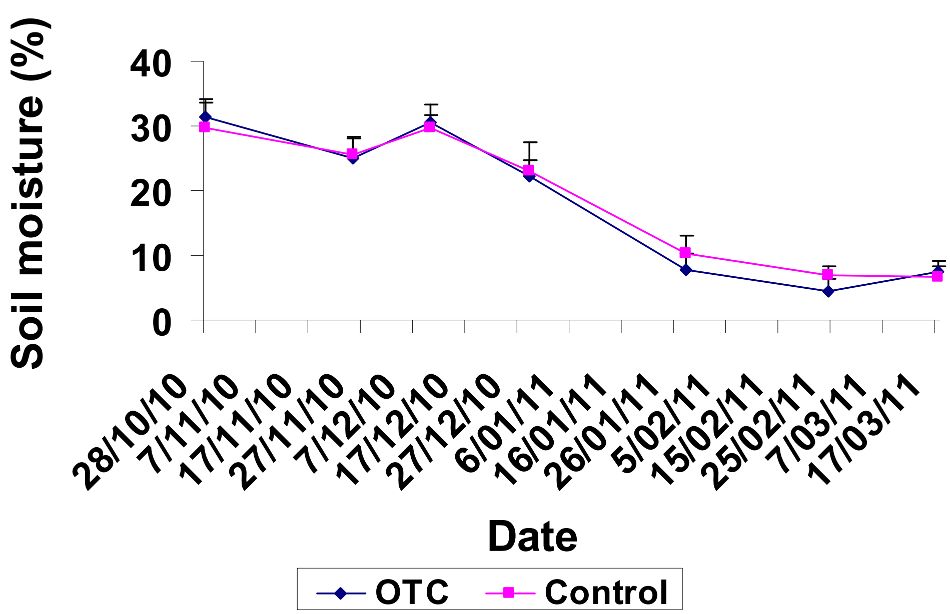
Appendix Figure 1. Climatic envelope (mean annual temperature (°C) and annual precipitation (mm)) for: (a) *Eucalyptus tereticornis* and (b) *Themeda australis.* Blue diamonds represent the species range, red rectangles represent the provenances selected and red arrows indicate location of common garden site (and local provenance). Note that the axes have different scales.



Appendix Figure 2. Daily 3pm air temperatures recorded by the Bureau of Meteorology (BOM), Campbelltown and by data loggers inside OTCs (plots 1 & 7) and control (plots 2 & 6) for the duration of the experiment 25/11/2010 – 17/3/2011.



Appendix Figure 3. Daily 3pm Relative Humidity recorded by the Bureau of Meteorology (BOM), Campbelltown and by data loggers inside OTCs (plots 1 & 7) and controls (plot 2 & 6) for the duration of the experiment 25/11/2010 – 17/3/2011



Appendix Figure 4 Average soil moisture (%) (± s.d.) within OTCs and control plots for the duration of the experiment 25/11/2010 – 17/3/2011.

CHAPTER 4

Intraspecific responsiveness to elevated CO_2 of two widespread native Australian species, *Acacia falcata* and *Eucalyptus crebra*

This chapter is intended for submission to *Australian Journal of Botany*

Abstract

Atmospheric carbon dioxide (CO_2) concentrations are rapidly rising. Whilst CO_2 enrichment generally enhances plant photosynthetic rates, interspecific variability in plant responses may help promote the development of novel plant communities. Intraspecific variation in responses to CO_2 enrichment may also alter vegetation assemblages by increasing the frequency of the fittest genotypes. In this study, intraspecific differences in responsiveness to elevated CO_2 were investigated for two widespread species that are commonly used in restoration projects in western Sydney: *Acacia falcata* and *Eucalyptus crebra.* Seedlings from three provenances of each species were grown at ambient and *c.* 550 ppm CO_2 to reflect projected atmospheric CO_2 levels in 2050. Irrespective of the CO_2 treatment, significant differences occurred between the provenances for all traits, except for survival, nodulation and C:N ratio for *A. falcata* and survival and presence of lignotubers for *E. crebra*. Despite substantial intraspecific variation, only one significant provenance x CO_2 interaction was detected.

Key words: Climate change, genotype, provenance, provenance x CO_2 interaction

Introduction

Atmospheric carbon dioxide (CO_2) concentrations have rapidly risen from *c.* 280 parts per million (ppm) in preindustrial times (IPCC 2007) to a current level of *c.* 397 ppm (Tans & Keeling 2012). Recent modelling predicts that concentrations will rise to between 540 and 1180 ppm by the end of this century (Sitch et al. 2008), with the high emissions trajectory more likely (Peters et al. 2012). CO_2 is a basic requirement for plant photosynthesis and elevated atmospheric CO_2 will affect plant species and their communities. It is generally acknowledged that elevated CO_2 (eCO_2) increases photosynthetic rates and improves water and light-use efficiencies of most plant species. Within this generalization, responses to eCO_2 by different species vary considerably, and several reviews and meta-analyses have summarised the factors associated with this variation (Saxe et al., 1998; Poorter & Navas, 2003; Ainsworth & Long, 2005; Körner, 2006).

These factors include resource availability (e.g. nutrients and water), temperature regime, soil type, functional group (C_3, C_4 or CAM), the degree of competition, nitrogen-fixing capability and the experimental design. The level at which the photosynthetic response becomes saturated by eCO_2 varies, and has been suggested to range from *c.*550 ppm (Ainsworth & Long 2005) to *c.*1000 ppm (Körner 2006). Whilst eCO_2 increases a plant's photosynthetic capability, there is not a simple linear translation to growth. Growth rates can range from negative to substantially positive, necessitating species-specific investigations under different environmental conditions (Körner 2006).

Predictions of plant responses to eCO_2 are usually derived by growing plants sourced from a single population or accession. However, there is growing recognition that substantial intraspecific variation in response to eCO_2 exists. A better understanding of intraspecific variation to eCO_2 may profoundly change the predictions of plant growth responses in general to CO_2 enrichment. Genetic variation in response to eCO_2 also has important implications for the growing field of restoration ecology. Traditionally, plant material has been sourced locally for revegetation activities because it is assumed to be adapted to local conditions, conferring survival and growth advantages over non-local material. Furthermore, 'preserving the genetic integrity' of the site by using locally-sourced material is often perceived to result in superior restoration outcomes. However, the climate is rapidly changing and plants that are locally-adapted now may not be locally-adapted in the future, negating any historical advantages (perceived or otherwise) of locally-sourced material. Intraspecific variation provides insurance against environmental change (Weeks et al. 2011) and genetic variation among and within species will determine how species physiologically respond to changes in CO_2 (Bradley & Pregitzer 2007). Selection may favour genotypes with a greater ability to compete for resources, leading to new species assemblages (Lüscher et al. 1998; Saxe et al. 1998; Körner 2006). For example, significant differential responses to eCO_2 have been demonstrated for genotypes of northern hemisphere C_3 tree species *Betula alleghaniensis* (Wayne & Bazzaz 1997), *Populus tremuloides* (when grown in a glasshouse) (Lindroth et al. 2001) and *Picea sitchensis* (Centritto et al. 1999), but not for

Fagus sylvatica (Leverenz et al. 1999), *Pinus ponderosa* (Callaway et al. 1994) or *Populus tremuloides* when grown in open chambers in the field (Zak et al. 2000). Variation between genotypes varies according to the species, the trait measured and the conditions under which the experiment is conducted (Zak et al., 2000; Bradley & Pregitzer, 2007 and references therein).

The literature is replete with experiments investigating impacts of enhanced CO_2 but the majority of research documented is for northern hemisphere plant species. Australian plant species may respond differently to eCO_2 compared to the northern hemisphere due to the nutrient-limited soils and periodic drought conditions (Medlyn et al. 2011). The impact of eCO_2 has been investigated for <0.5% of Australian vascular plants (Hovenden & Williams 2010) and research on intraspecific differences is almost non-existent. A search in several databases using the terms 'CO_2' in combination with 'intraspecific','population*' or 'genotyp*' found only two studies investigating the response of intraspecific differences of Australian plant species to eCO_2. The first study demonstrated genotypic differences in growth traits of *Nothofagus cunninghamii* when exposed to depleted CO_2 concentrations (Hovenden & Schimanski 2000). The second study found that plant defensive traits among populations of *Eucalyptus globulus* and *E. pauciflora,* and height (only measured for *E. pauciflora*) were largely unresponsive to eCO_2 (McKiernan et al. 2012).

The aim of this study was to investigate potential differences in survival and establishment of plants grown from different locations (provenances), when grown at current and eCO_2 levels. Seeds for *Acacia falcata* Willd and *Eucalyptus crebra* F.Muell. were sourced from three locations in New South Wales (NSW) and NSW and Queensland respectively (Figure 1). Both of the study species are C_3 plants and are widely used in Cumberland Plain Woodland (CPW) restoration projects in western Sydney. *Acacia falcata* is a fast-growing, nitrogen-fixing (legume) shrub. *Eucalyptus crebra* is one of the dominant CPW tree species and is a relatively slow grower compared to *A. falcata.* The CPW is highly fragmented and is listed as a Critically Endangered Ecological Community under the *NSW Threatened Species Conservation Act* (1995) (N.S.W. Government

Office of Environment & Heritage 2009) and under the federal *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) (Australian Government Department of Sustainability 2009).

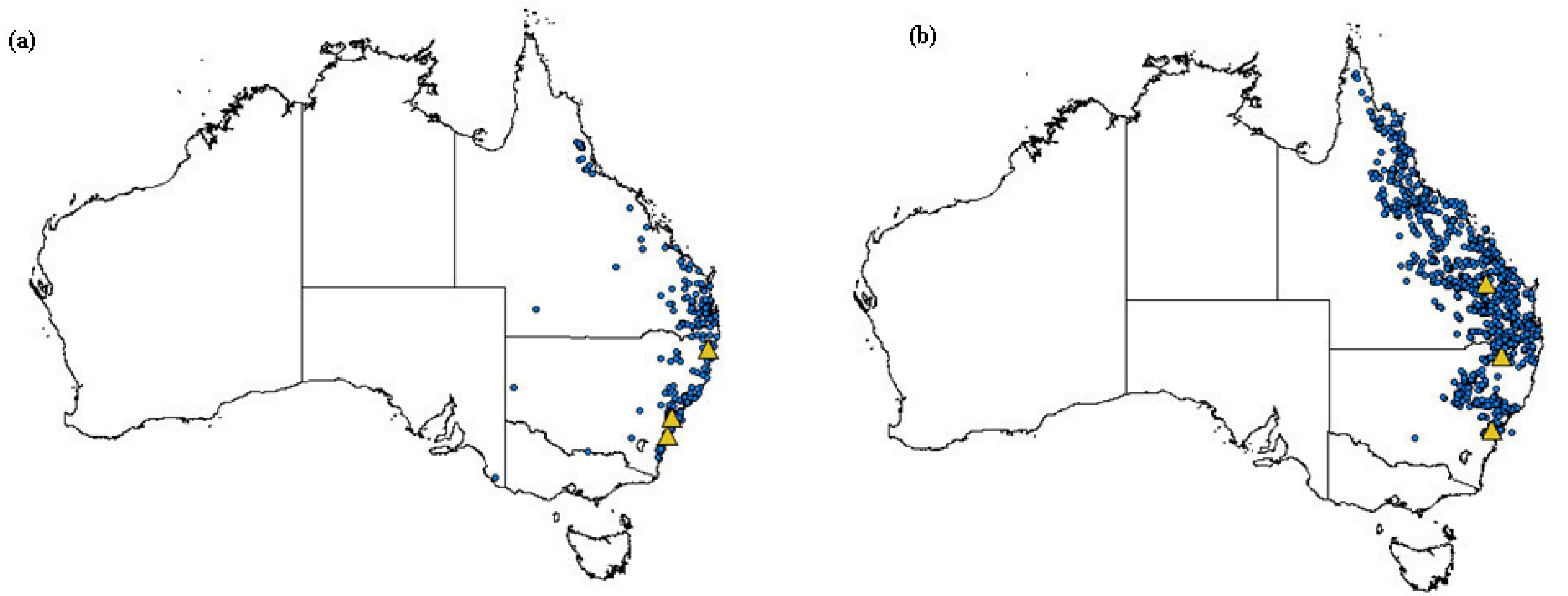


Figure 1. Distribution (dots) and approximate locations of the three provenances (triangles) from which seeds were sourced for: (a) *Acacia falcata* (b) *Eucalyptus crebra*. (Maps and distribution data sourced from Australia's Virtual Herbarium: http://www.rbg.vic.gov.au/cgi-bin/avhpublic/avh.cgi, accessed October 2008)

Methods

Plant material and growth conditions

Seeds from three provenances each of *A. falcata* and *E. crebra* were germinated (Table 1). The *Acacia s*eeds were placed in boiling water and soaked overnight, drained and then placed on top of soil mix in 1L pots and covered with vermiculite. The soil mix consisted of 1.5 buckets sand: 4.5 buckets *Debco Plugger 555 Striker - Low P* potting mix and 540g *Osmocote Plus Native Plant Fertilizer* (slow release) (N:17%, P:1.6%, K: 8.7%). The *Eucalyptus* seeds were sown in seedling trays on top of pre-mixed sand and peat (2:1 buckets) and *Nutricote Total + TE 140 day* (N:17.6, P: 2.9, K: 6.9) slow-release fertilizer (9 teaspoons), then covered lightly with sand. After germination, seedlings were transplanted into 1L pots containing premixed 80% *Debco Plugger 555 Striker - Low P* potting mix, 20% sand & 3gms/L *Osmocote Plus Native Plant Fertilizer* (slow release) (N:17%, P:1.6%, K: 8.7%). Ingredients for the soil mix were combined by using a cement mixer. All plants were germinated in glasshouses in ambient CO_2 conditions with natural light.

Acacia germination occurred over several days, with no obvious differences between provenances. Seedlings were randomly assigned to one of six growth cabinets, 2 - 3 days after germination. Of the *Eucalyptus* seedlings, the Queensland (Qld) and western Sydney (wSyd) provenance plants germinated one week before the western NSW (wNSW) provenance plants. All *Eucalyptus* seedlings were transferred to growth cabinets seven days after transplanting. Treatment commenced on the first two germinating provenances one week before the wNSW provenance. Equal growing time between the provenances was achieved by harvesting the wNSW provenance one week after the other two provenances. The CO_2 treatment commenced at the time of placement in the growth cabinets.

Table 1. Provenances (locations), approximate latitude and longitude of seed source, date seeds collected and seed suppliers for *Acacia falcata* and *Eucalyptus crebra.*

Provenance	Lat/Long (approx)	Date seed collected	Seed Supplier
	Acacia falcata		
South Coast, NSW (sNSW).	34.88/150.61	1/12/06	Australian Seed Company
Greendale, western Sydney, NSW (wSyd).	33.91/ 150.67	21/12/98	Botanic Gardens Trust
Grafton, North coast, NSW (nNSW).	29.45/ 152.55	28/11/2004	Botanic Gardens Trust
	Eucalyptus crebra		
Mt Annan, western Sydney, NSW (wSyd).	34.06/ 150.77	13/2/04	Botanic Gardens Trust
Gilgandra, western NSW (wNSW).	31.71/ 148.67	9/1/96	Australian Tree Seed Centre
Biloela, Queensland (Qld)	24.24/ 150.30	2/2009	Nindethana Seed Supply

A total of 360 plants were placed in six *Thermoline (Australia) Plant Growth Chambers* (10 pots per provenance per chamber), using a random block design. CO_2 (food grade 082) was dispensed and maintained in growth cabinets by a device fitted with a CO_2 monitoring sensor and a solenoid. CO_2 levels within three cabinets were set at 550 ±25 ppm (µL CO_2 L^{-1}) for the day time hours only (14 hours/day), while the other three cabinets were maintained at ambient CO_2 conditions. Temperatures varied slightly between the growth cabinets: daytime temperatures averaged from 25.5°C to 26.5°C and between 17.7°C to 18.6°C for the night time hours. Light levels within the cabinets were approximately 652 µmol m^{-2} s^{-1}. All plants were watered daily and were randomly rearranged within their cabinets each week. Fungicides and pesticides were applied equally to all plants as needed, according to the manufacturer's instructions. AzaMax™ was applied on two occasions and a Lime sulphur treatment (10mls/L) was applied once. PestOil (Yates) was applied to the *Acacia* plants on two occasions and on three occasions for the *Eucalyptus* plants.

Within a month of commencing the treatment, the eucalypts were moved from the chambers into glasshouses because their leaves were exhibiting severe curling, most likely due to insufficient light levels in the cabinets. The eucalypts that were in the three elevated CO_2 cabinets were grouped together and placed in one glasshouse under natural light and similarly for the ambient-CO_2 plants. The temperature and CO_2 levels in the glasshouses into which the eucalypts were transferred were set at the same levels as the corresponding cabinets. On a sunny day, natural light in the glasshouse averaged 745 compared to 1320 $\mu mol\ m^{-2}\ s^{-1}$ recorded outside.

Measurements (Plant responses)

Plants were subjected to a CO_2 treatment for an average of 71 days for *A. falcata* and 54 days for *E. crebra* (the *E. crebra* plants spent approximately 50% of the treatment time in the growth cabinets and 50% in the glasshouses). Before the plants were harvested, they were left in a glasshouse overnight to ensure that the leaves were fully illuminated for ≥ 2 hours (as per standardized protocols; Cornelissen et al. 2003). Survivorship was assessed as ‘alive’ (green leaves &/or stem) or ‘dead’ (no green anywhere on the plant). The primary stem of each plant was measured for height, for both species (from soil level to the tip of the terminal apex), and stem diameter, for *E. crebra* plants only (at soil level, below the lignotuber). Two fully expanded leaves were removed from each individual plant (as per standardized protocols; Cornelissen et al. 2003), then scanned and measured for Specific Leaf Area (SLA; leaf area per unit dry mass) using *ImageJ®* software. Lignotubers in *E. crebra* were recorded as present if they were visible to the naked eye or could be felt on the stem. After harvesting, roots and shoots were oven dried before weighing. Dried leaves were randomly selected from those used for SLA measurements, ground to a fine powder and C:N ratio measured on 108 subsamples with a CHN-900 analyser (Leco, USA). Estimates of rhizobium nodule abundance for *A. falcata* was measured by recording the following characteristics (as per Thrall et al. 2007): (i) presence/absence of nodules, (ii) nodule number (<10, 10 – 50, >50), (iii) nodule functionality based on colour and size (scores ranged from 1, small-non-N_2-fixing nodules with white centres to 5, large nodules with pink/red centres) and

(iv) nodule distribution (from 1, nodules distributed all or mostly within the root crown to 5, nodules more broadly distributed throughout the root system).

Statistical Analyses

Differences between the provenances were compared using a general linear model with provenance and CO_2 treated as fixed factors while growth cabinet (nested in CO_2 treatment) was treated as a random factor, with their interactions included in the model. An α level of 0.05 was used to determine significance in all tests. Where no growth cabinet affect was detected (p>0.25), data were pooled within CO_2 treatments and the data reanalyzed. *Post-hoc* multiple comparisons were carried out using the Bonferroni test. When required, data were transformed to satisfy assumptions of the model. There was a substantial departure from normality in the *E. crebra* SLA data; a non-parametric Kruskal-Wallis one way test was therefore performed on each factor and Mann-Whitney *post hoc* tests compared the means. Data from presence/absence of nodules and number of nodules present were combined and tested using Chi-Square tests. To account for the transfer of the *E. crebra* plants from the six growth cabinets to the two glasshouses, survival and height measurements were taken at the time of transfer and are reported separately.

Results

***Species responses to elevated* CO_2**

Growth rates increased substantially under eCO_2 for both species (Table 2). The mean increases for *A. falcata* were: stem height + 37% and above-ground biomass + 72% and for *E. crebra*: stem height + 17%, above-ground biomass + 51% and stem diameter +17% (data not shown). Only stem height for *E. crebra* was statistically significant between treatments. Percentage increases in growth were much higher for *A. falcata* than *E. crebra*, and the inconsistency in statistical significance was possibly due to the significant variability between *A. falcata* plants grown in different cabinets. Growth cabinet was a significant factor for all *A. falcata* traits, except for survival and nodulation, but only for stem diameter, total above-ground biomass and SLA for *E. crebra.* The magnitude of the effect of the growth cabinets varied considerably between the species (e.g.

above-ground biomass for *A. falcata* $F_{(4,163)}$=39.875, $p \leq 0.0001$: *E. crebra* $F_{(4,165)}$=3.704, p=0.0065).

Before the transfer of the *E. crebra* plants from the growth cabinets to the glasshouses, survival and height were measured. Under eCO_2, stem height for the species had increased by an average of 6.7% before the transfer and by 17% at the end of the experiment. Survival rates were high for both species, regardless of the CO_2 treatment. The direction of the change in allocation of roots to total biomass was different for the two species. *Acacia falcata* plants increased their allocation to roots by 11.9% whereas the *E. crebra* plants significantly reduced their allocation by 6.3% (Table 2). SLA decreased and C:N ratio increased for both species but was significant only for *E. crebra* (Table 2). Lignotuber counts and mean stem diameters for *E. crebra* were higher under eCO_2 but these differences were not statistically significant ($\chi^2_{(1)}$ =0.0022, p=0.9622 and $F_{(1,165)}$=4.401, p=0.1157 respectively) (data not shown).

The percentage of plants with nodules present and the function and distribution of nodules were higher under eCO_2 compared to ambient for the legume, *A. falcata,* but the differences were not statistically significant ($F_{(1,12)}$=3.571, p=0.0832: $\chi^2_{(4)}$=6.333, p =0.176 and $\chi^2_{(4)}$=4.309, p=0.366 respectively). However, the number of nodules per plant was significantly higher under eCO_2 compared to ambient ($\chi^2_{(2)}$=6.709, p=0.0349) (data not shown).

Table 2: Species responses (mean (±SE) to elevated *(*550 ±25 ppm) vs ambient CO_2 of *Acacia falcata* and *Eucalyptus crebra*. Significance levels: * *p*<0.05, ** *p*<0.01, *** *p*<0.001

Species	**CO_2**	**Survival** (%)	**Stem height** (cm)	**Above-ground biomass** (g)	**Root/ total biomass** (g)	**SLA** (mm^2mg^{-1})	**C:N**
Acacia falcata	Amb	94.4	16.80 (0.61)	2.361 (0.13)	0.2389 (0.01)	13.384 (0.22)	36.16 (3.41)
	Elv.	96.7	23.05 (0.94)	4.067 (0.24)	0.2673 (0.01)	12.537 (0.20)	50.53 (2.64)
Eucalyptus creba	Amb	97.0	27.28 (1.18)	2.753 (0.17)	0.2152 (0.01)	16.981 (0.72)	55.96 (4.28)
	Elv	100.0	31.86 (1.15) **	4.152 (0.24)	0.19105 (0.01)*	14.006 (0.53)***	87.20 (9.81)***

Provenance (intraspecific) responses

Regardless of the CO_2 treatment, significant differences occurred among the provenances for all traits, except for survival, nodulation and C:N ratio for *A. falcata* and survival and presence of lignotubers for *E. crebra*.

For *A. falcata*, the wSyd provenance was significantly taller ($F_{(2,8)}$=89.647, *p*≤0.0001) (Figure 2a) and heavier ($F_{(2,8)}$=12.197, *p*=0.0037) (Figure 2b) than the other two provenances and the nNSW provenance respectively, and allocated the most photosynthate to above-ground biomass rather than to roots ($F_{(2,8)}$=6.8761, *p*=0.0183) (Table 3). There were no significant differences between the provenances for any nodulation traits and all values increased under eCO_2 except for the sNSW provenance for which nodule function was unchanged. However, CO_2 enrichment did substantially increase the percentage of plants with nodules for the wSyd provenance; +22% compared to 8 and 10% for the other two provenances (data not shown).

Of the three *E. crebra* provenances, the wNSW provenance was significantly different to the other provenances for many traits. The wNSW provenance was the smallest (height $F_{(2,177)}$=26.06, $p\leq0.0001$, above-ground biomass $F_{(2,8)}$=31.308, p=0.0002) (Figure 2), its allocation of photosynthate to roots in relation to total biomass was the highest $F_{(2,177)}$=79.851, $p\leq0.0001$) and it had the lowest C:N ratio ($F_{(2,47)}$=18.133, $p\leq0.0001$) (Table 4). However, the wNSW provenance increased its above-ground biomass under eCO_2 by 117%, a considerably larger increase compared to the other provenances. The Qld provenance was the least responsive to eCO_2 for growth traits.

Table 3. General Linear Model and X^2 results for the effects of provenance and elevated CO_2 on survival, growth, allocation and nodulation traits of *Acacia falcata.* For X^2 tests, factors were analyzed individually (interactions were not analyzed).

	Provenance	**CO_2 x Provenance interaction**
Survival	$X^2_{(2)}$=3.50, p=0.1734	Ambient: $X^2_{(2)}$=1.92, p=0.3832
		Elevated: $X^2_{(2)}$=1.99, p=0.3695
Stem height	$F_{(2,8)}$=89.647, $\boldsymbol{p}$**=≤0.0001**	$F_{(2,8)}$=3.8315, p=0.0681
Above-ground biomass	$F_{(2,8)}$=12.197, $\boldsymbol{p}$**=0.0037**	$F_{(2,8)}$=1.4134, p=0.2981
Root/total biomass	$F_{(2,8)}$=6.8761, $\boldsymbol{p}$**=0.0183**	$F_{(2,8)}$=0.6713, p=0.5377
SLA	$F_{(2,8)}$=29.699, $\boldsymbol{p}$**=0.0002**	$F_{(2,8)}$=2.26, p=0.1667
C:N	$F_{(2,8)}$=1.4839, p=0.2831	$F_{(2,8)}$=0.6326, p=0.5558
Nodulation: % of plants nodulated	$F_{(2,8)}$=0.7471, p=0.4946	$F_{(2,8)}$=0.3368, p=0.7206
Nodulation: Number of nodules	$X^2_{(4)}$=2.71, p=0.6075	Ambient: $X^2_{(4)}$=4.54, p=0.3375
		Elevated: $X^2_{(4)}$=2.81, p=0.5903
Nodulation: Function	$X^2_{(8)}$=5.71, p=0.6796	Ambient: $X^2_{(6)}$=6.14, p=0.4081
		Elevated: $X^2_{(8)}$=5.40, p=0.7141
Nodulation: Distribution	$X^2_{(8)}$=11.86, p=0.1575	Ambient: $X^2_{(4)}$=2.34, p=0.6728
		Elevated: $X^2_{(8)}$=14.00, p=0.0818

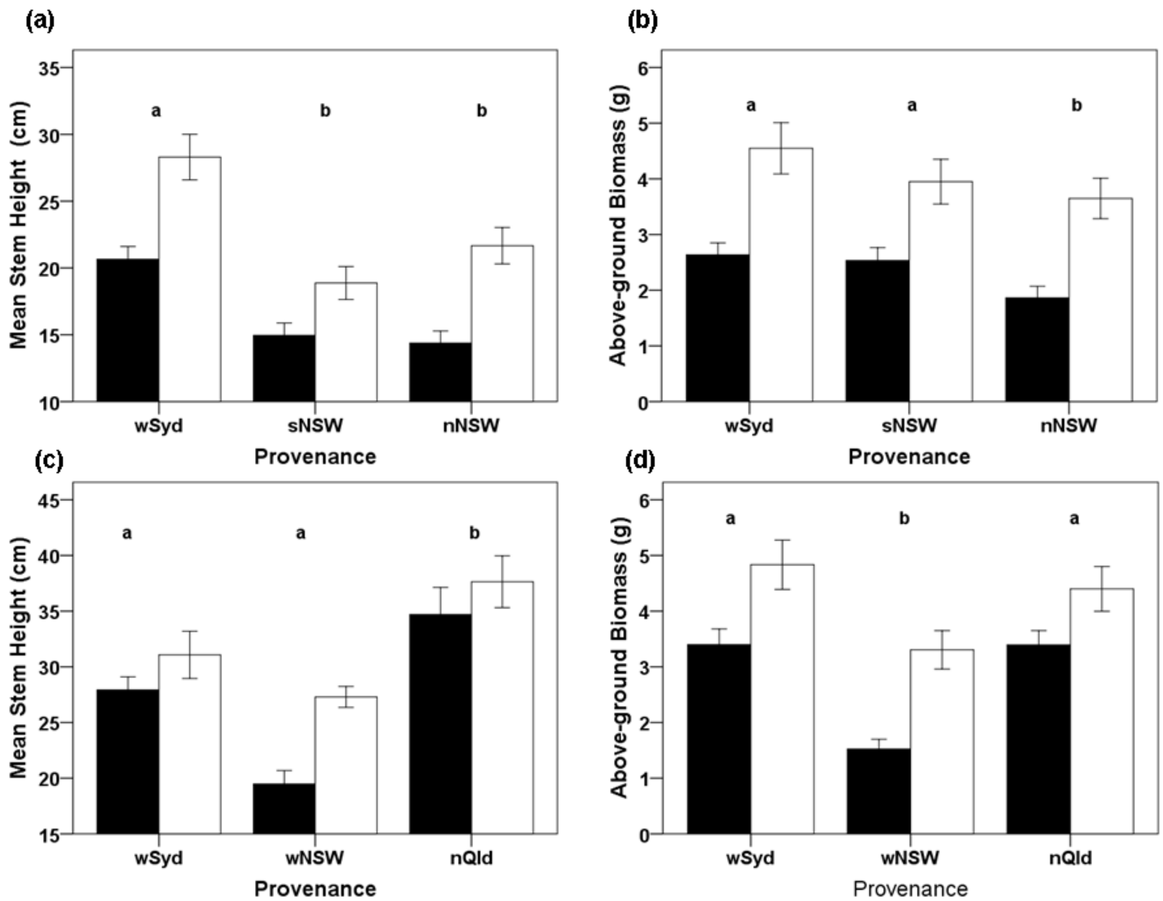


Figure 2. Growth responses to ambient (black bars) and elevated CO_2 (white bars) of three provenances of *Acacia falcata* for (a) stem height and (b) above-ground biomass and *Eucalyptus crebra* for (c) stem height and (d) above-ground biomass. Provenances sharing the same lower-case letters are not significantly different. Note the truncated y-axes.

Table 4. General Linear Model, X^2 and Kruskal-Wallis results for the effects of provenance and elevated CO_2 on survival, growth and allocation traits of *Eucalyptus crebra.* For X^2 and Kruskal-Wallis tests, factors were analyzed individually (interactions were not analyzed).

	Provenance	CO_2 x Provenance interaction
Survival: (January)	$X^2_{(2)}$=1.82, p=0.4025	Ambient: $X^2_{(2)}$=1.85, p=0.3964
		Elevated: No deaths
Survival: (December)	$X^2_{(2)}$=0. 97, p=0.6158	Ambient: $X^2_{(2)}$=0.98, p=0.6126
		Elevated: No deaths
Stem height (January)	$F_{(2,177)}$=26.06, $\mathbf{p \leq 0.0001}$	$F_{(2,177)}$=1.2259, p=0.2960
Stem height (December)	$F_{(2,178)}$=103.84, $\mathbf{p \leq 0.0001}$	$F_{(2,178)}$=0.0892, p=0.9147
Stem diameter	$F_{(2,8)}$=20.983, $\mathbf{p=0.0007}$	$F_{(2,8)}$=5.1062, $\mathbf{p=0.0372}$
Above-ground biomass	$F_{(2,8)}$=31.308, $\mathbf{p=0.0002}$	$F_{(2,8)}$=4.1386, p=0.0584
Root/total biomass	$F_{(2,177)}$=79.851, $\mathbf{p \leq 0.0001}$	$F_{(2,177)}$=1.0888, p=0.3389
SLA	$H_{(2)}$=17.04, $\mathbf{p \leq 0.001}$	Ambient: $H_{(2)}$=14.86, p=0.001
		Elevated: $H_{(2)}$=5.36, p=0.069
C:N	$F_{(2,47)}$=18.133, $\mathbf{p \leq 0.0001}$	$F_{(2,47)}$=0.6866, p=0.5082
Lignotuber:	$X^2_{(2)}$=0.83, p=0.6600	Ambient: $X^2_{(2)}$=3.44, p=0.1795
Presence/absence		Elevated: $X^2_{(2)}$=0.61, p=0.7387

CO_2 by provenance interactions

Interactions between CO_2 and provenance were significant only for stem diameter in *E. crebra* $F_{(2,8)}$=5.1062, p=0.0372 (Table 4). The CO_2 treatment had a negligible effect on the stem diameter of the nQld provenance compared to the response of the other two provenances (Figure 3). The interaction for total above-ground biomass for *E. crebra* was marginally non-significant ($F_{(2,8)}$=4.1386, p=0.0584) and is highlighted as the data is unbalanced and therefore possibly prone to Type 2 errors. The Qld provenance gained the least above-ground biomass under elevated CO_2.

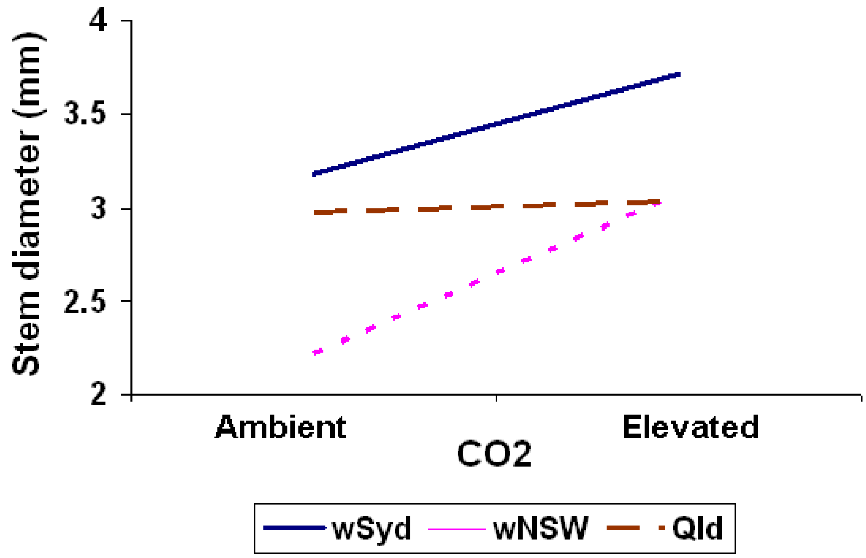


Figure 3. Interaction of provenance x CO_2 for stem diameter for three provenances of *E. crebra*. Note truncated y-axis.

Discussion

There are two major findings of this study. Firstly, on average, the provenances responded similarly to eCO_2, with stem diameter in *Eucalyptus crebra* the only parameter in which the provenance x CO_2 interaction was significant. Secondly, we found significant intraspecific variation for both species. For *Acacia falcata,* significant differences amongst provenances were found for the response to eCO_2 of stem height, above-ground biomass, root/total biomass and SLA. For *E. crebra*, differences between the provenances were found for stem height, stem diameter, above-ground biomass, root/total biomass, SLA and C:N ratio. There were no significant differences between the provenances for survival in either species.

Provenance (intraspecific) responses to elevated* CO_2 *and their interaction

Our study found significant variability in all growth traits among provenances but little difference in their responsiveness to eCO_2*.* Significant variation in growth traits among genotypes with little or no genotype x CO_2 interaction has also been demonstrated in 29 genotypes of *Picea glauca* (Mycroft et al. 2009), six genotypes of *Populus tremuloides* (Curtis et al. 2000) and for height in 10 populations of *Eucalyptus pauciflora* (McKiernan et al. 2012). In another study of *Populus tremuloides*, significant genotype x CO_2 interactions for photosynthetic responses were found but there was a poor correlation between photosynthesis and growth (Wang et al. 2000). In a 'reverse' experiment using clones from four genotypes of *Nothofagus cunninghamii*, when CO_2 was reduced from ambient to 170 µmol mol^{-1}, there was no genotype x CO_2 interaction for total weight (Hovenden & Schimanski 2000).

A significant provenance x CO_2 interaction detected for stem diameter in *E. crebra* plants, suggest that some provenances of *E. crebra* may perform better than others as atmospheric CO_2 levels continue to rise. The growth response to eCO_2 was consistently smaller for the nQld provenance.

For *E. crebra*, the western NSW (wNSW) provenance had the largest percentage response to CO_2 for all growth traits but was consistently the smallest of the three provenances, regardless of the measured trait. The ranking of the wNSW and wSyd provenances for height, stem diameter and above-ground biomass is consistent with the results from a 12 month field study undertaken in western Sydney (Chapter 2). The wNSW provenance also differed from the other two provenances, having the highest root to total biomass ratio and a significantly lower leaf C:N ratio. The C:N ratio finding is similar to McKiernan et al. (2012) who found significant differences among *E. globulus* and *E. pauciflora* populations for some secondary leaf chemical traits but no provenance by CO_2 interactions. The higher nitrogen ratio in the wNSW leaves suggests that herbivory levels will be higher for this provenance. Higher leaf C:N (and the accompanying increase in phenolics and condensed tannins) was demonstrated to increase mortality and reduce pupal body size of a common eucalypt herbivore, *Chrysophtharta flaveola* (Lawler et al. 1997). However, in a 12-month field trial in western Sydney, the wNSW provenance had the lowest rate of herbivory compared to four other provenances, one of which was the wSyd provenance (Chapter 2). The contrasting results of the two studies may be, in part, due to the differences in nutrient levels in the pot grown plants compared to the natural soil conditions.

For *A. falcata,* the performance of the western Sydney (wSyd) provenance was notably different to the other two provenances. Regardless of the CO_2 treatment, the wSyd provenance was significantly larger (for all growth traits) and allocated significantly fewer resources to roots than to above-ground biomass compared to the other two provenances. The height ranking of the three provenances is consistent with the results from a field study undertaken in western Sydney for approximately 12 months (height was the only growth measurement taken in both studies) (Chapter 2). Under ambient $CO_{2,}$ the wSyd provenance had substantially fewer nodulated plants compared to the other two provenances but in response to eCO_2, its percentage increase of nodulated plants was double that of the other two provenances.

Species responses to elevated CO_2

Eucalypts and Acacias dominate the Australian species investigated for response to eCO_2 (as reviewed in Hovenden & Williams 2010). However, this study is the first to investigate the responses of *A. falcata* and *E. crebra.* Interspecific comparisons of the response rates to eCO_2 cannot be made between the two species in this study because the nutrient levels and growing conditions differed for each species. The *E. crebra* plants were transferred from the growth cabinets to glasshouses during the experiment (spending approximately half of the treatment time in the glasshouses). Whilst the CO_2 levels and temperatures in the glasshouses and the growth cabinets were set at the same levels, other factors such as light intensity between two facilities differed.

The large increases in above-ground biomass for the legume *A. falcata* (+72%) was substantially higher than that generally found for legumes in FACE experiments (+24%) (Ainsworth & Long 2005) but at the lower end of the range found for six fast-growing Acacias (*Acacia dealbata, A. implexa, A. mearnsii, A. melanoxylon, A. irrorata* and *A. saligna*) (Atkin et al. 1999). In the current study and the study of fast-growing Acacias, it is unlikely that growth was constrained by water or nutrients, whereas the FACE study is a meta-analysis of many species under various conditions. The above-ground biomass response to eCO_2 of *E. crebra* (+51%) is consistent with increases demonstrated for other eucalypts (Medlyn et al. 2011) and with other studies of C_3 species (Poorter & Navas 2003; Wang et al. 2012) but the magnitude of the responses varied considerably, depending on nutrient and water availability.

Substantial increases in leaf C:N and a reduction in SLA under eCO_2 are commonly found for C_3 species (Lawler et al. 1997; Saxe et al. 1998; Roden et al. 1999; Poorter & Navas 2003; Ainsworth & Long 2005), and were confirmed in the current study (although the results were significant only for *E. crebra*). Contrary directions of the allocation of photosynthate between above- and below-ground biomass are common among different species (Curtis & Wang 1998; Saxe et al. 1998), and were demonstrated in the current study: *A. falcata* plants increased their biomass allocation to roots whereas the *E. crebra* plants reduced their allocation. CO_2 enrichment increased all aspects of nodulation of the *A. falcata* plants and is consistent with findings for other *Acacia* species (Schortemeyer et al. 2002).

There is need for caution when extrapolating these results to responses of plants in the field. Firstly, the plants in the current study were unlikely to be limited by nutrients or water availability and were grown under constant temperature, conditions not usually encountered in the field. The provision of nutrients has been demonstrated to significantly affect plant responses to eCO_2 (Poorter & Navis 2003) and it has been argued that differences in results are more affected by nutrient supply than differences between experimental conditions (e.g. glasshouse conditions vs FACE) (Körner 2006). Generally, CO_2 enrichment produces positive effects on growth under mild temperature increases but under high stress, the direction and the magnitude of the results vary (Wang et al. 2012). Secondly, the interactive affects of CO_2 with other environmental factors such as soil nutrients, water, temperature, competition and with herbivory were not investigated in the current study. Thirdly, plants will be subjected to continuous increases in CO_2, rather than the single-step change imposed on the seedlings in the current study. Generally, our results may also have been influenced by the high variability within the growth cabinets, significantly affecting the *A. falcata* results, with one cabinet in particular experiencing mechanical difficulties.

Our study did not control for environmental maternal effects, differences in seed age or size of the source populations and whilst these factors may have influenced the results, germination was not assessed. In regard to seed age, we note that seeds from species in the Myrtaceae and Fabaceae families remain viable for longer than for many other families (Martyn et al. 2009). There were no obvious differences between the *Acacia* provenances at the start of the treatment. We accounted for the later germination of one *Eucalyptus* provenance by delaying the harvest of that provenance. Whilst there is some debate as to whether pot size is a possible factor affecting the magnitude of photosynthetic responses to eCO_2 (Wang et al. 2012), the pot size used in the current short-term study (1 L) is larger than the 0.5 L pot size that has been found to significantly affect photosynthetic acclimation to eCO_2 (Curtis & Wang 1998).

Conclusion

Significant intraspecific variation was found for most traits in *Acacia falcata* (excluding survival, C:N ratio and nodulation) and in *Eucalyptus crebra* (excluding survival and presence of lignotuber). The significant findings were irrespective of the CO_2 treatment, except for one significant provenance by CO_2 interaction for stem diameter in *E. crebra*. The implications of these findings for restoration ecology are two-fold. The intraspecific variation that exists within the two study species presents an opportunity to enhance genetic diversity within revegetation sites and to provide the raw material on which selection can act (although factors other than eCO_2 may be the main drivers of the selection). If the intraspecific differences demonstrated in this study are replicated in the field, the use of mixed provenances may improve establishment success at some revegetation sites. The differences between the provenances for nodulation success (although differences were substantial, they were not statistically significant), allocation of photosynthate to roots rather than to biomass, and general growth variability, may confer higher establishment success under differing environmental conditions.

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CHAPTER 5

How far is it to your local? A survey on local provenance use in New South Wales.

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Abstract

The decision as to where to source seed is one of the most critical in restoration projects. Locally-collected seed is often recommended, or even contractually required, because it is assumed to be adapted to local conditions and therefore result in superior survival and growth rates, conferring a greater probability of restoration success. The perceived advantages, which include retaining the genetic 'integrity' of the site, are centred around the avoidance of outbreeding depression and hybridization. These traditional reasons for using locally-collected seed need to be reconsidered in the light of rapidly changing climatic and other environmental conditions; plants that are locally-adapted now, may not be locally-adapted in the future. Understanding the current usage of local provenance is pivotal to discussions on its appropriateness under climate change.

We present the results of a survey of restoration practitioners in New South Wales on attitudes and practices in relation to the use of local provenance. We found that while the majority of practitioners preferentially use local provenance seeds, the actual definition of local provenance varied amongst respondents. Whilst 80% of participants believe that projections of future climate change are relevant to restoration projects, there is an apparent reluctance to actively manage for this eventuality. However, many respondents are in favour of a review of seed-sourcing policy/guidelines to allow for the inclusion of non-local provenance material. Implications of the survey for potential changes to guidelines to better prepare for anticipated changing conditions are discussed.

Keywords: Climate change, local adaptation, local provenance, revegetation, seed-source

Introduction

Revegetation is a core activity of many restoration projects (See Box 1 for glossary). Increasingly, revegetation with native species is identified as a partial solution for many landscape and biodiversity problems. The recently commenced Biodiversity Fund, providing nearly $1 billion for carbon storage and biodiversity benefits, is one such example (Australian Government 2012).

An integral component of any revegetation project is the decision as to where to source the genetic material (including seeds or container-grown plants). Genetic material can either be sourced locally (hereafter referred to as local provenance), or from more distant locations within the species range. In recent decades, genetic material for revegetation projects has often preferentially been sourced locally. It has generally been assumed that locally-sourced plants are better adapted to local conditions and will therefore survive longer, grow faster and have reproductive advantages over non-local plants, thus providing the greatest chance of revegetation success. Exactly what constitutes 'local' is not always defined, but in many instances a maximum distance between the seed collection area and the proposed revegetation site is specified (generally 5-20 km). When environmental gradients are steep between the planting site and the seed source, locally-sourced plants may have a home-site advantage over non-local provenances (Hereford 2009). Examples of this include low vs high elevation, coastal vs inland, frost vs no frost and contaminated vs natural soils (see references in: Millar & Libby 1989; Linhart & Grant 1996; Davis & Shaw 2001). However, not all plants and/or populations are locally adapted and in some cases, non-local provenance plants survive and grow better than local provenance plants (e.g. Gordon & Rice 1998). The introduction of non-local material may also alter interactions between plants and their natural enemies or mutualists and these changes may be either beneficial or detrimental to either partner (Linhart & Grant 1996; Vander Mijnsbrugge et al. 2010 and references therein).

Box 1: Definitions used in the survey and this study

Genetic material: seed, cuttings, tubestock or other propagules
Local provenance: genetic material that has been sourced locally i.e. close to the proposed planting site
Inbreeding Depression: A reduction in survival, growth & reproduction in inbred offspring (caused by mating between relatives), relative to the parents
Outbreeding Depression: A reduction in survival, growth & reproduction of offspring from crosses between plants from different populations, relative to the parental populations i.e. sterile hybrids
Restoration (Ecological): the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed
Provenance: the geographic location/origin of the genetic material
Revegetation: adding plants to the ecosystem by planting, seeding or translocating
Threatened Ecological Community: a group of species that occur together in a particular area of the landscape that has been listed as threatened (either categorized as Critically Endangered, Endangered or Vulnerable) under the NSW Threatened Species Conservation Act, 1995: http://www.environment.nsw.gov.au/threatenedspecies/ &/or under the Commonwealth EPBC Act http://www.environment.gov.au/epbc/protect/species-communities.html

The use of local provenance material is also advocated as a strategy to preserve the genetic 'integrity' of the replanted site. Revegetation using non-local genetic material introduces new genetic material into an area. The consequences of such introductions can be positive or negative, depending on the species and the landscape. The introduction of inappropriate non-local provenance material may increase the possibility of outbreeding depression and hybridization, resulting in sterile offspring or unviable seed (Hufford & Mazer 2003). Risks of outbreeding depression and unwanted hybridization can often be predicted (and therefore avoided) (Byrne et al. 2011; Frankham et al. 2011).

Outbreeding depression is largely determined by the time period that populations have been separated and the degree of genetic difference between them (Frankham et al. 2011). A risk assessment protocol is now available to evaluate the likelihood of hybridization on surrounding plant populations (Byrne et al. 2011). The assessment is based on the taxonomy and reproductive biology of the species involved, the extent of pollen dispersal between the planting site and the surrounding vegetation and the differences in size of the sites (Byrne et al. 2011).

The traditional reasons for using local provenance are relevant for long-term restoration success under stable environmental conditions. But the climate is rapidly changing, populations are increasingly becoming more fragmented, land use practices are altering and exotic plants, animals and pathogens are invading. The advantages of being locally adapted will be determined, to a large extent, by the degree and the size of the change or disturbance (Lesica & Allendorf 1999; Jones & Monaco 2009); plants that are locally-adapted now may not be locally adapted in the future. Furthermore, if local populations are small and genetically-impoverished, sourcing seed from these populations may ultimately be detrimental to the long-term success of the revegetation project via inbreeding depression (Broadhurst & Young 2007). Under these conditions, the introduction of seed from genetically-diverse populations can increase the probability of revegetation success (Broadhurst et al. 2008). Genetic variation is fundamental to enable evolutionary adaptation to changing environments. Unless new genetic material enters the population (i.e. via pollen or propagule dispersal), the genetic diversity in small populations will eventually diminish and reduce species capacity to adapt to a changing environment (Weeks et al. 2011).

Seed-sourcing guidelines or seed zones are used by many organizations to delineate areas from which genetic material can be collected for revegetation projects with a minimal risk of maladaptation (Johnson et al. 2004; Krauss & He 2006). Ideally, studies assessing both genetic variation and local adaptation are used to provide accurate delineation (Wheeler et al. 2003). Such detailed information is rarely available and in its absence, different approaches have been taken to establish seed-sourcing guidelines. For example, seed zones in the UK are based on major climatic and geological regions, modified by altitude, while in Europe, various combinations of climate, soil and geomorphology are used to define collection zones (Vander Mijnsbrugge et al. 2010 and references therein). Other examples include the adoption of a 'precautionary' approach, with local provenance cited as 'best practice' (N.S.W. Department of Environment and Conservation 2005), or, the restriction of seed collection zones to a defined radius of the planting site (e.g. no further than 100 m from the site for herbs) (Linhart 1995, cited in Jones & Johnson 1998). Florabank guidelines, which often serve as an industry standard in the eastern states of Australia, provide information on seed collection methods and generalized advice for seed collection ranges for revegetation (Florabank 1999). The Florabank guidelines provide a range of approaches for provenance selection. The basic recommendation is to collect as locally as possible but to also maximize the genetic quality of the seed collected. When circumstances necessitate broader collection, it is suggested that consideration is given to matching the environmental conditions of the source population to the planting site and to be aware that these decisions are site and species specific (Florabank 1999).

The use of local provenance material is a requirement for the attainment of a Section 132C licence to bring in new plant material &/or to collect seed for revegetation in Threatened Ecological Communities and for threatened species in NSW. The use of local provenance material is also either recommended or required in many restoration projects and as a condition of granting agencies. Locally sourced material may sometimes be unavailable in sufficient quantities (Mortlock 2000), necessitating difficult decisions about how to best fulfil legal and contractual obligations and attain the best revegetation outcomes. Mixing different seed sources or 'composite provenancing' using high quality seed that is site- and species-specific, is increasingly being recommended as a method of incorporating a broad range of genetic material as an insurance policy for revegetation success (Lesica & Allendorf 1999; Broadhurst et al. 2008). Understanding the current usage of local provenance is critical for progressing discussions on its appropriateness in the face of a rapidly changing environment. We undertook a survey of restoration and revegetation practitioners and policy makers in New South Wales, Australia, to explore respondent's views on the following issues:

- What constitutes 'local' for local provenance revegetation activities?
- Is local provenance generally considered to be the best choice for revegetation activities?
- Are there supply or other constraints on the use of local provenance?
- Are current guidelines on the use of local provenance adequate under existing and/or anticipated future conditions?

Methods

Survey form

The survey was in the form of an on-line SurveyMonkey (https://www.surveymonkey.com/) (see Appendix A for a copy of the survey) and targeted participants in the restoration industry in New South Wales. Participants were broadly divided into practitioners (defined as anyone who undertakes revegetation using native seeds or seedlings as paid employment or for own business) and non-practitioners (policy makers, educators / researchers, nursery workers / seed collectors, volunteers or those interested in revegetation). The term 'respondent' is used when both practitioners and non-practitioners have answered the questions. 'Skip logic' was applied to the survey whereby respondents only answered questions that were relevant to their role in the industry. Specific questions were directed to: (1) practitioners who set their own policies or procedures regarding provenance, (2) practitioners who follow policies or procedures imposed upon them by another organization, (3) both (1) and (2), and (4) non-practitioners (e.g. policy makers and researchers). Respondents were not required to answer all questions and participation in the survey was voluntary.

Sampling Frame

The survey was largely distributed via the email networks of the Sydney Metropolitan Catchment Management Authority (SMCMA). These networks included the CMA offices, the Volunteer Co-coordinators Network (VCN), Natural Resource Managers (including the Local Government & Shires Associations (LGSAs) and Regional Landcare Facilitators, all in NSW. The survey was also sent out via the Australian Association of Bush Regenerators (AABR), National Parks & Wildlife Service (NPWS) the Ecological Consultants Association of NSW and the Australian Network of Plant Conservation (ANPC). The survey was also sent to representatives of the mining industry, contractors (non-bushland) who list revegetation with native species as a core activity, Indigenous Protection Areas, State Government Agencies, non-government organizations, project managers and native plant nurseries other than those on the AABR website. Apart from contractors (non-bushland), these contacts were either already known to the authors or had been recommended by contacts within the industry as potential respondents. The final number of recipients is unknown but we believe that coverage within the NSW restoration industry was comprehensive. The survey was completed by 144 respondents. The majority of participants were practitioners (56%) (Table 1) and many sectors of the restoration industry were represented (Table 2).

Table 1. Role of respondents in the restoration industry (n = 144 in total)

Role	**Response (%)**
Practitioner who undertakes revegetation using native seeds or seedlings as paid employment or for own business	56.3
Policy maker. This includes anyone who dictates which provenance(s) an organization will use	19.4
Nursery worker &/ or seed collector	6.9
Educator / Researcher	6.3
Volunteer	6.3
Interested in revegetation	4.9

Table 2. Organizations for which respondents represent or work (n = 132)

Organization	**Response (%)**
Local Government	41.7
State Government	15.9
Community group / Volunteer	10.6
Non-government Organization (NGO)	9.8
Contractor (bushland)	7.6
Mining Sector	6.1
Private individual land owner/lessee/manager	4.5
Commonwealth Government	2.3
Carer of Indigenous Protection Areas (IPA)	0.8
Contractor (non-bushland)	0.8

Results

Definition of 'local provenance'

There was little consistency between respondents as to how they defined local provenance but 31% noted that it *depends on the species* (i.e*. it depends on their pollination, dispersal or other traits*) (Figure 1). Twenty nine percent of respondents defined local provenance as *within the catchment of the proposed revegetation site*. Whilst this is a narrow preference, 56% of the comments that accompanied the answers made reference to their definition being species-dependent, further inflating the first preference. Many respondents commented that they consider the definition of local provenance to include other factors that were not listed in the survey, such as the condition of the planting site and the nature of the project.

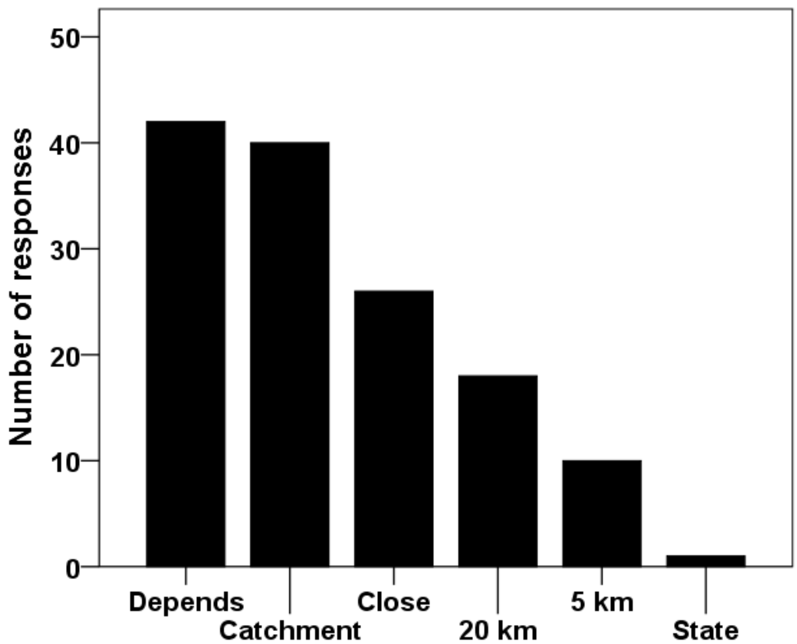

Figure 1. Respondents' definition of local provenance: Depends on the species (i.e. pollination, dispersal or other traits): Within the catchment of the proposed revegetation site; As close as possible to the proposed revegetation site; ≤ 20 km from the proposed revegetation site; ≤ 5 km from the proposed revegetation site; Within the state boundaries of the proposed revegetation site.

Current practice in regard to local provenance use

The majority of practitioners who make their own decisions regarding provenance selection (68%) responded that local provenance is their preferred choice. A further 30% choose a mix of local and non-local. When a mix is chosen, 11 out of the 17 practitioners who answered this question choose mostly local provenance. A clear majority of respondents regard the use of local provenance material as either important or very important for both Threatened Species or Threatened Ecological Communities (TS & TEC) (88%) and non-TS & TEC (80%) revegetation projects. The finding that local provenance use is important regardless of the vegetation status (threatened/endangered or common) was irrespective of the respondent's role within the industry, the organization for which they work or the Catchment Management Authority

(CMA) region in which they work (for the major groups only) (See Appendix B for CMA regions).

One third of practitioners indicated that they work with plant populations of less than 200 adult plants per species (a further one third were unsure of the number). Of the 23 practitioners who work with less than 200 plants per species, the majority use local provenance material exclusively.

Difficulties in obtaining a sufficient supply of local provenance plants had been experienced by 74% of practitioners. This shortage was overcome with a combination of strategies but the single largest response (33%) was to reduce the diversity of planting (Figure 2). Almost half (47%) of practitioners anticipate that their revegetation activities will increase over the next five years while 30% expect no change. In the past 12 months, 38% of practitioners planted less than 8,000 seedlings (or ≤ 5 kg seed), 20% planted between 8,000 - 25,000 seedlings (or between 5 & 20 kg seed) and 25% planted 25,000 seedlings or greater than 20 kg seed. Of the largest users of native stock, 55% responded that they use all local provenance seed (the remainder were unsure or the question was not applicable).

Current policies and guidelines

Two thirds of respondents indicated that they were in favour of a review of seed-sourcing policy/guidelines to allow for the inclusion of non-local provenances. Currently, almost half of the practitioners use a mix of procedures or specifications to determine their choice of provenance (Figure 3) with some respondents complying with up to four different sets of guidelines. The single most commonly used guidelines were those of Florabank (19%), followed by licence conditions (17%).

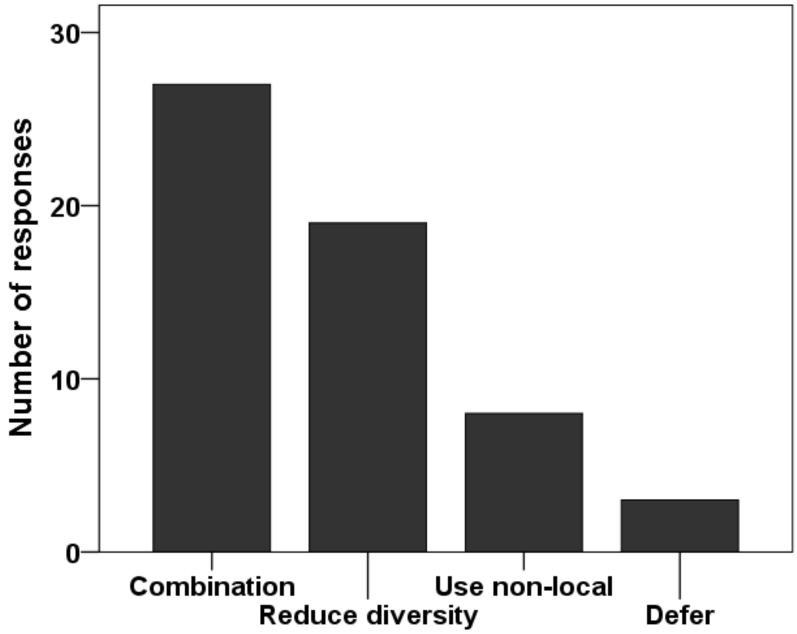

Figure 2. Practitioners' response to the difficulty in the obtainment of local provenance plants: A combination of the following; Reduced the diversity of planting to only those species for which local provenance is available; Used non-local provenance material; Deferred the project.

Of the practitioners who responded to the survey, 88% work in locations where both threatened species or Threatened Ecological Communities (TS & TEC) and non-TS& TEC are present. Accordingly, the majority of practitioners (54%) follow policies or procedures imposed upon them by another organization (i.e. licence conditions) and/or set their own policies. A further 18% solely follow policies or procedures imposed upon them by another organization.

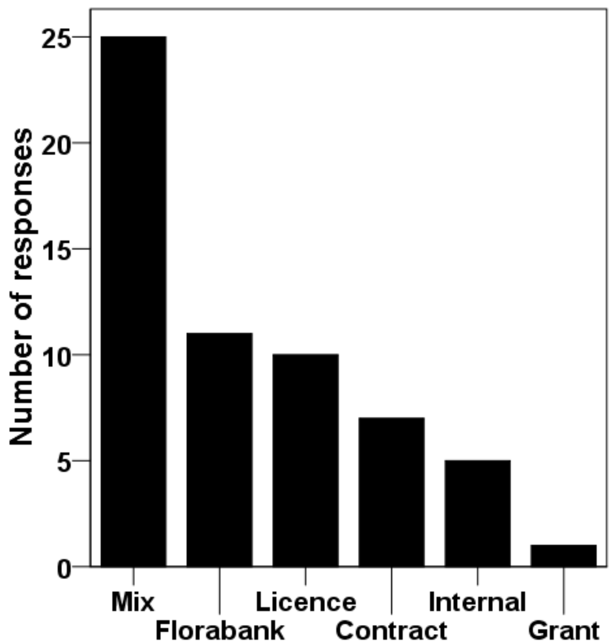

Figure 3. Procedures or specifications used by practitioners in determining the use of local vs non-local provenance for the majority of work undertaken: A mix of the following; FloraBank guidelines; Licence conditions; Contract conditions; Requirement of internal policy; Requirement of grant application or funding arrangement.

There was variation in understanding of the reason(s) that underpinned provenance guidelines (Table 3). The most common response (34%) was that the requirement was because of a combination of many factors, most notably, local knowledge and adherence to the precautionary principle.

Table 3. Opinions of respondents about why local provenance is required or recommended (n = 134**)**

Reason	**Response (%)**
A combination of reasons listed below	34.3
Practical experience of superior restoration results using local provenance (local knowledge)	26.1
Adherence to Precautionary Principle	20.1
Untested theory within the industry	9.0
Results from peer-reviewed scientific literature of provenance trials/research	6.7
Not sure	3.7

Future use of local provenance

A clear majority of respondents (80%) believe that projections of future climate change (e.g. more extreme weather events, increased temperatures, changes in rainfall patterns) are relevant to restoration/revegetation projects. However, *improving evolutionary potential to adapt to a changing environment* was not highly ranked as a reason for choice of provenance (Table 4). As a climate-change management strategy, 67% would consider using or advocating for the use of non-local provenance genetic material sourced from areas with current climatic conditions similar to those predicted for the proposed revegetation site. However, actual preparatory measures to deal with future climate-change impacts are being undertaken by less than half (45%) of respondents and less than one third of practitioners have a future-focus as their long-term restoration goal (Table 5).

Table 4. Ranked order of importance for choice of provenance (unimportant = 1 to very important = 5) . Numbers represent the response count in each category

Reason for choice of provenance	Unimportant	Not important	No opinion/don't know	Important	Very important	Rating Average
Match environmental conditions of source population to proposed planting site (temperature, rainfall, aspect, soil etc)	0	1	2	22	30	4.47
Increase genetic diversity	0	5	3	25	22	4.16
Avoidance of potential inbreeding depression	0	6	12	19	18	3.89
Size of source population/ recipient population	1	5	9	25	14	3.85
Limit the distance of the source population to proposed planting site	4	4	7	28	12	3.73
Improve evolutionary potential to adapt to a changing environment	2	6	12	23	13	3.70
Avoidance of potential outbreeding depression	0	8	18	16	13	3.62

Table 5. Long term restoration goals of practitioners' organizations (n = 76)

Goal	Response (%)
Restore to pre-European vegetation communities	27.6
Maintain current vegetation communities	44.8
Create and sustain new vegetation communities in anticipation of future environmental conditions	27.6

Discussion

This survey identified several inconsistencies of practice and belief within the restoration industry in New South Wales (NSW). We found that there is no consistent definition of 'local provenance' amongst practitioners and other participants in the industry. For many respondents, the definition is somewhat flexible, depending on the pollination, dispersal or other traits of the particular species involved. The majority of practitioners indicated that local provenance use is important, regardless of the status (threatened/endangered or common) of the vegetation being managed. Whilst 80% of respondents noted that projections of future climate change are relevant to restoration projects, only 45% are taking preparatory measures. However, a clear majority of respondents would like to see a review of seed-sourcing policy/guidelines to allow for the inclusion of non-local provenance material.

The lack of consistency in the definition of local provenance is possibly a reflection of the diversity of people working within the restoration industry. Many practitioners perform multiple roles, work in more than one location and under contrasting conditions and obligations. The survey questionnaire did not offer a definition for 'local' or for 'catchment' and this may have also contributed to the variability of the definition among respondents. In a 1999 survey of restoration practitioners (particularly seed collectors), the definition of 'local' was most often expressed as a distance (e.g. a 15 km radius) from the planting site or it was defined by a region (e.g. a catchment) (Mortlock 1999). The definition of local provenance appears to have undergone a subtle shift since the previous survey. In the current survey, slightly more respondents preferred to define local provenance as being species dependent, rather than identifying a consistent distance-based or regional definition. However, when the different categories of distance-based definitions were grouped together, the response was much higher and comparable to the 1999 survey of Mortlock. It should be noted, however, that the two surveys had different foci and the 1999 survey did not give respondents the option of choosing the definition *depends on the species.* Since the previous survey, recommendations based on factors other than a distance-based approach may have gained momentum. For example, Florabank guidelines 10 (Florabank 1999) suggest a range of approaches and more recently, CSIRO technical reports and research articles (e.g. Broadhurst 2007; Broadhurst et al. 2008) focus on the importance of using genetically diverse source material.

The prevailing preference and/or requirement for the use of local provenance stock is widespread in NSW and its use has increased since the previous survey (where 30% of respondents sourced all their seed locally and 44% collected ‘most’ seed locally) (Mortlock 1999). Demand for local provenance material (and a general increase in demand for native seed (Mortlock 2000)) may have contributed to 74% of all practitioners experiencing difficulty in obtaining a sufficient supply of local provenance material. This number is considerably higher than that found in the previous survey (49% usually or sometimes experienced supply difficulties in NSW) (Mortlock 1999). An increase in revegetation activities is expected by 47% of practitioners (and only 5% expect a decrease) over the next five years. A respondent noted that the mining sector, a large user of native seed, is ‘ramping up its collection of local provenance seed’ and that its use is becoming a regular condition for approval. This will further exacerbate supply problems already experienced by practitioners struggling to fulfil local provenance obligations. For most, the shortage is overcome by limiting planting to only those species for which sufficient quantities of local provenance seed is available. A consequence of this practice is that the diversity of restoration plantings may decline. On some occasions, the supply shortage has led to the sourcing of material of lesser quality and from unknown locations. Concerns were expressed by some respondents that seed collection is not always performed to industry standards (i.e. Florabank guidelines) and that guarantees of contractual obligations to use local provenance cannot always be made. A lack of time allowed for the collection of local provenance seed was also raised as an issue, usually with negative outcomes for the quality of the seed. Standardized industry accreditation for seed collection is a possible solution to the problem, and further discussion on this topic is warranted but is beyond the scope of this survey.

Overall, the response given for the reason(s) as to why the use of local provenance is required or recommended was equivocal. Many respondents noted that the requirement is due to the adherence to the precautionary principle. However, adherence to the precautionary principle may be counterproductive to successful restoration outcomes if the local seed is collected from genetically depauperate populations. Such collections may increase the chance of inbreeding depression and reduce adaptive potential, increasing the likelihood of restoration failure, especially in the face of a rapidly changing climate (Weeks et al. 2011). Many respondents stated that they use local provenance because of their practical experience of its superior restoration results. Most of these comments were anecdotal, with few respondents providing specific information. Of the examples provided, there was little to no evidence that the comparisons between local and non-local provenances had been rigorously tested. There are many factors that contribute to restoration outcomes that may be misconstrued as a problem of inferior seed. These factors include the variation in environmental conditions at the time of collection through to the quality of nursery conditions. There appeared to be little knowledge among the respondents about local provenance issues in the peer-reviewed scientific literature, suggesting a need for better dissemination of up to date scientific information through the restoration industry

A review of seed-sourcing policy/guidelines to allow the inclusion of non-local provenance(s) was sought by two thirds of the respondents. Respondents gave examples of guidelines that use a range of definitions of local provenance, from a narrow focus '*a licence condition that requires the use of local provenance within 300 m of the collection source'* to a broader approach of '*within the bioregion'*. There were large differences of opinion regarding the optimal collection range for specific functional groups of plants. As an example, comments ranged from '*as close as possible'* to '*up to 20 km'* as the appropriate seed collection range for tree species. Collection ranges for grasses were also very broad and variable. Guidelines and policies that limit local provenance to within a specific radius of the proposed revegetation site were not favoured by the majority of respondents. This finding is consistent with a review by McKay et. al. (2005) that concluded that it is impossible (and counterproductive) to impose a standard geographic distance as a scale for local adaptation (and therefore for seed collection). In summary, respondents indicated a desire that seed-sourcing policy/ guidelines: (1) reflect differences in species' traits, (2) allow for the matching of environmental conditions of the seed source to the revegetation site, (3) actively manage the avoidance of inbreeding depression and (4) alleviate supply problems. To assist with provenance selection, several new regional (NSW) *Seed Supply Strategies* have recently been produced that include the consideration of (2) and (3) and the condition (size and type of disturbance) of the proposed revegetation site (Vanzella & Greening Australia Capital Region 2012). The Society for Ecological Restoration Science and Policy Working Group (2004) also advocates a flexible approach to seed sourcing under certain conditions. Where substantial damage has altered physical environments, the introduction of "diverse genetic stock" is recommended. The definition of diverse is not specified but the aim is to promote genetically fit populations.

In contrast to the view that broader seed-sourcing guidelines and policies are needed, an overwhelming majority of the respondents nonetheless indicated that it is important to use only local provenance material for both TS & TEC and non-TS & TEC situations. The classification of endangered and threatened communities and species is partly based on population biology principles (Frankham et al. 2010); the risk of extinction increases when population size or reproduction success is reduced (N.S.W. Government Office of the Environment and Heritage 2007). Seed-sourcing protocols that limit seed collection to genetically-impoverished populations may reduce population size or reproduction success via inbreeding depression (Broadhurst 2007). Some respondents commented that inferior revegetation results are already occurring due to inferior seed collection protocols. The survey found that almost one third of practitioners work with small numbers (less than 200) of adult plants per species per population (a further one third were unsure of the number). Of those working with small numbers of adult plants, most use only local provenance material. The minimum population size from which seed can be collected without negative genetic consequences is the subject of much debate. A conservative estimate is that 100-200 reproductive adult plants is the minimum (Broadhurst 2007), but if adequate adaptive potential is the objective, a minimum of 1000 is suggested (Weeks et al. 2011). The appropriate number depends on the circumstances but this flexibility is not always recognized by those who set provenance policies or procedures. In reality, the costs involved in seed collection at such a scale may be prohibitive in many situations.

A significant inconsistency was noted in relation to respondents' views and actions on climate change. Climate change was largely acknowledged as happening but current practices and future restoration goals did not reflect this acceptance. Many respondents expressed the view that a lack of relevant information to guide revegetation work was a reason for the absence of preparatory actions. However, there was also a clear preference for climate change adaptation strategies that allow the flexibility to use non-local provenance material. Those who are undertaking preparatory actions gave examples of extending provenance boundaries, changing the mix of species, creating corridors and participating in plant research.

This survey has highlighted that adherence to inconsistent revegetation policies and contracts are engendering confusion within the restoration industry and possibly contributing to inferior revegetation outcomes. Research to test the validity of the assumptions about the benefits of using local seed is urgently needed to form the basis of new guidelines. Inbreeding and outbreeding problems are particular points of concern for threatened/endangered species but it is also important to delineate ‘seed sourcing zones’ for common or non-threatened species. Recently published decision trees can assist with strategies to avoid potential negative outcomes from the introduction of non-local provenance material (Byrne et al. 2011; Frankham et al. 2011). Guidelines are also available to assist with policies regarding genetic rescue and other types of translocations (Weeks et al. 2011). Access to and information on genetically diverse and climatically suitable non-local provenance material will become increasingly important as the climate rapidly changes.

Recommendations

- New seed-sourcing guidelines are sought to reflect current and future use of local provenance material. Incorporated into these guidelines, consideration should be given to allow for differences in species traits, for the inclusion of non-local provenance to allow for maximization of genetic diversity, and to match the future environmental conditions of the source population to the proposed planting site.
- Licensing systems by delegated authorities should mirror these guidelines (above) more closely.
- Access to down-scaled climate projections at a local level to enable management for climate change is required to assist with decision-making about sources of material that will have the highest probability of long-term sustainability.
- More research on the extent and magnitude of local adaptation is needed. Practitioners can assist in the improvement of guidelines by creating their own tests of ‘local is best’ when a revegetation project using mixed provenances is conducted. This would entail the documentation of all aspects of the collection of the stock used, the identification of each plant at the site, consistency of conditions under which the plants are grown and reporting the successes and failures.

- Further discussion is warranted on the advantages and disadvantages of an accreditation system for seed collectors/suppliers.
- Dissemination of relevant information needs to be improved to the seed industry and to those involved in revegetation work.

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Appendix A:

Local provenance survey

Aim of survey
The use of local provenance genetic material is a requirement for the attainment of a Section 132C licence to bring in new plant material &/or to collect seed for revegetation in Threatened Ecological Communities (TECs) and for threatened species in NSW. Its use is also a recommendation or specification of many restoration projects and grant applications.

This survey is aimed at collating information on the current use of local provenance plants in restoration projects in New South Wales and the appropriateness of current seed-sourcing guidelines. Participation is voluntary and is targeted towards people who select seeds or seedlings for revegetation or restoration projects.

Content of survey
This survey should take approximately 15 minutes. The first section of the survey asks questions about your use of local and non-local provenance plants and the conditions under which your decisions are made. The second section seeks your opinion on the future direction of current practice. The third section asks questions about you and your work role so that we can understand how practices may be associated with different organizations and decision-makers. Please choose the **best** answer in each case and if you answer "other" we would appreciate your further comments.

The results of this survey will be published in a peer-reviewed journal and will be made available to the wider community upon request. This survey is being conducted by Nola Hancock at Macquarie University and forms part of Nola's PhD thesis "*The role of plant provenance in restoration ecology under climate change".* Please contact Nola at nola.hancock@mq.edu.au or phone 0419 262 116 for further information or to receive a hard copy of the survey. The survey will be forwarded through several natural resource email networks. If you receive this survey from more than one source, please only complete the survey once.

The ethical aspects of this study have been approved by the Macquarie University Human Research Ethics Committee. If you have any complaints or reservations about any ethical aspect of your participation in this research, you may contact the Committee through the Director, Research Ethics (telephone (02) 9850 7854; email ethics@mq.edu.au). Any complaint you make will be treated in confidence and investigated, and you will be informed of the outcome.

For the purpose of this survey, the following definitions are used:

Ecosystem: the biota (plants, animals & microorganisms) within a given area, the environment that sustains it, and their interactions
Genetic material: seed, cuttings, tubestock or other propagules
Local provenance: genetic material that has been sourced locally or close to the proposed planting site
Inbreeding Depression: A reduction in survival, growth & reproduction in inbreed offspring (caused by mating between relatives), relative to the parents
Outbreeding Depression: A reduction in survival, growth & reproduction of offspring from crosses between plants from different populations, relative to the parental populations i.e. sterile hybrids
Restoration (Ecological): the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed
Provenance: the geographic location/origin of the genetic material
Revegetation: adding plants to the ecosystem by planting, seeding or translocating
Threatened Ecological Community: a group of species that occur together in a particular area of the landscape that has been listed as threatened (either categorized as Critically Endangered, Endangered or Vulnerable) under the NSW *Threatened Species Conservation Act, 1995* &/or under the Commonwealth EPBC Act http://www.environment.gov.au/epbc/protect/species-communities.html

1. Current Seed sourcing strategies

1. Please select which of the following **best** describes your role in the restoration industry:

a) Practitioner who undertakes revegetation using native seeds or seedlings as paid employment or for own business. Go to question 2
b) Policy maker. This includes anyone who dictates which provenance(s) an organizations will use. Go to question 14
c) Educator / Researcher. Go to question 14
d) Nursery worker &/ or seed collector. Go to question 14
e) Volunteer. Go to question 14
f) Interested in revegetation. Go to question 14
g) Other. Please specify. Go to question 14

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2. In regard to your revegetation activities:
   a) There are **no** threatened species or Threatened Ecological Communities where I work. Go to question 3
   b) I **only** work with threatened species &/or Threatened Ecological Communities. Go to question 4
   c) Both (a) & (b). I work with **both** threatened species &/or Threatened Ecological Communities **AND** in areas where there are **no** threatened species &/or Threatened Ecological Communities. Go to question 3 and answer all questions hereafter in regard to your practices for **non-**threatened species &/or Threatened Ecological Communities
   d) Don't know. Go to question 3

3. In regard to your use of provenance material for revegetation, does your Organization:
   a) Set its own policies or procedures regarding provenance use. Go to question 5
   b) Follow policies or procedures imposed upon it by another organization. Go to question 4
   c) Both (a) & (b). Go to question 4 and answer for when you are told what to use (b) and for questions 5 – 7 answer for when you make your own decisions (a)

4. For the majority of your work, what procedures or specifications does your Organization follow in determining your use of local vs non-local provenance:
   a. Licence conditions. Go to question 8
   b. Requirement of grant application or funding arrangement. Go to question 8
   c. Requirement of internal policy. Go to question 8
   d. Contract condition. Go to question 8
   e. Follow Florabank guidelines. Go to question 5
   f. A mix of the above. Please specify. Go to question 8
   g. Other. Please comment. Go to question 5

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5. Do you revegetate using:
    a. All local provenance. Go to question 7
    b. Mix of local & non-local provenance. Go to question 6
    c. All non-local provenance. Go to question 7
    d. Whatever is the cheapest, regardless of provenance. Go to question 7
    e. Whatever is available, regardless of provenance. Go to question 7
    f. Don't know because there is not enough information available from my supplier. Go to question 7
    g. Other. Please comment & go to question 7

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6. If a mix of local and non-local provenance is used what is the approximate ratio of local: non-local:
    a. Mostly local
    b. 50% local: 50% non-local
    c. Mostly non-local
    d. Not applicable
    e. Not sure

Please rank in the order of importance, the reason for your choice of provenance(s) (1 = Unimportant: 2 = Not important: 3 = No opinion/don't know: 4 = Important: 5 = Very important).

7.
    a. ( ) Limit the distance of the source population to proposed planting site
    b. ( ) Match environmental conditions of source population to proposed planting site (temperature, rainfall, aspect, soil etc)
    c. ( ) Size of source population/ recipient population
    d. ( ) Avoidance of potential outbreeding depression
    e. ( ) Avoidance of potential inbreeding depression
    f. ( ) Increase genetic diversity
    g. ( )Improve evolutionary potential to adapt to a changing environment
    h. ( ) Other. Please comment

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8. For the remainder of the survey, unless otherwise specified, please answer the questions with regard to all of your restoration activities i.e. if applicable, for both threatened species/ Threatened Ecological Communities (TECs) **and** non- threatened species/ TECs

Have you ever experienced difficulties in obtaining a sufficient supply of local provenance plants?

   a. Yes. Go to question 9
   b. No. Go to question 10

9. How have you responded to difficulties in obtaining local provenance plants?
   a. Cancelled the project
   b. Deferred the project
   c. Used non-local provenance
   d. Reduced the diversity of planting to only those species for which local provenance is available
   e. A combination of the above. Please specify
   f. Other. Please comment

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10. In the past 12 months, how many native seedlings did you plant or how much seed did you use if direct seeding (approximately)?
    a) < 8,000 seedlings or ≤ 5 kg seed
    b) Between 8,000 – 25,000 seedlings or 5 – 20 kg of seed
    c) 25,000 seedlings or > 20 kg seed
    d) Not applicable
    e) Unsure

11. Do you anticipate an increase or a decrease in your revegetation activities over the next five years?
    a. Increase
    b. Decrease
    c. Unchanged
    d. Unsure

12. For the **majority** of the plant species that you work with, is the approximate number of adult plants:
    a. < 200 adult plants per species
    b. > 200 adult plants per species
    c. Unsure

13. Which of the following statements **best** describes your Organization's long-term restoration goal. To:
    a. Restore to pre-European vegetation communities
    b. Maintain current vegetation communities
    c. Create and sustain new vegetation communities in anticipation of future environmental conditions
    d. Other. Please comment

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14. What do you generally consider "local" for sourcing genetic material for revegetation:
    a) As close as possible to the proposed revegetation site
    b) ≤ 5 km from the proposed revegetation site
    c) ≤ 20 km from the proposed revegetation site
    d) Within the catchment of the proposed revegetation site
    e) Within the state boundaries of the proposed revegetation site
    f) Depends on the species i.e. pollination, dispersal or other traits. Please specify
    g) Other. Please comment

15. In your opinion, for successful long-term revegetation projects in Threatened Ecological Communities &/or of threatened species, to what extent is it important to use **only** local provenance material:

1 = Unimportant
2 = Not important
3 = No opinion/don't know
4 = Important
5 = Very important

16. In your opinion, for successful long-term revegetation projects **that do not include** Threatened Ecological Communities &/or non-threatened species, to what extent is it important to use **only** local provenance material:

1 = Unimportant
2 = Not important
3 = No opinion/don't know
4 = Important
5 = Very important

17. In your opinion, when (or if) the use of local provenance is required or recommended, it is because of:
    a. Practical experience of superior restoration results using local provenance (local knowledge)
    b. Results from peer-reviewed scientific literature of provenance trials/research
    c. Adherence to the Precautionary Principal
    d. Untested theory within the industry
    e. Not sure
    f. A combination of the above. Please specify
    g. Other. Please comment
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2. Future Seed sourcing strategies

18. Do you believe that projections of future climate change are relevant (e.g. more extreme weather events, increased temperatures, changes in rainfall patterns) to restoration/revegetation projects?
    a. Yes. Go to question 19
    b. No. Go to question 21

19. Are any measures being taken by your Organization to prepare for these changes in relation to your restoration practices?
    a. Yes. Please comment on what these practices are?
    b. No. Go to question 21

20. As a climate change adaptation management strategy, would you consider using, or advocate for the use of, non-local provenance genetic material from areas with similar predicted climatic conditions?
    a. Yes
    b. No
    c. Comment
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21. Would you like to see a review of seed-sourcing policy/guidelines to allow for the inclusion of non-local provenance(s)?
    a. Yes
    b. No

3. General questions

22. Are you a representative of &/or work for:
    a) Commonwealth Government
    b) State Government
    c) Local Government
    d) Non-government Organization (NGO)
    e) Contractor in bushland
    f) Contractor in non-bushland i.e. road plantings
    g) Community Group/Volunteer i.e. Bushcare or Landcare
    h) Carer of Indigenous Protected Area
    i) Private individual land owner/lessee/manager i.e. agricultural sector/farmer
    j) Mining sector
    k) Other. Please specify

23. What is your age bracket:
    a. Under 20
    b. 21-30
    c. 31-40
    d. 41-50
    e. 51+

24. In which Catchment Management Authority (CMA) region is the majority of your revegetation work undertaken? For maps visit: http://www.cma.nsw.gov.au/:
    a. Border Rivers-Gwydir
    b. Central West
    c. Hawkesbury Nepean
    d. Hunter Central Rivers
    e. Lachlan
    f. Lower Murray Darling
    g. Murray
    h. Murrumbidgee
    i. Namoi
    j. Northern Rivers
    k. Sydney Metropolitan
    l. Southern Rivers
    m. Western
    n. Other/ don't know

25. The last question is optional. To avoid a duplication of responses to current seed-sourcing practices and to assist with the understanding of the geographic land industry spread of opinions, the name of the organization that you represented in completing this survey would be appreciated. This information will remain confidential and will not be identifiable in the analysis.

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This completes the survey. Thank you for your participation – your efforts are greatly appreciated. If you would like to add any further comments to any of the questions or to raise any other relevant issues, please use the space provided below.

Appendix B.

Table 1. Respondents' Catchment Management Authority (CMA) region. Note: Not all respondents answered all questions. For example, 12 respondents skipped the question in table 1.

CMA region	Response %	Response count
Sydney Metropolitan	34.1	45
Hunter Central Rivers	18.9	25
Hawkesbury Nepean	17.4	23
Northern Rivers	9.8	13
Southern Rivers	9.8	13
Murrumbidgee	3.8	5
Border Rivers-Gwydir	2.3	3
Central West	1.5	2
Murray	1.5	2
Namoi	0.9	1
Total	100	132

CHAPTER 6

Discussion and conclusion

The overarching aim of this thesis was to provide empirical support to underpin seed-sourcing guidelines in general, but particularly for the Cumberland Plain Woodland in western Sydney. Updated seed sourcing guidelines are urgently needed. The climate is rapidly changing and fitness advantages that locally-adapted plants may once have conferred, may not be relevant in the future. However, current seed-sourcing policies and guidelines often do not allow for the inclusion of non-local provenance material. This thesis explored a traditional assumption within the restoration industry. The 'local is best' paradigm (where local provenance propagules are assumed to be the best source of revegetation material and subsequently preferentially chosen), was investigated under current and potential future environmental conditions. The findings of a survey on attitudes and practices in relation to the use of local provenance of restoration participants within New South Wales are also presented. Extensive discussion has already been included in each chapter, and the aim of this discussion is to synthesize the main findings of the chapters and to provide suggestions for future research.

Investigating the local is best paradigm under current conditions (Chapter 2)

Six Cumberland Plain Woodland species (*Acacia falcata, Bursaria spinosa* ssp. *spinosa, Eucalyptus crebra, E. tereticornis, Hardenbergia violacea* and *Themeda australis*) were grown in a common garden experiment at two field sites on the Cumberland Plain. Local provenance plants rarely demonstrated superiority for establishment success (survival and growth) when compared to non-local plants of the same species. Of the six species investigated, only *Bursaria spinosa* ssp. *spinosa* plants consistently demonstrated significant local superiority compared to non-local plants. Significant differences among the provenances were found in all species for many traits including establishment success, phenology, leaf morphology and herbivory.

This study is the first to investigate the 'local is best' assumption for a number of community dominants that co-occur in an assemblage. Other field studies in the Northern Hemisphere that have investigated local adaptation for multiple species have focussed on major functional groups rather than more complex vegetation communities such as the Woodland investigated in this research. Grasses or grassland species such as herbs and forbs have predominately been the focus, with mixed results regarding the performance of local stock. For example, in a study of four wildflowers, large differences among provenances were found for fitness-related traits in all species but no general superiority was found for local provenances (Bischoff et al. 2010). In contrast, a separate study of two grasses and a forb, local adaptation was consistently found for many traits (Joshi et al. 2001).

The six species investigated in this thesis are all widely distributed throughout south-eastern Australia and are either community dominants and/or are commonly used in restoration projects. The findings in this thesis are therefore highly relevant to practitioners and also provide empirical evidence from which seed-sourcing guidelines can be based. With the exception of *Themeda australis,* local adaptation has not previously been investigated for any of these species (see Waters et al. 2005).

***Investigating the 'local is best' paradigm under future conditions* (Chapter 3 – High temperatures)**

To explore the role of local provenance under future temperature conditions, seedlings of *E. tereticornis* and *T. australis* (two of the species used in the field study, Chapter 2), were grown under ambient and higher temperatures in the field. There was no evidence of local superiority for survival and non-reproductive growth for either species. However, for *T. australis,* the local provenance plants demonstrated superiority for all reproductive growth traits, regardless of the temperature treatment.

Survival and growth were not affected by the provenance of the seeds in *E. tereticornis* under current conditions (Chapter 2) but there were significant differences between the provenances for growth under high temperature conditions (Chapter 3). A difference also occurred between the two studies where, in the high temperature study, locally-sourced *E. tereticornis* seedlings had significantly higher herbivory rates than non-local provenance plants, but only under ambient conditions. However, there was no significant difference in herbivore damage between the provenances in the current conditions study. The difference in the results of the two studies may be explained, in part, by the lack of herbivory in the most northerly provenance plants because this provenance was not used in the current-conditions study (Chapter 2). In the high temperature study, the local provenance plants were consistently amongst the slowest growing plants (but not in the current-conditions study). The results suggest that the higher rates of leaf damage on the local plants (caused by herbivores) may have resulted in the reduced growth of the local plants compared to the non-local plants.

For *Themeda australis*, the results of the two studies were inconsistent. It should be noted that the seeds for the local provenance plants for each study were collected from different local populations that may have affected their comparisons. In addition, the ploidy level of the populations (both local and non-local) has not been determined but it is known that diploids and tetraploids exist within the taxa (Hayman 1960). Differences in ploidy and populations have been demonstrated to explain most of the variation in morphological traits of another widespread Australian grass species, *Austrodanthonia caespitose* (Waters et al. 2011). It has also been demonstrated in four *Austrodanthonia* species that dry weights tend to increase with increasing ploidy (Waters et al. 2011). In the current-conditions study (Chapter 2), the local provenance plants survived significantly longer than non-local plants whereas all plants survived in the high temperature experiment (Chapter 3). The differences between the two studies may be due to the different duration of the studies but it implies that local plants outperform non-local plants over time. This rationale, however, does not apply when comparing the non-reproductive growth results of the two studies. In the high temperature study, the local provenance plants were clearly superior to non-local plants for all measurements of non-reproductive growth. However, in the current-conditions study, there was no significant difference between the provenances for the percentage of plants that flowered after two years. This result suggests that over time, non-local provenances perform equally well.

Local plants flowered earlier than non-local plants, regardless of duration and temperature conditions, but it is not clear from these experiments if early reproduction is actually advantageous. Modelling has demonstrated that under changing conditions, it is beneficial for annual plants to delay reproduction and grow larger (Johansson et al. in press). However, in a study investigating the change in the abundance of flowering species in Thoreau's Woods (USA), populations of species with flowering times that did not track seasonal temperatures were found to have declined substantially over the past 100 years (Willis et al. 2008).

***Investigating the 'local is best' paradigm under future conditions* (Chapter 4 – elevated CO_2)**

The effects of elevated CO_2 on plants from different provenances of *Acacia falcata* and *Eucalyptus crebra* (two of the species used in the field study, Chapter 2) were investigated (Chapter 4). Seedlings were grown in growth cabinets and glasshouses under ambient and ~ 550 ppm CO_2. Significant differences among the provenances for both species were found for all traits except survival, nodulation and C:N ratio for *A. falcata*, and survival and presence of lignotubers for *E. crebra*. The differences were regardless of the CO_2 treatment except for stem diameter in *E. crebra*. Results from the two studies (Chapters 2 and 4) were consistent for survival for both species (no significant differences between the provenances) and for stem height and stem diameter in *E. crebra* (significant differences between the provenances). However, there were no significant differences between the provenances in *A. falcata* for stem height in the current conditions study (Chapter 2) but differences were statistically significant in the CO_2 study (height was the only growth trait measured in both studies).

The studies that were conducted under projected future conditions did not account for co-occurring environmental changes (and their interactions), some of which are known to affect the outcome of intraspecific comparisons such as precipitation (Beierkuhnlein et al. 2011). However, these findings make an important contribution to our understanding of the role of local provenance in restoration ecology under climate change.

Current practice and attitudes towards local provenance use in New South Wales

Exploration of restoration participants' views on local provenance issues in NSW was conducted through a survey (Chapter 5) and the findings have been published in a local 'applied' journal. Restoration scientists have been criticized for failing to adequately disseminate their results (Vander Mijnsbrugge et al. 2010) and this article was written, in part, to address this issue. The survey revealed that the participants' preference to use local provenance is not based on empirical evidence. With the exception of some knowledge of the pitfalls of revegetation from seed sourced from small populations, there appears to be a lack of knowledge on current provenance issues among the sectors of the industry.

Whilst the majority of the practitioners surveyed indicated that they preferentially use local provenance propagules, the majority of respondents were in favour of changing seed sourcing guidelines to include the use of non-local provenance material. In particular, the inclusion of non-local provenance material from areas with similar predicted climatic conditions (to the proposed revegetation site) was viewed as an appropriate adaption management strategy as the climate changes. This is an important finding because it was apparent from the survey results that there is a reluctance to actively manage for climate change, even though the majority of respondents believe that climate change impacts are relevant for restoration/revegetation projects. Seed sourcing guidelines that consider attributes other than geographic distance from the donor site to the recipient site already exist in other parts of the world. Examples of seed 'transfer zones' include the 38 seed zones in Ontario's forests (based on climatic parameters, particularly day length) (Ontario Ministry of Natural Resources 2009), the ecoregion concept (in relatively homogenous landscapes) in the Pacific Northwest of North America (Miller et al. 2011), and the planting of seedlings adapted to future climate in British Columbia (O'Neill et al. 2008).

An update on the current beliefs and practices on the use of local provenance by participants in the restoration industry is timely. It is my hope that the results from the survey will create vigorous debate among policy makers, practitioners and scientists about the appropriateness of seed-sourcing guidelines in this age of a rapidly changing environment.

Future research directions

It is important to determine if the results of the experiments described in this thesis are maintained when the plants are grown for longer periods. Longer growing times would subject the different provenances to a wider range of climatic and biotic conditions, and allow long-term fitness, herbivory and disease assessments to be made. Further studies on *Busaria spinosa* ssp. *spinosa* are particularly recommended because this was the only species to show consistent local superiority. In this thesis, comparisons could only be made between the local and one other provenance of this species at one of the sites, due to high mortality rates. Genetic investigation to confirm *B. spinosa* ssp. *spinosa's* taxonomic status, as currently determined by its morphological classification (Cayzer et al. 1999), is also warranted. Generally, there is a higher risk of hybridization, threatening species' persistence, if species from different taxonomical units (or genetically distinct populations) interbreed (Byrne et al. 2011). Whilst the taxonomic status of *T. australis* is "resolved", the inconsistency in the use of *T. australis* and *T. triandra* among the States is perplexing.

Further exploration of the results was limited by the lack of information regarding the nature of some of the populations from which the seeds were sourced. The field study was a large experiment and seeds could not be collected by the author from the geographical scale required in the time available. Seeds were therefore purchased commercially. Not all commercial suppliers could provide information on the size of the source population, and in some cases, the exact location of the seed collection sites could not be ascertained. As a consequence, inferences could not be made concerning the differences in the population sizes or the environmental distances between the planting site and the donor sites. Future research into local provenance should include analysis of the relationship between these factors and local adaptation to allow generalizations to be made for seed-sourcing guidelines. Furthermore, information regarding these two fundamental factors is critical for practitioners to confidently select the most appropriate seed source. An accreditation system for seed collectors and suppliers is a possible solution to this problem.

The delineation of populations of the taxa with different ploidy levels is also suggested as an important area for future research, particularly for *T. australis*. *T. australis* is widespread throughout Australia and is an important species for both restoration activities and agriculture (Waters & Shaw 2003). Different ploidy levels and breeding systems are known to exist between populations at both small and large scales (Hayman 1960; Evans & Knox 1969). The scale of the cytological differences between populations is required to define boundaries within which populations can be mixed to reduce the risk of maladapted offspring. Fixed chromosomal differences between parents may increase the risk of outbreeding depression (Frankham et al. 2011). Furthermore, populations with cytological variations may possess fitness or competitive advantages, swamping some genotypes and reducing genetic diversity (Prentis et al. 2008). Further research should also seek to investigate any link between the species' propensity to produce large quantities of unviable seed and the scale at which cytology differs (personal observation). Morphological differences and performance of the different provenances of *Hardenbergia violacea* were grouped in such a way to suggest that cytological differences may also occur in this taxon, and also warrants further investigation.

Conclusion

The climate is rapidly changing and many plant populations and species are likely to be threatened by the speed and magnitude of these changes (Burrows et al. 2011; Hughes 2011). Recent modelling predicts that by 2070, most places in Australia will have environments that are more ecologically different from current conditions than they are similar (Dunlop et al. 2012). Increasingly, an adaptation strategy to use a mix of seed sources in restoration projects to maximize genetic diversity is being recommended (Broadhurst et al. 2008; Jones & Monaco 2009; Hoffmann & Sgró 2011). A provenance 'mix' strategy aims to increase the speed at which adaptation to future climates can occur.

This study found that for multiple species, regardless of the environmental conditions investigated, the local stock rarely outperformed non-local stock for establishment success, when grown on the Cumberland Plain. The overall findings of this research highlight the unpredictability and complexity of the performance of locally-sourced seed, with no simple responses across species traits or environmental conditions. However, this study suggests that establishment success will not be impaired by the introduction of non-local seed sources in restoration projects for the Cumberland Plain Woodland. In particular, the inclusion of non-local *E. crebra* and *E. tereticornis* in revegetation projects on the Cumberland Plain may assist restoration success by promoting the introduction of genetic diversity and thereby increasing adaptive potential. The eucalypts are highlighted as their taxonomy and cytological boundaries (Grattapaglia et al. 2012) are believed to be largely resolved, thereby reducing any potential negative effects of hybridization (Byrne et al. 2011). The inclusion of non-local provenances of *E. tereticornis* may lessen the potential of herbivore damage, as demonstrated in Chapter 3.

In NSW, the practice of using local provenance material currently takes precedence over the introduction of non-local seed sources. The overall findings of this research suggest that establishment success will not be impaired by the introduction of non-local seed sources in restoration projects for the Cumberland Plain Woodland. These findings therefore provide grounds for a change in seed-sourcing guidelines. A rapidly changing climate and the advocacy of restoration participants for the inclusion of non-local provenance material in seed-sourcing guidelines, suggests that the time is right to reconsider this traditional paradigm of restoration ecology.

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Appendix:

Papers accepted for publication during candidature

Two papers from this thesis were accepted for publication during my candidature and copies are attached:

Hancock, Nola, Leishman, Michelle R. and Hughes, Lesley (in press), Testing the “local provenance” paradigm: a common garden experiment in Cumberland Plain Woodland, Sydney, Australia, *Restoration Ecology.*

Hancock, N., and L. Hughes. 2012. How far is it to your local? A survey on local provenance use in New South Wales. *Ecological Management & Restoration* **13:**259-266.

Ethics Approval for Survey: Macquarie University, Faculty of Science Human Research Ethics Sub-Committee

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	Journal Name	Manuscript No.		Author Received:	No of pages: n/a	TS: Karthik

RESEARCH ARTICLE

Testing the "Local Provenance" Paradigm: A Common Garden Experiment in Cumberland Plain Woodland, Sydney, Australia

Nola Hancock,[1,2] Michelle R. Leishman,[1] and Lesley Hughes[1]

Abstract

Seed for restoration projects has traditionally been sourced locally to "preserve" the genetic integrity of the replanted site. Plants grown from locally sourced seeds are perceived to have the advantage of being adapted to local conditions, and the use of local provenance is a requirement of many restoration projects. However, the processes of climate change and habitat fragmentation, with the subsequent development of novel environments, are forcing us to reconsider this basic tenet of restoration ecology. We tested the "local provenance is best" paradigm, by comparing the performance of plants grown from local with non-local seed sources within a common garden experiment. We selected six species representing a range of growth forms (*Acacia falcata*, *Bursaria spinosa* ssp. *spinosa*, *Eucalyptus crebra*, *E. tereticornis*, *Hardenbergia violacea* and *Themeda australis*) from an assemblage known as the Cumberland Plain Woodland, a threatened community in western Sydney. Multiple provenances were collected from within the range of each species and grown at two field sites on the Cumberland Plain. Growing time varied between species and ranged from 7 months to 2 years. With the exception of *B. spinosa*, and to a lesser extent *T. australis*, we found little evidence that local provenance plants were superior to distant provenances in terms of survival and establishment.

Key words: **adaptive potential, home-site advantage, local adaptation, local superiority, restoration, seed source.**

Introduction

AQ1

The changing environment poses many challenges for restoration ecology. Small populations in fragmented landscapes will be particularly vulnerable to rapid change in the future. Genetic diversity within these populations is a key consideration for climate change adaptation strategies, but many seed-sourcing guidelines used by restoration practitioners do not allow for the incorporation of a broad range of genotypes in restoration projects (N.S.W. Department of Environment and Conservation 2005; State of Minnesota 2010).

Traditionally, it has been considered desirable to use seeds collected within a defined radius of the restoration site to "preserve" the genetic integrity of the replanted site. Plants sourced from local seed (hereafter referred to as "local provenance") are generally assumed to be better adapted to local conditions, with superior survival and faster growth rates conferring a greater probability of restoration success. In addition, the use of non-local provenance is considered to increase the potential for the negative effects of outbreeding depression (Edmands 2007) and to initiate unplanned gene flow into neighboring populations either by hybridization between subspecies (Sampson & Byrne 2008; Millar et al. 2012), or by "cryptic" invasions (Hufford & Mazer 2003 and references therein). This may result in maladapted offspring and altered trophic interactions with associated organisms (Vander Mijnsbrugge et al. 2010 and references therein). Increasingly, these potential negative impacts are being weighed against the positive effects of avoiding inbreeding depression (Broadhurst et al. 2008; Lopez et al. 2009) and a recent review concluded that current concerns about outbreeding depression are excessive (Frankham et al. 2011). Furthermore, there is a growing recognition that the exclusive use of local material may hinder adaptive potential in the face of a rapidly changing climate (Weeks et al. 2011 and references therein). A broader approach to seed sourcing has been adopted by some restoration practitioners (Corangamite Seed Supply & Revegetation Network 2007; Native Seed Network 2011), but empirical evidence is needed to underpin improved guidelines.

Local adaptation of plants has previously been demonstrated over strong environmental gradients: altitudinal (Gimenez-Benavides et al. 2007), latitudinal (Davis & Shaw 2001 and references therein), and in novel environments such as polluted soils (Antonovics & Bradshaw 1970). A recent meta-analysis found that local adaptation is common, but if large environmental gradients between sites are used, the frequency and magnitude of local adaptation may be overestimated due

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Table 1. Nomenclature and life form details for six perennial species used in the common garden experiment (*Themeda australis* is a synonym of *T. triandra*).

	Acacia falcata Willd	*Bursaria spinosa* ssp *spinosa* Cav	*Eucalyptus crebra* F. Muell and *E. tereticornis* Sm	*Hardenbergia violacea* (Schneev.) Stearn	*Themeda australis* (R.Br.) Stapf
Family	Fabaceae: Mimosoideae	Pittosporaceae	Myrtaceae	Fabaceae: Faboideae	Poaceae
Life form	Shrub	Shrub	Tree	Vine	Grass

T. australis is commonly used in NSW and is used in this paper.

to a sampling bias caused by a priori expectations (Hereford 2009).

The few empirical studies of local adaptation undertaken in Australia have generally produced equivocal results, ranging from weak or no evidence of home-site advantage (Price & Morgan 2006; Byars & Hoffmann 2009) to evidence of selective local adaptation (Byars et al. 2007; O'Brien & Krauss 2010; Waters et al. 2011). Forestry provenance trials on Australian eucalypts have confirmed significant intra-specific variation for some traits and species (Duncan et al. 2000) and have also found that for breeding purposes, the local seed source may not be the best performer at its home site (Raymond & Namkoong 1990).

We compared survivorship and early growth of plants grown from local versus non-local seed sources using six species typical of the Cumberland Plain Woodland (CPW) community in western Sydney, NSW. The species selected [Hickory wattle (*Acacia falcata*), Blackthorn (*Bursaria spinosa* ssp. *spinosa*) hereafter referred to as *B. spinosa*, Narrow-leaved ironbark (*Eucalyptus crebra*), Forest red gum (*E. tereticornis*), False sarsparilla (*Hardenbergia violacea*), and Kangaroo grass (*Themeda australis*)] are either community dominants and/or are commonly used in CPW revegetation programs and represent a range of different life history traits (Tables 1 & S1). All six species have a wide geographic distribution along the east coast of Australia; across seasonal and biotic boundaries (Fig. S1). Their ranges include tropical to temperate and mesic to dry conditions and therefore, would be expected to express clinal patterns in phenotype that may be due to genetic differences and possibly local adaptation. *B. spinosa* and *H. violacea* extend into South Australia and Tasmania, although discontinuously for the latter. A prostrate and a climbing form of *H. violacea* are known (Harden 1991). *T. australis* is a polyploid complex (Hayman 1960) found throughout the continent and different nomenclature is used in different states.

The Cumberland Plain is Australia's fastest growing and most populous region. Only 13% of its native vegetation remains distributed in highly fragmented landscapes. This vegetation has attracted unprecedented investment in recovery efforts (N.S.W. Department of Environment Climate Change and Water 2010). As part of the Cumberland Plain Recovery Plan, 2011, a research priority is to investigate the benefits, or otherwise, of introducing new genetic material into the fragmented remnants through restoration (N.S.W. Department of Environment Climate Change and Water 2010). This study aims to test the "local is best" paradigm and to contribute to the identification of seed collection areas suitable for revegetation efforts.

Methods

Study Sites and Species

The Cumberland Plain, western Sydney (33°30–34°30 S and 150°30–151°30 E) is an undulating landscape, ranging in elevation from just above sea level to approximately 350 m (N.S.W. Government Office of Environment & Heritage 2009). The deep clay soils are derived from the Wianamatta Group of shales and alluviums that retain moisture and have higher nutrient levels than the surrounding landforms (Benson & Howell 1990). Average annual rainfall is 700–900 mm, most of which falls during summer. Maximum daily temperatures of 44.8°C and minima of −1°C have been recorded in western Sydney (Bureau of Meteorology 2011).

CPW typically consists of an open tree canopy of large, mostly *Eucalyptus* species (often dominated by *E. crebra*, *E. tereticornis*, and *E. moluccana)*, a diverse grassy groundcover (often dominated by *T. australis* and *Microlaena stipoides)* and depending on the local fire regime, a shrub layer (often dominated by *B. spinosa)* (Benson & Howell 1990). The CPW and Cumberland Plain Shale Woodlands are listed as Critically Endangered Ecological Communities under the *NSW Threatened Species Conservation Act* (1995) (N.S.W. Government Office of Environment & Heritage 2009) and under the federal *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) (Australian Government Department of Sustainability 2009) respectively, which provides some legislated protection for its conservation.

Common garden experiments were established at two field sites on the Cumberland Plain, approximately 20-km apart: the Australian Botanic Garden, Mount Annan (MtA) (34°04′ S, 150°46′ E) and at western Sydney Parklands, Cecil Hills (CH) (33°53′ S, 150°49′ E). The soils on both sites are derived from Bringelly shale of the Wianamatta Group (Clark & Jones 1991) and are broadly similar (analysis performed at Sydney Environmental & Soil Laboratory, Sydney, data not shown). Seeds of each species were obtained commercially from five different geographical locations (provenances); one from the Cumberland Plain (hereafter referred to as the local provenance) and the rest from widely distributed locations within each species' geographic range (Fig. S1; Table S2). Information regarding seed collection procedures, that is size of population, number of mother plants, and the exact location of the original seed collection sites was not available from all of the commercial suppliers. Provenances were largely selected due to availability, but they are generally representative of the lower temperature and rainfall areas of the core climatic envelopes that these widespread species occupy, especially for *E. crebra* and *T. australis* (Fig. S2).

For all species, multiple seeds were germinated and plants were established from the largest and most-closely timed early germinants at the Macquarie University glasshouses. The method used during the processes from germination (including potting into 50 × 125-mm pots) until field transplantation was uniform for each species and was broadly in line with that used by commercial native plant nurseries. Only three of the four non-local provenances of *B. spinosa* germinated successfully. The sites were prepared by slashing the existing weed cover and spraying with Roundup herbicide (Monsanto Company, St. Louis, MO, USA), according to the manufacturer's instructions. Pre-swollen water crystals and a slow release fertilizer (Osmocote Plus Native Gardens, NPK: 17:1.6:9.7, Scotts Australia P/L, Sydney, NSW, Australia) were placed in the bottom of the hole before planting. Planting occurred in April and May 2009, at an average age of 3 months. Each species was planted as a group, with provenances randomly distributed within the group, approximately 1-m apart. Minor flooding occurred at the CH site and necessitated the replanting of *A. falcata*, *B. spinosa*, and *H. violacea*, completed by late August 2009. Plants that died within 1 month of planting were replaced. All plants were watered at the time of planting with minimal supplementary watering. Both sites were mulched and occasionally hand weeded during the first 12 months of the experiment. Unless otherwise specified, 100 replicates of each species (20 per provenance) were planted (Table S2). Experiment duration varied between species, ranging from 7 to 24 months, to avoid shading or in the case of the vine, *H. violacea*, from intermingling with other plants (Table S3).

Data Collection

Survivorship. Plants were assessed as "alive" (green leaves and/or stem) or "dead" (no green anywhere on the plant or plant missing). Plants were scored on a weekly basis during 2009, monthly during 2010, and quarterly during 2011. Survivorship was the only measurement taken for the following provenances: Illabo (*Bursaria spinosa*) at CH, Denver (*Hardenbergia violacea*), and Manilla *(Themeda australis)* at CH due to high mortality.

Growth Traits.

- *Stem height*. Plants were measured in situ from ground to apical meristem. Leaf length was measured for *T. australis* by averaging the five longest leaves (non-culms), measured from base to the tip.
- *Stem diameter*. Stems were cut at ground level and two measurements were taken at right angles and averaged. The in situ basal circumference of the clump was measured for *T. australis*.
- *Aboveground total biomass*. After harvest, plant material was dried for at least 2 days at 70°C then weighed.

Phenology.

- *Percentage of flowering plants*: At harvest, plants were scored as "flowered" if inflorescences, capsules, or pods were visible. This was cross referenced with data collected periodically for "time to flowering."
- *Time to flowering*: Plants were scored on a weekly basis from June to July 2010 for *A. falcata* when stamens were visible and from September 2009 to May 2010 for *T. australis* when seed heads were visible.

Morphology.

- *Specific Leaf Area (SLA)*: Fresh leaves were collected as per Cornelissen et al. (2003), scanned and their area measured using ImageJ software (http://rsb.info.nih.gov/ij/download.html) before being oven-dried and weighed. SLA was calculated as leaf area (mm^2) per unit biomass (mg).
- *Leaf width:length ratio*: Lamina length along the midvein and lamina width at the widest point was measured with ImageJ using the same leaves as for SLA.
- *Lignotuber*: Plants were scored as having a lignotuber present if the lignotuber was visible aboveground or could be felt at the base of the stem.
- *Branching*: The number of branches arising from the main stem that were greater than 10 cm in length were counted. If the main branch split into two, only the branches from a predetermined stem were counted.

Herbivory. The total amount of defoliation and leaf necrosis per plant was visually assessed at the time of harvest and scored: 0–1%, 1–5%, 5–25%, 25–50%, 50–75%, and >75% for all plants except for *H. violacea*. Due to the prostrate habit of *H. violacea*, the data presented here are from a random sample of 10 mature leaves per plant, visually assessed for the percentage of area lost due to chewing and sucking herbivores.

Details of measurements taken for each species are shown in Table S3.

Statistical Analyses

The local population was defined as being superior to or having better performance than non-local provenances if there was a significant difference between the provenances such that the local provenance had the highest survival or growth compared to non-local provenances. This definition was also used to calculate the frequency of local superiority relative to the number of measurements taken. For example, the frequency of local superiority for survival = N/T, where N is the number of times local provenance significantly survived the longest and T is the total number of survival measurements.

Differences in performance between provenances were compared using a general linear model with provenance as a fixed and site as a random factor, with their interaction included in the model. A Dunnett's post hoc test was used to compare

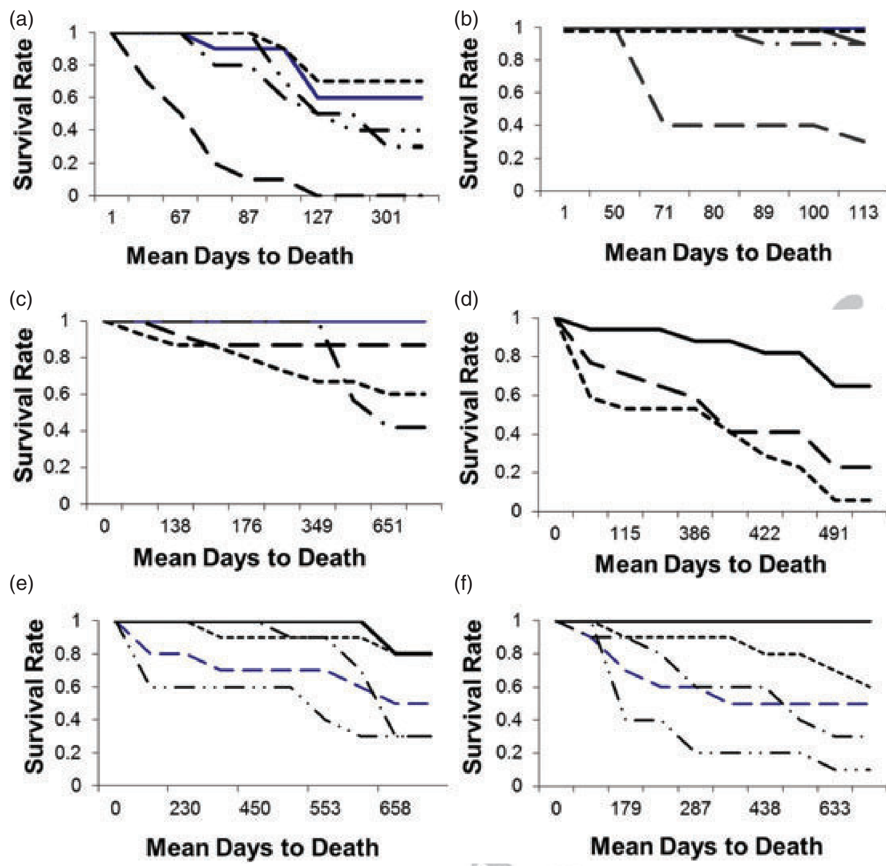

Figure 1. Proportion of surviving plants (mean days to death) at the Mount Annan (MtA) and Cecil Hills (CH) sites, respectively, for provenances of: *Hardenbergia violacea* (a) $p < 0.001$ and (b) $p < 0.001$. ___Local, _ _Denver, _._Bathurst, ----Nabiac, _.._ Manilla.(N.B. Nabiac and Local both had 100% survivorship at CH). *Bursaria spinosa*: (c) $p < 0.012$ and (d) $p < 0.001$. ___Local, _ _Port Campbell, ----Illabo, _._ Jerilderie. *Themeda australis*, (e) $p < 0.014$ and (f) $p < 0.001$. ___Local, _ _Gower, ----Bethungra, _._Fern Bay, _.._ Manilla. p value = log rank χ^2 test statistic. (Data not shown for non-significant differences between provenances of *Acacia falcata*, *Eucalyptus crebra* and *E. tereticornis*).

means where the provenance effect was significant. Data were transformed where necessary to meet the assumptions of normality and homoscedasticity. When this was not achievable, sites were analyzed separately using a Kruskal–Wallis test. There were only two provenance comparisons for *B. spinosa* at the CH site, so each site was analyzed separately for this species. Survival and time to flowering distributions were compared using Kaplan–Meier estimates (log rank χ^2 test). Chi-square tests were used to test for differences in herbivory, presence of lignotuber, and percentage of flowering plants. For all analyses, significance was determined at $p < 0.05$ for provenance and site was removed as a factor when $p > 0.25$.

Results

Survivorship

There were no significant differences in survivorship between provenances of *Acacia falcata*, *Eucalyptus crebra*, and *E. tereticornis*: all *E. tereticornis* and 97% of *E. crebra* plants survived until harvest. This contrasted with only 2% survival of *A. falcata* plants, possibly due to root rot caused by water-logging (P. Cuneo 2010, The Australian Botanic Garden, personal communication). Whilst there were significant differences between the provenances for *Hardenbergia violacea*, (Fig. 1a & 1b), the differential mortality was due to plants sourced from one non-local provenance dying significantly earlier at both sites, compared to the other four provenances. The local provenance was equal longest survivor at the CH site and second longest survivor at the MtA site. For the remaining two species, *Bursaria spinosa* and *Themeda australis*, plants sourced from local provenances survived significantly longer than those from non-local provenances at both sites (Fig. 1c–f). Overall, the frequency of local superiority was 0.33, that is the local provenance survived significantly longer only four times out of a possible 12 counts.

Growth: Stem Height, Stem Diameter, and Total Aboveground Biomass

Local provenance plants exhibited consistently superior growth in only one of the six species (Table 2). Locally

Table 2. Growth data by species.

	Provenance Mean (±SE) Raw Data					df	F/H *Value*	p-*Value*
Acacia falcata								
Stem height	Broke	**Local**	Sth NSW	Grafton	Nanango	4,4	2.96	0.159
	174 (6)	162.17 (7)	148.2 (8)	137.44 (9)	133 (9)			
Bursaria spinosa								
Stem height	**Local**	Illabo	PCampbell	Jerilderee				
Mt A	**176.1 (9.0)**	162.9 (14.1)	*104.7 (8.61)*	*92.7 (5.2)*		3,36	13.04	0.001
C Hills	**130.2 (7.9)**		*87.8 (7.4)*			1,13	9.07	0.010
Stem diameter	Illabo	**Local**	Jerilderee	PCampbell				
Mt A	**19.4 (2.2)**	18.3 (1.3)	*10.3 (1.2)*	*10.0 (0.8)*		3,36	12.63	0.001
C Hills		**12.7 (0.9)**		*8.3 (0.4)*		1,13	8.47	0.012
Biomass	Illabo	**Local**	Jerilderee	PCampbell				
Mt A	**249.8 (46.4)**	211.2 (28.3)	*66.5 (17.6)*	*63.5 (11.8)*		3,36	13.50	0.001
C Hills		**75.2 (11.0)**		*30.23 (6.9)*		1,13	6.20	0.027
Eucalyptus crebra								
Stem height	Halcomb H	Ashford	**Local**	Gilgandra	Manilla	4,4	8.76	0.029
	95.15 (6)	93.83 (6)	80.88 (7)	70.81 (5)	*60 (9)*			
Stem diameter	Halcomb H	**Local**	Gilgandra	Ashford	Manilla	4,4	19.364	0.007
	13.56 (0.7)	12.15 (.7)	10 (.5)	9.9 (.7)	*8.72 (0.7)*			
Biomass	Halcomb H	**Local**	Gilgandra	Ashford	Manilla	4,86	1.32	0.268
	58.3 (7)	54.45 (7.3)	53.47 (5)	48.5 (8)	41.9 (7)			
E. tereticornis								
Stem height	Selection Fl	Dungog	Sth NSW	**Local**	Tenterfield	4,4	2.18	0.235
	144.97 (8.1)	132.3 (4.16)	131.95 (5.5)	131.25 (7.0)	117.8 (6.51)			
Stem diameter	Sth NSW	Selection Fl	**Local**	Tenterfield	Dungog	4,4	6.09	0.054
	27.33 (1.58)	27.13 (1.94)	26.68 (1.52)	22.29 (1.19)	24.36 (1.27)			
Biomass	Sth NSW	**Local**	Dungog	Tenterfield	Selection Fl	4,4	2.50	0.198
	242.2 (30.8)	210.3 (26.6)	187.8 (21.7)	180.3 (22.8)	162.3 (23.8)			
Hardenbergia violacea								
Biomass	Nabiac	**Local**	Manilla	Bathurst		3,3	35.83	0.008
	122.7 (24.5)	107.1 (20.7)	71.9 (19.7)	*42.3 (10.6)*				
Themeda australis[b]								
Leaf length	Gowar	Bethungra	Fern Bay	**Local**		3,3	1.128	0.462
	64.22 (6)	57.71 (5)	55.2 (6)	54.28 (2)				
Clump circumference	Gowar	**Local**	Bethungra	Fern Bay	Manilla			
Mt A	***70.1 (8)***	42.5 (1.7)	37.6 (3)	37.0 (6)	36.0 (4)	4,22	8.36	0.000
C Hills	38.3 (10)	**38.9 (3)**	31.8 (2)	*16.3 (3)*		3	8.33	0.04
Biomass	Gowar	**Local**	Manilla	Bethungra	Fern Bay			
Mt A	**318.7 (86)**	134.4 (14)	114.8 (8)	108.0 (4)	85.6 (11)	4	8.47	0.076
C Hills	**111.7 (64)**	83.5 (23)		55.84 (9)	6.67 (2)			

Provenance mean is italicized if significant differences were found between local and non-local provenances. Sites (MtA & CH) were analyzed separately for *B. spinosa* due to only two provenances surviving at the CH site and for *Themeda australis* when the data could not be transformed for ANOVA analysis and Kruskal–Wallis analysis was used. Only one plant from the Manilla provenance of *T. australis* survived and this was removed from the growth analysis. Measurement units: stem height, stem diameter, leaf length & clump circumference (cm) and aboveground total biomass (biomass) (g).

sourced *B. spinosa* plants showed superior performance for all three growth measurements at the CH site, but this comparison was made against only one non-local provenance. At the MtA site, where the local provenance was compared to three non-local provenances, the local was the largest only for mean stem height. The local provenance of *T. australis* had the largest mean basal circumference at one site only, with a non-local provenance having superior growth for all other measurements. Significant differences between the provenances were recorded for *E. crebra* and *H. violacea*, but the local provenance did not display the greatest growth in either case. For *H. violacea*, only biomass was measured due to its prostrate growth habit. Overall, the frequency of local superiority was calculated at 0.26, that is the local provenance grew significantly larger only five times out of a possible 19 counts.

Phenology

Percentage of Flowering Plants. Three of the species flowered during the course of the experiment, but only one demonstrated local superiority. On average, the local provenance plants of *B. spinosa* had significantly more flowering plants than two non-local provenances at the MtA site and one non-local at the CH site (Fig. 2a). There were significant differences between the provenances for *A. falcata*, but the local provenance, on average, did not have the most flowering plants (Fig. 2b). There were no significant differences between

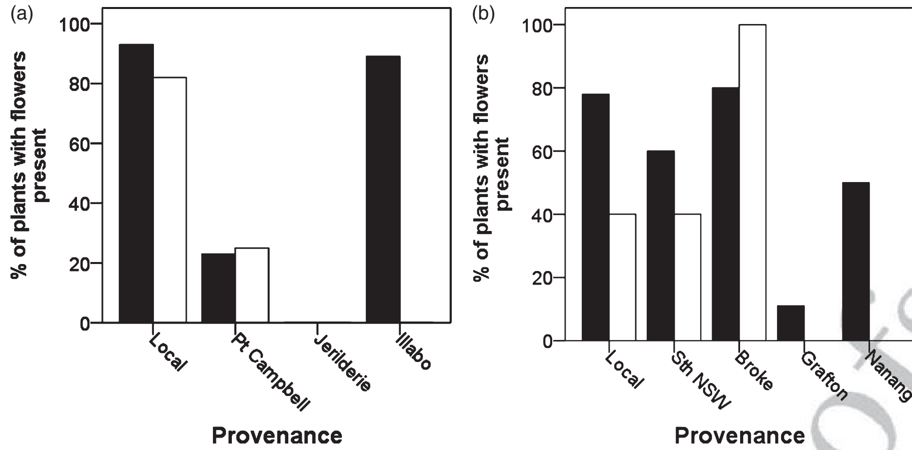

Figure 2. Percentage of plants of the different provenances that flowered (flowers present or absent) at Mount Annan (MtA) (black bars) and Cecil Hills (CH) (white bars) for (a) *Bursaria spinosa* (MtA: χ^2_3 = 22.38, $p \leq 0.001$; CH, χ^2_1 = 4.261, p = 0.039) and (b) *Acacia falcata* (MtA, χ^2_4 = 11.64, p = 0.0203; CH, χ^2_4 = 25.85, $p \leq 0.001$). No plants from the Jerilderie provenance of *B. spinosa* were planted at the CH site.

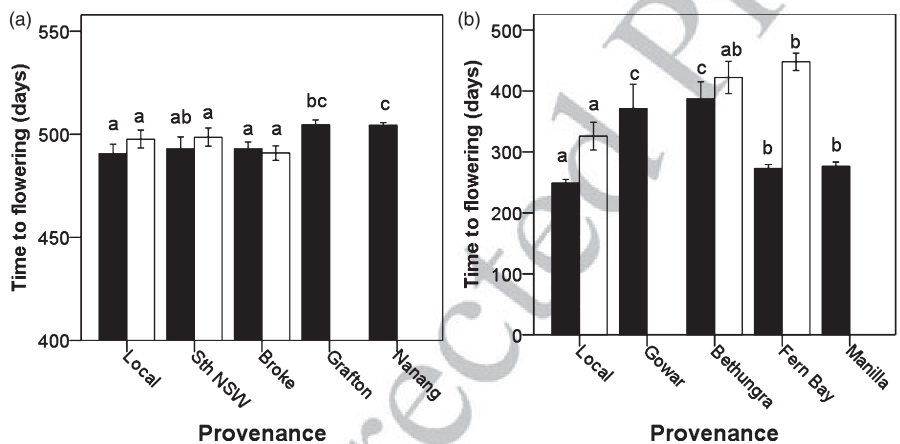

Figure 3. Mean (±SE) time to flowering for (a) *Acacia falcata* (MtA: χ^2_4 = 12.501, $p = 0.014$; CH: $\chi^2_4 = 21.63$, $p < 0.001$) and (b) *Themeda australis* (MtA: $\chi^2_4 = 29.15$, $p \leq 0.001$; CH: $\chi^2_4 = 19.54$, $p \leq 0.001$) provenances grown at Mount Annan (MtA) (black bars) and Cecil Hills (CH) (white bars). Provenances sharing the same lower-case letters are not significantly different as indicated by the overlap of 95% confidence intervals. There were no flowering plants at the CH site for *A. falcata* provenances Grafton and Nanango and for *T. australis* provenances Gowar and Manilla. Note the truncated y-axis in (a).

the provenances of *T. australis* ($\chi^2_4 = 6.577$, $p = 0.1600$) with plants from two provenances, including the local, achieving 100% flowering at both sites. Overall, the frequency of local superiority was calculated at 0.40.

Time to Flowering. Time to flowering was recorded only for *A. falcata* (Fig. 3a) and *T. australis* (Fig. 3b). For *A. falcata* at the MtA site, the southerly provenances flowered, on average, significantly earlier than the northerly provenances with the local provenance plants the earliest to flower. Only the southerly provenances flowered at the CH site and in contrast to the MtA site, there were no significant differences between the provenances and the local provenance plants were not the earliest to flower. For *T. australis* at the MtA site, the local provenance plants flowered significantly earlier than all of the non-local provenance plants. At the CH site, only three provenances flowered and whilst the local provenance plants flowered the earliest, in contrast to the MtA site, it was only significantly earlier than one non-local provenance.

Morphological Traits

Significant differences were recorded between the provenances for many morphological traits but, with the exception of *B. spinosa*, the local provenances were always within the range of variation. The local plants and those from one non-local provenance had significantly smaller leaf width:length ratios than plants from two non-local provenances (Table 3).

Herbivory

A significant difference in herbivory between provenances was found only for *Eucalyptus crebra* ($\chi^2_{16} = 28.46$, $p = 0.028$;

Table 3. Variation in morphological traits: leaf width:length ratio (W:L), SLA, Lignotuber present or absent and number of main branches for *Bursaria spinosa, Eucalyptus crebra*, *E. tereticornis* and *Hardenbergia violacea*.

	W:L	*SLA*	*Lignotuber (p/a)*	*Number of Branches*
Bursaria spinosa	$F_{[3,36]} = 29.035^a$, $\boldsymbol{p < 0.001}$ $F_{[1,13]} = 2.22^b$, $\boldsymbol{p < 0.001}$	$F_{[3,35]} = 1.36^a$, $p = 0.272$ $F_{[1,13]} = 0.11^b$, $p = 0.751$	n.a.	n.a.
Eucalyptus crebra	$F_{[4,82]} = 14.41$, $p < 0.001$	$F_{[4,4]} = 8.17$, $p = 0.061$	$\chi^2{}_4 = 3.89^a$, $p = 0.422$: $\chi^2{}_4 = 3.48^b$, $p = 0.481$	$F_{[4,4]} = 8.33$, $p = 0.032$
E. tereticornis	$F_{[4,89]} = 33.78$, $p < 0.001$	$F_{[4,89]} = 5.39$, $p = 0.001$	$\chi^2{}_4 = 12.22$, $p = 0.016$	$F_{[4,94]} = 3.47$, $p \leq 0.011$
Hardenbergia violacea	$F_{[3,45]} = 26.42$, $p < 0.001$	$F_{[3,45]} = 2.38$, $p = 0.082$	n.a.	n.a.

Significant differences between local and non-local provenances indicated in bold ($p < 0.05$). Sites (MtA and CH) were analyzed separately for *Bursaria spinosa* due to only two provenances surviving at the CH site and for *E. crebra* when sites were analyzed separately using a χ^2 test.
[a,b] Mount Annan and Cecil Hills sites, respectively.

data not shown). Leaves from plants sourced from the non-local provenance with the smallest leaf width:length ratio had the least amount of herbivory (approximately 5%) and plants from the slowest growing non-local provenance suffered the most damage (15–20%).

Site

There were significant differences between the two sites in almost all cases ($p < 0.25$). Generally, survival and growth performances were lower at the CH site, most likely due to occasional water-logged conditions. There was only one significant interaction between provenance and site which was for basal circumference in *T. australis* ($F_{[4,42]} = 3.35$, $p = 0.018$). This was due to a non-local provenance doing particularly poorly at the wet site (CH) and another non-local provenance growing comparatively well at the MtA site.

Discussion

This study found equivocal evidence for superiority of local provenance plants during the establishment phase. Whilst the local provenance plants were sometimes equal top performers (and therefore not superior), we found that with the exception of *Bursaria spinosa*, and to a lesser extent *Themeda australis*, the local provenance plants rarely demonstrated superiority overall. The frequency of local superiority was 0.33 for survivorship, 0.26 for growth, and 0.40 for percentage of flowering plants (although not all species flowered). With the exception of leaf W:L ratio in *B. spinosa*, the local provenance plants were always within the range of variation for morphological traits and herbivory.

Intra-specific variation was evident within each species, with significant differences between the provenances demonstrated for many traits. The only published provenance trials conducted in Australia for any of the species investigated are for *T. australis*. Waters et al. (2005) found mixed home-site advantage for survival, and Groves (1975) found few significant differences between provenances in the number of tillers between five populations spread across a 14° longitudinal band in south-eastern Australia. Differences between provenances for flowering time in this widespread species are well known (Evans & Knox 1969; Groves 1975) and this was confirmed in our study. The local provenance plants of *T. australis* flowered significantly earlier than all non-local provenance plants at the MtA site. Whilst earlier flowering time does not necessarily confer a fitness advantage, it may have implications for trophic interactions (Vander Mijnsbrugge et al. 2010 and references therein).

The probability of detecting local superiority in our study may have been influenced by the timing of the census. The two species in which the local provenance demonstrated superior survival to non-local provenances (*B. spinosa* and *T. australis*) were also grown for the longest period (approximately 2 years). The results for *E. crebra* and *E. tereticornis* can be compared to longer-term forestry trials. Our findings are consistent with nine forestry trials spanning 3.3–13 years for five eucalypt species in which none of the local provenance trees had higher survival than those sourced from non-local provenances when grown at a common site (Johnson & Stanton 1993). However, a comparison of survival rates in two *E. pilularis* trials (for 38 and 104-months) found that although the local provenances did not survive the longest, over time, their performance improved (Raymond 2010), a trend which was not detected in our relatively short-term study.

The two species that demonstrated local superiority, *B. spinosa* and *T. australis*, were also the species with a history of taxonomic uncertainty and/or different ploidy levels. This raises the possibility that our comparisons were between subspecies or varieties within these species, rather than between provenances. This has implications for seed-sourcing zones and restoration success as gene flow between populations of species with ploidy differences and taxonomic uncertainly increases the likelihood of outbreeding depression and hybridization (Byrne et al. 2011; Frankham et al. 2011). For *T. australis*, it is beyond the scope of this paper to expand on its polyploid status, but it has been established that some Sydney populations are tetraploid whilst many other populations in Australia are diploid (Hayman 1960). It is highly likely that provenances with different ploidy numbers were used in our study. *B. spinosa* has long been the subject of taxonomic debate and its taxonomic status, based on morphology, was reviewed in 1999, which resulted in two subspecies currently being recognized (Cayzer et al. 1999). We obtained confirmation that all of the provenances used were of the same subspecies, *spinosa* (B. Wiecek 2011, National Herbarium of New South Wales, personal communication), but we found that most of the traits we measured separated into two fairly distinct groups. In addition, differences in unrecorded traits (germination requirements, stem flexibility, and habit differentials), also applied to this grouping. Whilst the local provenance of *H. violacea* did not show clear superiority, there is also some taxonomic uncertainty about this species; a prostrate and a climbing form are known (Harden 1991). From the provenances we studied, there appeared to be three morphological forms: prostrate round-leaved, climbing long-linear leaved, and climbing round-leaved. These three forms were significantly differentiated, suggesting different ploidy levels may also exist within this species (L. Broadhurst 2010, CSIRO, personal communication). Further investigation into taxonomic uncertainties and potential differences in ploidy levels and reproductive methods that may create problems in subsequent generations is warranted.

Several other factors may also explain our findings of equivocal evidence for superiority of local provenance plants. First, there was a lack of statistical power for some analyses, including survivorship, and these results therefore need to be interpreted with caution. This includes the comparisons of *B. spinosa* plants at the CH site where the local provenance plants were only compared to one non-local provenance. Second, the suboptimal performance of some provenances may have been due to seeds being sourced from small populations. Information on population size was not available from all of the commercial seed suppliers and in some instances, only large regional areas could be given as the seed source location. This is a problem for practitioners who need this level of detail to enable informed decisions about appropriate seed sourcing. Third, environmental maternal effects may have influenced the results of all species, particularly *H. violacea* because this species had the shortest growing period (8 months) (Roach & Wulff 1987). Finally, it is possible that the results were affected by differences in seed age between provenances. However, germination was not assessed in our study and we should note that seed age varied most for Myrtaceae and Fabaceae species, two families in which seeds remain viable for longer time than for many other families (Martyn et al. 2009).

The long-term sustainability of restoration projects is under threat from a rapidly changing climate. This will present new challenges for restoration practitioners already faced with dwindling seed supplies, increasing fragmentation, and the associated risks of sourcing seed from small populations. These challenges raise the need for a different approach to the traditional practice of the exclusive use of genetic material that has been collected within a defined radius of the restoration site. We found little evidence that restricting provenance choice to locally collected seed will be detrimental to the establishment success for several CPW species. Longer-term field trials that investigate the transition from establishment to persistence will progress our findings. The inclusion of non-local stock (from populations with the same ploidy levels and taxon lineage) may provide potential for evolutionary adaptation in the highly fragmented CPW landscape in the face of climate change.

Implications for Practice

- The common garden experiment did not detect strong evidence of local superiority for establishment success. This implies that the inclusion of non-local provenance material could be considered as an adaptation strategy to mitigate the effects of a changing environment by increasing evolutionary potential for the CPW.
- A lack of seed collection detail will hinder informed decision-making on suitable non-local seed sources. For example, details of population size and environmental conditions of the seed source and correct identification to subspecies and variety level will assist in the maximization of restoration success.
- Careful consideration should be given to seed sourcing in species known to have differing ploidy levels.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Distribution of species, location of provenances and common garden sites.

Figure S2. Climatic envelope for six species used in the common garden study.

Table S1. Life history details for six species used in the common garden study.

Table S2. Locations and climatic conditions of field collections of provenances.

Table S3. Measurements recorded for each species.

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How far is it to your local? A survey on local provenance use in New South Wales

By Nola Hancock and Lesley Hughes

***Nola Hancock** is a PhD candidate (Macquarie University, North Ryde, NSW 2109, Australia; Tel: 0419262116; Email: nola.hancock@mq.edu.au, nolahancock@hotmail.com). **Lesley Hughes** is an ecologist (Macquarie University, North Ryde, NSW, 2109; Tel: 9850 8195; Email: lesley.hughes@mq.edu.au). This study forms part of Nola Hancock's thesis on the role of provenance in restoration ecology under climate change.*

Summary The decision as to where to source seed is one of the most critical in restoration projects. Locally collected seed is often recommended, or even contractually required, because it is assumed to be adapted to local conditions and therefore result in superior survival and growth rates, conferring a greater probability of restoration success. The perceived advantages, which include retaining the genetic 'integrity' of the site, are centred around the avoidance of outbreeding depression and hybridization. These traditional reasons for using locally collected seed need to be reconsidered in the light of rapidly changing climatic and other environmental conditions; plants that are locally adapted now may not be locally adapted in future. Understanding the current usage of local provenance is pivotal to discussions on its appropriateness under climate change. We present the results of a survey of restoration practitioners in New South Wales on attitudes and practices in relation to the use of local provenance. We found that whilst the majority of practitioners preferentially use local provenance seeds, the actual definition of local provenance varied amongst respondents. Whilst 80% of participants believe that projections of future climate change are relevant to restoration projects, there is an apparent reluctance to actively manage for this eventuality. However, many respondents are in favour of a review of seed-sourcing policy/guidelines to allow for the inclusion of non-local provenance material. Implications of the survey for potential changes to guidelines to better prepare for anticipated changing conditions are discussed.

Key words: *climate change, local adaptation, local provenance, revegetation, seed source.*

Introduction

Revegetation is a core activity of many restoration projects (See Box 1 for glossary). Increasingly, revegetation with native species is identified as a partial solution for many landscape and biodiversity problems. The recently commenced Biodiversity Fund, providing nearly $1 billion for carbon storage and biodiversity benefits, is one such example (Australian Government, 2012).

An integral component of any revegetation project is the decision as to where to source the genetic material (including seeds or container-grown plants). Genetic material can either be sourced locally (hereafter referred to as local provenance), or from more distant locations within the species range. In recent decades, genetic material for revegetation projects has often preferentially been sourced locally. It has generally been assumed that locally sourced plants are better adapted to local conditions and will therefore survive longer, grow faster and have reproductive advantages over non-local plants, thus providing the greatest chance of revegetation success. Exactly what constitutes 'local' is not always defined, but in many instances a maximum distance between the seed collection area and the proposed revegetation site is specified (generally 5–20 km). When environmental gradients are steep between the planting site and the seed source, locally sourced plants may have a home-site advantage over non-local provenances (Hereford 2009). Examples of this include low versus high elevation, coastal versus inland, frost versus no frost and contaminated versus natural soils (see references in: Millar & Libby 1989; Linhart & Grant 1996; Davis & Shaw 2001). However, not all plants and/or populations are locally adapted and in some cases, non-local provenance plants survive and grow better than local provenance plants (e.g. Gordon & Rice 1998). The introduction of non-local material may also alter interactions between plants and their natural enemies or mutualists, and these changes may be either beneficial or detrimental to either partner (Linhart & Grant 1996; Vander Mijnsbrugge *et al.* 2010 and references therein).

The use of local provenance material is also advocated as a strategy to preserve the genetic 'integrity' of the replanted site. Revegetation using non-local genetic material introduces new genetic material into an area. The consequences of such introductions can be positive or negative, depending on the species and the landscape. The introduction of inappropriate non-local provenance material may increase the possibility of outbreeding depression and hybridization, resulting in sterile offspring or unviable seed (Hufford & Mazer 2003). Risks of outbreeding depression and unwanted hybridization can often be predicted (and therefore avoided) (Byrne *et al.* 2011; Frankham *et al.* 2011). Outbreeding depression is largely determined by the time period that populations have been separated and the degree of genetic difference between them (Frankham *et al.* 2011). A

Box 1. Definitions used in the survey and this study.

Genetic material: seed, cuttings, tubestock or other propagules.
Local provenance: genetic material that has been sourced locally, that is, close to the proposed planting site.
Inbreeding Depression: a reduction in survival, growth and reproduction in inbred offspring (caused by mating between relatives), relative to the parents.
Outbreeding Depression: a reduction in survival, growth and reproduction of offspring from crosses between plants from different populations, relative to the parental populations, that is, sterile hybrids.
Restoration (Ecological): the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed.
Provenance: the geographic location/origin of the genetic material.
Revegetation: adding plants to the ecosystem by planting, seeding or translocating.
Threatened Ecological Community: a group of species that occur together in a particular area of the landscape that has been listed as threatened (either categorized as Critically Endangered, Endangered or Vulnerable) under the NSW Threatened Species Conservation Act, 1995: http://www.environment.nsw.gov.au/threatenedspecies/and/or under the Commonwealth EPBC Act http://www.environment.gov.au/epbc/protect/species-communities.html

risk assessment protocol is now available to evaluate the likelihood of hybridization on surrounding plant populations (Byrne *et al.* 2011). The assessment is based on the taxonomy and reproductive biology of the species involved, the extent of pollen dispersal between the planting site and the surrounding vegetation and the differences in size of the sites (Byrne *et al.* 2011).

The traditional reasons for using local provenance are relevant for long-term restoration success under stable environmental conditions. But the climate is rapidly changing, populations are increasingly becoming more fragmented, land use practices are altering and exotic plants, animals and pathogens are invading. The advantages of being locally adapted will be determined, to a large extent, by the degree and the size of the change or disturbance (Lesica & Allendorf 1999; Jones & Monaco 2009); plants that are locally adapted now may not be locally adapted in future. Furthermore, if local populations are small and genetically impoverished, sourcing seed from these populations may ultimately be detrimental to the long-term success of the revegetation project via inbreeding depression (Broadhurst & Young 2007). Under these conditions, the introduction of seed from genetically diverse populations can increase the probability of revegetation success (Broadhurst *et al.* 2008). Genetic variation is fundamental to enable evolutionary adaptation to changing environments. Unless new genetic material enters the population (i.e. via pollen or propagule dispersal), the genetic diversity in small populations will eventually diminish and reduce species capacity to adapt to a changing environment (Weeks *et al.* 2011).

Seed-sourcing guidelines or seed zones are used by many organizations to delineate areas from which genetic material can be collected for revegetation projects with a minimal risk of maladaptation (Johnson *et al.* 2004; Krauss & He 2006). Ideally, studies assessing both genetic variation and local adaptation are used to provide accurate delineation (Wheeler *et al.* 2003). Such detailed information is rarely available and in its absence, different approaches have been taken to establish seed-sourcing guidelines. For example, seed zones in the UK are based on major climatic and geological regions, modified by altitude, whilst in Europe, various combinations of climate, soil and geomorphology are used to define collection zones (Vander Mijnsbrugge *et al.* 2010 and references therein). Other examples include the adoption of a 'precautionary' approach, with local provenance cited as 'best practice' (N.S.W. Department of Environment and Conservation, 2005), or, the restriction of seed collection zones to a defined radius of the planting site (e.g. no further than 100 m from the site for herbs) (Linhart 1995, cited in Jones & Johnson 1998). Florabank guidelines, which often serve as an industry standard in the eastern states of Australia, provide information on seed collection methods and generalized advice for seed collection ranges for revegetation (Florabank, 1999). The Florabank guidelines provide a range of approaches for provenance selection. The basic recommendation is to collect as locally as possible but to also maximize the genetic quality of the seed collected. When circumstances necessitate broader collection, it is suggested that consideration is given to matching the environmental conditions of the source population to the planting site and to be aware that these decisions are site and species specific (Florabank, 1999).

The use of local provenance material is a requirement for the attainment of a Section 132C licence to bring in new plant material and/or to collect seed for revegetation in Threatened Ecological Communities and for threatened species in NSW. The use of local provenance material is also either recommended or required in many restoration projects and as a condition of granting agencies. Locally sourced material may sometimes be unavailable in sufficient quantities (Mortlock 2000), necessitating difficult decisions about how to best fulfil legal and contractual obligations and attain the best revegetation outcomes. Mixing different seed sources or 'composite provenancing' using high-quality seed that is site and species specific, is increasingly being recommended as a method of incorporating a broad range of genetic material as an insurance policy for revegetation success (Lesica & Allendorf 1999; Broadhurst *et al.* 2008). Understanding the current usage of local provenance is critical for progressing discussions on its appropriateness in the face of a rapidly changing environment. We undertook a survey of restoration and revegetation practitioners and policy makers in New South Wales, Australia, to explore respondent's views on the following issues:

- What constitutes 'local' for local provenance revegetation activities?
- Is local provenance generally considered to be the best choice for revegetation activities?
- Are there supply or other constraints on the use of local provenance?
- Are current guidelines on the use of local provenance adequate under existing and/or anticipated future conditions?

Methods

Survey form

The survey was in the form of an on-line SurveyMonkey (https://www.surveymonkey.com/) (see Data S1 for a copy of the survey) and targeted participants in the restoration industry in New South Wales. Participants were broadly divided into practitioners (defined as anyone who undertakes revegetation using native seeds or seedlings as paid employment or for own business) and non-practitioners (policy makers, educators/researchers, nursery workers/seed collectors, volunteers or those interested in revegetation). The term 'respondent' is used when both practitioners and non-practitioners have answered the questions. 'Skip logic' was applied to the survey whereby respondents only answered questions that were relevant to their role in the industry. Specific questions were directed to (1) practitioners who set their own policies or procedures regarding provenance, (2) practitioners who follow policies or procedures imposed upon them by another organization, (3) both (1) and (2), and (4) non-practitioners (e.g. policy makers and researchers). Respondents were not required to answer all questions and participation in the survey was voluntary.

Sampling frame

The survey was largely distributed via the email networks of the Sydney Metropolitan Catchment Management Authority (SMCMA). These networks included the CMA offices, the Volunteer Co-coordinators Network (VCN), Natural Resource Managers (including the Local Government & Shires Associations (LGSAs) and Regional Landcare Facilitators, all in NSW). The survey was also sent out via the Australian Association of Bush Regenerators (AABR), National Parks & Wildlife Service (NPWS), the Ecological Consultants Association of NSW and the Australian Network of Plant Conservation (ANPC). The survey was also sent to representatives of the mining industry, contractors (non-bushland) who list revegetation with native species as a core activity, Indigenous Protection Areas, State Government Agencies, Non-government Organizations, project managers and native plant nurseries other than those on the AABR website. Apart from contractors (non-bushland), these contacts were either already known to the authors or had been recommended by contacts within the industry as potential respondents. The final number of recipients is unknown but we believe that coverage within the NSW restoration industry was comprehensive. The survey was completed by 144 respondents. The majority of participants were practitioners (56%) (Table 1) and many sectors of the restoration industry were represented (Table 2).

Table 1. Role of respondents in the restoration industry (n = 144 in total)

Role	Response (%)
Practitioner who undertakes revegetation using native seeds or seedlings as paid employment or for own business	56.3
Policy maker. This includes anyone who dictates which provenance(s) an organization will use	19.4
Nursery worker and/or seed collector	6.9
Educator/Researcher	6.3
Volunteer	6.3
Interested in revegetation	4.9

Table 2. Organizations for which respondents represent or work (n = 132)

Organization	Response (%)
Local Government	41.7
State Government	15.9
Community group/Volunteer	10.6
Non-government Organization (NGO)	9.8
Contractor (bushland)	7.6
Mining sector	6.1
Private individual land owner/lessee/manager	4.5
Commonwealth Government	2.3
Carer of Indigenous Protection Areas (IPA)	0.8
Contractor (non-bushland)	0.8

Results

Definition of 'local provenance'

There was little consistency between respondents as to how they defined local provenance but 31% noted that it *depends on the species* (i.e. it *depends on their pollination, dispersal or other traits*) (Fig. 1). Twenty-nine per cent of respondents defined local provenance as *within the catchment of the proposed revegetation site*. Whilst this is a narrow preference, 56% of the comments that accompanied the answers made reference to their definition being species dependent, further inflating the first preference. Many respondents commented that they consider the definition of local provenance to include other factors that were not listed in the survey, such as the condition of the planting site and the nature of the project.

Current practice in regard to local provenance use

The majority of practitioners who make their own decisions regarding provenance selection (68%) responded that local provenance is their preferred choice. A further 30% choose a mix of local and non-local. When a mix is chosen, 11 of the 17 practitioners who answered this question choose mostly local provenance. A clear

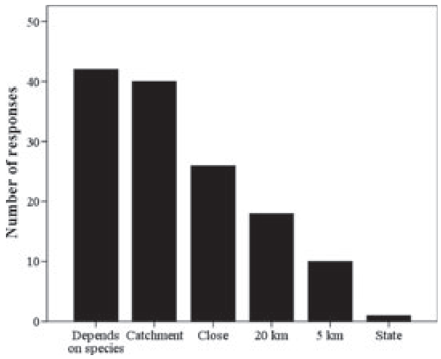

Figure 1. Respondents' definition of local provenance: Depends on the species (i.e. pollination, dispersal or other traits); Within the catchment of the proposed revegetation site; As close as possible to the proposed revegetation site; ≤20 km from the proposed revegetation site; ≤5 km from the proposed revegetation site; Within the state boundaries of the proposed revegetation site.

majority of respondents regard the use of local provenance material as either important or very important for both Threatened Species or Threatened Ecological Communities (TS & TEC) (88%) and non-TS and TEC (80%) revegetation projects. The finding that local provenance use is important regardless of the vegetation status (threatened/endangered or common) was irrespective of the respondent's role within the industry, the organization for which they work or the Catchment Management Authority (CMA) region in which they work (for the major groups only) (See Table S1 for CMA regions).

One-third of practitioners indicated that they work with plant populations of <200 adult plants per species (a further one-third were unsure of the number). Of the 23 practitioners who work with <200 plants per species, the majority use local provenance material exclusively.

Difficulties in obtaining a sufficient supply of local provenance plants had been experienced by 74% of practitioners. This shortage was overcome with a combination of strategies but the single largest response (33%) was to reduce the diversity of planting (Fig. 2). Almost half (47%) of the practitioners anticipate that their revegetation activities will increase over the next 5 years, whilst 30% expect no change. In the past 12 months, 38% of practitioners planted <8000 seedlings (or ≤5 kg seed), 20% planted between 8000 and 25 000 seedlings (or between 5 and 20 kg seed) and 25% planted 25 000 seedlings or >20 kg seed. Of the largest users of native stock, 55% responded that they use all local provenance seeds (the remainder were unsure or the question was not applicable).

Current policies and guidelines

Two-thirds of respondents indicated that they were in favour of a review of seed-sourcing policy/guidelines to allow for the inclusion of non-local provenances. Currently, almost half of the practitioners use a mix of procedures or specifications to determine their choice of provenance (Fig. 3) with some respondents complying with up to four different sets of guidelines. The single most commonly used guidelines were those of Florabank (19%), followed by licence conditions (17%).

Of the practitioners who responded to the survey, 88% work in locations where both threatened species or Threatened Ecological Communities (TS & TEC) and non-TS and TEC are present. Accordingly, the majority of practitioners (54%) follow policies or procedures imposed upon them by another organization (i.e. licence conditions) and/or set their own policies. A further 18% solely follow policies or procedures imposed upon them by another organization.

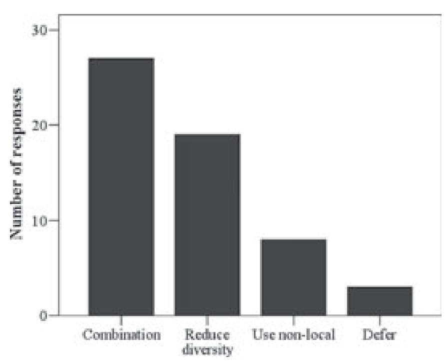

Figure 2. Practitioners' response to the difficulty in the obtainment of local provenance plants; A combination of the following: Reduced the diversity of planting to only those species for which local provenance is available; Used non-local provenance material; Deferred the project.

There was variation in understanding of the reason(s) that underpinned provenance guidelines (Table 3). The most common response (34%) was that the requirement was because of a combination of many factors, most notably, local knowledge and adherence to the precautionary principle.

Future use of local provenance

A clear majority of respondents (80%) believe that projections of future climate change (e.g. more extreme weather events, increased temperatures, changes in rainfall patterns) are relevant to restoration/revegetation projects. However, *improving evolutionary potential to adapt to a changing environment* was not highly ranked as a reason for the choice of provenance (Table 4). As a climate-change management strategy, 67% would consider using or advocating for the use of non-local provenance genetic material sourced from areas with current climatic conditions similar to those predicted for the proposed revegetation site. However, actual preparatory measures to deal with future climate-change impacts are being undertaken by less than half (45%) of the respondents, and less than one-third of practitioners have a future focus as their long-term restoration goal (Table 5).

Discussion

This survey identified several inconsistencies of practice and belief within the restoration industry in New South Wales (NSW). We found that there is no consistent definition of 'local provenance' amongst practitioners and other participants in the industry. For many respondents, the definition is somewhat flexible, depending on the pollination, dispersal or other traits of the particular species involved. The majority of practitioners indicated that local provenance use is important, regardless of the status (threatened/endangered or common) of the vegetation being managed. Whilst 80% of respondents noted that projections of future climate change are relevant to restoration projects, only 45% are taking preparatory measures. However, a clear majority

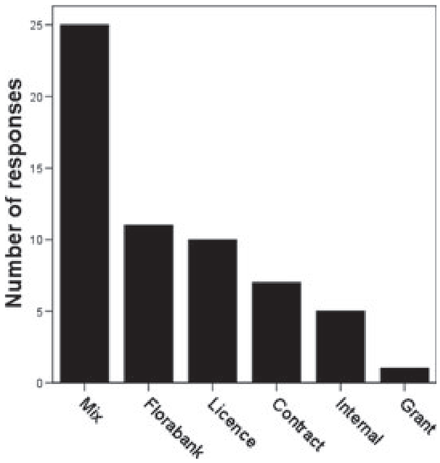

Figure 3. Procedures or specifications used by practitioners in determining the use of local versus non-local provenance for the majority of work undertaken; A mix of the following; FloraBank guidelines; Licence conditions; Contract conditions; Requirement of internal policy; Requirement of grant application or funding arrangement.

of respondents would like to see a review of seed-sourcing policy/guidelines to allow for the inclusion of non-local provenance material.

The lack of consistency in the definition of local provenance is possibly a reflection of the diversity of people working within the restoration industry. Many practitioners perform multiple roles, work in more than one location and under contrasting conditions and obligations. The survey questionnaire did not offer a definition for 'local' or for 'catchment', and this may have also contributed to the variability of the definition amongst respondents. In a 1999 survey of restoration practitioners (particularly seed collectors), the definition of 'local' was most often expressed as a distance (e.g. a 15-km radius) from the planting site, or it was defined by a region (e.g. a catchment) (Mortlock 1999). The definition of local provenance appears to have undergone a subtle shift since the previous survey. In the current survey, slightly more respondents preferred to define local provenance as being species dependent, rather than identifying a consistent distance-based or regional definition. However, when the different categories of distance-based definitions were grouped together, the response was much higher and comparable to the 1999 survey of Mortlock. It should be noted, however, that the two surveys had a different foci and the 1999 survey did not give respondents the option of choosing the definition *depends on the species*. Since the previous survey, recommendations based on factors other than a distance-based approach may have gained momentum. For example, Florabank guidelines 10 (Florabank, 1999) suggest a range of approaches and more recently, CSIRO technical reports and research articles (e.g. Broadhurst 2007; Broadhurst *et al.* 2008) focus on the importance of using genetically diverse source material.

Table 3. Opinions of respondents about why local provenance is required or recommended (n = 134)

Reason	Response (%)
A combination of reasons listed below	34.3
Practical experience of superior restoration results using local provenance (local knowledge)	26.1
Adherence to precautionary principle	20.1
Untested theory within the industry	9.0
Results from peer-reviewed scientific literature of provenance trials/research	6.7
Not sure	3.7

Table 5. Long-term restoration goals of practitioners' organizations (n = 76)

Goal	Response (%)
Restore to pre-European vegetation communities	27.6
Maintain current vegetation communities	44.8
Create and sustain new vegetation communities in anticipation of future environmental conditions	27.6

Table 4. Ranked order of importance for choice of provenance (unimportant = 1 to very important = 5). Numbers represent the response count in each category

Reason for choice of provenance	Unimportant	Not important	No opinion/do not know	Important	Very important	Rating average
Match environmental conditions of source population to proposed planting site (temperature, rainfall, aspect, soil, etc.)	0	1	2	22	30	4.47
Increase genetic diversity	0	5	3	25	22	4.16
Avoidance of potential inbreeding depression	0	6	12	19	18	3.89
Size of source population/ recipient population	1	5	9	25	14	3.85
Limit the distance of the source population to proposed planting site	4	4	7	28	12	3.73
Improve evolutionary potential to adapt to a changing environment	2	6	12	23	13	3.70
Avoidance of potential outbreeding depression	0	8	18	16	13	3.62

The prevailing preference and/or requirement for the use of local provenance stock is widespread in NSW, and its use has increased since the previous survey (where 30% of respondents sourced all their seed locally and 44% collected 'most' seed locally) (Mortlock 1999). Demand for local provenance material (and a general increase in demand for native seed (Mortlock 2000) may have contributed to 74% of all practitioners experiencing difficulty in obtaining a sufficient supply of local provenance material. This number is considerably higher than that found in the previous survey (49% usually or sometimes experienced supply difficulties in NSW) (Mortlock 1999). An increase in revegetation activities is expected by 47% of practitioners (and only 5% expect a decrease) over the next 5 years. A respondent noted that the mining sector, a large user of native seed, is 'ramping up its collection of local provenance seed' and that its use is becoming a regular condition for approval. This will further exacerbate supply problems already experienced by practitioners struggling to fulfil local provenance obligations. For most, the shortage is overcome by limiting planting to only those species for which sufficient quantities of local provenance seed is available. A consequence of this practice is that the diversity of restoration plantings may decline. On some occasions, the supply shortage has led to the sourcing of material of lesser quality and from unknown locations. Concerns were expressed by some respondents that seed collection is not always performed to industry standards (i.e. Florabank guidelines) and that guarantees of contractual obligations to use local provenance cannot always be made. A lack of time allowed for the collection of local provenance seed was also raised as an issue, usually with negative outcomes for the quality of the seed. Standardized industry accreditation for seed collection is a possible solution to the problem, and further discussion on this topic is warranted but is beyond the scope of this survey.

Overall, the response given for the reason(s) as to why the use of local provenance is required or recommended was equivocal. Many respondents noted that the requirement is because of the adherence to the precautionary principle. However, adherence to the precautionary principle may be counterproductive to successful restoration outcomes if the local seed is collected from genetically depauperate populations. Such collections may increase the chance of inbreeding depression and reduce adaptive potential, increasing the likelihood of restoration failure, especially in the face of a rapidly changing climate (Weeks *et al.* 2011). Many respondents stated that they use local provenance because of their practical experience of its superior restoration results. Most of these comments were anecdotal, with few respondents providing specific information. Of the examples provided, there was little to no evidence that the comparisons between local and non-local provenances had been rigorously tested. There are many factors that contribute to restoration outcomes that may be misconstrued as a problem of inferior seed. These factors include the variation in environmental conditions at the time of collection through to the quality of nursery conditions. There appeared to be little knowledge amongst the respondents about local provenance issues in the peer-reviewed scientific literature, suggesting a need for better dissemination of up to date scientific information through the restoration industry.

A review of seed-sourcing policy/guidelines to allow the inclusion of non-local provenance(s) was sought by two-thirds of the respondents. Respondents gave examples of guidelines that use a range of definitions of local provenance, from a narrow focus 'a licence condition that requires the use of local provenance within 300 m of the collection source' to a broader approach of 'within the bioregion'. There were large differences of opinion regarding the optimal collection range for specific functional groups of plants. As an example, comments ranged from 'as close as possible' to 'up to 20 km' as the appropriate seed collection range for tree species. Collection ranges for grasses were also very broad and variable. Guidelines and policies that limit local provenance to within a specific radius of the proposed revegetation site were not favoured by the majority of respondents. This finding is consistent with a review by McKay *et al.* (2005) that concluded that it is impossible (and counterproductive) to impose a standard geographic distance as a scale for local adaptation (and therefore for seed collection). In summary, respondents indicated a desire that seed-sourcing policy/guidelines (1) reflect differences in species' traits, (2) allow for the matching of environmental conditions of the seed source to the revegetation site, (3) actively manage the avoidance of inbreeding depression and (4) alleviate supply problems. To assist with provenance selection, several new regional (NSW) *Seed Supply Strategies* have recently been produced that include the consideration of (2) and (3) and the condition (size and type of disturbance) of the proposed revegetation site (Vanzella and Greening Australia Capital Region 2012). The Society for Ecological Restoration Science and Policy Working Group (2004) also advocates a flexible approach to seed sourcing under certain conditions. Where substantial damage has altered physical environments, the introduction of 'diverse genetic stock' is recommended. The definition of diverse is not specified but the aim is to promote genetically fit populations.

In contrast to the view that broader seed-sourcing guidelines and policies are needed, an overwhelming majority of the respondents nonetheless indicated that it is important to use only local provenance material for both TS & TEC and non-TS & TEC situations. The classification of endangered and threatened communities and species is partly based on the population biology principles (Frankham *et al.* 2010); the risk of extinction increases when population size or reproduction success is reduced (N.S.W. Government Office of the Environment and Heritage, 2007). Seed-sourcing protocols that limit seed collection to genetically impoverished populations may reduce population size or reproduction success via inbreeding depression (Broadhurst 2007). Some respondents commented that inferior revegetation results are already occurring because of inferior seed collection protocols. The survey found that almost one-third of practitioners work with small numbers (<200) of adult plants per species per population (a further one-third were unsure of the number). Of those working with small numbers of adult plants, most use only local provenance material. The minimum population size from which seed can be collected without negative genetic consequences is the subject of much debate. A conservative estimate is that 100–200 reproductive adult plants is the minimum (Broadhurst 2007), but if adequate adaptive potential is the objective, a minimum of 1000 is suggested (Weeks *et al.* 2011). The appropriate number depends on the circumstances but this flexibility is not always recognized by those who set provenance policies or procedures. In reality, the costs involved in seed collection at such a scale may be prohibitive in many situations.

A significant inconsistency was noted in relation to respondents' views and actions on climate change. Climate change was largely acknowledged as happening but current practices and future restoration goals did not reflect this acceptance. Many respondents expressed the view that a lack of relevant information to guide revegetation work was a reason for the absence of preparatory actions. However, there was also a clear preference for climate change adaptation strategies that allow the flexibility to use non-local provenance material. Those who are undertaking preparatory actions gave examples of extending provenance boundaries, changing the mix of species, creating corridors and participating in plant research.

This survey has highlighted that adherence to inconsistent revegetation policies and contracts are engendering confusion within the restoration industry and possibly contributing to inferior revegetation outcomes. Research to test the validity of the assumptions about the benefits of using local seed is urgently needed to form the basis of new guidelines. Inbreeding and outbreeding problems are particular points of concern for threatened/endangered species but it is also important to delineate 'seed sourcing zones' for common or non-threatened species. Recently published decision trees can assist with strategies to avoid potential negative outcomes from the introduction of non-local provenance material (Byrne *et al.* 2011; Frankham *et al.* 2011). Guidelines are also available to assist with policies regarding genetic rescue and other types of translocations (Weeks *et al.* 2011). Access to and information on genetically diverse and climatically suitable non-local provenance material will become increasingly important as the climate rapidly changes.

Recommendations

- New seed-sourcing guidelines are sought to reflect current and future use of local provenance material. Incorporated into these guidelines, consideration should be given to allow for differences in species traits, for the inclusion of non-local provenance to allow for maximization of genetic diversity, and to match the future environmental conditions of the source population to the proposed planting site.
- Licensing systems by delegated authorities should mirror these guidelines (above) more closely.
- Access to down-scaled climate projections at a local level to enable management for climate change is required to assist with decision-making about sources of material that will have the highest probability of long-term sustainability.
- More research on the extent and magnitude of local adaptation is needed. Practitioners can assist in the improvement of guidelines by creating their own tests of 'local is best' when a revegetation project using mixed provenances is conducted. This would entail the documentation of all aspects of the collection of the stock used, the identification of each plant at the site, consistency of conditions under which the plants are grown and reporting the successes and failures.
- Further discussion is warranted on the advantages and disadvantages of an accreditation system for seed collectors/suppliers.
- Dissemination of relevant information needs to be improved to the seed industry and to those involved in revegetation work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Local provenance survey.

Table S1. Respondents' Catchment Management Authority (CMA) region.

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MACQUARIE UNIVERSITY

Nola Hancock <nola.hancock@mq.edu.au>

FW: Ethics Application - 5201200068 Hughes - Approved

1 message

Lesley Hughes <lesley.hughes@mq.edu.au> Mon, Mar 5, 2012 at 7:59 PM
To: Nola Hancock <nola.hancock@mq.edu.au>

--
Prof. Lesley Hughes
Department of biological Sciences
Macquarie University
North Ryde, NSW, 2109
Australia

Ph: (612) 9850 8195
Fax: (612) 9850 8245
Email: lesley.hughes@mq.edu.au

On 5/03/12 3:10 PM, "Ms Cathi Humphrey-Hood"
<cathi.humphrey-hood@mq.edu.au> wrote:

>Dear Professor Hughes,
>
>RE: Ethics project entitled: "The Role of Plant Provenance in Restoration
>Ecology under Climate Change."
>Ref number: 5201200068
>
>The Faculty of Science Human Research Ethics Sub-Committee have reviewed
>your responses to the issues raised in your original application and have
>confirmed final approval, effective 24th February 2012. You may now
>commence your research.
>
>The following personnel are authorised to conduct this research:
>Professor Lesley Hughes
>Ms Nola Hancock
>
>NB. STUDENTS: IT IS YOUR RESPONSIBILITY TO KEEP A COPY OF THIS APPROVAL
>EMAIL TO SUBMIT WITH YOUR THESIS.
>
>Please note the following standard requirements of approval:
>1. The approval of this project is conditional upon your continuing
>compliance with the National Statement on Ethical Conduct in Human
>Research
>(2007).
>
>2. Approval will be for a period of five (5) years subject to the
>provision
>of annual reports. Your first progress report is due on 24th February
>2013.
>
>If you complete the work earlier than you had planned you must submit a
>Final Report as soon as the work is completed. If the project has been

>discontinued or not commenced for any reason, you are also required to
>submit a Final Report for the project.
>
>Progress reports and Final Reports are available at the following website:
>
>http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approva
>l/
>human_research_ethics/forms
>
>3. You may not renew approval for a project lasting more than five (5)
>years. You will need to complete and submit a Final Report and submit a
>new
>application for the project. (The five year limit on renewal of approvals
>allows the University Human Research Ethics Committee to fully re-review
>research in an environment where legislation, guidelines and requirements
>are continually changing, for example, new child protection and privacy
>laws).
>
>4. All amendments to the project must be reviewed and approved by the
>Faculty Sub-Committee before implementation. Please complete and submit a
>Request for Amendment Form available at the following website:
>
>http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approva
>l/
>human_research_ethics/forms
>
>5. Please notify the Faculty Sub-Committee immediately in the event of any
>adverse effects on participants or of any unforeseen events that affect
>the
>continued ethical acceptability of the project.
>
>6. At all times you are responsible for the ethical conduct of your
>research in accordance with the guidelines established by the University.
>This information is available at the following websites:
>
>http://www.mq.edu.au/policy/
>
>http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approva
>l/
>human_research_ethics/policy
>
>If you will be applying for or have applied for internal or external
>funding for the above project, you are responsible for providing the
>Macquarie University's Research Grants Management Assistant with a copy of
>this email as soon as possible. Internal and External funding agencies
>will
>not be informed that you have final approval for your project and funds
>will not be released until the Research Grants Management Assistant has
>received a copy of this email.
>
>If you need to provide a hard copy letter of Final Approval to an external
>organisation as evidence that you have Final Approval, please do not
>hesitate to contact the Faculty of Science Research team at
>sci.ethics@mq.edu.au.
>
>Please retain a copy of this email as this is your official notification
>of
>final ethics approval.
>
>Yours sincerely,
>Richie Howitt, Chair
>Faculty of Science Human Research Ethics Sub-Committee

>Macquarie University
>NSW 2109
>
>