Seed germination traits of two common floodplain eucalypts: optimum germination temperatures and influence of leaf litter

Vincent, BE[1], Smith, R[1], Good, M[2] and Clarke, PJ†
1 Ecosystem Management, School of Environmental and Rural Science, University of New England, Armidale, NSW 2351.
2 School of Applied and Biomedical Science, Faculty of Science and Technology, Federation University Australia, Ballarat, VIC 3353.
† Deceased author

Key Points

- Alternating temperatures increase seed germination in coolibah and black box.
- Leaf litter inhibited germination of both coolibah and black box seed.
- Coolibah had a lower optimal germination temperature regime but a greater range of optimal germination temperatures when compared with black box.

Abstract

Eucalyptus coolabah subsp. *coolabah* (coolibah) and *E.largiflorens* (black box) are two dominant floodplain tree species of Australia's Murray–Darling Basin. Over the past 200 years, widespread clearing and altered flood regimes have greatly reduced the abundance of these species in floodplain landscapes. Germination events are rare and establishment requirements for these species are poorly understood. Little is known regarding the life history of these two species, and until recently, mass recruitment events of coolibah were considered to be an invasive response activated by flood events. It is now thought that these rare recruitment events are an evolutionary response, and therefore part of the species' reproductive strategy. We investigated the recruitment of coolibah and black box by conducting a series of germination trials to 1) determine the optimum temperature for germination, and 2) investigate the effect of leaf litter on germination. We found that both species required alternating temperatures for maximum germination, with coolibah exhibiting a wider range of optimal germination temperatures than black box. Leaf litter was found to inhibit germination in both eucalypt species due to the direct effect it has on light availability and temperature conditioning. The long-term survival of floodplain eucalypts depends on a thorough understanding of their life history. This study builds upon basic ecology information for both coolibah and black box and can be used to inform management and conservation directions. Future field studies should be directed at the effects of flooding timing and regulated flow regimes on the microsite conditions and recruitment strategies of floodplain vegetation.

Keywords

Seed germination, coolibah, black box, temperature, leaf litter, flood regimes, floodplain vegetation.

Introduction

Coolibah, *Eucalyptus coolabah* subsp. *coolabah*, and black box, *E. largiflorens*, are two of three dominant floodplain tree species in the Murray-Darling Basin (MDB). These species have distinct distributions that marginally overlap at the northern and southern limits of their range (Figure 1) (Roberts & Marston 2000). It is generally accepted that recruitment events of floodplain eucalypts are driven by natural flood regimes (Jensen et al. 2008; Good et al. 2017). However, germination requirements for coolibah and black box are largely unknown (Roberts & Marston 2000; Good 2012). Agricultural practices such as grazing and cropping, combined with water diversions, have highly modified the landscape with the original Coolibah-Black Box floodplain woodlands reduced by over 60% of their former size (Keith et al. 2009). While clearing has resulted in the reduction of coolibah and black box distribution, river regulation has altered the extent, frequency, timing, and duration of flooding events which may adversely affect the ability of floodplain species to regenerate (Robertson et al. 1999; George et al. 2005; Good 2012).

Water flow from natural flood regimes serves as an important ecological function (Kingsford 2000; George et al. 2005) and is widely recognised as a key driving force in the dynamics of floodplain vegetation due to the critical role it plays in cycling nutrients, renewing water supplies, and assisting germination (Roberts & Marston 2000; Boulton et al. 2014). Extensive changes to the natural water regime may have profound long-term impacts on the plant communities which they support, affecting the integrity and function of floodplain systems (Robertson et al. 1999).

Figure 1. The distribution of coolibah (left) and black box (right). Overlap occurs in Western and north-western New South Wales and southern Queensland (images sourced from AVH 2018).

Coolibah occur on riverbanks and seasonally inundated alluvial plains, and are the dominant tree species of the North-west Floodplain Woodlands, supporting a predominantly grassy understorey with chenopods becoming more abundant as aridity increases (Keith 2004; Good 2012). Although coolibah trees flower annually, in the past 110 years there have been just six widespread regeneration events in the MDB (Good 2012), suggesting germination is both rare and dependent on specific environmental cues (Roberts & Marston 2011). Black box typically occurs higher in the landscape than coolabah where the two species co-occur, on occasionally inundated, heavy alluvial clay floodplains (Roberts & Marston 2000; Keith 2004; Smith 2010). Black box are the dominant species of the Inland Floodplain Woodlands, typically supporting a mixed shrub and grass understorey with chenopods also present (Keith 2004). Black box woodlands are in decline due to a lack of regeneration thought to be caused by a lack of flooding following river regulation (Roberts & Marston 2011). Where they co-occur, coolibah/black box woodlands are listed as an endangered ecological community (NSW Scientific Committee 2011), yet they are one of the most poorly conserved vegetation groups in NSW (Benson 2006). Drivers of the distribution of both eucalypt species are thought to be a combination of positioning in the landscape with respect to species tolerance of flooding events (frequency, intensity and timing), drought, soil chemical and textural requirements, optimal temperatures, and micro-site conditions for recruitment (Roberts & Marston 2011).

Temperature is widely considered as one of the most important environmental variables influencing seed germination (Probert 2000), and has previously been found to dictate the distribution of a number of eucalypt species (Glossop et al. 1982). Germination success is also governed by microsite conditions, where microsite refers to a seed's immediate environment affecting water, light, temperature and humidity levels (Facelli & Ladd 1996). Eucalypt leaf litter alters the physical, chemical and biotic environment and can have a significant effect on seed germination (Facelli & Ladd 1996). In a leaf litter study on eucalypts, Facelli and Ladd (1996) found eucalypt leaf litter enhanced germination rates in two of the four species studied. Understanding the germination requirements of dominant floodplain tree species is important for the management of floodplain ecosystems, especially in heavily modified landscapes where river regulation has altered flood regimes (Kingsford 2000; Roberts & Marston 2011). Optimal temperature requirements and micro-site conditions should therefore be considered when determining recruitment limitations in coolibah and black box.

Vincent, B.E., Smith, R., Good, M., Clarke, P.J., (2018). Seed germination traits of two common floodplain eucalypts: optimum germination temperatures and influence of leaf litter, in ………, Proceedings of the 9th Australian Stream Management Conference. Hobart, Tasmania. Pages XXX-XXX.

We investigated the effect of both temperature and micro-site environment, in the form of leaf litter, on seed germination response in coolibah and black box. We hypothesised that;

1) temperature was the driving force behind the distribution of coolibah and black box, and due to its southwestern distribution, expected black box to have a cooler optimal germination temperature than coolibah, and

2) leaf litter may provide favourable environmental conditions that enhance seedling emergence, and that seeds applied after litter deposition would have less chance of survival than those applied before litter deposition, due to poorer micro-site positioning.

Methodology

Study Area, Seed and Litter Collection:

The coolibah and black box seed used for both the thermogradient plate and the leaf litter experiments was collected from the region bounded by Walgett, Wee Waa, Moree and Collarenebri in the Darling Riverine Plains Bioregion of northern New South Wales, Australia. Leaf litter was collected in the field from coolibah woodland, located on floodplains five kilometres west of Wee Waa, New South Wales. The climate in this region is semi-arid and supports a summer dominant average annual rainfall of 540 mm, peaking in January (Australian Bureau of Meteorology (BOM) 2018). Soils on the floodplains were generally recognised as self-mulching grey vertosols or cracking clays (McKenzie et al. 2004). Collected seed was separated from chaff using an air seed-sorter machine and stored in air tight packages in the dark at room temperature. Large twigs, > 5mm diameter, and grasses were removed from leaf litter which was then air-dried on tarpaulins in full sun following collection to reduce moisture content.

Experiment 1. Optimal Temperature:

Multiple diel (daily) alternating temperature combinations were simultaneously tested on a two-way thermogradient plate to determine optimal germination temperatures for each species (Larsen 1971; Tarasoff et al. 2005). A total of nine day and nine night temperature combinations simulating eighty-one diurnal temperature combinations, ranging from 5ºC to 45ºC, in 5ºC increments, were employed in the study. Eighty-one aluminium trays (50 mm square by 20 mm deep) with convex plastic covers each containing twenty-five seeds sitting on a blotch pad designed for germination experiments and a sponge to retain deionised water, were placed in moist sand on the temperature gradient plate to accurately record the germination response to each range of temperatures (Figure 1). Two fluorescent light bulbs (irradiance approximately 25µmol-2s-1) on a twelve hour timer switch were utilised to imitate sunlight. Thermocouples were inserted into seventeen locations along the gradient plate and temperatures were logged at one-hour intervals. The two-way thermogradient plate experiment ran for fifteen days, during which time both germination events and seeds affected by fungus were recorded and removed from each tray at the same time each day.

Experiment 2. Leaf Litter:

We tested litter presence, litter depth, and whether application of seeds below or above litter had any effect on germination response. Levels of litter were chosen to mimic the levels of litter observed in floodplain woodlands by Good (2012). We used a factorial design with four levels of litter: (1) no litter; (2) medium litter (100 g); (3) high litter (200 g), and (4) artificial litter (shredded waterproof paper, at medium density (25 g), and two levels of seed sowing: (1) after litter application, and (2) before litter application to test for germination response in coolibah and black box seeds. Plastic mesh bottom trays (30 cm × 35 cm × 8 cm) were used to contain the litter and a layer of Kimpak® hydro-crepe™ tissue was laid in the bottom of each tray to retain moisture (Figure 1). A light sterilised sand layer (0.5 mm particle size) was then spread across the tissue surface. Trays were placed in aluminium water beds, so that the water level was high enough to infiltrate each tray, therefore keeping Kimpak® tissues moist, and best imitating post-flood, saturated soil conditions. An artificial litter treatment was applied in order to isolate the physical barrier effect of leaf litter on germination from any biological effect (including possible allelopathy).

Four replicates for each leaf litter level were set up for each treatment of seed application. A control tray with no litter had eight replicates in order to balance out the model. One hundred seeds were counted out and evenly scattered over each tray for each eucalypt species, with the leaf litter glasshouse experiment lasting for seventeen days. Glasshouse temperatures were recorded at an average of between 19°C and 22°C. Seeds in both experiments were considered germinated once the root radicle was >2 mm in length.

Figure 1. Thermogradient plate used to assess optimal temperature (left) and an example of the leaf litter trays used to assess seed application and litter presence and depth (right).

Data Analysis

Optimal Temperature: Using methods developed in previous germination studies, two-way thermogradient plate results were converted into a raster file using the kriging method and presented using ESRI® ArcGIS™ software. Diurnal temperatures were represented on the XY axis and a Z value for each tray was assigned, i.e. the proportion of germinated seeds (Lodge & Whalley 2002; Tarasoff et al. 2005).

Leaf Litter: Following seed viability testing conducted during the two-way thermogradient plate experiment, germination responses to leaf litter were expressed as a proportion of the total number of viable seeds, as each germination event was treated as a test of the binomial function. By finding the viable seed average for the thermogradient trays with the highest germination response (>10 germinates), the binomial n was calculated as $n = 13$ for coolibah, and $n = 14$ for black box. A one-factor generalised linear model (GLM) was used to test for a possible biological effect of leaf litter, relative to artificial litter on seed germination using a binomial error structure, and a Pearson's Chi-squared scale estimate. Two-factor GLM analyses were then used to determine effect size of litter (medium and high) and seed application (before and after) treatments on germination. A binomial error structure and a Pearson's Chi-squared scale estimate were used in our two-factor GLM analysis.

Results

Optimal Temperature: Alternating temperatures were found to enhance germination response for both species. Optimal germination temperature for coolibah (Figure 2) was determined to be 15:30°C night:day respectively, while black box responded to an increased temperature range of 20:35°C, night:day respectively (Figure 2). In both species, reduced germination was observed for seeds subjected to constant temperature regimes (diel fluctuation ≤5 ºC), than for seeds in fluctuating temperature treatments (diel fluctuation ≥10 ºC). However, coolibah appeared to be more tolerant than black box, having higher germination through constant temperatures. A greater optimal temperature range was apparent for coolibah when compared to black box (Figure 2).

Leaf Litter: There was no evidence of a biological effect on seed germination for either of the two *Eucalyptus* species (coolibah [$p > 0.25$], and black box [$p > 0.25$]). *Coolibah:* both seed application and leaf litter cover were found to have a significant effect on seed germination response ($p < 0.001$ and $p < 0.001$ respectively). A significant interaction observed between cover and seed application ($p < 0.05$) indicated that seeds applied after litter application experienced higher germination rates, and germination increased further if seeds were applied following medium litter application (Figure 3). *Black box:* leaf litter cover had a significant effect on seed germination response ($p < 0.001$), however, seed application did not ($p > 0.25$) (Figure 3).

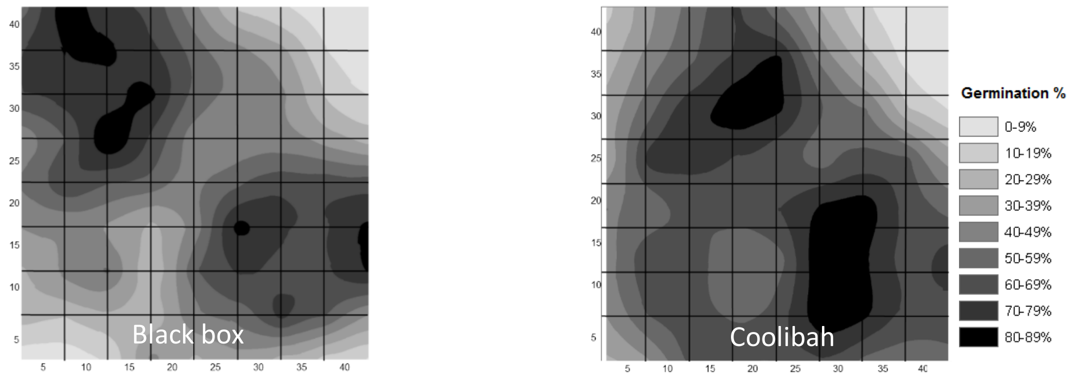

Figure 2. Optimal germination temperatures for black box (left) and coolibah (right) are represented by dark plateaus (>80% germination). Both species required alternating temperatures for optimal germination response, while narrow dark plateaus indicate that black box was more restricted than coolibah with respect to optimal germination temperature range.

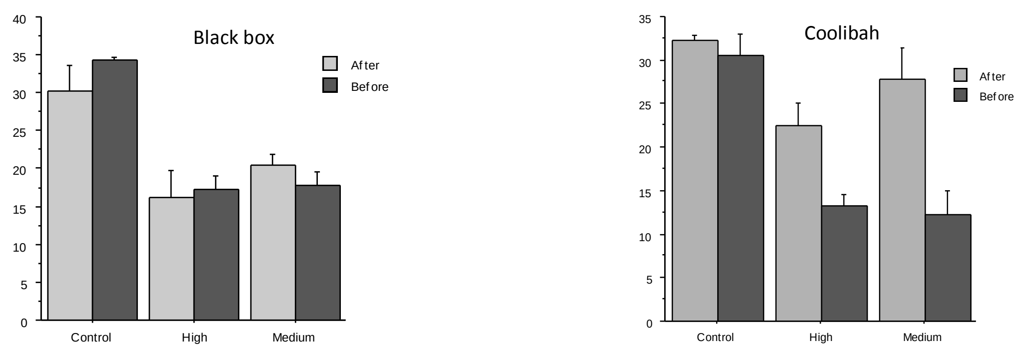

Figure 3. The germination responses of coolibah (left) and black box (right) to seed application treatments (after, before) and leaf litter densities (control, high and medium). Where leaf litter was applied before seed application mean germination rates were significantly different between the control and high leaf litter ($p < 0.001$) and the control and medium leaf litter ($p < 0.001$) for both coolibah and black box. Germination rates between the control and high leaf litter applied after seed application were significantly different for black box ($p < 0.005$).

Discussion

Optimal temperature: Findings from this study indicated that seeds of coolibah and black box require different alternating temperatures at relatively high regimes (≥15 ºC) as an optimal temperature germination requirement. Constant temperatures have reportedly been found to suppress germination in many plant species (e.g. MacDonald 2008), including the genus *Eucalyptus* (Yates et al. 1996). In this study, constant temperatures reduced germination responses in both coolibah and black box which both responded positively to combinations of alternating temperatures, a common trait in floodplain species known as ‘homothermophobia’ (MacDonald 2008).

While eucalypt seeds are known to germinate over a range of temperatures, an optimal germination temperature within this range exists, and germination may be depressed or delayed outside this range (Mott & Groves 1981; Yates et al. 1996). Rates of both seed mortality and dormancy increased either side of the optimal temperature range for germination in both species. Mortality due to high temperatures was likely attributed to fungal infection, the growth of which is promoted by such conditions (Facelli & Ladd 1996), while temperatures below 10ºC appeared to induce dormancy, a trait which was previously observed in black box by Jensen et al. (2008). While neither of these floodplain species are known to have a long-lived soil seed bank, both coolibah and black box reportedly exhibit serotiny (Roberts & Marston 2000; Jensen et al. 2008), therefore temporary dormancy may assist in keeping seeds viable in such conditions.

Temperature has long been regarded as one of the most influential environmental factors determining species distribution (Woodward 1990). Unexpectedly, black box responded to a higher optimal temperature regime than the more northerly distributed species, however, coolibah exhibited a greater range of optimal germination temperatures when compared with the narrower optimal range observed in black box. One potential explanation for the higher optimal temperature range observed in black box may be the slight temperature gradient, c.1ºC annually (BOM 2018), that occurs between the two seed collection sites; coolibah from Wee Waa, and black box from Collarenebri, NSW. This may indicate that optimal temperatures for germination could be a plastic trait that is influenced by local environmental conditions (Woodward 1990). However, additional factors such as seasonality of rainfall should be considered. The greater range of optimal germination temperatures observed in coolibah may directly reflect the greater geographical distribution of this sub-species while the narrow range observed in black box may indicate that geographical distribution for this species is restricted by a narrower set of environmental conditions, as has been observed in other floodplain vegetation (MacDonald 2008).

Leaf litter: The application of leaf litter was found to have a negative effect on the germination response of both coolibah and black box. In addition there was an interaction between method of seed application and level of leaf litter density in coolibah which was not present in black box. Leaf litter has been found to promote a light limiting environment which may inhibit those species which have positive germination responses to light exposure (Grose & Zimmer 1958; Grime 1979; Facelli & Picket 1991), as has been reported in several species of eastern-Australian eucalypts (Bell 1999). Despite this, previous studies have also found that leaf litter can enhance water availability for plants and result in increased seedling establishment in arid and semi-arid systems (Fowler 1985). While we observed leaf litter 'soaking-up' moisture from the wetted substrate, it did not translate to an increase in germination. Both coolibah and black box germinated very poorly when seed was applied beneath leaf litter cover indicating light was a limiting factor. Despite this, for coolibah we observed a negative interaction between leaf litter density and seed application; as leaf litter density increased, germination response decreased. Seeds applied before leaf litter had a lower germination response than seeds applied after leaf litter, and seeds applied after a medium density leaf litter treatment had a significantly higher germination response than seeds applied after a high density leaf litter treatment. While black box also displayed this trend, it was not statistically significant and infers that black box may be more light-demanding than coolibah. The difference in germination response between medium and high density leaf litter treatments in coolibah adds further support to the notion of light and temperature being limiting factors, the availability of which is suppressed by leaf litter (Facelli & Picket 1991).

On the floodplains west of Wee Waa, Good (2012) observed that there was an absence of recruitment within woodlands, indicating that there might be an inhibitory effect of litter on seed germination. The most likely effect of eucalypt litter on conspecific seed germination is in its effect on the physical microclimate of seeds. Despite suggestions that eucalypts might exhibit allelopathy which could inhibit seed germination, our results suggest that this was not the case – artificial litter effects on germination were not significantly different to actual litter. The physical effects of litter on microsite conditions have been well demonstrated (e.g. Facelli & Pickett 1991). Leaf litter not only limits the availability of light, it also reduces the thermal amplitude in the soil reducing maximum soil temperatures and changing the range of temperature alternations experienced, which may inhibit germination in species that display homothermophobia (Grime 1979; Facelli & Picket 1991), such as coolibah and black box. Litter acts as a thermo-regulator by intercepting solar radiation and using it to insulate the soil, thereby reducing the overall range of temperatures experienced (Facelli & Picket 1991). Litter accumulation decreases with increasing rainfall and the removal of litter increases soil temperatures (Facelli & Picket 1991), which creates a vacant space that enhances germination (George et al. 2005). These factors in combination with light and water availability might best explain why the controls in our leaf litter experiment exhibited the highest germination rates for both coolibah and black box.

Conclusions

The long-term survival of floodplain eucalypts depends on a thorough understanding of their life history. This study highlights the influence that temperature and leaf litter has on the seed germination success of both coolibah and black box and builds upon the basic ecology and flooding timing requirements of both floodplain species. These results can inform site management and conservation directions. However, if we are to make well-informed management decisions that favour recruitment in these endangered ecosystems, our recommendation is that future studies be directed at the effects of flood timing and regulated flow regimes on the microsite conditions and recruitment strategies of floodplain vegetation.

References

Australian Bureau of Meteorology (BOM). 2018. Climate data online, http://www.bom.gov.au/climate/data/

AVH. 2018. Australia's Virtual Herbarium, retrieved on the 13th of March, 2018, from http://chah.gov.au/avh/

Benson, J. S., Allen, C. B., Togher, C., and Lemmon, J. 2006. New South Wales vegetation classification and assessment. Part 1 Plant communities of the NSW western plains. Cunninghamia **9**: 383-450

Bell, D. T. 1999. Turner Review No. 1 - The process of germination in Australian species. *Australian Journal of Botany* **47**: 475-517.

Boulton, A.J., Brock, M.A., Robson, B.J., Ryder, D.S., Chambers, J.M., and Davis, J.A. 2014. Australian Freshwater Ecology. Wiley & Sons, New York. ISBN: 978 1 118 56822 4.

Facelli, J. M., and S. T. A. Pickett. 1991. Plant litter - light interception and effects on an old-field plant community. *Ecology* **72**: 1024-1031.

Facelli, J. M., and B. Ladd. 1996. Germination requirements and responses to leaf litter of four species of eucalypt. *Oecologia* **107**: 441-445.

Fowler, N. L. 1985. Microsite requirements for germination and establishment of three grass species. *American Midland Naturalist* **115**: 131-145.

George, A. K., K. F. Walker, and M. M. Lewis. 2005. Population status of eucalypt trees on the River Murray floodplain, South Australia. *River Research and Applications* **21**: 271-282.

Glossop, B.L., Van De Moezel, P.G., and Michaelson, D. 1982. *Seed germination trials on eleven Eucalyptus species*. Environmental Research note No.9, ALCOA of Australia Ltd.

Good, M.K. 2012. *Plant community patterns and population dynamics of coolibah woodlands*. Ph.D. thesis. University of New England.

Good, M., Smith, R. and Pettit, N. (2017). Forests and Woodlands of Australia's Rivers and Floodplains. In D. A. Keith, ed., *Australian Vegetation*, 3rd edn. Cambridge: Cambridge University Press, pp. 516-43.

Grime, J. P. 1979. Plant strategies and vegetation processes. John Wiley & Son, New York.

Grose, R.J., and Zimmer, W.J. 1958. Some laboratory germination responses of the seeds of river red gum Eucalyptus camaldulensis Dehn., syn. Eucalyptus rostrata Schlecht. Australian Journal of Botany **6**: 129–158.

Jensen, A. E., K. F. Walker, and D. C. Paton. 2008. The role of seedbanks in restoration of floodplain woodlands. *River Research and Applications* **24**: 632-649.

Keith, D.A. 2004. *Ocean shores to desert dunes: The native vegetation of New South Wales and the ACT*. Department of Environment and Conservation, Hurstville, New South Wales.

Keith, D.A., Orscheg, C. Simpson, C.C., Clarke, P.J., Hughes, L., Kennelly, S.J., Major, R.E., Soderquist, T.R., Wilson, A.L. and Bedward, M. 2009. A new approach and case study for estimating extent and rates of habitat loss for ecological communities. *Biological Conservation* **142**: 1469-1479.

Kingsford, R.T. 2000. Ecological impacts of dams, water diversions and river management on floodplain wetlands in Australia. *Austral Ecology* **25**: 109-127.

Larsen, A.L. 1971. *The thermogradient plate for seed germination research: Construction plans and procedures*. Washington, DC: US Government Printing Office. US Department of Agriculture, ARS 51-41.

Lodge, G. M., and R. D. B. Whalley. 2002. Fate of annual pasture legumes seeds on a two-way thermogradient plate. Rangeland Journal **24**: 227-241.

MacDonald, M.J. 2008. *Ecology of Phyla canescens (Verbenaceae) in Australia*. Ph.D. thesis, University of New England.

McKenzie N., Jacquier D., Isbell R., and Brown K. 2004. *Australian soils and landscapes: An illustrated compendium*. CSIRO publishing, Collingwood.

Mott, J.J., and Groves, R.H. 1981. Germination strategies. Pages 307-341, In. Pate, J.S., and A. J. McComb (eds.), *Biology of Australian plants*. University of Western Australia Press, Nedlands.

New South Wales (NSW) Scientific Committee, 2011. *Coolibah – Black Box Woodland in the Darling Riverine Plains, Brigalow Belt South, Cobar Peneplain and Mulga Lands Bioregions – endangered ecological community listing - final determination*. NSW Scientific Committee, Sydney. Accessed 10 March 2018. Available online at: http://www.environment.nsw.gov.au/determinations/coolibahblackboxdrpbbscpmlbiofd.htm

Probert, R. J. 2000. The role of temperature in the regulation of seed dormancy and germination. Seeds: the ecology of regeneration in plant communities: 261-292.

Roberts, J., and Marston, F. 2000. *Water regime of wetland and floodplain plants in the Murray-Darling Basin: A source book of ecological knowledge*. CSIRO Land and Water. Technical Report 30/00. October 2000. Canberra.

Roberts J., and Marston, F. 2011. Water regime for wetland and floodplain plants: a source book for the Murray–Darling Basin, National Water Commission, Canberra.

Robertson, A. I., S. E. Bunn, P. I. Boon, and K. F. Walker. 1999. Sources, sinks and transformations of organic carbon in Australian floodplain rivers. *Marine and Freshwater Research* **50**: 813-829.

Smith, R. 2010. Chpt 2: What Drives Landscape Scale Vegetation Patterns on the Fertile Semi-arid Floodplains of Northern NSW? In *Biodiversity and Ecosystem Services Associated with Remnant Native Vegetation in an Agricultural Floodplain Landscape*. Ph.D. thesis, University of New England.

Tarasoff, C.S., Louhaichi, M., Mallory-Smith, C., and Ball, A. 2005.Using Geographical Information Systems to Present Nongeographical Data: An Example Using 2-Way Thermogradient Plate Data. *Rangeland Ecology and Management*, **58**: 215-218.

Woodward, F. I. 1990. The impact of low-temperatures in controlling the geographical-distribution of plants. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **326**: 585-593.

Yates, C. J., R. J. Hobbs, and R. W. Bell. 1996. Factors limiting the recruitment of *Eucalyptus salmonophloia* in remnant woodlands .3. Conditions necessary for seed germination. *Australian Journal of Botany* **44**: 283-296.