Cotton Catchment Communities CRC

SUMMER SCHOLARSHIP
Final Report

Part 1 - Summary Details

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1. Project Title: Coolibah (*Eucalyptus coolabah*) recruitment after flooding and implications for environmental water management

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Part 3 – Scholarship Report

The points below are to be used as a guideline when completing your final report.

1. Background:

Coolibah, *Eucalyptus coolabah* subsp. *coolabah*, and black box, *E. largiflorens*, are two of three dominant floodplain tree species in the Murray-Darling Basin (Roberts & Martson2000). While the third dominant species, river red gum, *E. camaldulensis* is widespread, found along river banks and surrounding floodplains throughout the Murray Darling Basin (Stefano 2001), coolibah and black box have distinct distributions, marginally overlapping at the northern and southern limits of their range (Figure 1) (Roberts& Marston 2000; AVH 2010). It is generally accepted that recruitment events of floodplain eucalypts are driven by natural flood regimes (Jensen *et al.* 2008; Good 2012), however germination requirements for coolibah and black box are largely unknown (Roberts & Marston 2000; Good 2012).

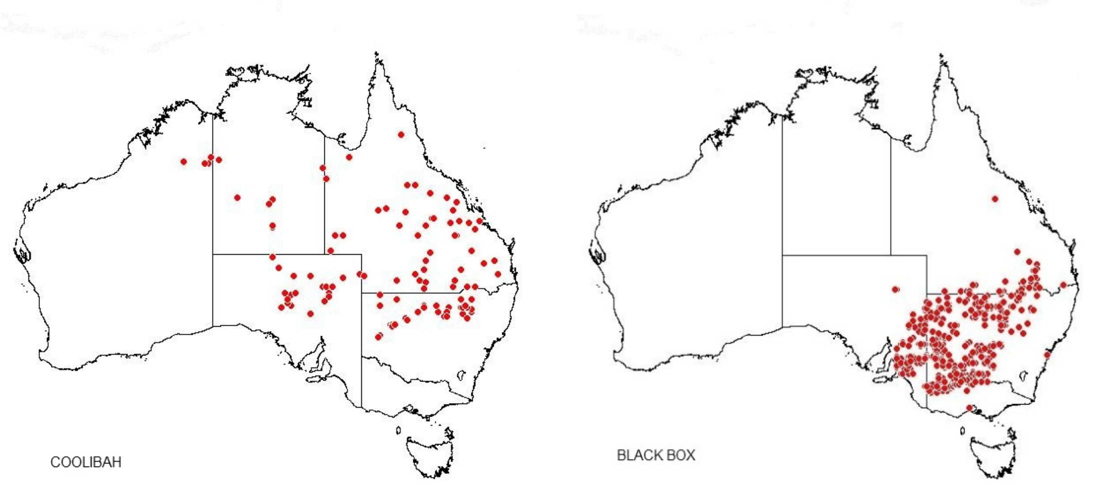

Figure 1. The distribution of coolibah and black box. Overlap occurs in Western and North-western New South Wales (sourced from AVH 2012).

The Murray-Darling Basin covers an area which equates to approximately 14% of Australia's total landmass (Arthington *et al.* 2003; George *et al.*2005) and supports the fertile floodplains on which coolibah and black box are found (Roberts & Marston 2000). In Australia, the past 200 years of intensive clearing of woodlands has been compounded by river regulation and has had profound effects on the functioning and productivity of natural ecosystems (Benson 1991; Robertson *et al.* 1999; Stefano 2001; George *et al.* 2005). Agricultural practices such as grazing and cropping, combined with water diversions, have highly modified the landscape with the original floodplain woodlands of the Murray-Darling Basin reduced by up to 60% of their former size (Benson 1991). While clearing has resulted in the contraction 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; Zubrinich *et al.* 2000; Stefano 2001; George *et al.* 2005; Jensen *et al.* 2008; Good 2012).

Floodplains hold a clear agricultural importance (Benson 1991; Robertson *et al.* 1999; Tockner & Stanford 2002), however they have also been recognised as being amongst the most biologically diverse ecosystems in the world (Kingsford 2000; Tockner & Stanford 2002) owing their high productivity to the continual accruement of nutrient-rich sediments from the higher reaches of their catchments (Tockner & Stanford 2002). Floodplain ecosystems, such as those within the Murray-Darling Basin, boast rich alluvial soils and provide habitat and resources for a plethora of plants and animals (Kingsford 2000; Tockner & Stanford 2002).

Water flow from natural flood regimes serves as an important ecological function (Kingsford 2000; Gerge l*et al.* 2002; Leyer 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 (Boulton & Brock 1999; Roberts & Marston 2000; Keith 2004). 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; Leyer 2005; Sheil *et al.* 2006). Tolerances and life-cycle requirements of plant species determine where they occur along a gradient of flood frequency and inundation duration, thus understanding the germination requirements of dominant floodplain tree species is important for the management of floodplain ecosystems (Kingsford 2000).

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 105 years there have been just six major regeneration events in the Murray-

Darling Basin, suggesting germination is both rare and dependent upon specific environmental cues (Roberts & Marston 2011). The widespread flooding in 2010-2012 in the semi-arid regions of NSW has prompted germination of coolibah at several locations in northern NSW (Pers. Comm. Meg Coleman, Park ranger, Culgoa National Park). Black box typically occurs higher in the landscape than coolibah, on occasionally inundated, heavy alluvial clay floodplains (Roberts & Marston 2000; Keith 2004; Smith 2010). They are the dominant species of the Inland Floodplain Woodlands, typically supporting a shrubby grassy 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 due to an altered water regime (Roberts & Marston 2011). What determines the distribution of these species is thought to be a combination positioning in the landscape with respect to species tolerance of flooding events, frequency and intensity, drought, soil property requirements, optimal temperatures, and micro-site conditions for recruitment (Roberts & Marston 2000; Roberts & Marston 2011).

Where they co-occur, coolibah/black box woodlands are listed as an endangered ecological community (NSW Scientific Committee 2009), yet they are one of the most poorly conserved vegetation groups in NSW (Benson 1991). However, because of their episodic, dense recruitment strategies, both coolibah and black box have also been listed as 'Invasive Native Scrub' in New South Wales, allowing clearing of both species where an improvement in biodiversity values can be demonstrated (http://www.environment.nsw.gov.au/resources/vegetation/INSdiscussionpaper.pdf , accessed 14th April 2012). Recent studies by Good (2012) suggests that dense recruitment of coolibah did not have an effect on biodiversity values and attributed episodic dense recruitment to the species evolutionary strategies, proposing that coolibah naturally thins-out as the stand ages.

The regeneration niche of *eucalyptus* is species specific and dependent upon suitable environmental conditions such as the interaction of light and temperature to induce germination (Bell & Williams 1997). Optimal temperature requirements and micro-site conditions should therefore be considered when determining recruitment limitation in coolibah and black box. Temperature is widely considered as one of the most important environmental variables influencing seed germination (Mott and Groves 1981; Probert 2000), and has previously been found to dictate the distribution of certain eucalypt species. (Glossop *et al.* 1982; Yates *et al.* 1995). Temperatures outside optimum germination requirements may induce in dormancy (Yates *et al.* 1995), and when combined with altered flooding regimes may have negative consequences for successful germination events, given that both coolibah and black box do not form a soil seed bank (Jensen *et al.* 2008; Roberts & Marston 2011).

Germination success is also governed by microsite conditions. 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 being studied. Good (2012) observed little or no recruitment beneath remnant stands of coolibah, and they proposed that leaf litter deposition may inhibit seed germination response in coolibah, and possibly other floodplain eucalypts given their similarity in recruitment patterns (Roberts & Marston 2000).

2. Aims and Objectives:

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). Further research into germination requirements such as optimal temperatures and micro-site conditions, may provide insight on what limits the distribution and abundance of coolibah and black box. We therefore 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.

1. We hypothesised that temperature was the driving force behind the distribution of coolibah and black box. Due to its South-western distribution, we expected black box to have a cooler optimal germination temperature than coolibah which is predominantly distributed in higher latitudes.

2. We expected leaf litter to provide favourable environmental conditions that enhance seedling emergence. We hypothesised that seeds applied after litter deposition would have less chance of survival than those applied before litter deposition as they would be offered less security in the form of micro-sites than seed applied before leaf litter.

To test these hypotheses two glasshouse experiments were conducted, a two-way thermogradient plate experiment to establish the optimal germination temperature of the two species (Tarasoff *et al.* 2005), and a leaf litter experiment, where we tested litter presence, litter depth, and whether application of seeds below or above litter had any effect on germination response.

3. Methodology:

Study Area, *Seed and Litter Collection*

The seed used for both the thermogradient plate and the leaf litter experiments was collected from the region bounded by Walgett, Wee Waa and Moree and Collarenebri in the Darling Riverine Plains Bioregion of New South Wales, Australia (148°80'E, 29°70'S) (Good 2012). The climate in this region is semi-arid with an average annual rainfall of 500 mm (Australian Bureau of Meteorology 2009) and a slight rainfall peak in January. Soils are self-mulching grey vertosols or cracking clays (McKenzie *et al.* 2004).

Coolibah seed was collected and donated by the property owners of Bilary, Wee Waa, New South Wales. Black box seed was collected from Collarenebri, located 140kms North-west of Wee Waa New South Wales. Leaf litter was collected in the field from coolibah woodland, located on floodplains five kilometres west of Wee Waa, New South Wales.

Collected seed was stored in air tight packages and kept in the dark and at room temperature. A Threshing Machine manufactured by the Science and Engineering Workshop at the University of New England, was used to separate the chaff from the seed, the separated seed was then counted out and placed into envelopes. Leaf litter was air-dried on tarpaulins in full sun following collection, to reduce any moisture content which might have been present in the litter. Large twigs, > 5mm diameter, and grasses were removed, leaving predominantly coolibah leaf litter which was then kept inside the glass house under tarpaulin. Glass house temperatures were recorded at an average of between 19^{o}C and 22^{o}C.

Experiment 1: Temperature Gradient Measurements

Multiple diel (daily) alternating temperature combinations can be simultaneously tested on a two-way thermogradient plate (Larsen 1971) to test for optimal germination temperatures. A two-way thermogradient plate built by the University of

New England was used to study germination response to a range of different temperatures (Figure 2). A total of nine day and nine night temperature combinations were employed in the study.

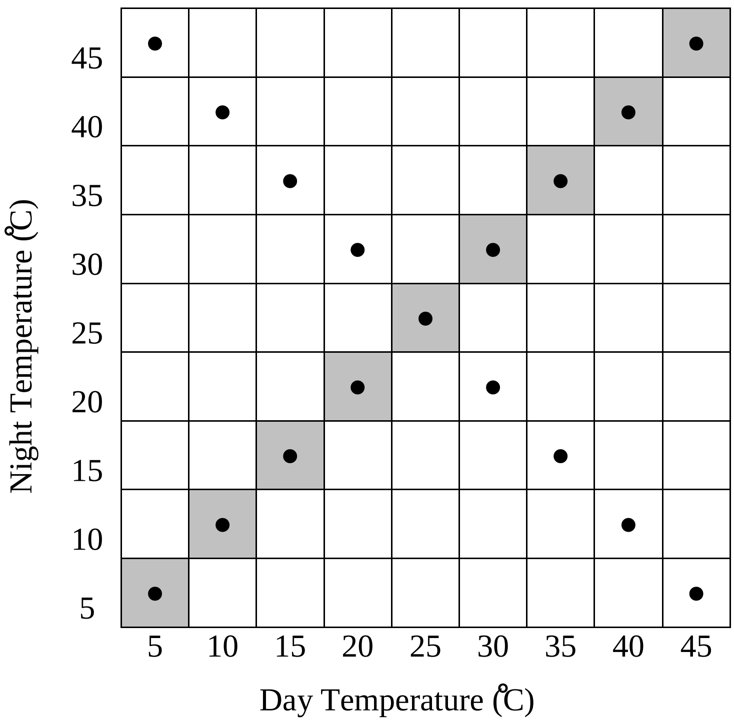

Figure 2. Temperature combinations used in the two-way thermogradient experiment.

The two-way thermogradient plate simulated eighty-one diurnal temperature combinations which ranged from 5ºC to 45ºC, in 5ºC increments. Eighty-one aluminium trays (50mm square by 20mm deep) with convex plastic covers were utilised across the plate to accurately record the germination response to each range of temperatures. Within each tray, twenty-five seeds were placed on top of blotch pads designed for germination experiments. Beneath each blotch pad a sponge was placed to retain moisture, and best simulate requirements for seed germination. Two fluorescent light bulbs (irradiance approximately 25μmol^{-2}s^{-1}) were set up on a twelve hour timer switch to imitate sunlight. Thermocouples were inserted into seventeen locations along the gradient and temperatures 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. To maintain moisture content deionised water was added every four days. Upon the conclusion of the fifteen day experiment the remainder of the seed was tested for viability by putting each of the eighty-one blotch pads into separate Petri-dishes and placing them in an incubator set at the optimal temperature for germination.

Viable seeds were determined by either those seeds which germinated in the growth cabinet or by testing remaining seeds under microscope for dormancy. Dormant seeds were determined as those seeds which were firm and did not exude coloured ooze that is associated with infected seeds (Wal Whalley, Pers. Comm. Botany Dept. University of New England).

Experiment 2: Leaf Litter Germination Experiment

We used a factorial design with four levels of litter: no litter, medium litter, high litter, and artificial litter, and two levels of seed sewing: after litter application, and before litter application. The levels of litter chosen mimic the levels of litter observed in floodplain woodlands by Good (2012). We used this design to test for germination response in coolibah and black box seeds.

Leaf litter was mixed by hand and weighed out using scales to satisfy our two treatments, 1) medium litter density, 100gms, and 2) high litter density, 200gms. Dimensions of the plastic trays used in our leaf litter glasshouse experiment to contain each replicate, were 30cm x 35cm x 8cm. A layer of Kimpak® hydro-crepe™ tissue was laid out at the bottom of each tray to retain moisture content and best simulate lying floodwater conditions. A light sand layer (0.5mm) was then spread across the tissue surface, using sand sterilised in an autoclave. 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 floodplain conditions.

In addition to the two levels of litter (Medium and High), artificial litter was included to detect presence/absence of any alleopathic response by the seedlings to coolibah leaf litter. Waterproof paper was shredded to mimic litter and applied at medium density (25gms) using image analysis software, *ASSESS* © (*Image Analysis Software for Plant Disease Quantification*. Seeds were applied to each leaf litter treatment either before (B), or after (A) litter application. 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 our model. One hundred seeds were counted out and scattered over each tray using a container with small holes in the lid and Pleistocene sand to ensure an even distribution of seeds across the leaf litter tray. The leaf litter glasshouse experiment was run once for each of our two eucalypt species for a period of seventeen days.

In both experiments, seeds were considered germinated once the root radicle was >2mm in length. In experiment 1) germinants were recorded and discarded on a daily basis, while in experiment 2) seedlings were left in place and marked with a coloured pins.

GIS Data Presentation of Two-Way Thermogradient Plate Results

Following the work of previous optimal germination temperature researchers (Lodge &Whalley 2002; Tarasoff *et al.* 2005), two-way thermogradient plate results were presented using ESRI® ArcGIS™ software, a method which allows users easier presentation of results by summarising and converting tabulated data into a visual interpretation. *XY* axis represented diurnal temperatures, while a *Z* value allowed us to assign each tray a single number; the proportion of germinated seeds. Data collected was placed into three columns, 1) germination percentage, 2) X values, and 3) Y values. X and Y values served as grid coordinates and essentially represented temperature values (Tarasoff *et al.* 2005). The data was then interpolated into a raster file. Kriging was employed as the most suitable mathematical formula to interpolate our germination data because it, 1) generates values that do not exceed the highest and lowest values, 2) is good for sample points which are close together but which have extreme differences in values, and 3) it is well suited for dense, evenly spaced sample point sets (Johnson *et al* 2001).

Leaf Litter Data Analysis

When determining germination response we assessed the number of viable seeds for both species while conducting the two-way thermogradient plate experiment. Our leaf litter germination response could then be expressed as a proportion of the total number of viable seeds, as we treated each germination event as a test of the binomial function. Therefore the total number of seeds was represented as the binomial *n*. The binomial *n* was determined by finding the viable seed average for the thermogradient trays with the highest germination response (>10 germinates). Out of 25 seeds our bi-nomial n was calculated as n = 13 for coolibah, and n = 14 for black box.

A one-factor general linear model (GLIM) was used to test for an alleopathic effect of leaf litter on seed germination. This was performed by comparing both medium and artificial leaf litter applications using a binomial error structure, and a Pearsons *Chi*-squared scale estimate.

A two-factor generalised linear model (GLIM) analysis was then used to determine the effect size of the different litter applications (medium and high) and whether different seed sewing arrangements (before and after) had any significant effect on germination. Germination was our response variable while litter cover and seed application were treated as fixed factors. A binomial error structure and a Pearsons *Chi*-squred scale estimate were used in our two-factor GLIM analysis.

4. Results:

Thermogradient plate:

Germination responses for both eucalypt species slowed dramatically after two weeks on the two-way thermogradient plate. Optimal temperature for coolibah (Figure 3.) was determined to be 15:30^{o}C day:night respectively, while black box responded to an optimal temperature of 20:35^{o}C, day:night respectively (Figure 4.).

Reduced germination was observed for seeds subjected to constant (diel fluctuation $\leq 5^{o}$C) temperature regimes for both species, than for seeds from fluctuating temperature treatments (diel fluctuation >10^{o}C) however coolibah appeared to be more tolerable than black box, having higher germination through constant temperatures. A greater optimal temperature range was apparent for coolibah when compared to black box which is indicated by darker broader plateaus (Figures 3 & 4). Alternating temperatures were found to enhance germination response for both species.

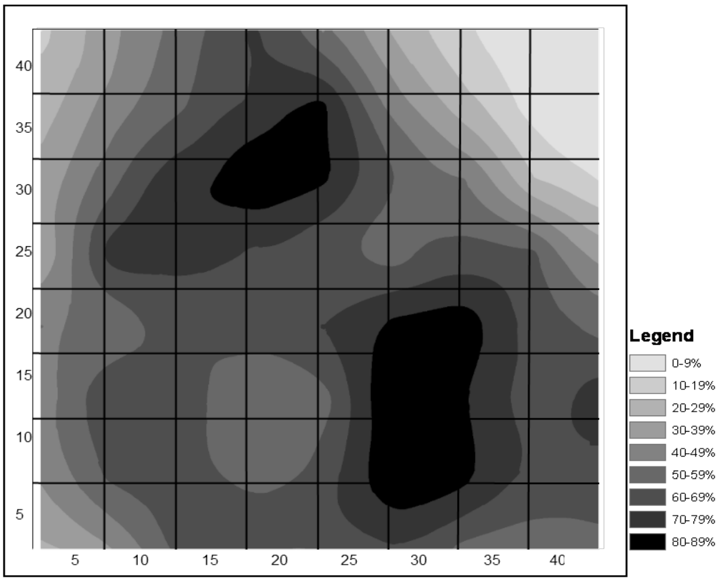

Figure 3. Coolibah was found to have low germination response at a constant temperature, requiring alternate temperatures for optimal germination response. The presence of two broad plateaus suggests that coolibah has a greater optimal germination temperature range than black box.

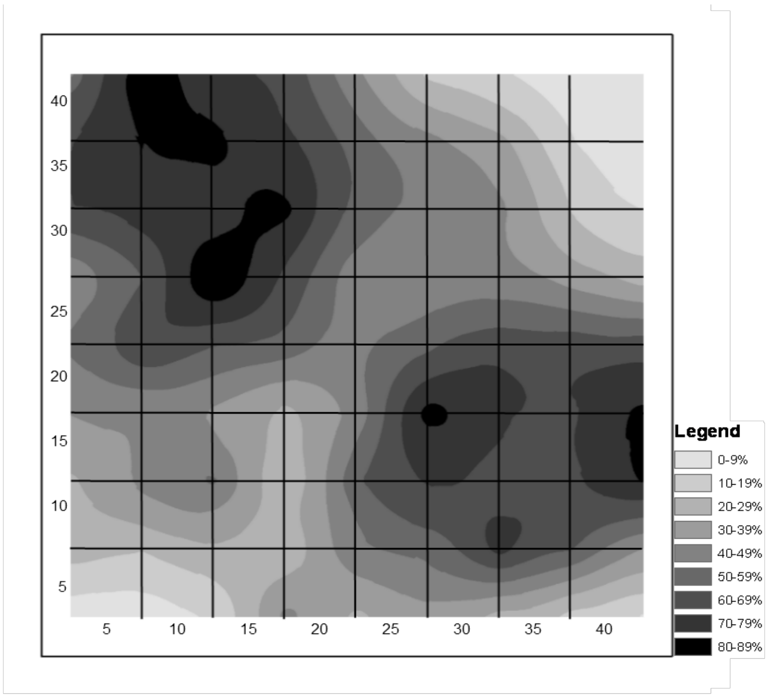

Figure 4. Black box was found to have low germination response at a constant temperature, requiring alternate temperatures for optimal germination response. Narrow plateaus suggest that optimal germination temperatures are more restricted than coolibah.

Leaf Litter:

There was no evidence of an alleopathic effect on germination for either of the two eucalyptus species. When tested alongside medium leaf litter density, artificial litter was found not to have an effect on seed germination of coolibah ($F_{1,12}$ = 0.85, P>0.25), and black box ($F_{1,12}$ = 1.7, P>0.25). Overall germination rates in artificial leaf litter trays were not found to be significantly different from medium leaf litter trays, suggesting no alleopathic response, and therefore justifying the removal of artificial leaf litter trays from the data analysis

Coolibah–

For coolibah the effect of seed application on germination was found to be significant ($F_{1,18}$ = 18.8, P<0.001). Cover was also found to be significant ($F_{2,18}$ = 15.9, P<0.001), as was the interaction between cover and seed application ($F_{2,18}$ = 3.9, P<0.05). A lower germination rate resulted from high litter application than the medium litter treatment. Seeds applied after litter application were more likely to germinate and even higher still was the likelihood of germination if seeds were applied after to a medium litter treatment given the significant interaction (figure 5.).

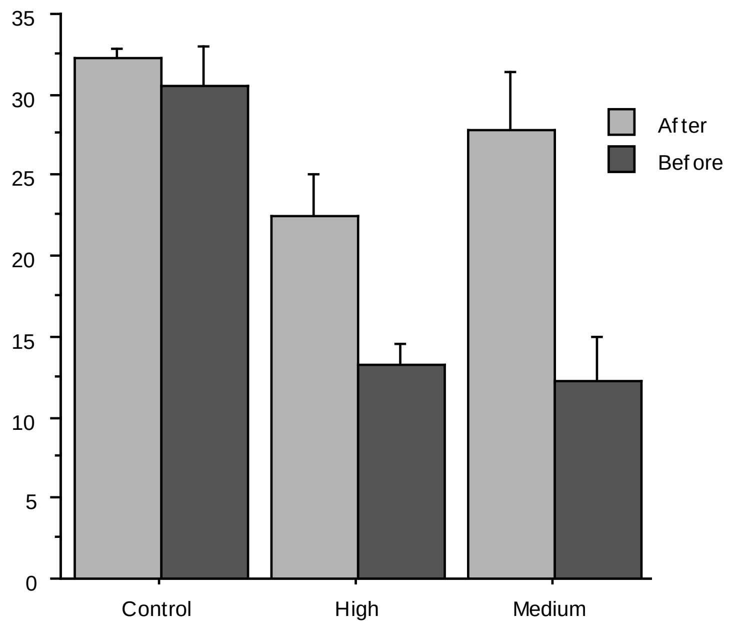

Figure 5. The response of coolibah to different levels of leaf litter applications, and seed sewing treatments. Outside the control, i.e. bear ground, the germination rates were found to increase with a combination of medium

density leaf litter treatment and seed application after litter fall, than any other combination of treatments.

Black Box –

For black box, type of seed application was found not to have a significant effect on seed germination ($F_{1,18}$ = 0.16, P>0.25). Level of leaf litter cover did have a significant effect on germination response ($F_{2,18}$ = 1.02, P<0.001). The application of leaf litter regardless of its density was found to reduce germination response when compared to the control, in black box (Figure 6.).

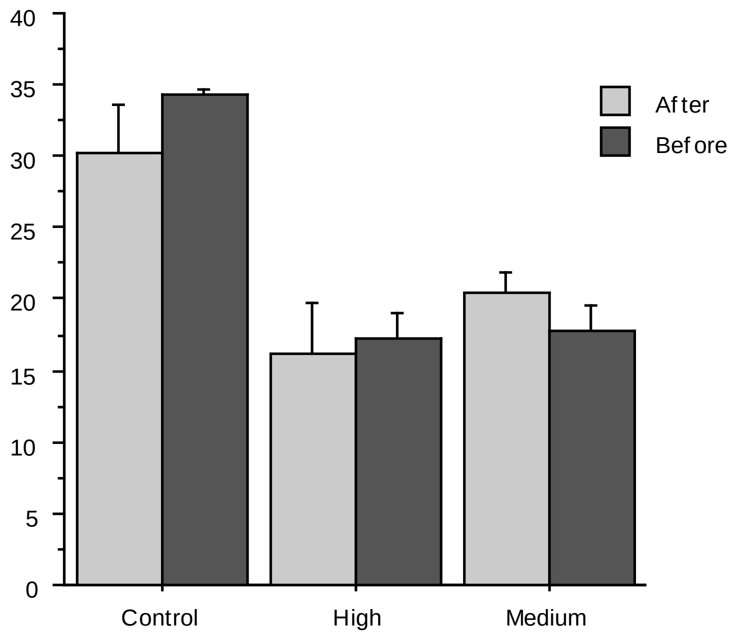

Figure 6. The response of black box to different levels of leaf litter applications, and seed sewing treatments. Leaf litter was found to negatively affect germination response in black box.

5. Conclusion:

This study establishes that seeds of coolibah and black box require alternating temperatures at relatively high regimes as an optimal temperature germination requirement.

While eucalypt seeds germinate over a range of temperatures an optimal germination temperature within this range exists, with temperatures outside this range either depressing or delaying germination (Mott & Groves 1981; Yates *et al.* 1996). Observations made in this study on the optimal germination response for coolibah and black box highlight important characteristics of the germination physiology of each of the two species.

As expected coolibah and black box were found to have differing optimal temperatures for germination, however black box responded to higher optimal temperatures than coolibah at 20:35^{o}C, and 15:30^{o}C respectively. Although displaying a lower optimal germination temperature, coolibah had a higher range of optimal germination temperatures when compared with black box, which displayed a narrower optimal temperature range (Figures 5 & 6). Constant temperatures were found to reduce germination response with both eucalypt species exhibiting the same requirement for a 15^{o}C temperature alteration from within their optimal range. As temperatures progressed to the outer edges of the two-way thermogradient plate margins (<10^{o}C, and >40^{o}C), mortality and dormancy were promoted in both coolibah and black box seeds.

With temperature having long been regarded as one of the environmental factors determining species distribution (Woodward 1990), our expectation was that due to its predominant southerly distribution, black box would have a lower optimal germination temperature than coolibah, however this was found not to be the case. The difference between the two species can likely be attributed to the presence of a slight temperature gradient, circa 1^{o}C annually (ABM 2012), between the two collection sites, coolibah from Wee Waa, and black box from Collarenebri, NSW, and may be further evidence that optimal temperatures for germination can be determined by local environmental conditions (Woodward 1990), however, factors such as seasonality of rainfall should be considered.

No specific optimal temperature data could be found in the literature for black box however, Doran and Boland (1984) reported that coolibah germinated optimally at 35^{o}C. Our study found coolibah exhibited a larger range of optimal germination temperatures than black box which may reflect the greater distribution of the sub-species coolibah and suggests environmental conditions are of higher importance than temperature alone, a trend which has been reported in other eucalypt species (Woodward 1990). Our findings indicate that the distribution of black box may therefore be restricted due to a narrower set of environmental conditions as has been observed in other floodplain vegetation (Macdonald 2008), with germination predominantly cued by temperature.

Constant temperatures have reportedly been found to suppress germination in many plant species (Macdonald 2008), including the genus *Eucalyptus* (Yates *et al.* 1996). In our study constant temperatures reduced the germination response in both coolibah and black box with both species responding positively to combinations of alternating temperatures. The requirement for alternating temperatures in promoting germination has been termed ‘homothermophobia’, appears to be a common trait in floodplain species (Macdonald 2008), and has been considered by some authors as a normal environmental factor necessary for germination (Rice 1985; Baskin &Baskin 2004).

Rates for seed mortality and dormancy increased either side of the optimal temperature range for germination. 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 10^{o}C typically induced dormancy. Bellairs and Bell (1990) reported similar patterns in Western Australian plant communities, while Wellington (1981) found that seeds of *Eucalyptus incrassata* became temporarily dormant at temperatures above the optimal germination range. Jensen *et al.* (2008) also observed dormancy in black box seeds but suggested the dormancy period was short and was not beneficial to the soil seed bank. Although dormancy was recorded in both species the length of dormancy was not established as it was outside the scope of this study, however as both coolibah and black box reportedly hold canopy stored seed bank (Roberts & Marston 2000; Jensen *et al.* 2008), temporary dormancy may assist in keeping seeds viable in such conditions.

Leaf Litter

We found that leaf litter had a negative effect on the germination response of coolibah and black box. An interaction existed between leaf litter density and seed application in coolibah, and promotes observations made by Glossop *et al.* (1982) on the importance of both light and temperature in the germination requirements of eucalyptus.

Fowler (1985) reported that leaf litter enhances water availability for plants and results in increased establishment in arid and semi arid systems. While we observed the ability of leaf litter to act as a sponge, ‘soaking-up’ moisture from the wetted substrate the overall effect of litter on coolibah and black box seed germination was negative despite our expectations. 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. Allelopathic effects of the eucalypt litter were tested for and found not to attribute to the negative germination response of coolibah and black box. When studying the effects of leaf litter on germination response of four species of eucalypt, Facelli and Picket (1991) reported that micro-habitat conditions of the top soil are altered by the presence of leaf litter, which effects the light environment, soil temperature, water dynamics, and acts as a mechanical barrier impeding soil substrate contact. The reduction in germination success of both coolibah and black box in response to leaf litter application can likely be attributed to a combination of these factors.

On the floodplains west of Wee Waa, Good (2012) observed that remnant stands of coolibah suppressed germination. Leaf litter promotes a light limiting environment and may inhibit those species which have positive germination responses to light exposure (Grose& Zimmer 1957; Grime, 1979; Sydes & Grime 1981; Vazquez-Yanes *et al*. 1990; Facelli & Picket 1996),as has been reported in several species of eastern-Australian eucalypts (Bell 1999). Both coolibah and black box germinated very poorly when seed was applied beneath leaf litter cover indicating light was a limiting factor. Coolibah displayed a higher germination response when seeds were applied after rather than before leaf litter treatments, subjecting seeds to increased light availability. While black box also displayed this trend, it was not statistically significant and infers that black box may be more light-demanding than coolibah.

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; Thompson *et al*. 1977; Fenner 1985; Facelli & Picket 1996) 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 (McKinney 1929; Facelli & Picket 1996). The accumulation of litter decreases with increasing rainfall (Mentmeyer *et al.* 1982; Facelli & Picket 1996) and the removal of litter increases soil temperatures (Hulbert 1969; Facelli & Picket 1996) which creates a vacant space that enhances germination (Smith *et al* 1997; George *et al.* 2004). 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.

In coolibah we observed an interaction between leaf litter density and seed application, where as leaf litter density was 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. 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). Our findings suggest that medium litter density affords greater light gaps and that as litter density increases temperature regulation increases, reducing the temperature alterations required for optimal germination.

6. Highlights:

Light and temperature requirements and the interaction of the two assist in piecing together the life-history of coolibah and black box and may be useful in the future management and conservation of the two species. Our study indicates that a combination of factors explain the limitations and absence of recruitment of coolibah and black box throughout their range in the Murray-Darling Basin. Bell *et al.* (1993)illustrated that germination success is directly correlated to those environmental conditions which support maximal survival, which for coolibah and black box, is greatest following natural flooding events during warmer months (Roberts & Marston 2000; Jensen *et al.* 2008; Good 2012). Altered flow regimes in the Murray-Darling Basin has reduced the frequency, extent and altered the timing of floods, so that germination is followed by unsuitable conditions for establishment success (Jensen *et al.* 2008). Our findings support the model that natural flooding regimes assist in the removal of leaf litter and occur during the warmer months which coincide with peak seed release and optimal germination temperatures. Subsequently seeds are exposed to high light, sufficient alternating temperatures, and a wetted soil substrate, all of which are required for promoting optimal germination in coolibah and black box. The long-term survival of floodplain eucalypts depends upon a thorough understanding of their life history, however if we are to make well informed management decisions it is our recommendation that future studies be directed at the current regulated flow regimes and their effects on recruitment strategies of floodplain vegetation.

7. Presentations and public relations:

N/A

8. References:

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9. Executive Summary:
(provide a one paragraph summary of the Summer Scholarship, suitable for posting on the Cotton CRC web site)

Eucalyptus coolabah subsp. *coolabah* (coolibah) and *E.largiflorens* (black box) are two dominant floodplain species of Australia’s Murray-DarlingBasin. Over the past 200 years widespread clearing and altered flood regimes have greatly restricted the distribution of both species, and recruitment events are rare and poorly understood. Little is known regarding the life history of each of the two species, for instance, until recently mass recruitment events of coolibah were considered to be an invasive response activated by flooding events, however, it is now thought that these rare recruitment events are part of the species natural reproductive strategy. To further our knowledge and fill the existing gaps regarding the recruitment of coolibah and black box, we conducted a series of germination trials to 1) determine the optimal temperature for germination, and 2) establish the effect of leaf litter on germination. We found that both species required alternating temperatures for optimal germination, with coolibah exhibiting a wider range of optimal germination temperatures than black box. Leaf litter was found to inhibit germination in both eucalyptspecies due to the direct effects it has on light availability and temperature conditioning. The long-

term survival of floodplain eucalypts depends upon a thorough understanding of their life history. The results from this study can be used to assist in making well informed management and conservation decisions.