



Seedling Tolerance of Three Eucalyptus Species to a Short-term Flooding Event: Tolerance and Physiological Response

E. Farifr¹ and S. Aboglila^{1*}

¹Department of Geochemistry and Environmental Chemistry, Azzaytuna University, Tripoli, Libya.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Research was conducted at Curtin University (Western Australia) to assess the seedling tolerance of three species *Eucalyptus gomphocephala* DC (Myrtaceae) (common name 'Tuart'), *Eucalyptus marginata* Sm. (common name 'Jarrah') and *Corymbia calophylla* (Lindl.) K.D. Hill & J. A. S. Johnson (common name 'Marri') to soil-induced stressor, namely water logging (flooding). Flooding treatment was achieved by filling the tubs with water, approximately 1 cm above the soil surface and control treatment had the same method except with drainage holes. Study assessment was attained by statistical change in seedling growth, leaf allocation and leaf physiology after 70 days of seed germination. Tolerance was assessed by measuring changes in seedling growth, leaf allocation and leaf physiology after 70-80 days. *C. calophylla* was the most tolerant to prolonged waterlogging (80% survival, no difference in transpiration rates); *E. marginata* was the least tolerant (10% survival, 95% decrease in transpiration rate). *E. marginata* was the least tolerant to the three soil stresses. *E. marginata* prefers habitats that are not excessively wet on well-drained soils. *C. calophylla* was the most tolerant, occurring and tolerating wet, well-drained soils and thus demonstrated better tolerance to prolong waterlogging. Knowing the seedling growth and

*Corresponding author: E-mail: salem.aboglila@gmail.com;

physiological responses of three prominent studied eucalypts to soil-induced stresses provides us with invaluable knowledge for rehabilitating and restoring urban bush land.

Keywords: *Eucalypt flood tolerance; eucalypt physiological response; eucalypt growth.*

1. INTRODUCTION

Eucalyptus is a species of plant has been distributed to a lot of tropical and subtropical region of the world. The *Eucalyptus* plant has been shown economic significance and planted in many areas of the world as a part of the agro-industry to manufacture raw material for furniture, poles and fuel wood. Through water logging the top soil become saturated which reduces the distribution of oxygen in the soil near a factor of 10^4 [1]. The oxygen needs of the roots are not reduced through flooding, oxygen quickly turns into depleted and anoxic soil be resulted. Anoxia circumstance changes metabolic and physiological processes in plant which eventually lead to a decline in plant growth [2]. As usual the primary response by plant species (including eucalyptus) to anoxic circumstances is stomata closure [3-5]. Waterlogging can reason stomatal closure under together non-saline case [6] and saline conditions. The stomatal closure for example has been noticed in 6 week old and 22 month old greenhouse seedlings after 30 and 62 days of flooding, respectively [3,4]. Recent search have studied the short term plants response to waterlogging [7] and the seedling response to flooding [3,4] of *E. largiflorens*, indicated that stomatal closure did not happen after 32 days of flooding. The oxygen rate of roots seedling was significantly depleted by flooding to approximately 0.3 m mol in 6 weeks old seedling and the root system was able to uptake oxygen at very low soil concentrations at a critical O_2 pressure of 0.015 mol O_2 m^{-3} [3]. Low levels of oxygen in the root zone trig root tips and begin to die within a few days of waterlogging and change in plant water availability because to the flood result, therefore no raise in tree transpiration after flooding can be observed [7]. Water loggings have been shown to influence major physiological functions such as photosynthesis which is rapidly decreased, with a following decline in carbohydrate production and therefore the growth rate mostly reduced [8]. The waterlogging especially for long period is reason for a decreasing in photosynthesis and somewhat changes in the carboxylation enzymes. These reactions led to a nutrient shortage in the plants which depleted the chlorophyll content of the leaves, abscission and

causing early leaf senescence [9] and decreased leaf area [6]. [2] suggested the waterlogging in long term make the plants switched from an aerobic-respiration pathway to an anaerobic way. The anaerobic respiration is cause for a fermentation pathway led to synthesis decreasing and plants reduced their photosynthesis and their growth rate [10]. Measuring chlorophyll fluorescence is a suitable, not destructive and non-invasive method to examine photosynthetic actions at diverse functional levels. Leaf-gas exchanges are factors have been employed to study the photosynthetic capacity of trees during waterlogging. The *Eucalypt* plant is extremely sensitive to extreme flooding circumstances, with reduced growth and increase leading to reduce biomass production. This study investigates the impact of waterlogging response on seedling growth and physiological characteristics in three tree species; namely, *Corymbia calophylla*, *Eucalyptus marginata* and *Eucalyptus gomphocephala*. As discussed, physiological characteristics are greatly influenced by environmental condition in which plant is grown, also this study aimed to compare waterlogging tolerance of these three seedling tree species under the same nursery condition.

2. METHODOLOGY

2.1 Experimental Design

Seeds of the three eucalypt species (*C. calophylla*, *E. gomphocephala* and *E. marginata*), were purchased from Nindethana Seed Service (Albany, Western Australia). Seeds were germinated in shallow trays filled with white sand in a naturally lit glasshouse at Curtin University (Western Australia). Trays were initially partly submerged in a larger tray of water containing pervicur fungicide (2 mL L^{-1}) to minimize seedling death resulting from fungal infection. Every 3-4 days the trays were rewatered. Seedlings remained in these trays until they had obtained a height of approximately 3 cm. In the August 2009, about 85 germinated seeds per species were planted directly into individual square pots (7 cm wide and 7 cm long by 8 cm deep) filed with soil at a ratio of four parts white sand to every two parts peat, each

pot containing one seedling. Transplanted seedlings were watered twice weekly using tap water until the seedlings had 4-6 leaves or were approximately 6 cm tall. Five seedlings of each species were then randomly selected for harvesting, with each seedling divided into stem, root and leaf components. These were dried in a drying oven at 80°C for 48 hours, and the biomass recorded. After pre-harvest the flooding trial started and finished 80 days later. 80 seedlings per species were residual, which placed in three 60 L clear plastic tubs each containing 40 pots per species; flooding (F), the plants were allowed to establish in a glasshouse for 11 weeks prior to the application treatments. The pots were randomly ordered within tubs. Flooding was achieved by filling the tubs with tap water so the water level was approximately 1 cm above the soil surface. Another three tubs had the same method except with drainage holes, were watered three times a week for the duration of the flooding treatment, and used as control (C). After three weeks all the seedlings were removed and all the tubs drained and cleaned with tap water to remove the algae which constructed on the tub walls. Seedlings were returned in to the tubs and refilled with fresh tap water.

2.2 Physiology and Growth Measurements

In the final harvest, approximately ten plants per species and treatment were chosen randomly for chlorophyll and physiological measurements. Stomata conductance (steady state porometer, LI-1600, Li-Cor, Nebraska, USA), Chlorophyll content (SPAD-502 meter, Konica Minolta, Japan), and photochemical yield (modulated chlorophyll flour meter), were measured on the youngest fully expanded leaf. All measurements were recorded during the mid-morning in full sunlight. Stomata conductance was measured a second time 14 days afterwards to assess for physiological recovery after watering with tap water. Midday measurements of potential photochemical efficiency of Photo system (Fv/Fm) were recorded after 10 min as leaf dark by leaf clips which are attached to fluoro meter manufacturer. Percentage relative water content was measured on a different subset of seedlings as $[(\text{saturation weight} - \text{dry weight}) / (\text{fresh weight} - \text{dry weight})] \times 100$ of the youngest fully expanded leaves. Seedling height and leaf number were measured in the end of the experiment. Ten seedling per species and treatment were harvest at the end of the

experiment, each seedling was separated into stem, leaf and root components. For each seedling all leaves were digitally scanned fresh and total leaf area measured using the image J software (<http://rsb.info.nih.gov/ij>). All plant material was oven dried at 80°C for 48 hours, and the dry weights of each component recorded. Various growth, biomass and leaf area allocation parameters were then calculated for each treatment and species. These included leaf area ration (LAR), leaf weight ratio (LWR), specific leaf area (SLA), total dry weight, shoot to root ratio and relative growth rates (RGR) were calculated as defined by McGraw and Garbutt (1990).

2.3 Statistical Analysis

Data was analysed using Independent t-test with the statistical program SPSS. Means were determined to be significantly different between treatments (control and flooded) at P values <0.05.

3. RESULTS

Seedlings for three Eucalyptus species under flooding stress for 70 days treatment period were affect seedlings survival began from the seventh day of the flooded in *E. marginata* followed by *E. gomphecaphata*. After 10 weeks of the experiment *E. marginata* was essentially lethal by 90% of seedlings were killed followed by *E. gomphecaphata* with 65% and *C. calophylla* with only 20% of seedlings killed.

3.1 *Corymbia calophylla*

Seedlings under flooded condition about 70 days period of treatment had significant affected of some growth parameters, total dry weight of flooded *C. calophylla* seedlings were significantly lesser than the control seedlings (P= 0.001), as well the height of the control seedlings were significantly great comparing with flooded seedlings (0.002) as shown (Fig. 1). This difference between control and flooded seedlings is affected by RGR (Table 1). There was no significant difference between flood and control seedlings in all other growth parameters measured (LAR, LWR, SAL, leaf number and Root: Shoot ratio), however the leaf area ratio of flooded seedlings were declined by 48% compare with control seedlings. Control seedlings had a greater relative growth ratio than flooded seedlings (Table 1). The relative chlorophyll content and F_v/F_m were significant

decreasing in flooded *C. calophylla* seedlings, comparing with control seedlings (Table 2). Although there was no significant different in the other physiology parameters measured of seedlings after 70 days from flooding compared with control seedlings (Table 2).

3.2 *Eucalyptus gomphocephala*

After 70 days of flooding experiment the total dry weight and height of the seedlings were decreased significantly by 60% (0.18) and 40% (9.80) compared to the control (Fig. 1) (Table 3). However, there was no significant difference in LAR, LWR, SAL, leaf number and root: shoot ratio between control and flooded after 70 days of flooding (Table 3). Relative growth ratio of flooded seedlings was decreased comparing with the control seedlings in the same treatment period. For the physiological measurements there was only significant difference in the relative chlorophyll content ($P=0.000$) (Table 4) between control and flooded seedlings. There was no significant difference in stomata conductance, transpiration, maximum F_v / F_m and relative water content (Table 4) between control and flooded seedlings after 70 days of flooding.

3.3 *Eucalyptus marginata*

The data carried out, seedlings of *E. marginata* has more influences after 70 days of flooding, comparing with studied species, nearly all growth parameters of the flooded seedlings were significantly smaller than the control seedlings after 70 days of flooded (Table 5), as root to shoot ratio and seedlings height reduced by about 75% (Fig. 1), total dry weight and leaf number smaller by 65% than the control seedlings. The difference between control seedlings and flooded of LWR and SLA was not significant (Table 5). There were significant declines in relative chlorophyll content, stomata conductance, and transpiration, F_v / F_m and RWC recorded from flooded after 70 days (Table 6). The greater physiological parameter reduction by flooded was transpiration with 90% ($P = 0.001$).

4. DISCUSSION

Three of *Eucalyptus* species were grown under two treatments of control and subjected to waterlogging conditions for 70 days. "Flood tolerance varies greatly with plant species and genotype, rootstock, age of plants, time and duration of flooding, and condition of the floodwater" [2,11]. The effect of waterlogging on

growth parameters, stomata conductance, relative chlorophyll content, transpiration, F_v/F_m and relative water content was studied. According to growth and physiology data of the three species examined, *E. Marginata* seedlings were the lowest floods tolerance than *C. calophylla* and *E. gomphocephala* seedlings, with all parameters measuring a weekly declining in water flooding treatment [12,13]. The *E. marginata* species was unable to survive more than 10 weeks of flooding treatment, and showed many negative influences on the growth and physiological characteristics. These involve stomatal closure, which has been correlated with a decrease in root hydraulic conductivity [14] decline in photosynthesis process, decrease growth and increase in mortality symptoms on the seedlings. Although, increasing mortality rate of *E. marginata* seedlings under flooded condition may be related to the roots inability to survive and function under less-oxygen conditions. Conversely, *C. calophylla* and *E. gomphocephala* demonstrated a decrease in stomatal conductance under flooded stress, however less impact than *E. marginata* and more likely that the tolerant genus of seedling may able to recommence photosynthetic activity behind the flooding case ended. In all Species, gs and E were lowered under flooded condition, especially in the *E. marginata* Waterlogging can cause stomatal closure under non-saline [6].

A good indicator of the effects of environmental stresses on photosynthesis is chlorophyll fluorescence F_v/F_m [15]. Through the experiment we find the significant difference in F_v/F_m between control and flooded treatments in the studied species (except *E. gomphocephala* $P=0.25$). This showed that there were negative effects or damage to photochemical reactions, which is supported gas exchange results Table (4,6). Relative water content were decreased in all three species, on the other hand the difference was insignificant except in *E. marginata* $P=0.04$ (Table 6).

Fig. 1 shows the growth (in height) of all three species in two treatment conditions. A trend of increased growth can be seen for well watered plants across all three species. The increase in height of well watered of three species was significantly different to waterlogged, this difference supported the results from Table (1,3,5), there is significant difference between control and flooded of dry weight in all species. This could imply that both species are adversely affected by waterlogging (as a result shedding of

extensive leaf). There are major factors causal CO_2 intake, decrease in stomatal opening and reduced carbon uptake and reduced whole plant its, followed by a decrease in total leaf area [16]. biomass in flooded seedlings which are curb on

Table 1. Growth parameters of *C. calophylla* seedlings under control and flooding condition
 Values are mean \pm SE for ten seedlings. P value based on a t- test comparing control and flooded data. LAR=Leaf area ratio; LWR=Leaf weight ratio; SLA = specific leaf area and RGR=Relative growth ratio

Parameters	70 days of flooding		P
	Control	Flooded	
Total dry weight (g)	2.35 \pm 0.22	0.86 \pm 0.08	0.001
seedling height (cm)	30.20 \pm 2.13	18.00 \pm 1.14	0.002
LAR (m ² g ⁻¹)	119.12 \pm 13.1	62.97 \pm 10.3	0.101
LWR (g g ⁻¹)	0.61 \pm 0.01	0.60 \pm 0.10	0.908
SLA (m ² g ⁻¹)	193.08 \pm 38.23	153.28 \pm 38.25	0.139
Leaf number	15.66 \pm 1.45	11.66 \pm 0.88	0.092
Root: shoot ratio g ⁻¹	5.85 \pm 1.43	4.51 \pm 1.04	0.135
RGR %	35.7	21.5	

Table 2. Stomata conductance (g_s), greatest (F_v/F_m), transpiration (E), relative chlorophyll content and relative water content of *C. calophylla* seedling under control and flooded. Values are mean \pm SE for ten seedlings

Parameters	70 days of flooding		P
	Control	Flooded	
Relative chlorophyll content (SPAD units)	45.32 \pm 1.81	25.28 \pm 2.22	0.000
Stomata conductance Mmol/m ² /s	0.07 \pm 0.01	0.05 \pm 0.01	0.89
Transpiration (mmol/m2/s)	1.57 \pm 0.35	1.43 \pm 0.31	0.77
F _v / F _m	0.78 \pm 0.005	0.70 \pm 0.01	0.05
RWC	88.81 \pm 3.88	86.98 \pm 3.47	0.82

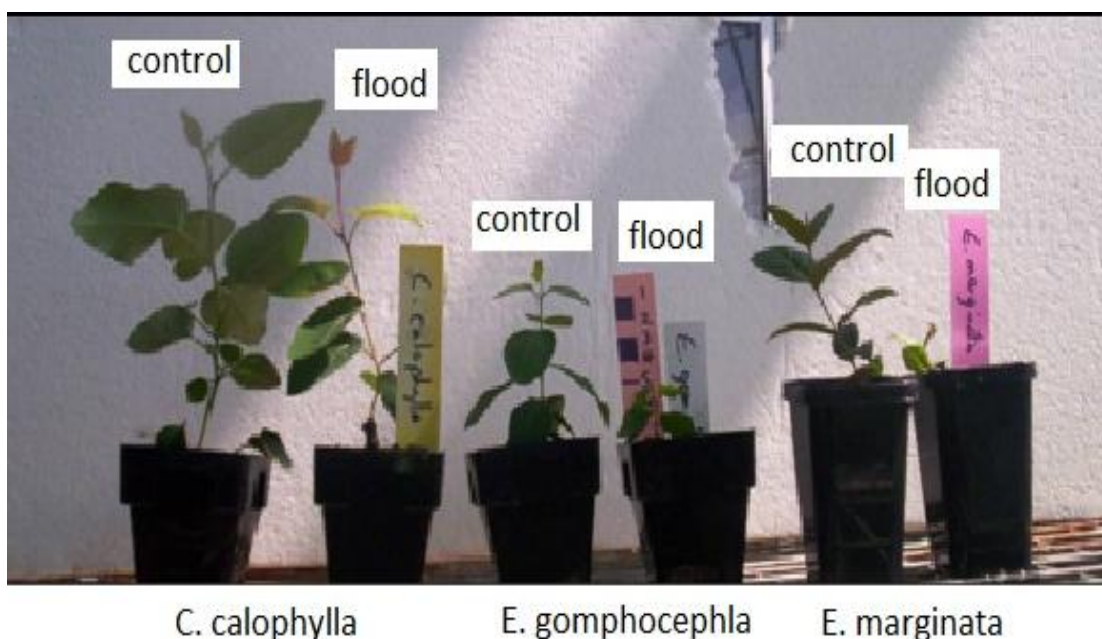


Fig. 1. Height comparison between control and flooded in the three *Eucalyptus* seedlings

Table 3. Growth parameters of *E. gomphecapkata* seedlings under control and flooding condition. Values are mean \pm SE for ten seedlings. P value based on a t- test comparing control and flooded data.LAR=Leaf area ratio; LWR=Leaf weight ratio; SLA = specific leaf area and RGR= Relative growth ratio

Parameters	70 days of flooding		P
	Control	Flooded	
Total dry weight (g)	0.39 \pm 0.06	0.18 \pm 0.02	0.005
seedling height (cm)	16.60 \pm 1.07	9.80 \pm 0.66	0.001
LAR (m ² g ⁻¹)	122.94 \pm 14.63	131.20 \pm 75.37	0.816
LWR (g g ⁻¹)	0.66 \pm 0.05	0.65 \pm 0.04	0.684
SLA (m ² g ⁻¹)	185.88 \pm 20.13	200.38 \pm 108.43	0.783
Leaf number	13.33 \pm 2.40	18.66 \pm 1.76	0.155
Root: Shoot ratio g ⁻¹	7.70 \pm 3.26	5.26 \pm 1.69	0.189
RGR %	43.1	29.4	

Table 4. Stomata conductance (g_s), greatest (F_v/F_m), transpiration (E), relative chlorophyll content and relative water content of *E. gomphecapkata* seedling under control a flooded. Values are mean \pm SE for ten seedlings

Parameters	70 days of flooding		P
	Control	Flooding	
Relative chlorophyll content (SPA units)	39.78 \pm 1.28	19.24 \pm 1.45	0.000
Stomata conductance Mmol/m ² /s	0.23 \pm 0.08	0.08 \pm 0.03	0.12
Transpiration (mmol/m ² /s)	7.68 \pm 2.68	2.64 \pm 1.23	0.12
F _v / F _m	0.76 \pm 0.007	0.72 \pm 0.02	0.25
RWC	81.60 \pm 6.48	68.94 \pm 7.00	0.22

Table 5. Growth parameters of *E. marginata* seedlings under control and flooding condition. Values are mean \pm SE for ten seedlings. P value based on a t- test comparing control and flooded data.LAR=Leaf area ratio; LWR=Leaf weight ratio; SLA = specific leaf area and RGR= Relative

Parameters	70 days of flooding		P
	Control	Flooded	
Total dry weight (g)	0.49 \pm 0.06	0.18 \pm 0.02	0.005
seedling height (cm)	13.25 \pm 1.37	4.75 \pm 0.47	0.001
LAR(m ² g ⁻¹)	119.05 \pm 13.18	83.30 \pm 9.99	0.006
LWR(g g ⁻¹)	0.73 \pm 0.02	0.60 \pm 0.07	0.132
SLA(m ² g ⁻¹)	161.85 \pm 15.00	142.15 \pm 34.36	0.334
Leaf number	10.33 \pm 0.33	4.33 \pm 0.88	0.012
Root: shoot ratio g ⁻¹	14.95 \pm 3.21	3.53 \pm 1.36	0.003
RGR %	39.36	18.78	

Table 6. Stomatal conductance (g_s), greatest (F_v/F_m), transpiration (E), relative chlorophyll content and relative water content (RWC) of *E. marginata* seedling under control a flooded. Values are mean \pm SE for ten seedlings

Parameters	70 days of flooding		P
	Control	Flooded	
Relative chlorophyll content (SPAD units)	43.62 \pm 3.20	25.38 \pm 2.13	0.000
Stomata conductance Mmol/m ² /s	0.33 \pm 0.11	0.01 \pm 0.00	0.02
Transpiration (mmol/m ² /s)	9.95 \pm 3.19	0.47 \pm 0.31	0.001
F _v / F _m	0.78 \pm 0.004	0.72 \pm 0.01	0.03
RWC	82.54 \pm 1.67	65.32 \pm 5.83	0.04

Shoot to root ratio were decreased in all three species, however the difference was insignificant except in *E. marginata* $P=0.003$ (Table 5), it is due to the relationship between flooding and shoot growth is adverse" by suppressing leaf formation and expansion of leaves and internodes, causing premature leaf senescence and abscission, and inducing shoot dieback" [11] and the amount of investment in growing tall or growing adventitious roots differ in this species. Each species the RGR is highest for control seedlings and lowest for flooded which is expected, the efficiency of leaves in generating biomass different between species and treatments. Flooded *E. marginata* seedlings recorded the lowest percentage of RGR, which can be linked to reduction in photosynthetic activity with significant differences in biomass allocation; also the number of leaves may affect the RGR.

Across all three eucalypt species there are significant differences in the leaf weight ratio of control and waterlogged seedlings. In addition, there were no significant differences in the leaf area ratio of every species (except *E. marginata*). So although mass is significantly different, the allocation of mass is not. Table (1,2) shows that specific leaf area in control *C. calophylla* and *E. Gomphecaphata* are similar to waterlogged indicating that due to its flooding tolerance it can keep adjustments minimal to reduce plant stress. Table 5 illustrated the significant differences shown by *E. marginata* between control and flooded seedlings which contradicts the trend of *E. marginata* coping well with flood stress. The lack of significant difference between control and waterlogged leaf area for *C. calophylla* and *E. gomphecaphata* indicates that these plants can tolerate flooding better than *E. marginata*.

The light energy absorbed by chlorophyll enables photosynthesis so the more chlorophyll a plant has; the better it is at photosynthesising. The chlorophyll content of *C. calophylla* (Table 1), *E. gomphecaphata* (Table 3) and *E. marginata* (Table 5) appear to be more severely affected by waterlogging as there was a significant difference between the chlorophyll content of control and waterlogged seedlings of each species. This suggests that the photosynthetic rate of these species would have been reduced meaning their energy levels would have decreased, resulting in slower growth rates. Comparisons of the control and waterlogged height measurements of *C. calophylla*, *E. gomphecaphata* and *E. marginata* (Fig. 1) support this assumption.

5. CONCLUSION AND RECOMMENDATIONS FOR FUTURE WORK

The research has been established that the waterlogging issue in ecosystem will ultimately has a harmful effect on physiological functions of seedling, on the other hand this effect was diverse among the species, appropriate to the information that the study demonstrated the highest tolerance level of flood was of *C. calophylla* compared to the tolerance level of *E. marginata* but not as low as *E. gomphecephala*. Novel consequences are obtainable within this manuscript and a great deal models can be proposed for simulation by this method. The conclusions of paper prove complication of this form of simulated models on open field, but gives a novel approaches for such application. An additional focus on further environments conditions and flora could recognize the resolution for other environmental troubles.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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