British Journal of Applied Science & Technology
18(2): 1-8, 2016; Article no.BJAST.30185
ISSN: 2231-0843, NLM ID: 101664541

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Seedling Tolerance of Three Eucalypt Species to Changes in Soil Alkalinity Due to Limestone Addition

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Authors' contributions

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

Article Information

DOI: 10.9734/BJAST/2016/30185
Editor(s):
(1) Rares Halbac-Cotoara-Zamfir, Hydrotechnical Engineering Department, "Politehnica" University of Timisoara, Romania.
Reviewers:
(1) Eugenio Cazzato, University of Bari Aldo Moro, Italy.
(2) Amouri Adel Amar, University of Oran, Algeria.
(3) Lianxuan Shi, Northeast Normal University, China.
Complete Peer review History: http://www.sciencedomain.org/review-history/17165

Short Research Article

Received 22nd October 2016
Accepted 29th November 2016
Published 8th December 2016

ABSTRACT

The present research was implemented to analyses 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 soil alkalinity (limestone). Seeds germinated in shallow trays filled with white sand in a naturally lit glasshouse (control treatment). A liming treatment was conducted with 20% w/w crushed and sifted Tomala limestone adds to potting mix to increase soil pH. The experiment was conducted over 82 days. *E. gomphocephala* is restricted soils overlying limestone on study area and according to total seedling dry weight data and calculated relative growth rates coped best in a limestone-enriched soil. However, when examining all the growth and physiological data collected *C. calophylla* appeared to be the middling tolerant, with no significant difference in leaf allocation or leaf water loss between the well-watered controls and the limestone-enriched treatments whereas the *E. marginata* was the least tolerant with a 14% reduction in stomatal conductance.

Keywords: Eucalypt limestone tolerance; growth and physiology; limestone stresses.

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1. INTRODUCTION

Western Australian soils of calcareous origin are high in pH [1] Moore. The alkalinity-acidity salt of Western Australia soils have been categorised according to their plant toxicity [2] Szabolcs. The common field crops have a preference to a neutral or slightly acidic soil (pH 7) whereas some plants, however, prefer more acidic or alkaline conditions. The pH of soil solution is dependent relative on mineral weathering conditions, and mineral weathering raising pH by releasing base cations (Ca, Mg and K), and therefore the soils that rich in simply weather able minerals tend mostly have higher pH and higher soil solution concentration of Ca, Mg and K [3 Jarvan]. Several nutrient cations such as aluminum (Al^{3+}), zinc (Zn^{2+}), copper (Cu^{2+}), iron(Fe^{2+}), manganese (Mn^{2+}) and cobalt (Co^{2+}) are soluble and available for absorbing by plants when pH value below 5.0, even though their availability may result in ion toxicity as soil pH decreases [4]. These cations are less available in soil in more alkaline environment and usually the soil shows indications of nutrient deficiency, comprise thin plant stems, yellowing and mottling of leaves, and slow and/or short growth. Some elements require a specific pH range. Phosphorus (P) uptake requires a soil pH between 6.0 and 7.5; otherwise it becomes chemically immobile, forming insoluble compounds with iron (Fe) and aluminum (Al) in acid soils and with calcium (Ca) in calcareous soils [4]. Soils that contain calcium sulphate (gypsum), sodium bicarbonate and calcium carbonate can impact of seedling growth because of the effect of increasing soil pH, on micro and macro nutrient availability, particularly phosphorus, nitrogen, copper, zinc, manganese and iron [5]. The pH (in soil solution) of the soils have been documented as being either slightly acid (calcium sulphate), low alkaline (carbon carbonate) and alkaline (sodium bicarbonate) [2].

An excessive addition of calcium carbonate in the soil PH can cause calcium phosphates precipitation phenomenon. It plays an important role in controlling phosphorus activity and its availability in soil solution. The general reaction that explains the interaction of a liming material such as $CaCO_3$ with water to form OH- ions is as follows [6]. $CaCO_3 + H_2O$ (in soil) → $Ca^{2+} + HCO^{-}{}^{3} + OH^{-}$

The overall reaction of lime with an acid soil can be expressed by the following $2Al^{+}\ 3\ CaCO_3 + 3\ H_2O \rightarrow 3\ Ca^{+}\ 2Al\ (OH)_3 + 3CO_2$

Species from the genus *Eucalyptus* naturally occur in a range of different soil characteristics from alkaline, calcareous or acidic soils acid [7,8,9]. Characteristically, differing tolerance to calcareous circumstances in *Eucalyptus* has been investigated in relationship between transpacific populations of plants raised from seed collected from trees growing on acidic or alkalinised soils [10,11,12]. For example, *Eucalyptus obliqua* has received particular attention to assess the response of potentially useful species to the pH of the growing medium [10,13,14].About 35 seedlings of *Eucalyptus* species were grown under amended medium with limestone and dolomite (1:1) and pH range of 5.1 to 8.9 to study a response of ornamental eucalypts from acidic and alkaline habitats [15]. Results indicated that the seedlings growth was generally greater under acidic (pH 5.1-5.6) than under more alkaline conditions. Species demonstrated a range of responses to changes in soil pH, including species that were unaffected even at pH 8.9 such as *E. erythrocorys* and *E. extensa.* The tolerance to high pH is associated with a capability to maintain relatively low Ca and Mg and P and Fe concentration ratios < PH 5. The tolerance of six clones from five provenances of *E. camaldulensis* was examined under controlled conditions of waterlogged, highly saline and highly alkaline soils in a greenhouse where leaves produced under salinity and alkalinity impacts were similar in ion content to those produced prior to the test condition [16,17,18]. Some *Eucalyptus* species that show growth in soils of relatively high pH, high bicarbonate (HCO^{3-}), and low iron (Fe) concentration has potential to live in calcareous or alkaline soils [19,8]. The tolerance of 5 semi-arid Western Australia species (*E*. *gracilis*, *E*. *halophila*, *E*. *kondininensis*, *E*. *loxophleba*and, *E*. *platypus* var. *heterophylla*) to alkaline conditions, bicarbonate, and low iron availability was examined by [8]. The study showed that seedlings growing in medium of pH 9, caused a reduction in plant height and leaf production for most of the study species, compared with a species growing in system of pH 6. Iron concentrations in the youngest fully expanded leaves were reduced for seedlings in the pH 9 solution. *Eucalyptus halophila* was an exception to this. *Eucalyptus* species may be important in the amelioration of salt-affected [20,21] and alkaline lands [19], the treatment of limestone quarries [20] and mine waste process that often very alkaline, acidic or saline [22,23,24,25]. The research presented in this paper focuses on the tolerance of three eucalypts species to soils enriched by natural limestone, as measured by changes in growth and physiology over 80 days.

2. METHODOLOGY

2.1 Experimental Design

Seeds of the three eucalypt species were germinated in shallow trays filled with white sand in a naturally lit glasshouse. Trays were initially, partly submerged in a larger tray of water containing Pervicur® fungicide (2 *m*L 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. One hundred and twenty seedlings of each species were there transplanted into square individual pots (7 cm wide and 7 cm long by 8 cm deep) filled with soil at a ratio of four parts white sand to two parts peat, each pot containing one seedling. Transplanted seedlings were watered twice weekly until the seedlings had 4-6 leaves or were approximately 6 cm tall. The day before applying the alkalinity treatment, 10 seedlings of each species were randomly selected for harvesting, with each seedling divided into stem, root and leaf components. Biomass of stem, root and leaves were recorded after drying the samples in a drying oven at 80℃ for 48 hours, or until constant mass was achieved.

For each species, the remaining seedlings were randomly divided into two treatments. The first treatment had no additional limestone added, and was the control. For the second treatment (the 'limestone' treatment) pots were filled with crushed local Tamala limestone, which had been passed through a 3 mm sieve, and mixed at a rate of 20% by soil weight. Overall 55 seedlings per treatment were used per species. Seedlings in both treatments were watered twice a week with fresh tap water for 12 weeks experiment period.

2.2 Physiological and Growth Measurements

At the end of experiment, about ten seedlings per treatment and species were randomly chosen for chlorophyll and physiological measurements. Chlorophyll content (SPAD-502 meter, Konica Minolta, Japan), stomatal conductance and transpiration (steady state porometer, LI-1600, Li-Cor, Nebraska, USA) were measured on the youngest fully expanded leaf. All measurements were recorded during the mid-morning in full sunlight. The number of leaves and seedling height were measured for ten plants which chosen randomly at the end of the experimental. An additional ten seedling per species and treatment were harvest at the end of experiment, each seedling was divided 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.hih.gov/ij). All plant material was oven dried at 80ºCfor 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 total dry weight, shoot to root ratio, leaf area ration (LAR), leaf weight ratio (LWR), Specific leaf area (SLA) and growth rate (RGR), as defined by McGraw and Garbutt (1990). Dried leaf material were then ground separately through a 40 µm mesh and 2 g subsamples were analysed by the Western Australia's Chemistry Centre for macro and micro element concentrations N was analysed by Kjeldahl digestion and titration the remaining elements were digested by a concentrated HNO3: HClO3: H2SO4 solution. Other elements were analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (e.g. Cu, Fe, Mg, Mn, Zn) and ion exchange chromatography (Cl, B, S, P, K).

2.3 Statistical Analysis

Data was analysed using independent *t*-tests with the statistical program SPSS. Means were determined to be significantly different between treatments (control and limestone) at *P* values <0.05.36

3. RESULTS

3.1 *Corymbia calophylla*

After 82 days of the limestone treatment the total seedling dry weight (*P*=0.008), plant height (*P*=0.001) and specific leaf area (*P*=0.004) were negatively impacted by the presence of limestone in *C. calophylla* seedlings (Table 1). *C. calophylla* seedlings growing in the limestone enhanced soils had a lower relative growth rate than the well-watered controls by approximately 20% (Table 1). Limestone seedlings had no significant difference in all Physiology parameters compared with control (Table 1).

Table 1. Growth and physiological parameters of *C. calophylla, E. gomphocephala* and *E. marginata* seedlings after 82 days under well watered (control) and limestone-enhanced conditions. Values are mean ±SE for 10 seedlings. LAR=leaf area ratio; LWR=leaf weight ratio; SLA = specific leaf area and RGR= relative growth ratio

Parameters	Control	Limestone	P (*t*-test)
C. calophylla			
Total dry weight (g)	2.35 ± 0.22	1.37 ± 0.16	**
Seedling height (cm)	30.2 ± 2.1	15.83 ± 1.30	**
LAR (mm ^{2} g^{-1})	119.1 ± 13.1	67.90 ± 2.41	NS
LWR (g g^{-1})	0.61 ± 0.01	0.69 ± 0.02	NS
SLA (mm ^{2} g^{-1})	193.1 ± 17.1	97.80 ± 4.76	**
Leaf number	15.7± 1.4	10.00 ± 1.18	NS
Root: shoot ratio (g g-1)	5.8 ± 1.4	5.89 ± 0.75	NS
RGR (mg g^{-1}g day^{-1})	30.5	24.3	NA
Chlorophyll content (relative units)	44.28 ± 1.76	40.67 ± 2.22	NS
Stomatal conductance (mol m^{-2}s^{-1})	0.06 ± 0.01	0.03 ± 0.01	NS
Transpiration (mmol m^{-2} s^{-1})	2.02 ± 0.38	1.11 ± 0.76	NS
E. gomphocephala			
Total dry weight (g)	0.39 ± 0.06	0.35 ± 0.01	*
Seedling height (cm)	16.6 ± 1.1	8.33 ± 0.9	**
LAR (mm ^{2} g^{-1})	122.9 ± 6.5	87.21 ± 7.3	**
LWR (g g^{-1})	0.66 ± 0.02	0.72 ± 0.01	NS
SLA (mm ^{2} g^{-1})	185.9 ± 9.0	121.0 ± 11.5	**
Leaf number	13.3± 2.4	17.0 ± 1.34	**
Root: shoot ratio (g g-1)	7.7 ± 1.5	7.29 ± 0.25	NS
RGR (mg g^{-1}g day^{-1})	36.8	32.9	NA
Chlorophyll content (relative units)	42.92 ± 1.63	37.62 ± 1.43	*
Stomatal conductance (mol m^{-2}s^{-1})	0.33 ± 0.09	0.09 ± 0.02	*
Transpiration (mmol m^{-2} s^{-1})	6.95 ±3.13	1.97 ± 0.59	NS
E. marginata			
Total dry weight (g)	0.70 ± 0.05	0.32 ± 0.02	**
Seedling height (cm)	10.8 ± 1.6	7.50 ± 0.9	NS
LAR (mm ^{2} g^{-1})	118.8 ± 5.1	72.0 ± 1.8	**
LWR (g g^{-1})	0.73 ± 0.01	0.58 ± 0.03	**
SLA (mm ^{2} g^{-1})	161.5 ± 5.8	142.1 ± 34.4	*
Leaf number	8.0 ± 0.6	8.2 ± 0.6	NS
Root: shoot ratio (g g-1)	33.6	23.5	NA
RGR (mg g^{-1}g day^{-1})	46.47 ± 1.62	30.05 ± 2.85	*
Chlorophyll content (relative units)	0.49 ± 0.10	0.07 ± 0.02	*
Stomatal conductance (mol m^{-2}s^{-1})	14.29 ± 2.71	4.79 ± 2.58	NS

(= 0.05, ** = 0.005, *** = 0.0005, NS= not significant). (NA= not available).*

3.2 *Eucalyptus gomphocephala*

E. gomphocephala seedlings exhibited a negative effect in most of the growth parameters measured in relation to the occurrence of limestone in the soil, except for an increase in leaf number (Table 1). This is supported by a decrease in the relative growth rate. Limestone-effected seedlings had significantly less leaf chlorophyll content (P=0.02) and a decreased stomatal conductance (*P*=0.05), but not transpiration (*P*=0. 19) than well watered seedlings.

3.3 *Eucalyptus marginata*

Seedling total dry weight (*P*= 0.001), leaf area ratio (*P*= 0.001), leaf weight ratio (P= 0.004) and specific leaf area (P= 0.01), and root: shoot ratio (*P*= 0.001) were negatively affected after growing in limestone enriched soils for 82 days (Table 1) with an approximately a 30% decrease in relative growth rate. Limestone-affected seedlings a significant decrease in leaf chlorophyll content (*P*= 0.01) and stomata conductance (*P*= 0.05). There was also a decrease in transpiration rate (approximately 70%).

3.4 Leaf Chemistry

Minerals such as boron, iron and manganese were the only mineral nutrients that showed an overall significant decrease in leaf content resulting from liming compared with the control treatment. The decrease boron mineral was greatest for *C. calophylla* seedlings (18% compared with control) whereas decline of iron varied from 56 to 67% and manganese's fall was varied from 31 to 43%. There was no significant increase in leaf calcium concentration in response to liming in either *C. calophylla* or *E. gomphocephala* (Table 2). Leaf magnesium concentrations decreased with increasing alkalinity in all species. There was a significant effect ($P < 0.05$) of pH on leaf Fe content in three tested *Eucalyptus* examined (Table 2). There was no effect of pH on leaf K concentration in all *Eucalyptus* species.

4. DISCUSSION

The most tolerant species, *E. gomphocephala* has demonstrated significant decline in adult crown health and numbers since the mid and is the most restricted in calcareous soils). However *E. gomphocephala* wasn't the most tolerant to an increase is soil alkalinity, although it displayed the least change in seedling dry weight and relative growth rate. This may due to the limestone mixture used for the experiments

Table 2. Leaf chemistry of three *Eucalyptus* seedlings after 82 days growing under well-watered (control) and limestone-enhanced conditions. Data represents chemical analysis of ground dried material

Minerals	B mg/kg	Ca %	Cu mg/kg	Fe mg/kg	K %	Mg %	Mn mg/kg	Na %	P %
C. calophylla									
Control	27	0.86	4.8	84	1.36	0.23	120	1.06	0.11
Limestone	5	0.87	1.8	50	1.8	0.12	42	0.57	0.08
E. gomphocephala									
Control	33	0.92	22	89	1.48	0.2	220	0.83	0.19
Limestone	11	1.4	30	50	1.52	0.18	95	0.79	0.18
E. marginata									
Control	21	1.38	17	61	1.56	0.27	140	1.04	0.14
Limestone	13	1.18	37	41	1.59	0.23	43	1.46	0.08
P-value	*	NS	NS	*	NS	NS	*	NS	NS

(*= 0.05, ** = 0.005 , *** = 0.0005 , NS= not significant.)

Fig. 1. Height comparison between the control and alkalinity treatments of the three *Eucalyptus* seedlings

wasn't reflective of the natural subsurface calcium carbonate concentration experienced by tuart plants in the field. It may also be because alkalinity tolerance is developed over longer periods than the experiments were conducted. Nevertheless the coastal distribution of *E. gomphocephala* suggests an ability of this species to tolerate soil alkalinity. The differences in species high pH tolerance in experiments has been reported in *Eucalyptus* consisted with [10].

The *E. gomphocephala* is restricted soils overlying limestone on Perth's Swan Coastal Plain, and according to total seedling dry weight data and calculated relative growth rates was the most tolerant to the limestone-enriched soils. All the growth and physiological data pointed out that the *C. calophylla* is the most tolerant, with no significant difference in leaf allocation or leaf water loss between the well-watered controls and the limestone-enriched treatments. The research showed that the *E. Marginata* was the least tolerant with a 14% due to reduction in stomatal conductance values. Liming (i.e. the presence of bicarbonate) increases soil pH and is well known to decrease the growth of Eucalypts in agree with studies of [14,22,26,27] and was represented by decreased relative growth rate, seedling height and reduced leaf production in the three target species of this study.

The leaf chemistry analyses for the high pH tolerant *C. calophylla* and the intolerant *E. marginata* gives an insight into the basis of discrepancy reaction to changing pH. Liming causes an increase in soil pH, and affects the ability for the seedlings to uptake some mineral nutrients. According to the leaf chemistry results an increase in Ca and decrease in Mg concentrations in the liming trial in the intolerant species, it is probable have been negative effect on some physiological functions and growth ratio on seedlings studied. It has previously been show that a significant increase in soil pH relates to a decrease total Fe content in plant tissues [28,29,30]. James et al. [9] found that lower Fe concentrations of newly produced leaves of *Eucalyptus* species growing in a growth media of pH 9 demonstrates that the availability and translocation of Fe within seedlings is reduced as a result of both the presence of bicarbonate and the reduced concentration of soil Fe, ultimately limiting seedling growth rate. Similar results were obtained in the present study. Manganese and Boron are both made unavailable to plants with an increase in soil pH, and *Eucalyptus* species are known to tolerate a range of soil acidity-alkalinity [20].

5. CONCLUSION AND RECOMMENDATIONSFOR FUTURE WORK

The research has been established that the alkalinity case in the natural environment will ultimately has a negative influence on physiological functions of plantlet, on the other side this influence was varied between the species, applicable to the information that the scholar confirmed the highest tolerance level of Limestone Addition was of *E. gomphocephala* compared to the tolerance level of *C. calophylla* and *E. marginata* was the least tolerant. Novel consequences are obtainable within this manuscript and a great deal models can be proposed for simulation by this method. 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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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17165