Differential Fitness of *Eucalyptus* Defense Phenotypes Under Altered Nutrient and Light Conditions

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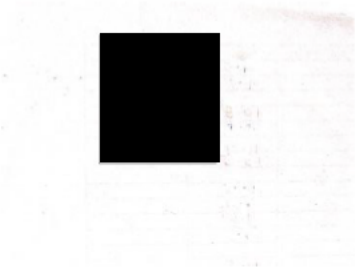
Declaration

This report describes the original work of the author except where otherwise stated. It has not been submitted previously in whole or in part for any degree or diploma in any university or institution.



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11th October 2021

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List of Abbreviations

AG – Aboveground mass

LT – Lignotuber mass

LT% - Lignotuber mass percentage

ML – Mean leaf mass

PSM – Plant secondary metabolites

RT – Root biomass

RT% - Root biomass percentage

SLA – Specific leaf area

TS – Total sideroxylonal concentration

TP – Total phenolics concentrations

Abstract

Producing defensive chemicals is a cost for a plant. Scientists have hypothesized that there is always a trade-off between investment in chemical defence and plant physiological and developmental growth processes. It has been proposed that plants growing under high resource availability should use their carbon budget for plant growth rather than defence and that when plants are growing under resource-limited environmental conditions plants use their available carbon budget for the differentiation processes such as the production of defensive chemicals, rather than plant growth. *Eucalyptus* is the dominant genus of trees in Australian forests and their leaves are the main food source for many herbivores including insects and some arboreal marsupials. However, trees from the genus *Eucalyptus* possess a complex mixture of plant secondary metabolites (PSM), including formylated phloroglucinol compounds such as sideroxylonal, and a range of water-soluble phenolics. These compounds vary qualitatively and quantitatively between species and quantitatively within species. These plants are extensively studied for the great chemical variation they possess. However, few experiments have been conducted to test for the existence of trade-offs between growth and defence in these plants. The defensive chemistry of *Eucalyptus* species is mainly constitutively determined and phenotypic plasticity across the environmental variation is less well understood. I aimed to find out under which environmental circumstances this proposed trade-off will take place in *Eucalyptus* and also the nature of such trade-offs, under different conditions of light and nutrient availability. *Eucalyptus melliodora* and *Eucalyptus camaldulensis* seeds were grown under three different light conditions and *E. melliodora* seedlings were grown under two nutrient conditions and changes in growth parameters and sideroxylonal and total phenolic concentrations of seedlings were observed. Plants under different light and nutrient treatments varied greatly in their growth parameters and foliar PSM concentrations as well. Plants that were grown under higher light levels contained more sideroxylonal and more total phenolics than plants under low light conditions. Plants grown under higher nutrient levels possessed higher sideroxylonal but lower total phenolics compared to low nutrient plants. However, no trade-offs were identified between growth parameters and defensive chemical concentrations under any environmental conditions. Contrary to expectations, positive relationships between growth and concentrations of sideroxylonal were identified between and within many environmental treatments.

Chapter 1- General Introduction

1.1 Plant secondary Metabolites

Plant secondary metabolites (PSM) are a group of molecules that are derived from the differentiation of primary metabolites (Lerdau, Litvak and Monson, 1994). They are not involved in primary biochemical pathways, which are those involved in cell growth and reproduction. However, they are involved in the adaptation of plants to their growing environment (Makkar, Siddhuraju and Becker, 2007). As these chemicals did not seem to have been involved in primary metabolism, they were named 'Secondary' (Stamp, 2003). In contrast to primary metabolites which are common to all plants, secondary metabolite profile varies considerably between different plant species. PSMs are believed to play an important role in plant defence against herbivores (Siemens et al. 2002). PSMs also influence a wide range of ecological interactions as well, including acting against competing plants, mediating interactions with pollinators and seed-dispersing animals, influencing leaf litter decomposition rates, and giving protection against abiotic stresses such as UV-B radiation, frost, and drought (McKiernan et al. 2014; Wink, 2003; Moore et al. 2014). However, among all these functions, defence against herbivores is the most prominent role of PSMs (McKiernan et al. 2014).

Most PSMs are carbon-based compounds. However, some plants produce PSM compounds containing nitrogen, which is a limiting and valuable resource for most plants (Massad, Dyer and Vega 2012). On the other hand, N-containing precursors and enzymes are involved in chemical reactions producing C-based PSM. Therefore, considering compounds as either C-based or N-based may create an oversimplification and confuse our understanding of their exact mode of reacting (Massad, Dyer and Vega 2012). According to Lambers (1993), there are poisonous secondary metabolites whose concentration is relatively low within the plant, but they are very toxic such as alkaloids, cyanogenic glucosides, and cardenolides. However, some PSM accumulate within the plant in high concentrations making the less digestible and less palatable to herbivores (Lavola and Julkunen, 1994) such as PSM having phenolic origin

especially including tannins, lignin, and other phenolics compounds (Lambers, 1993). The concentration of the PSM including lignin, condensed tannin, and volatile terpenoid may take up to 10% to 30% of leaf dry weight which is a considerably high concentration (Lambers and Pooter, 2004). However, many herbivores prefer to eat leaves with high concentrations of proteins and water, leaves with low toughness, and leaves with low concentrations of anti-herbivory compounds (Lambers, 1993).

PSMs are present in all higher plants, with great structural diversity throughout the plant kingdom. Often, there is a great variation of PSM composition within and between species (Moore et al. 2004), and even within a population, there is a variation in the concentration of particular secondary metabolite (Simens et al. 2002). The PSM concentration is believed to vary with the age of the plant leaf resulting higher concentration of PSM accumulates within young leaves than older leaves (Wallace and Eigenbrode, 2002; Kouki and Manetas, 2002). This great phenotypic variation of plant defence is controlled by both genetic and environmental factors (Kulheim et al. 2011). To identify the broader effects of PSMs on ecosystem changes, it is necessary to understand which PSMs have a particular biological activity, and how these PSMs vary qualitatively and quantitatively between plants (Kulheim et al. 2011). Normally, a single PSM such as sideroxylonal, Marcocarpal, or a small subset of the PSMs such as phenols, tannins in a plant is responsible for the main deterrent effects against specific herbivore species (Moore et al. 2004). Although, the process of determining which PSM is effective can be complicated by various biotic and abiotic reasons (Moore et al. 2004). A particular PSM might be effective against a particular herbivore species than others and sometimes different PSM groups in the same plant may affect differently on different herbivore species (Moore et al. 2004).

1.2 Cost of Defence

Plants are exposed to different levels of herbivory throughout their life as they develop from seed to seedling then juvenile to mature stages in their life (Boege and Marquis, 2005). Therefore, the level of herbivory pressure on plant defensive qualities may vary during the development stages of plants which are then affecting the type and the amount of defensive compound they produce throughout their life. The level of the defensive compound they produce may also be affected by establishment, growth, reproduction, and resource

allocation as well (Boege and Marquis, 2005). Although higher concentrations of PSM give more resistance to the plant, the production of PSM is believed to be costly and this cost will reduce plant growth and reproduction while maintaining genetic variation of defence within the population (Siemens et al. 2002).

It is believed that the cost associated with the plant defence is more apparent under stressful conditions created by the low soil nutrients, water, and low light conditions (Siemens et al. 2002). According to Tuomi (1992) plants have a limited pool of resources to allocate for different plant functions such as growth, differentiation of cells, and defence of plants. However, if the environment is favorable vegetative growth generally receives higher resource priority than plant defence or storage (Tuomi, 1992). The cost associated with plant defence is because of the allocation of limited resources for the PSM production process that can be used in plant growth and reproduction-related functions thus reduces the growth and other competing physiological processes (Ballhorn et al. 2014). Plants must efficiently distribute the limitedly available photosynthates between plant growth and plant defence processes (Ballhorn et al. 2014). Furthermore, plants commonly use the same precursors and intermediate molecules for their primary and secondary metabolic pathways (Ballhorn et al. 2014). Therefore, PSM costs to a plant because production, transport, storage, activation of these compounds reduce the plant fitness due to reduced growth and reproduction (Marak, Biere and Van Damme, 2003).

In addition, functional prioritisation of growth, development, storage, resistance, and reproduction change during different life stages of the plant when they develop and these differences require changes in resource allocation (Koricheva, 2002). For an instance, when plants reached reproductive age flowers and fruits acquire more resources for their production and defence that were previously stored or that were allocated for the shoot and root production. However, there is always sequential growth and development of tissues within the plant, and this receives a high priority over plant defence (Koricheva, 2002). Plant life-history changes, environment, and within plant and cell tradeoffs are also important in determining resource allocation for plant defence (Boege and Marquis, 2005). Therefore, investing plant resources in producing, transporting, and storing plant secondary metabolites is a cost to a plant and should only occur when and where these protective efforts could be pay off the cost (Tuomi, 1992).

There are numerous hypotheses and concepts to explain how external changes like environmental conditions can affect the intraspecific variation of the amount and the types of PSM plant produces (Berenbaum 1995, Koricheva, 2002). These hypotheses explain the quantitative and qualitative patterns of variation of PSM (Holopainen et al. 1995). All these hypotheses generally predict that under resource-rich habitats plants are less defended by C-based secondary metabolites than resource-poor environments because they can compensate for herbivory by the higher growth plants acquired in resource-rich environments (Price, 1991). Both of the below hypotheses assume that defence allocation will increase under conditions of limited growth while photosynthesis remains at the same level (Price, 1991).

1.3 Hypotheses on Plant Defence

1.3.1 Carbon nutrition balance hypothesis

The Carbon nutrition balance hypothesis (CNBH) by Bryant et al (1983), predicts how carbon and the nutrient content in the environment can influence the phenotypic expression of defence of plants and this theory was developed to explain the impact of soil nutrients and shade on plant defensive chemistry through the effect on the carbon: nutrient ratio of the plant (Stamp, 2003). According to this hypothesis if the carbon: nutrient ratio acquired by the plants controls the allocation of photosynthate to different plant functions then the phenotypic expression of the plant defensive will be affected (Stamp, 2003). This hypothesis predicts that the concentration of C-based secondary metabolites will be positively correlated with C: nutrient ratio of the plants (Herms and Mattson, 1992). On the other hand, N-based PSM will be inversely correlated with the C: nutrient ratio of the plant (Herms and Mattson, 1992).

The nutrient-deficient conditions limit plant growth more than reduces photosynthesis (Herms and Mattson, 1992). Therefore, if the environment is nutrient-deficient carbohydrates will accumulate within the plant and it will increase the C: nutrient ratio within the plant (Herms and Mattson, 1992). The excess carbohydrates that accumulate than growth requirements will be allocated to C-based secondary metabolites (Herms and Mattson, 1992). In nutrient deficient conditions plant photosynthesis rate also gets reduced because RuBP carboxylase, chlorophyll, and phospholipid contents also getting reduced. Under increased

nutrient availability as growth getting the higher priority, it reduces the C: nutrient ratio within the plant which will result in decreased C-based secondary metabolites (Herms and Mattson, 1993). Nutrients accumulating more than growth requirements will be allocated to N-based secondary metabolite production (Herms and Mattson, 1992).

The light intensity can also affect the C: nutrient balance within the plant and thereby PSM concentration as well (Herms and Mattson, 1992). According to Herms and Mattson (1992) shade level reduces C accumulation within plants more than it reduces nutrient absorption. As the available carbohydrates have been allocated to growth, the C: nutrient ratio within the plant will decrease which will result in a decrease concentration of C-based secondary metabolite concentration. In shade conditions nitrogen that has been accumulated more the growth rate will be converted to N-based secondary metabolites (Herms and Mattson, 1992). Under higher light levels photosynthesis get increases and results in a higher C: nutrient ratio which will increase C-based secondary metabolites (Herms and Mattson, 1992). As available N will allocate to photosynthesis and growth the concentration of N-based secondary metabolites get reduces (Herms and Mattson, 1992).

Because of the failure of this concept to consider the adaptive changes in PSM this hypothesis is considered as narrowed and refined (Moore et al. 2004). Therefore, this concept now only use to predict the plastic responses of particular plant genotypes to variation of resources and only for few C-based PSMs (Moore et al. 2004).

1.3.1 Growth differentiation balance hypothesis

This is a conceptual model explaining plant resource allocation to growth-related functions and differentiation-related functions under various environmental conditions (Stamp, 2003). Further, it concludes that physiological limitations result in a trade-off between the plant growth and differentiation processes and gives resistance again herbivores (Price, 1991). According to Herms and Mattson (1992), there is always a trade-off taking place between growth and plant defence. Herms and Mattson (1992) further mentioned that any factor which reduces growth more than it reduces photosynthesis increases the resource pool available to allocate secondary metabolism. Growth-related functions include the production of roots, leaves, fruits, and other plant parts and any function requires cell division and elongation (Stamp, 2003). Differentiation includes the modification of existing structures of

cells and functions. An example of a differentiation function is the production of secondary metabolites and the production of their storage organs including trichomes and glands repelling herbivores (Herms and Mattson, 1992).

However, photosynthesis is less affected by the environmental constraints, and therefore there will be more carbohydrates available excess than growth requirements within the plant to be converted into plant secondary metabolites (Herms and Mattson, 1992; Massad et al. 2012). Under resource limiting conditions occurred by extreme environments will result in I accumulation of carbohydrates and thus increased concentrations of PSM (Herms and Mattson, 1992). The growth and the photosynthesis of the plants under higher resource availability are not limited and therefore allocate a larger portion to growth than plant defense (Stamp, 2003).

This hypothesis was developed based on the empirical observations from plant species in boreal and temperate systems (Hattas, Scogings and Julkunen-Tiitto, 2017). Even though, this hypothesis is using to describe the resource allocation of different species under different abiotic and biotic environments making its predictions questionable and problematic (Hattas, Scogings and Julkunen-Tiitto, 2017). An experiment conduct on African savanna woody species showed that PSMs under nutrient limitation showed a compound-specific response across the N gradient (Hattas, Scogings and Julkunen-Tiitto, 2017). They found that condensed tannin concentration was not affected by N treatments. But flavonol glycoside concentration showed a decreasing logarithmic response and quercetin glycoside showed a decreasing logarithmic response with N treatments. However, allocation to individual components are not correlated to the total allocation of PSMs (Hattas, Scogings and Julkunen-Tiitto, 2017). Another study conducted on *Pentaclethra macroloba* which is a dominant tree species in tropical forests in Costa Rica discovered that the production of flavans and saponins was not costly to the plant and the trade-off predicted by GDBH was only present between flavans and biomass of sun-grown plants (Massad, Dyer and Vega C., 2012).

However, none of these hypotheses appear to provide universal explanations of plant allocation to defense. According to Donaldson, Kruger and Lindroth (2006), the number of experiments that had been conducted to understand the cost associated with the plant defense is equal to the number of trials that fail to explain this cost. Although most of the

studies are focusing on identifying the cost, the factors which cause this cost, to what extent the environmental factors can mediate these costs, and where and under what circumstances these costs occur, are still unclear for most plant species (Donaldson, Kruger and Lindroth, 2006).

1.4 The genus *Eucalyptus*

The genus *Eucalyptus* belongs to the family Myrtaceae and is a species-rich genus that contains more than 800 different plant species divided into 13 subgenera (Santos et al, 2019). Although only a few species are distributed across Australian forests and woodlands, the genus *Eucalyptus* plays an important role in Australian vegetation by dominating more than 90% of the Australian forests and woodlands (Lawler et al. 2016). These species have limited dispersal ability and higher genetic variation within and between populations (Drake et al. 2017). *Eucalyptus camaldulensis* is the most widespread species in all mainland states in Australia (Butcher, McDonald and Bell, 2009). These species are now successfully introduced worldwide where the climate is favorable and also used in lowering the water table as well (Brezáni and Šmejkal, 2013).

Many of the *Eucalyptus* plants can survive and recover from fire and other damaging factors by posing special adaptations such as lignotubers that stop their growth under unfavorable conditions and successfully resume their growth under suitable environmental conditions (Moore, 2015). Lignotubers can replace the root system if they get damaged as well. Normally, most of the seedlings produce lignotubers as part of their growth and some Eucalyptus species do not have a visible seen swollen lignotuber (Moore, 2015). Lignotubers may be present under the soil level, or they may be incorporated into the trunk and when the plant grows it also increases the diameter. According to Moore (2015), there is an intraspecific variation of the size and the responses of the lignotuber for environmental variations.

1.5 PSMs in *Eucalyptus*

Leaves of the genus *Eucalyptus* have a great diversity of PSMs which varies qualitatively and quantitatively between species and quantitatively within species (Butcher, McDonald, and Bell, 2009; McKiernan et al. 2012; McKiernan et al. 2014; Henery et al. 2008). An experiment on volatile oils of *Eucalyptus* species resulted that volatile oil was quantitatively different

between juvenile and old leaves (Li, Madden and Potts, 1996). Another experiment resulted in a significant difference in foliar oil concentration between *E. pauciflora* leaves from different populations (McKiernan et al. 2012). However, phenotypic expression of these compounds is under strong genetic control (Freeman et al. 2008). According to King et al. (2004), PSMs of genus *Eucalyptus* such as UBFs, terpenes, and sideroxylonals are stored in the foliar glands, but other phenolics are likely to be stored in vacuoles of cells. Most of the PSMs of *Eucalyptus* species produces are constitutively expressed and there are some PSMs induced by herbivores and pathogens (Andrew et al. 2010). Both of these defensive responses require resources away from growth and reproduction processes and are considered to be costly to plant (Keeling and Bohlmann, 2006). However, inducible defense is believed to be less costly because it uses the resources only in the presence of herbivores and their attack on plants (Keeling and Bohlmann, 2006). However, plant defense of genus *Eucalyptus* is highly constitutional and inducible defense has not been successfully demonstrated yet (Rapley et al. 2007; Henery et al. 2008). Henery et al. (2008) observed no induced defense was presented in *E. grandis* and concluded that constitutive defense is important in giving defence against the herbivore on *Eucalyptus* species. In another experiment by Rapley et al. (2008), foliar tannins of *E. globulus* gives rapid constitutive defense and not a delayed induced defense, and no induced defense was detected in *E. globulus*.

PSM concentration is the main determinant of the diet choice of the folivores such as Koalas and Possums (Marsh, Wallis and Foley, 2003; Moore et al. 2004). A single PSM compound or a small subset of PSM compounds act as the main deterrent against both vertebrate and invertebrate herbivores (Marsh et al. 2019). A study by Wallis, Watson and Foley (2002), showed that sideroxylonal concentration is the main feeding inhibitor for the brushtail possums, and the intraspecific variation of the concentration of sideroxylonals determines the susceptibility of the *E. melliodora* leaves to herbivores. They further observed that possums prefer the leaves with a lower concentration of sideroxylonals. *Eucalyptus* species belong to the subgenus *Eucalyptus* contain the antifeedant compound unsubstituted B-ring flavanones (UBF) making them less palatable to Koalas and common brushtail possums than the *Eucalyptus* species belong to subgenus *Symphyomyrtus* (Marsh et al. 2019). Different *Eucalyptus* species contain a different subset of UBFs and different concentrations of UBFs between different individuals which can affect the feeding behaviors of marsupial folivores

(Marsh et al. 2019). Another study by Lawler et al. (1998) found that both tannins and phloroglucinol of *E. ovata* and *E. viminalis* leaves act as antifeedant compounds against mammalian herbivore ringtail possum. Higher concentrations of PSM also reduces the availability of nutrients to herbivores by reducing the protein availability within leaves, and some PSM acts as toxins as well (Moore et al. 2004). Normally, higher marsupial folivores can be observed in *Eucalyptus* communities with leaves containing higher nutrient concentrations grow on fertile soil (Moore et al. 2004).

1.5.1 Formylated phloroglucinol compounds

Formylated phloroglucinol compounds (FPC) are a class of C-based constitutive defensive compounds unique to plants in the family Myrtaceae, especially in the *Eucalyptus* species (Eyles, Davies and Mohammed, 2003; Eschler et al. 2000). Among the plants belonging to the genus *Eucalyptus*, this form of the compound is only present in plants belonging to the subgenera *Symphyomyrtus* and *Alveolata* (the latter contains a single species, *E. microcorys*). Eschler et al. (2000) reported that all the observed *Eucalyptus* species from the subgenus *Eucalyptus* lack euglobals, macrocarpals and sideroxylonals in their foliar extracts and this is not a result of polymorphism. A study from Anekonda et al. (1999) proposed that *Symphyomyrtus* species are more successful in adapting to diverse environments and highly resistant to insect attack compared to the subgenus *Eucalyptus* (Eschler et al. 2000).

According to Moore et al. (2004) and Moore et al. (2005), these lipophilic phenolic compounds act as antifeedant compounds for most of the marsupial folivores (Lawler et al. 2000) and this is the most important factor which determines the number of leaves eats by the marsupials from a single *Eucalyptus* tree (Moore et al. 2005; Lawler et al. 2000; Wallis et al. 2002). Even within the same *Eucalyptus* species, there is a great variation in the type and the quantity of the FPC present (Moore et al. 2005; Lawler et al. 2000). According to Eschler et al. (2000), *Eucalyptus* plants growing closer to each other within the same population can also greatly vary in their FPC concentrations.

FPCs are formylated phloroglucinol-based derivatives with an attached monoterpene or sesquiterpenes moiety to a formylated phloroglucinol ring (Eyles, Davies and Mohammed, 2003; Santos et al. 2019). Eyles, Davies and Mohammed (2003) further described that there are two main classes of FPCs known as marcocarpals and euglobals (Figure 1). The simplest

FPC is the jensenone, a fully substituted formylated acyl phloroglucinol with no terpene moiety attached (Moore et al. 2004). This structure makes the basic unit for some FPCs, dimeric acyl phloroglucinols that do not have an attached terpene moiety such as sideroxylonals, and also, jensenones combine with mono or sesquiterpenes to make other complex FPCs such as marcocarplas and euglobals (Eyles, Davies and Mohammed, 2003). Moore et al. (2004) found that jensenone is very rare in *Eucalyptus* plant extracts because it acts only as a precursor of large FPCs.

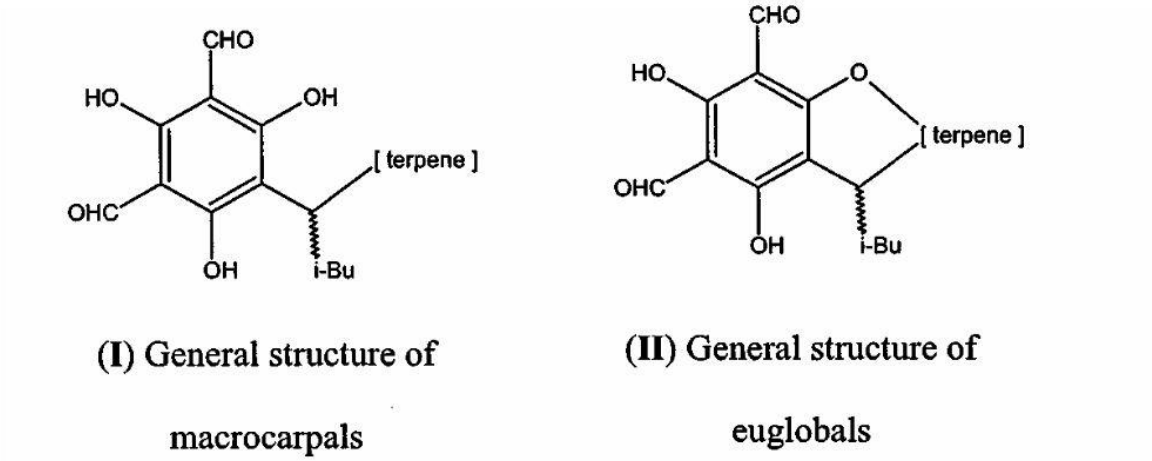

Figure 1: Different Structures of two main classes of FPCs in *Eucalyptus* species (Eyles, Davies and Mohammed, 2003).

Sideroxylonal is a simple form of FPCs with two isopentyl di formyl phloroglucinols without any attached terpene group to it and can be found in leaves and buds of some species of the genus *Eucalyptus* (Eyles, Davies and Mohammed, 2003). Sideroxylonal A, Sideroxylonal B, and Sideroxylonal C are the three isomers identified (Figure 2) (Wallis et al. 2002; Eschler et al. 2000). Among these isomers, Sideroxylonal A is the most dominant form followed by the Sideroxylonal C and Sideroxylonal B occurs only in traces (Wallis and Foley, 2005). There is a great intraspecific variation of Sideroxylonal concentration (Wallis and Foley, 2005). For example, Lawler et al (2000) reported that Leaves of *E. polyanthemos* contain 0 to 13 mg of Sideroxylonal per 1 g of dry matter. According to Wallis et al. (2002), *E. melliodora* leaves contain high sideroxylonal concentrations ranging from 0 to 52 mg per 1g of dry matter. Further, they mentioned that the ratio of Sideroxylonal C to Sideroxylonal A is constant for all plants.

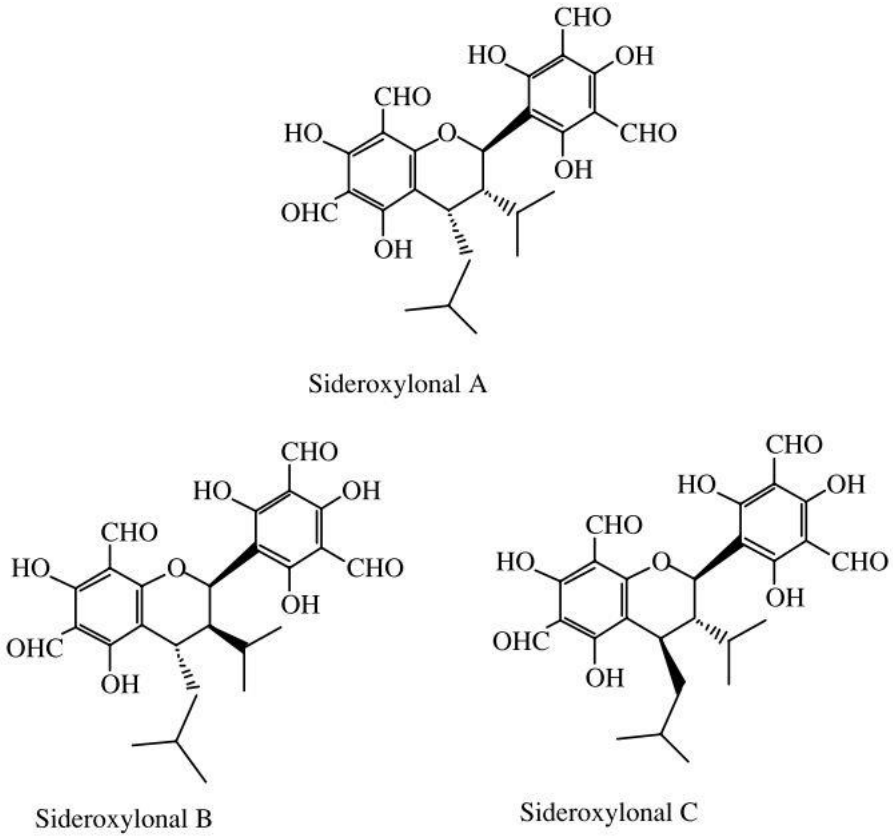

Figure 2: Different Sideroxylonal structures present in *Eucalyptus* species (Wallis and Foley, 2005).

1.5.2 Terpenes

Terpenes are the most diverse group of plant secondary metabolites in the genus *Eucalyptus*. Nearly, all *Eucalyptus* species produce terpenes, which are volatile organic compounds. Most abundant terpenes in plants are stored in various specialized structures which differ in their form and their location between species (King et al. 2004). For example, resin ducts in leaves and woods of conifers, glandular trichomes in *Mentha* species and *Salvia* species, and internal foliar oil glands in the Myrtaceae family (King et al. 2004). Genus *Eucalyptus* are higher in terpenoids concentrations and also contain a higher amount of cavities that store various mixtures of mono and sesquiterpenes (Goodger et al. 2013). For example, the foliar terpene content of the Australian *Eucalyptus* species is more than 7% of the fresh weight of the leaves (King et al. 2004). Therefore, terpenes possess the highest cost for the plant in terms of biosynthesis because of their high level of chemical reduction and also in terms of storage as allocation of space to specialized structures is costly (King et al. 2004).

According to Bustos-Segura et al. (2017), these terpenes could be one of the reasons which determine the success of *Eucalyptus* in Australia. Bustos-Segura et al. (2017) observed four dominant chemotypes in *E. camaludensis* known as 1,8-cineole, g-terpinene, α- and β-phellandrene and the 1,8-cineole chemotype is the most abundant of them all. In another experiment by Stone and Bacon (1994), identified two distinct *E. camaludensis* populations based on their terpenoid and cineole content and population with higher genetically determined 1,8-cineole concentration experienced a low level of herbivory than other population. Wallis et al. (2011) observed three different chemotypes characterized by specific terpenes which reflected the difference between three closely related *E. globulus* species.

1.5.3 Unsubstituted B-ring flavanones

Unsubstituted B-ring flavanones (UBFs) are another group of antifeedant compounds found in *Eucalyptus* leaves especially in plants belongs to subgenus *Eucalyptus* (Saraf et al. 2015) which is the most recently identified PSM present in *Eucalyptus* (Tucker et al. 2010). Marsh *et al* (2019) found that there is a great variation of total UBF concentration between *Eucalyptus* species and even within species. For an instance, the mean UBF concentration of *Eucalyptus muelleriana* was 0.2mgg^{-1} per dry weight and it was 105.7 mgg^{-1} per dry weight for *E. mediocris* with a range of 78.2 mgg^{-1}to 141.3 mgg^{-1}. According to Marsh et al. (2019), different *Eucalyptus* species contain a different subset of UBFs and some species show chemotypic variation between individuals within species that change the feeding preferences of marsupials. Goodger et al. (2016) found that flavanones are specifically located in foliar glands rather than distributed through leaf tissue. According to Goodger et al. (2016), from the glandular extracts of *Eucalyptus* species, flavanone pinocembrin was found as the main constituent of non-volatile foliar gland extracts of the subgenus *Eucalyptus*. The presence of pinocembrin and other unsubstituted B ring flavanones in the subgenus *Eucalyptus* is identified as a consistent chemical difference between the two largest *Eucalyptus* subgenera Symphyomyrtus and *Eucalyptus* (Saraf et al. 2015).

1.5.4 Tannins

Tannins are one of the abundant PSM produce by *Eucalyptus* mostly ranging from 5% to 10% of the dry weight of the leaves (Barbehenn and Constabel, 2011). They are the most chemically complex plant polyphenols with varying molecular sizes and complexity (Marsh et al. 2017). It

was believed that their protein precipitation ability decides the anti-herbivore activity as high tannin concentration reduces the nutritional value of the *Eucalyptus* foliage thus making it less palatable for animals (DeGabriel et al. 2009). Further, it was also believed that the oxidative ability of tannins gives the anti-herbivore qualities for tannins (Salminen et al. 2011; Barbehenn and Constabel, 2011). These compounds have widely distributed in various cells and tissues all over the plants and often accumulating in or near to external surfaces of plants organs in keeping with their defensive role (Hutzler et al. 1998).

Tannins are classified into two classes known as condensed tannins (CT), which are also known as proanthocyanidins (PA), and Hydrolysable Tannins (HT) (Barbehenn and Constabel, 2011). Among them, HT is divided into two main subgroups as gallic acid derivatives and ellagitannins (Barbehenn and constable, 2011). As mentioned in Marsh et al. (2017) biosynthesis of both HT and CT use the shikimic acid pathway. Therefore, the production of large quantities of HT might directly reduce the production of CT in large quantities due to the competition for the precursors. Large CT can precipitate proteins easily and, in contrast, HT especially ellagitannins can be oxidized very fast than CT. According to the findings of Marsh et al. (2017), HT makes the large proportion of tannins for most of the *Eucalyptus* species. They further mentioned species with the same concentrations of total tannins can have different combinations of CT and HT.

1.5.5 Cyanogenic glucosides

Finally, cyanogenic glucosides are the only example of N-containing PSM compounds in *Eucalyptus* which provides the constitutive defence for the plant (Gleadow and Woodrow, 2000). All of the species that have been identified to produce cyanogenic compounds so far are from the subgenus Symphyomyrtus (Gleadow et al. 2008). According to Gleadow et al. (2008), only 23 *Eucalyptus* species are known to be cyanogenic approximately representing 4% of the genus. Cyanogenesis is a process mediated by an enzyme that releases toxic hydrogen cyanide from cyanide-containing precursors in response to cell damage (Selmar, 1993). This means they produce defensive compounds only after wounding the tissue. Tissue disruption initiated the cleavage of sugar moiety from the cyanogenic glucoside followed by the degradation of the cyanohydrin to produce HCN and an aldehyde or a ketone (Gleadow and Woodrow, 2000). These products, especially HCN are highly toxic for herbivory animals.

The amount of HCN release is directly correlated to the amount of tissue damage (Ballhorn et al. 2011). As this cyanide precursor contains nitrogen within its structure, cyanogenesis is considered to be more costly for the plants than C-based secondary metabolites (Ballhorn et al. 2014). Although the production of these compounds limits the resources available for growth and reproduction, 10% of vascular plants species produce cyanogenic glucosides as their defensive compound (Ballhorn et al. 2014). According to a study conducted by Gleadow and Woodrow (2000), they found that *E. cladocalyx* allocates up to 20% of leaf nitrogen to cyanogenic glucosides and high concentrations of cyanogenic glucosides were observed in young and developing reproductive and vegetive tissues compared to mature tissues.

1.6 Research question and Hypothesis

Abiotic environmental factors of the habitat decide the resource availability within the plant, then affect the plant growth. The development of plant defensive traits also varies with resource availability and plant growth (Yamawo and Hada, 2010). Light condition is an important factor in determining plant growth as it directly affects photosynthesis and impacts the development of defensive traits (Herms and Matson, 1992).

Many experiments have been conducted to examine how various environmental factors affect plant secondary metabolite concentration in *Eucalyptus*. Yet none to date have identified trade-offs against the defence in *Eucalyptus*. The exact location where this trade-off occurs, and under what environmental condition does this trade-off become apparent, which are still unclear. Plants tend to minimize the cost of plant secondary metabolite production as it is highly cost for the plant.

Further, as natural selection is acting upon plants the surviving plants contain the preferred level of the defensive profile which is favored by the environment. This study aims to identify test the presence of trade-offs under different nutrient and light availability. Also, this study aims to identify the location where these trade-offs occur under nutrient deficient and shade environments. One hypothesis of this study is that trade-off between plant growth parameters and defensive chemicals should occur under low light and low nutrient conditions and the plant growth parameters such as above groundmass, below groundmass, lignotuber mass, and root mass should have a negative relationship with plant secondary chemical concentrations.

According to Agrawal (2007), the defense is pre-determined by the genetic material. FPCs, in particular, are constitutively determined and the initial experimental design had been to identify chemotype individuals before allocating them to experimental treatments, but COVID lockdowns prevented it.

Chapter 2- Impact of light on resource allocation of *Eucalyptus*

2.1 Introduction

Light conditions in forests and woodlands are highly variable and can create a great intraspecific variation in seedlings and saplings growing under different light conditions (Nichols-Orians, 1991). For an instance, light conditions in forest understory are ten to twenty times less than the light conditions in treefall gaps. Changes in light conditions can strongly impact the physical growth, secondary chemical concentration, and leaf nutritional quality of plants (Barber and Marquis, 2011; Hirano et al. 2019). The plant genotypes that have been distributed through an environmental gradient show gradual changes in their phenotype according to the environment (Humphrey et al. 2018). These modified phenotypes arise when natural selection acts upon the genotypes that have been favored by the gene flow (Humphrey et al. 2018). Janzen-Connell's hypothesis predicts that the diversity of plant communities is maintained by specialist natural enemies like herbivores and pathogens (Pommerening, Wang and Zhao, 2020). According to this hypothesis, the survival rate of the seedlings from the same species is reduced if they are located close to reproductive adults and in areas with high conspecific diversity where shade is more likely high and the survival rate is high when they are far away (Pommerening, Wang and Zhao, 2020; Comita et al. 2014; Zhu et al. 2013).

The effect of light on the phenotypic variation of plant growth and plant chemistry phenotypes is hypothesized in the C: nutrition balance hypothesis (Bryant et al. 1983). This hypothesis predicts that light intensity can change the C: nutrient balance within the plant and thereby it changes the PSM concentration within the plant. According to Herms and Matson (1992), shade conditions decrease the C: nutrient ratio within the plant by reducing C accumulation than nutrient uptake. Therefore, the concentration of C-based secondary metabolites reduces because limitedly available C is allocating to growth. In contrast, higher light intensity is expected to increase the photosynthesis and thereby increases the C: nutrient ratio within the plant which will increase the C-based secondary metabolites. Shading generally decreases the plant resistance to herbivores (Stamp, 2003). Shade increases the concentration of N-based secondary metabolites and decreases C-based secondary metabolites. However, under high light intensity. the concentration of N-based PSM decrease as N is allocated to photosynthesis and growth processes (Burns, Gleadow and Woodrow, 2002).

According to GDBH light limitation has a more negative effect on photosynthesis than plant growth (Stamp, 2003). Growth and secondary metabolism processes compete for available photosynthate, and priority is given to plant growth. Therefore, under the source limitation caused by shade conditions the predictions of GDBH behave the same way as the C: nutrient balance hypothesis (Herms and Mattson, 1992). GDBH predicts concentrations of C-based PSM decrease under low light levels because of the lack of C compared to other nutrients such as nitrogen (Hirano et al. 2019). Under high light level, both photosynthesis and plant growth are not limited and a large proportion of available photosynthate will allocate to photosynthesis (Herms and Mattson, 1992) and secondary metabolism will increase proportionally with growth (Stamp, 2003).

Plants exhibit adaptations to different stresses including light availability, competition and herbivory with a potential to express a tradeoff within the plant (McGuire and Agrawal, 2005). Both the shade avoidance response and defense against herbivory are considered important adaptations to mitigate the intrinsic effect from external stresses (McGuire and Agrawal, 2005). Under low light levels, plant changes their physiological and morphological responses to increase photosynthetic production and to maintain defensive ability against herbivores (Takahashi et al. 2001). Compared to sun-grown plants shade plants are thought to have thinner, less tough, less trichomes, higher specific leaf area, and higher N content (McGuire and Agrawal, 2005). Both plant photosynthesis and allocation pattern for growth and differentiation processes depend on the availability of resources such as light (Agrell, McDonald and Lindroth, 2000). Differences in resource availability can create a plant-to-plant variation in defensive chemistry in many plants (Wilkens, 1997). Although light intensity can alter the PSM concentration among different plant species (Agrawal, 2007; Yamawo and Hada, 2010) the response varies depending on the plant species and the type of the PSM (Estell et al. 2016). In an experiment conducted by Agrell, McDonald and Lindroth (2000), condensed tannin concentrations of aspen, birch and maple were increased with increased light intensity and the phenolic glycoside concentration in aspen also increased as predicted by CNB and GDBH as a response to increased resource availability. In another experiment, foliar C-based secondary metabolites such as phenolics were increased under high light environments and the results were consistent with C: nutrient theory (Hemmin and Lindroth, 1999).

According to Barber and Marquis (2011), leaves of plants exposed to high light levels were thicker, contained lower N and higher phenolics content than the leaves in plants under shade. Although the high light leaves are less in nutritional quality with less foliar N making them less palatable to herbivores, the number of herbivores and leaf damage was higher in sun plants than shade plants (Barber and Marquis, 2011). Dudt and Shure (1994) in their experiment found that shade-tolerant *Cornus florida* seedlings contained higher levels of total phenolics and hydrolysable tannins than shade intolerant *Liriodendron tulipifera* seedlings. However, *Cornus florida* contained less condensed tannin giving consistent results with the C: nutrient balance hypothesis. These results were parallel to the theory explained by C: nutrient hypothesis. Chacón and Armesto (2006), observed that although seedlings in open canopy gaps in a temperate rain forest at Chiloé Island, Chile produced higher concentration of phenolics than the seedlings in forest interior still seedlings in open canopy gaps received higher herbivore density than the seedlings in forest interior. Leaf phenolic concentration seems to increase frequently under high light conditions (Close and McArthur, 2002). This may be evidence for the antioxidant capacity of range of phenolics by protecting plants from photodamage under cold conditions causing photoinhibition (Close and McArthur, 2002). Chacón and Armesto (2006), also proposed that increased phenolic concentrations in seedlings at canopy gaps than forest interior is because of the phenolics are involving in protecting plant from photo damage, rather than acting as a anti-herbivory compound.

Plants' responses to their light environment are expressed within the plant at several Levels (Evans and Pooter, 2001). Firstly, at the whole plant level by changing the fraction of biomass allocated to a different part of the plant like leaves, stems, and roots. Secondly, at leaf level by changing their leaf anatomy such as changing the leaf area per unit biomass invested in leaves. Finally, changing the investment of leaf carbon and nitrogen between leaf components like photosynthetic enzymes, phytochrome system, and PSM Levels (Evans and Pooter, 2001). In an experiment done by Kruse et al. (2020), both *E. grandis* and *E. regnans* produced thicker leaves with increased light intensity, and the Leaf mass per area (LMA) of both species was also increased with irradiation. Leaves growing under full sunlight have larger LMA and thicker leaves than leaves growing under shader environments (Evans, 2006). King et al. (2006) and King et al. (2004) found that there is a positive correlation between the leaf mass-based and leaf area-based oil content and LMA in the oil mallee, *Eucalyptus polybractea*. King et al.

(2004) further found that when leaf LMA increases the height, and the width of the glands also increases suggesting that increase in leaf thickness results in an increase in oil gland height and volume and thereby terpene content of leaves as well. This may not be representative of all eucalypt leaves however, as *E.* polybractea accumulates unusually high terpene concentrations and has exceptionally large oil glands. Furthermore, leaf phenolic content was increased with increasing LMA in *Eucalyptus polybractea* leaves when expressed as area basis but not on a mass basis suggesting that phenolics are not stored in trichomes on the leaf surface (King et al. 2004). Localization of phenolic compounds like tannins, sideroxylonals in subdermal glands on *Eucalyptus* leaves is known to be a support for their role as a defensive compound for herbivores (Salminen and Karonen, 2011). However, the way light availability can affect the growth and the vegetative phase changes of the *Eucalyptus* leaves is very lightly described in the literature (James and Bell, 2000).

Therefore, this study focuses on a) how *E. melliodora* and *E. camaldulensis* seedlings respond to three different light conditions by changing their growth parameters b) how intraspecific variation of sideroxylonal and phenolics concentrations in *E. melliodora* and *E. camaldulensis* seedlings occur under three different light conditions c) to identify the trade-off offs in growth and storage parameters associated with intraspecific variation in constitutive defence levels in *E. melliodora* and *E. camaldulensis* under varying light environments. I have hypothesized in this study that a) full sunlight seedlings will exhibit higher plant growth and plant growth reduces with decreasing light level b) both *E. melliodora* and *E. camaldulensis* seedlings grown under full sunlight level result in higher total phenolics and higher sideroxylonal concentration and the concentrations to be reduced with reducing light level c) trade-off between growth and defence is present in low light seedlings for both *Eucalyptus* species. To test these hypotheses seedlings from *E. melliodora* and *E. camaldulensis* were grown in three different light levels and their growth parameters and total phenolics and sideroxylonal concentrations were recorded to identify the patterns.

2.2 Materials and Methods

2.2.1 Selecting plant Species

Seeds of *E. melliodora* (CSIRO seedlot provenance 21387), *E.camaldulensis* (CSIRO seedlot provenance 20437), and *E. diversifolia* (CSIRO seedlot provenance 20690) were selected based on the foliar secondary chemical variation of seedlings. *E. melliodora* and *E. camaldulensis* are well known for FPCs and *E. diversifolia* is known for UBFs. All these *Eucalyptus* seeds were acquired from CSIRO, Tree Seed Centre, Australia. From each *Eucalyptus* species, approximately 200 seeds were selected. Cold stratification was used to overcome the seed dormancy where the seeds from each species were sowed on moist tissue paper, and this was put into a clear resealable bag. The bag was sealed well to prevent it from drying off and stored in a refrigerated environment at 4^0C on 6th of February 2019 for three weeks to facilitate germination. Plastic bags were observed regularly to prevent fungus formation and watered if necessary.

2.2.2 Planting

Figure 3: *Eucalyptus* seeds growing in seed trays.

After the stratification period seeds from three different species were planted on seed trays using a commercially available "Native" premium commercial potting mixture (Debco® Pty. Ltd., Tyabb, Vic.) on the 4th of March 2019 (Figure 3). After the germination when seedlings have emerged, healthy seedlings were transferred to plastic pots (65 x 65 x 150 mm) on the

25th of March 2020. The "Native" premium commercial potting mixture (Debco® Pty. Ltd., Tyabb, Vic.) was used for transferring the seedlings. However, *E. diversifolia* was not very successful, and very few seedlings survived. Only *E. melliodora* and *E. camaldulensis* were successful and many of the seedlings were survived. Therefore, *E. melliodora* and *E. camaldulensis* were selected for the experiment. From each species, 300 seedlings were transplanted. Plants were initially kept in growth chamber with 25^0C temperature and 12 hours light-dark photoperiod. Plants were manually watered daily or as required. Pots were kept in the controlled growth chambers for further growth before the experiment started.

Figure 4: *E. melliodora* and *E. camaludensis* seedlings in pots growing in a growth chamber.

By the 14th of April 2020, most of the seedlings appeared to be suffering from blistering and eventually senescence that occurred on the leaves. This is because the leaves were growing under artificial light conditions (Pinkard, Gill and Mohammed, 2006). Therefore, the plants were transferred to a greenhouse for the remainder of the experiments, where plants recovered, and normal leaf growth was recommenced. The intention had been to screen seedlings for individual expression of chemical defence traits (FPCs and UBFs) before the allocation of seedlings to experimental treatments, under the assumption that constitutive defence levels were genetically pre-determined for each individual. Unfortunately, the commencement of the COVID-19 pandemic and severe restrictions on access to the university prevented this from taking place, and instead, plants were allocated to treatments without chemical screening taking place. Subsequently, plants were left to continue growing, with only minimal attention for maintenance (watering).

2.2.3 Experiment Setup

The light experiment was conducted in the glasshouse premises (25°C) in the university. For the light experiment, three different light conditions were selected. Full ambient sunlight, 30% of the light, and 10% of the light were three light levels selected for the experiment. For full sunlight treatment, plants were arranged under full sunlight in the glasshouse without any shading. For the other two light levels, 30% and 10% reduced light treatments were imposed using commercially available sunblock shade cloth green shade nets with 70% and 90% shading percentages (Coolaroo, Melbourne, Australia). Shade house frames were prepared using plastic tubes and covered with shade cloth of the prepared using plastic tubes and covered using shade meshes with two different shading levels. For each treatment, 100 pots from *E. melliodora* and 25 pots from *E. camaldulensis* species were randomly assigned to each treatment.

Figure 5: *E. melliodora* and *E. camaldulensis* seedlings growing under full sunlight, 30% light and 10% light conditions.

2.2.4 Data Collection

At the end of the growing period data collection was started on the 17th of August 2020. First, plant height was recorded for each plant from the soil surface to the tip of the plant. All the leaves were harvested, and the fresh weight of the leaves was recorded. Five leaves were randomly selected from each plant and their leaf area was recorded using the LI-3100C leaf area meter (LI-COR Biosciences, Lincoln, USA). Then those leaves were put in a drying oven of

70^{o}C to determine dry mass. All the plants' remaining leaves were collected into plastic zip-lock bags with their label on them and stored in the freezer for future chemical analysis.

Stems of all plants were harvested, and fresh mass was measured. Then they were put into separate paper bags with their label and put them into the oven of 70^{o}C to determine dry weight. Lignotubers, the swollen root crown areas just before the root system, were carefully separated and fresh weight was measured. They were collected into separate paper bags with names and put in the oven (70^{o}C) for drying. The remaining root system attached to soils was taken out carefully from the pots and excess soil was removed. The roots system was separated from the soil with the minimum disturbance to the fine roots. Then they were washed gently. These washed roots were then patted dry using tissues and put into labeled paper bags. They were dried after measuring the fresh weight of root systems. All the plant parts stored in the dry oven were dried until they reached a constant weight and dry weight measurements of the leaf area leaf, stems, lignotuber (LT), and roots (RT) were recorded.

Specific leaf area (SLA) was calculated by dividing leaf area measurements of the 5 selected leaves by the dry weight of those leaves. Aboveground mass (AG) was calculated by adding the dry mass of leaves and stems together. Below ground biomass (BG) was calculated by adding the masses of dried roots and lignotubers together. Total plant biomass was calculated by adding the mass of dried leaves, stems, lignotubers, and roots together. Lignotuber mass percentage (LT%) was calculated by dividing the dry mass of lignotuber by total plant mass and multiplying it by 100 to take the percentage value [(LT/total plant mass) x100] and the root mass percentage (RT%) was calculated the same way by dividing root dry mass by total plant dry mass and multiplying it by 100 [(RT/total plant dry mass) x100]. Root: shoot ratio (Root: shoot) was calculated by dividing the dry mass of the roots by the integrated dry mass of the rest of the plant parts (RT/(AG+LT).). Mean Leaf mass (ML mass) was calculated from the dry mass of the five leaves used for leaf area determination.

2.2.5 Chemical Analysis

2.2.5.1 Sideroxylonal and other FPCs

2.2.5.1.1 Extraction

From the leaves saved for the chemical analysis three randomly selected samples were first freeze-dried and then ground to a fine powder using a ball mill (Mixer Mill MM200, Retsch GmBH, Germany). Extraction was carried out according to the rapid extraction method mentioned by Wallis and Foley (2005). First, 50 mg of leaf powder was weighed into a small glass vial containing 8 g of extraction solution. Following Wallis and Foley (2005), 7% water in acetonitrile containing 0.1% v/v trifluoracetic acid solution was used for the extractions. These samples were allowed to extract for 15 minutes and then sonicated for 5 minutes. Then they were filtered through a syringe filter into autosampler vials and analysed using HPLC together with standard solutions.

2.2.5.1.2 HPLC Technique

Sideroxylonal Analysis – *E. melliodora*

Three samples from the same plant resulted from the rapid extractions with 7% water in acetonitrile were retained in their autosampler vials, stored refrigerated (4°C) and analysed again by HPLC on a Poroshell 120 EC-C18 column (4.6x75 mm 2.7 Micron) and the column temperature was 37^0C. The isocratic elution method was used with 7% water in acetonitrile, with 0.1% trifluoroacetic acid at a flow rate of 0.75 mL/min for optimal separation of sideroxylonal compound. Sideroxylonal A, C, and B were detected at 3.72, 3.89, and 4.88 minutes respectively at 275 nm for all the *E. melliodora* samples. Total sideroxylonal concentration (TS) was calculated for each sample using a previously established standard curve and the final total sideroxylonal concentration for each plant was taken by calculating the mean value of three samples.

Sideroxylonal Analysis – *E. camaldulensis*

The analytical column for FPC analysis was an SGE GL Wakosil II 3C18RS column (250x 4.00 mm 3µm) and the column temperature was 37^oC. The extracts were eluted under gradient conditions with 0.1% trifluoracetic acid (TFA) in Acetonitrile (A) and 0.1% TFA in water(D) as follow: 60% A and 40% D for % minutes to 90% A and 10% D at 60 minutes hold for 10 minutes

with the flow rate of 0.75 ml $min^{-1.}$ A portion of 20 µl from each E. *camaldulensis* sample was injected into the system. Absorbance values were measured at 275 nm. Total Sideroxylonal concentration (TS) was calculated for each sample using the absorbance values. Total sideroxylonal concentration (TS) was calculated for each sample using a previously established standard curve and the final total sideroxylonal concentration for each plant was taken by calculating the mean value of three samples.

2.2.5.2 Total Phenolics Analysis

A portion of 50 mg of finely ground freeze-dried leaf tissue from each sample was extracted using 500 µl of 70% (v/v) of Acetone solution. This mixture was shaken for 3 minutes in a shaker with 30 Hz frequency then centrifuged (5000 rpm, 1 minute, 4^0C) for 1 minute. A 10µl of a portion of extraction was then tested for total phenolics with Folin-Ciocalteu reagent with gallic acid as the standard as described by Burns, Gleadow and Woodrow (2002). Total phenolics concentrations (TP) of each sample were calculated using the absorbance value. The final total phenolics concentration for each plant (GA equivalents; TP) was taken by calculating the mean value of three samples.

2.2.6 Statistical Analysis

Because sideroxylonal and total phenolic concentrations are traits subject to major gene effects (Andrew et al. 2007 and 2010) and are considered to be constitutive defences, I considered it to be an independent variable in models of seedling growth/morphological traits (e.g. plant height; aboveground biomass, etc). The models were only developed for full sunlight and 30% light level and 10% light was not considered due to insufficient sample sizes. To test the effects of constitutive chemical defenses and light availability on these variables, I first constructed linear models (e.g. height ~ sideroxylonal) separately for each light level. I then ran a model including both predictor variables (e.g. height ~light + sideroxylonal) including plants from both the 100% and 30% light levels, and where both terms were retained, I ran a further model including a term for the interaction of light and chemical defence. Because both FPC and TP concentrations were influenced by light levels, I assessed the relationship between these variables separately for the 100% and 30% light levels by calculating Pearson's product-moment correlations. All analyses were performed in R (Version 4.0.2).

2.3 Results

2.3.1 *Eucalyptus melliodora*

E. melliodora seedlings grown under three different light levels were different in their growth performance. From the 50 seedlings that have been grown in 10% shade level, only 16 plants survived at the end of the experiment. Plants that survived in this shade condition were very small in size and short compared to the plants in other light conditions. Stems were very fragile and green in color compared to other seedlings from light levels. Seedlings grown under full sunlight and 30% light conditions were healthy and successfully grown compared to the plants grown under 10% of light condition.

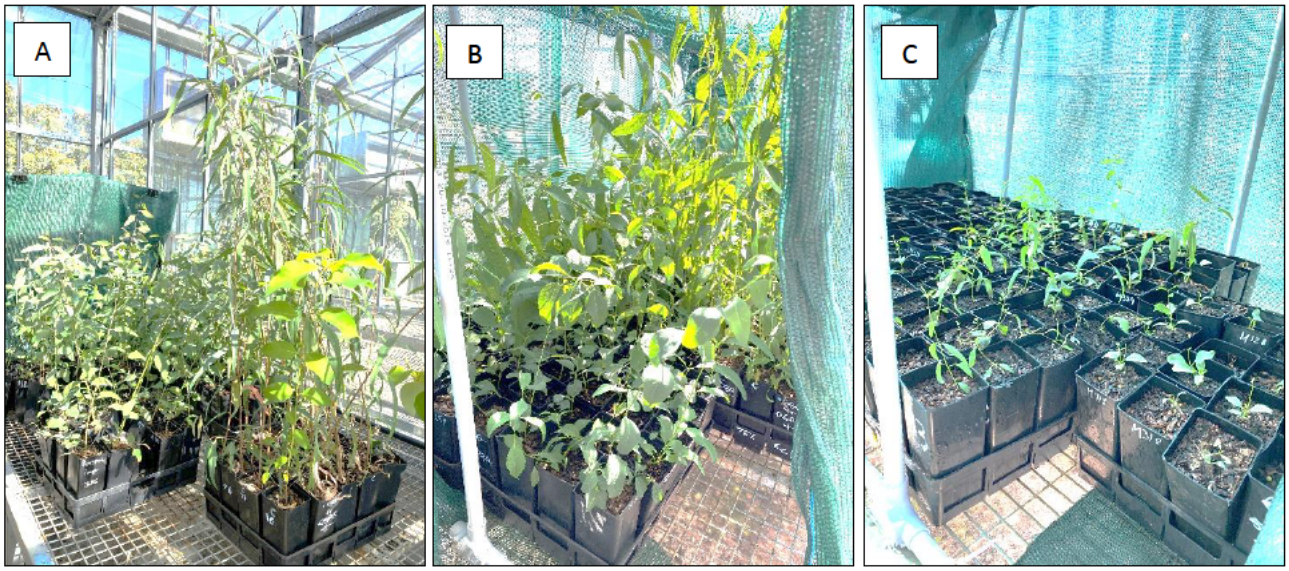

Figure 6: *E. melliodora* seedling growth at the end of the experiment. A) *E. melliodora* seedlings grown under full sunlight condition B) *E. melliodora* seedlings grown under 30% light condition C) *E. melliodora* seedlings grown under 10% light condition

During the project development stage, it was planned to identify the constitutive chemical defence composition of the seedlings and to separate them into high and low defence plants and then to introduce these high and low defence seedlings to different light and nutrient conditions to test how these high and low defence genotypes express their defence phenotype under different light and nutrient conditions. However, during the development stage of the experiment in 2020, just a few weeks after the seeds have been planted covid 19

lockdown conditions were imposed and no access was granted for laboratories for few months. During the whole growing period, watering was done by the university on a roster basis, and by the time the access was granted all the plants have already passed the planned harvesting period. Therefore, separation of seedlings into high and low defence groups could not be completed due to unexpected lockdown and the plants were kept inaccessible and could not be monitored for some time due to an unexpected Covid outbreak. As a result, the experiments had to be redesigned to identify the tradeoffs of *Eucalyptus* seedling grown under different environmental conditions.

Table 1: Total sideroxylonal and total phenolics concentration variation of *E. melliodora* seedlings under three light levels.

	Full sunlight (n=101)	30% Light (n=100)	10%Light (n=16)
Total Sideroxylonal ($mgg^{-1)}$	13.00±0.5[a]	2.94±0.2[b]	0.02±0.01[c]
Total Phenolics(mgg^{-1})	61.65±2.9[a]	39.62±2.9[b]	42.06±8.0[b]

Within a row, different letters indicate significant differences (P < 0.05) from the Least significant difference test (Mean concentration± 1 Standard error) of one-way ANOVA

Both foliar chemical concentrations differed among different light treatments of *E. melliodora* seedlings. TS was significantly different between different light conditions ($F_{1,200}$ = 300.6, p < 0.0001, R^2_{adj} = 0.59). The highest TS was observed in seedlings in brighter lights and the lowest TS was observed in 10% light seedlings (Table 1). Similarly, total phenolics concentration showed a significant difference between light treatments ($F_{1,200}$ = 27.29, p < 0.0001, R^2_{adj} = 0.11) and the highest TP was observed in full sunlight seedlings among treatments (Table 1). Higher foliar chemical concentrations were observed in plants under higher light levels and lower concentrations were observed in lower light levels.

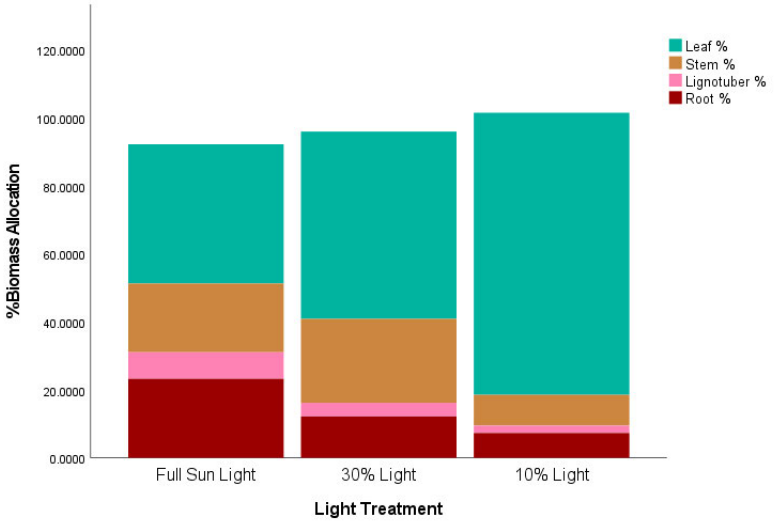

Figure 7: Biomass allocation percentage for different plant parts of *E. melliodora* seedlings.

Plants under full light conditions showed higher above ground and higher belowground masses compared to the plants under the other two light conditions. However, when comparing the biomass allocation percentages of three light treatments 10% light seedlings showed the highest percentage of allocation for leaves while full sunlight seedlings allocated the lowest percentage for leaves (Figure 7). However, 10% light seedlings have allocated the lowest percentages for roots while full light seedlings showed the highest allocation percentage for roots. Similarly, full sunlight showed highest biomass allocation percentage for lignotubers compared to the 10% light seedlings.

2.3.1.1 Plant height

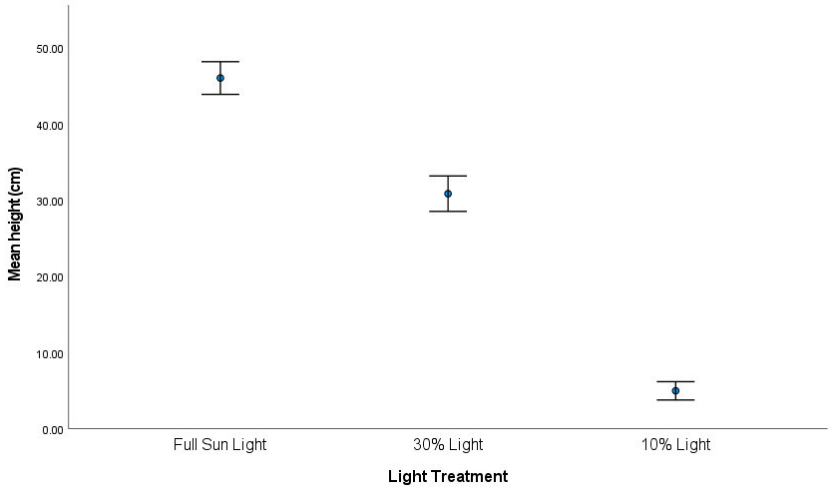

Figure 8: Plant height variation of *E. melliodora* seedlings under three different light levels
Error bars represent the means ± standard error

Mean plant height values were significantly different between three different light levels ($F_{1,200}$ = 90.7, p < 0.0001) (Figure 8). The model of the plant height explained the 30.8% (R^2_{adj}) variation in plant height observed in different light treatments. Seedlings that were grown under full sunlight were the tallest among the seedlings under different light levels and the seedlings grown under 10% of light level were the shortest (Figure 8).

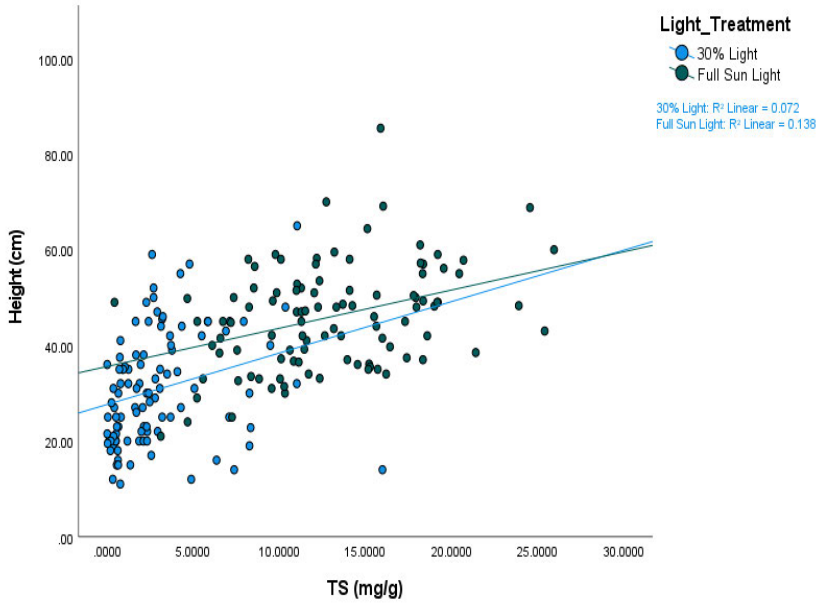

Figure 9: The relationship between the height and the total sideroxylonal concentration of *E. melliodora* seedlings of full sunlight and 30% light treatments.

According to models of plant height resulted plant height was very weakly, significantly, and positively related to TS among full sunlight treatment (slope= 0.79±0.20, $F_{1,99}$ = 15.81, p < 0.0001, R^2_{adj} =0.12) and 30% light treatment as well (slope= 1.07±0.38, $F_{1,99}$ = 7.69, p < 0.05, R^2_{adj} =0.06) (Figure 9). The model of plant height suggested that there was a very weak significant positive relationship between plant height and TS across two light levels ($F_{2,199}$ = 61.25, p < 0.0001) (Table 2). This model explained the 37% ($R^2_{adj)}$ of the plant height variation and showed that plants were taller under brighter light and when they contained higher FPC concentrations. But no significant interaction between plant height and TS across light levels was identified by the model as TS was not retained in the model.

Table 2: Estimated regression parameters, standard errors, F-values, and P-values for a linear model describing the height of seedlings in the 100% and 30% light treatments (height ~ sideroxylonal + Light).

	Estimate	Standard error	t-value	P-value
Intercept	34.6757	2.6289	13.19	<0.0001 ***
Light 30%	-6.4221	2.396	-2.68	0.008 **
TS	0.8696	0.8696	0.1846	<0.0001 ***

The estimate for the effect of light is for the 30% treatment relative to the full light treatment. *$P < 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$
The light effect size describes the effect of 30% light relative to full sunlight.

The model of the plant height resulted that there was no significant relationship between plant height and total phenolics concentration of full sunlight seedlings ($F_{1,99} = 2.72$, $p > 0.05$, $R^2_{adj} = 0.01$). But there was a very weak, significant negative relationship between plant height and TP among 30% light seedlings ($F_{3,198} = 7.52$, $p < 0.0001$, $R^2_{adj} = 0.37$) (Figure 10). The model of plant height described the 33% of the variation (R^2_{adj}) of plant height and showed that plant height is weakly significantly and negatively related to TP under different light conditions ($F_{2,199} = 52.25$, $p < 0.0001$) (Table 3). But there was no effect of any significant interaction between plant height and TS across two light levels as they were dropped off from the model.

Table 3: Estimated regression parameters, standard errors, F-values, and P-values for a linear model describing the height of seedlings in the 100% and 30% light treatments (height ~ phenolics + Light).

	Estimate	Standard error	t-value	P-value
Intercept	51.02849	1.95217	26.139	<0.0001 ***
Light 30%	-16.9698	1.66158	-3.131	0.002 **
TP	-0.08182	0.02614	-10.213	<0.0001 ***

The estimate for the effect of light is for the 30% treatment relative to the full light treatment. *$P < 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$
The light effect size describes the effect of 30% light relative to full sunlight.

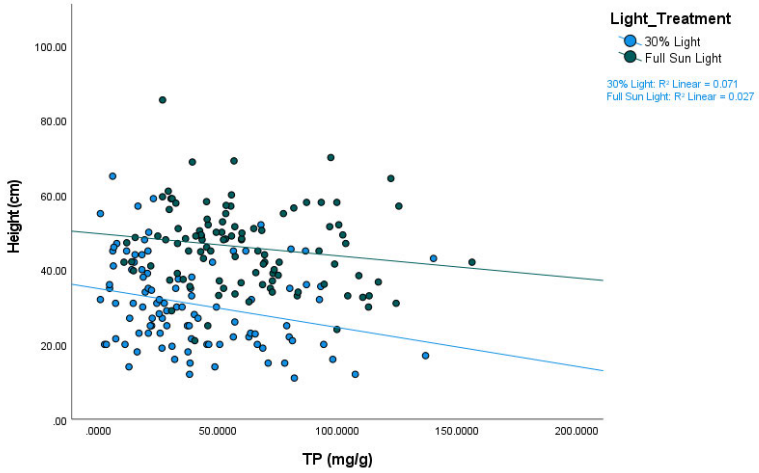

Figure 10: The relationship between the height and the total phenolic concentration of *E. melliodora* seedlings of full sunlight and 30% light treatments

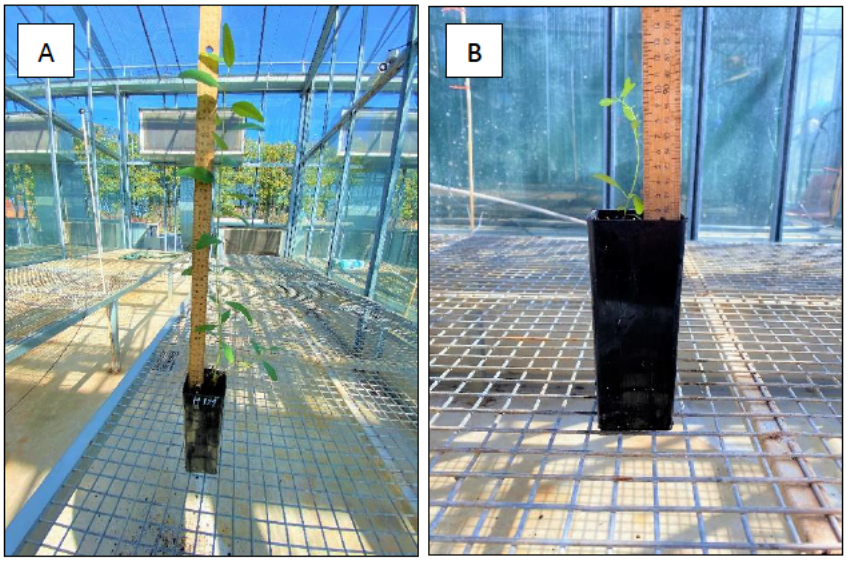

Figure 11: Growth comparison of *E. melliodora* seedlings grown under different light conditions. A) A well-grown taller seedling grown under full sunlight condition B) A weak seedling grew under 10% light condition

2.3.1.2 Aboveground biomass

AG mass was varied under different light levels ($F_{1,200}$ = 276, p < 0.0001, R^2_{adj} =0.01). *E. melliodora* seedlings produced the highest AG under full sunlight and the lowest AG under 10% light conditions (Figure 11). According to the model of AG mass, there was a very weak significant and positive relationship between AG and TS for seedlings in full light level ($F_{1,99}$ = 6.6, p < 0.05, R^2_{adj} =0.05). Plants grown under 30% light condition also showed a very weak significant and positive relationship between AG and TS as well ($F_{1,99}$ = 4.14, p < 0.05, R^2_{adj} =0.03) (Figure 12 a). The model of the AG explained the 59% (R^2_{adj}) of the variation of AG biomass ($F_{2,199}$ = 151.6, p > 0.0001) suggesting that AG of *E. melliodora* seedlings showed a moderate positive relationship with TS under different light conditions (Table 4). According to the regression models, *E. melliodora* seedlings had higher AG under brighter light when they contained higher TS. However, the model of AG suggested that there was no significant interaction between AG mass and TS across two different light levels in *E. melliodora* seedlings as they were dropped out of the model.

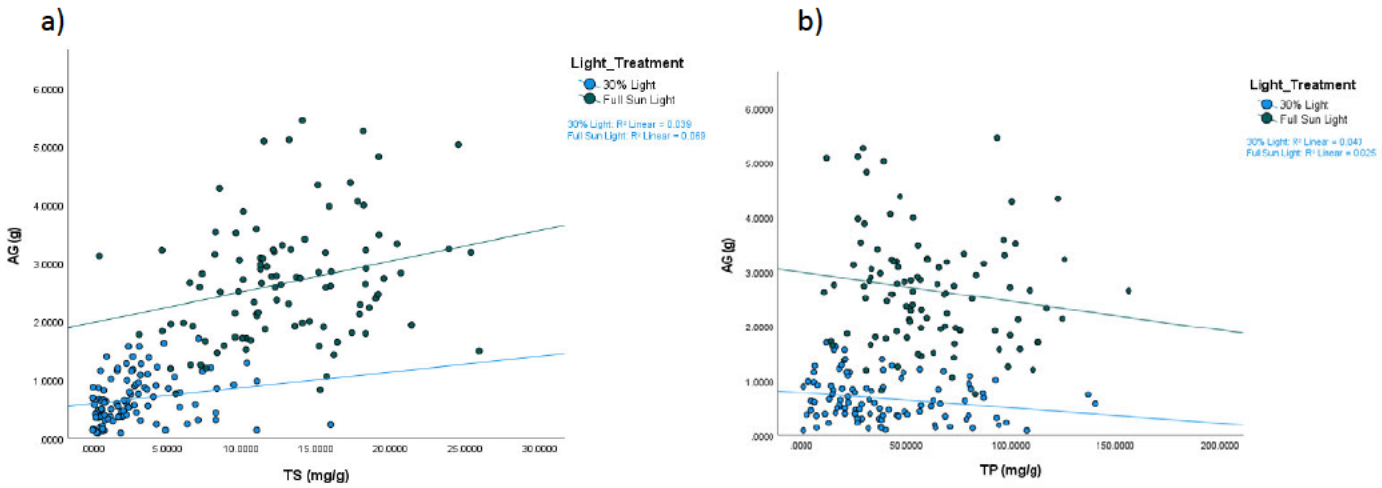

Figure 12: The relationship between the aboveground biomass and the foliar defence chemicals

a) The relationship between the aboveground biomass and the total sideroxylonal concentration of *E. melliodora* seedlings of full sunlight and 30% light treatments. b) The relationship between the aboveground biomass and the total phenolics concentration of *E. melliodora* seedlings of full sunlight and 30% light treatments.

Table 4: Estimated regression parameters, standard errors, F-values, and P-values for a linear model describing the aboveground biomass of seedlings in the 100% and 30% light treatments (aboveground mass ~ sideroxylonal + Light).

	Estimate	Standard error	t-value	P-value
Intercept	1.80	0.17	10.32	<0.0001 ***
Light 30%	-1.29	0.15	-8.10	0.00065 ***
TS	0.04	0.01	3.46	<0.0001 ***

The estimate for the effect of light is for the 30% treatment relative to the full light treatment. *$P < 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$
The light effect size describes the effect of 30% light relative to full sunlight.

The model of AG resulted that there was no relationship between AG and TP in full sunlight seedling ($F_{1,99} = 2.1$, $p < 0.05$, $R^2_{adj} = 0.01$). But TP showed a very weak, significant and negative relationship between AG and TP in 30% light seedlings ($F_{1,99} = 4.2$, $p < 0.05$, $R^2_{adj} = 0.03$) (Figure 12 b). The model of AG explained 58% of the variation of AG and showed AG was moderately and negatively related to the TP under different light levels ($F_{2,199} = 142.6$, $p < 0.0001$) (Table 5). TP dropped out of the full model and no significant interaction resulted between AG mass and TP across two different light levels. Results of the model of AG suggested that plants had higher AG under higher light and when they contained lower TP and no significant interaction between light level and TS was observed in varying AG.

Table 5: Estimated regression parameters, standard errors, F-values, and P-values for a linear model describing the aboveground biomass of seedlings in the 100% and 30% light treatments (aboveground mass ~ phenolics + Light).

	Estimate	Standard error	t-value	P-value
Intercept	2.58	0.12	20.08	<0.0001 ***

Light 30%	-1.003	0.001	-2.10	<0.05 *
TP	-1.80	0.19	-16.44	<0.0001 ***

The estimate for the effect of light is for the 30% treatment relative to the full light treatment. *$P < 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

2.3.1.3 Lignotuber biomass

Table 6: Dry mass allocation for lignotubers of *E. melliodora* seedlings under three different light conditions.

	Full sunlight (n=101)	30% Light (n=100)	10%Light (n=16)
Lignotuber dry weight (g)	0.29±0.02[a]	0.030±0.002[a]	0.002±0.0005[a]
Dry weight allocation for lignotuber (%)	7.81±0.4[a]	3.5±2.8[b]	2.20±0.3[b]

Within a row, different letters indicate significant differences (P < 0.05) from the Least significant difference test

(Mean value± 1 Standard error) of one-way ANOVA

Lignotuber biomass was significantly different between three different light conditions ($F_{1,200}$ = 170.1, $p < 0.0001$, $R^2_{adj=}$ 0.45) with highest LT mass in full sunlight and lower LT mass in low light levels (Table 6). Similarly, higher LT% was also observed in higher light levels and lower LT% was observed in low light levels. The model of LT mass identified that no significant interaction resulted between LT mass and TS under both full sun light ($F_{1,99}$ = 1.2, $p > 0.005$, $R^2_{adj=}$ 0.002) and 30% light level ($F_{1,99}$ = 0.5, $p > 0.005$, $R^2_{adj=}$ -0.004). Very weak negative interaction was observed between LT mass and TP in 30% light conditions ($F_{1,99}$ = 4.5, $p < 0.05$, $R^2_{adj=}$ 0.03). No relationship was identified between LT mass and TP in full sunlight seedlings ($F_{1,99}$ = 0.8, $p > 0.05$, $R^2_{adj=}$ -0.001). From the model of LT mass, TP dropped out of the model and resulted no interaction between LT mass and TP across different light level ($F_{3,198}$= 57.2, $p < 0.0001$, $R^2_{adj=}$ 0.45).

2.3.1.4 Root biomass

Table 7: Dry mass allocation for roots of *E. melliodora* seedlings under three different light conditions

	Full sunlight (n=101)	30% Light (n=100)	10%Light (n=16)
Root dry weight (g)	0.9±0.04[a]	0.1±0.008[b]	0.006±2.1[b]
Dry weight allocation for Root (%)	23.2±0.4[a]	12.1±0.5[b]	7.2±0.3[c]

Within a row, different letters indicate significant differences (P < 0.05) from the Least significant difference test
(Mean value± 1 Standard error) of one-way ANOVA

Root biomass was significantly different between three different light conditions ($F_{2,215}$ = 200.7, $p < 0.0001$, $R^2_{adj=}$ 0.64) with highest RT mass in full sunlight and lower LT mass in 10%

light level (Table 7). Similarly, higher RT% was also observed in higher light levels and lower RT% was observed in low light levels. According to the model of RT mass, there was a very weak significant but positive relationship was observed between RT mass and TS for seedlings in full light level ($F_{1,99}$ = 4.5, p < 0.05, R^2_{adj} =0.03). Plants grown under 30% light condition did not show a relationship between RT mass and TS ($F_{1,99}$ = 2.7, p > 0.05, R^2_{adj} =0.01). The model of the RT mass explained the 63% (R^2_{adj}) of the variation of RT mass ($F_{2,199}$ = 177, p < 0.0001) suggesting that RT mass of *E. melliodora* seedlings showed a strong positive relationship with TS under different light conditions. According to the results, *E. melliodora* seedlings showed that plants had higher RT mass under brighter light and when they contained higher TS and no significant interaction between light level and TS was observed in varying RT mass. However, the model of RT mass resulted that there was no significant interaction between RT mass and TS across two different light levels as they dropped out of the model. The model of RT mass suggested that there was no relationship between RT mass and TP in full sunlight seedling ($F_{1,99}$ = 0.15, p > 0.05, $R^2_{adj=}$ -0.008). But TP showed a very weak, significant but negative relationship between RT mass in 30% light seedlings ($F_{1,99}$ = 8.5, p < 0.05, $R^2_{adj=}$ 0.07). The model of RT mass expressed 62% (R^2_{adj}) of the variation of RT mass ($F_{2,199}$ = 177, p < 0.0001) suggesting that RT mass of *E. melliodora* seedlings showed a weak negative relationship with TP under different light levels. The model of the RT mass showed that there was no significant interaction between RT mass and TP across two light levels ($F_{2,199}$ = 167.8, p < 0.0001) as TP dropped out of the model.

2.3.1.5 Root: shoot ratio

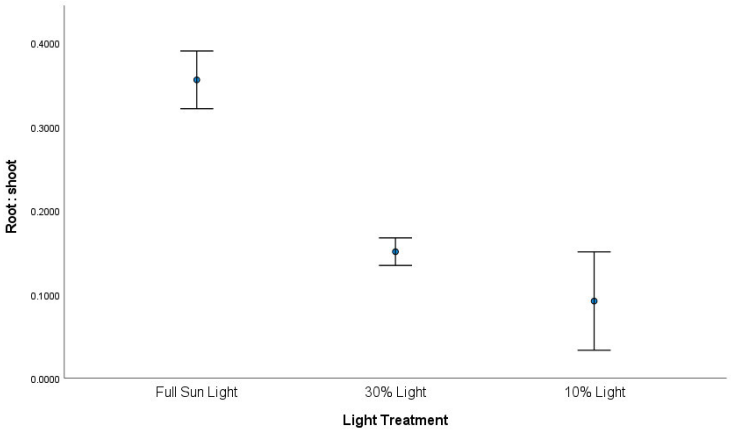

Figure 13: Mean root: shoot ratio variation of *E. melliodora* seedlings under three different light levels. Error bars represent the means ± standard error

Root: shoot ratio was significantly different between light treatments ($F_{1,200}$ = 157.7, p < 0.0001, $R^2_{adj=}$ 0.43) (Figure 13). Light levels had affected the differential allocations of biomass for root and shoot by resulting highest root: shoot ratio for the seedlings grown under full sunlight and the lowest root: shoot ratio was observed in plants grown in 10% light conditions. The model of root: shoot ratio revealed that there was no relationship between root: shoot ratio and TS in both full sunlight ($F_{1,99}$ = 0.05, p > 0.05, $R^2_{adj=}$ -0.009) and 30% light ($F_{1,99}$ = 1.59, p > 0.005, $R^2_{adj=}$ 0.005) as well. The model of root: shoot ratio and TP found that there was no relationship between the root: shoot ratio and TP among full sunlight seedlings ($F_{1,99}$ = 0.0008, p > 0.05, $R^2_{adj=}$ -0.01). But there was a very weak significant negative relationship between root: shoot ratio and TP in 30% light seedlings ($F_{1,99}$ = 5.46, p < 0.05, $R^2_{adj=}$ 0.04). Model of the root: shoot ratio explained 43% ($R^2_{adj=}$ 0.43) variation of the root: shoot ratio and resulted that there was no significant interaction between TP and light levels.

2.3.1.6 Specific Leaf area

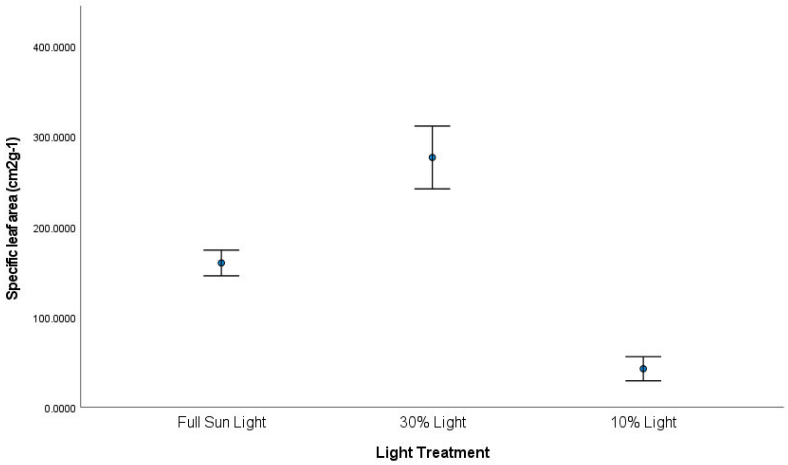

Figure 14: Specific leaf area (cm^2g^{-1}) variation of *E. melliodora* seedlings under three different light conditions. Error bars represent the means ± standard error

SLA of seedlings were significantly different under three different light conditions ($F_{1,200}$ = 38.67, $p < 0.0001$, $R^2_{adj=}$ 0.15) (Figure 14). Seedlings of 30% sunlight had the highest SLA and 10% seedlings resulted the lowest SLA. The model of SLA did not show any relationship between SLA and TS for both full sunlight ($F_{1,99}$ = 2,29, $p > 0.05$, $R^2_{adj=}$ 0.01) and 30% light seedlings ($F_{1,99}$ = 0.51, $p > 0.05$, $R^2_{adj=}$ -0.004). The model of the SLA resulted no significant interaction between SLA and TS across two light levels. The model of the SLA resulted that there was no relationship between SLA and TP in both full sunlight seedlings ($F_{1,99}$ = 0.27, $p >$ 0.05, $R^2_{adj=}$ -0.007) and 30% light as well ($F_{1,99}$ = 1.80, $p > 0.05$, $R^2_{adj=}$ 0.007). The model of SLA resulted there was no significant interaction between SLA and TP across two light level ($F_{3,198}$ = 14.02, $p < 0.0001$, $R^2_{adj=}$ 0.16). According to the results of the model SLA of *E. melliodora* seedlings did not show any relationship with foliar secondary chemical concentration at different light levels.

2.3.1.7 Mean leaf mass

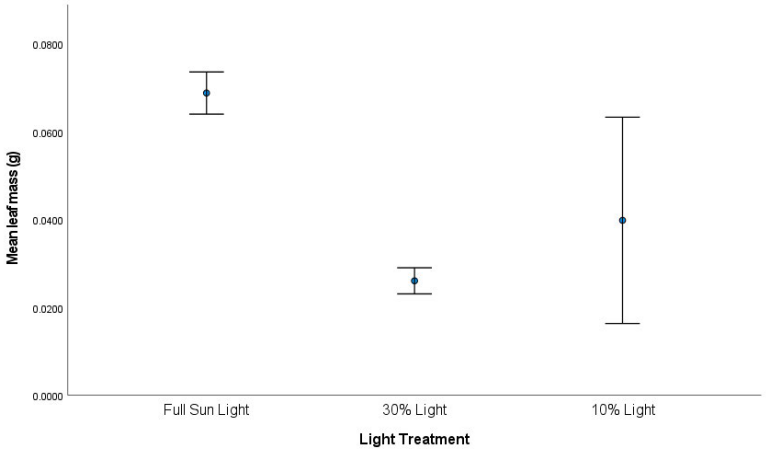

Figure 15: Mean leaf mass variation (g) of *E. melliodora* seedlings under three different light levels. Error bars represent the means ± standard error

ML mass was significantly different between light treatments ($F_{1,200}$ = 229.3, p < 0.0001, $R^2_{adj=}$ 0.53) and highest ML mass was resulted in full sunlight plants and the lowest ML mass in 30% light condition (Figure 15). No relationship was found between ML mass and TS in both full sun light seedlings ($F_{1,99}$ = 1.55, p > 0.05, $R^2_{adj=}$ 0.005) and 30% light seedlings ($F_{1,99}$ = 1.65, p > 0.05, $R^2_{adj=}$ 0.006). Model of the ML mass resulted that TS dropped out of the model and there was no significant interaction between ML mass and TS across two light levels ($F_{1,99}$ = 76.6, p < 0.0001, $R^2_{adj=}$ 0.53).The model of ML mass resulted that ML mass did not show any relationship with TP in both full light seedlings ($F_{1,99}$ = 0, p > 0.05, $R^2_{adj=}$ -0.01) and 30% light seedlings ($F_{1,99}$ = 2.56, p > 0.05, $R^2_{adj=}$ 0.01). Model of the ML mass resulted no significant relationship between ML mass and TP across light levels ($F_{3,198}$ = 76,66, p > 0.05, $R^2_{adj=}$ 0.53). Model outputs of ML mass suggest that no relationship was observed in ML mass and foliar secondary metabolite concentration under any light treatments.

2.3.2 *Eucalyptus camaldulensis*

All the *Eucalyptus camaldulensis* seedlings that were grown under full sunlight and 30% light conditions successfully survived to the end of the experiment period. From the 50 seedlings that were grown under 10% light level, only 8 were survived at the end of the experiment. The surviving plants were small compared to the plants in other light conditions and were very fragile. Plants that were grown under the other two light conditions were large and healthy compared to the 10% light condition (Figure 16).

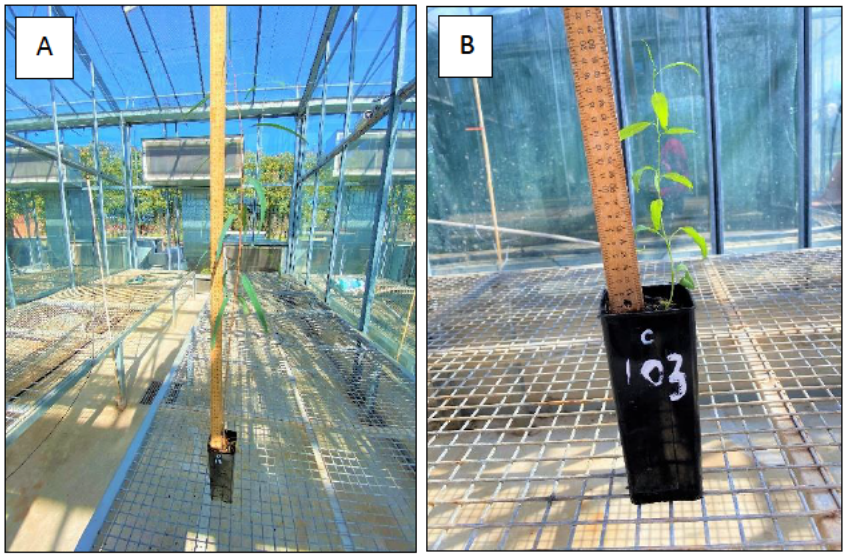

Figure 16: Growth comparison of *E. camaldulensis* seedlings grown under different light conditions. A). Well-grown *E. camaldulensis* seedling grown under full sunlight B) A weak *E. camaldulensis* seedling grown under 10% light condition

Both foliar chemical concentrations of *E. camaldulensis* seedlings differed between three light treatments (Table 8). TS concentration was significantly different ($F_{2,61} = 5.16$, $p < 0.005$, $R^2_{adj} = 0.11$) among three light levels with the highest TS in full sunlight and the lowest in 10% light level. Similarly,TP was also varied among light treatments ($F_{2,61} = 11.8$, $p < 0.0001$, $R^2_{adj} = 0.25$) resulting highest TP in full sunlight and the lowest in 10% light level (Table). According to the results, constitutive defense chemical concentrations of *E. camaldulensis* seedlings were higher in seedlings grown in high sunlight levels and lower at low light levels.

Table 8: Mean concentration variation of *E. camaldulensis* seedlings grown under three different light levels.

	Full sunlight (n=20)	70% Light (n=36)	10%Light (n=8)
Total Sideroxylonal ($mgg^{-1)}$	3.80 ± 0.3^a	3.7 ± 0.4^a	1.22 ± 0.4^b

Total Phenolics(mgg^{-1})	113.18±12.4[a]	78.95±3.2[b]	43.95±11.2[c]

Within a row, different letters indicate significant differences (P < 0.05) from the Least significant difference test (Mean concentration± 1 Standard error) of one-way ANOVA

2.3.2.1 Plant height

Plant height was significantly different under three different light levels ($F_{2,61}$ = 126.7, P < 0.0001, R^2_{adj}=0.79) resulting taller plants in brighter lights and shorter plants under less lighted environments (Figure 17). The model of the plant height suggests that there was no relationship between plant height and TS among 100% light plants ($F_{2,61}$ = 0.53, p > 0.05, R^2_{adj} = -0.025) and 30% light seedlings ($F_{1,34}$ = 0.003, p > 0.05, R^2_{adj} = -0.042). The model of the plant height identified no relationship between plant height and TP for both 100% light seedlings ($F_{1,18}$ = 0.2127, p > 0.05, R^2_{adj} = -0.029) and 30% light seedlings ($F_{1,34}$ = 0.003, p > 0.05, R^2_{adj} = -0.042) as well. Results from the models of the plant height revealed that there was no relationship between constitutive defensive chemical concentrations and plant height under different light conditions.

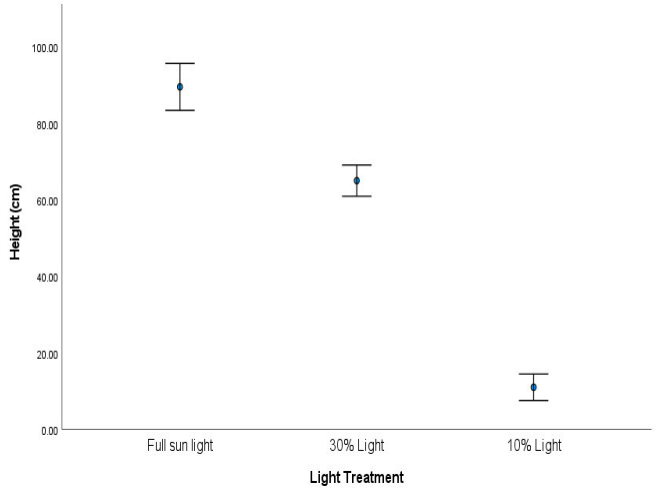

Figure 17: Height variation of *E. camaldulensis* seedlings grown under three different light conditions. Error bars represent the means ± standard error

2.3.2.2 Aboveground biomass

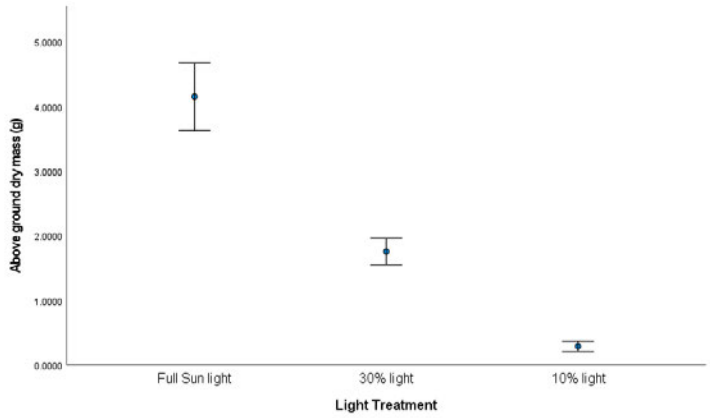

Figure 18: Aboveground mass variation of *E. camaldulensis* seedlings grown under three different light levels. Error bars represent the means ± standard error

Above-ground biomass of the plants were significantly different under three different light conditions ($F_{1,54}$= 100.3, $p < 0.0001$, $R^2_{adj} = 0.6$) (Figure 18). The highest above-ground biomass was observed in full sunlight seedlings and the smallest above-ground biomass was observed in 10% light seedlings. The model of the AG biomass suggested that there was no relationship between AG biomass and TS of full sunlight seedlings ($F_{1,18}$= 0.008, $p > 0.05$, $R^2_{adj} = -0.05$) and seedlings grown under 30% light conditions as well ($F_{1,34} = 0.28$, $p > 0.05$, $R^2_{adj} = 0.59$). The model of AG biomass revealed that there was no relationship identified between the AG biomass and TP for both full sunlight seedlings ($F_{1,18} = 0.22$, $p > 0.05$, $R^2_{adj} = 0.63$) and 30% light seedlings ($F_{1,34} = 0.95$, $p > 0.05$, $R^2_{adj} = -0.001$). According to the model of AG, it was clear that there was no relationship between the AG mass and foliar chemical concentration under different light environments.

2.3.2.3 Lignotuber mass

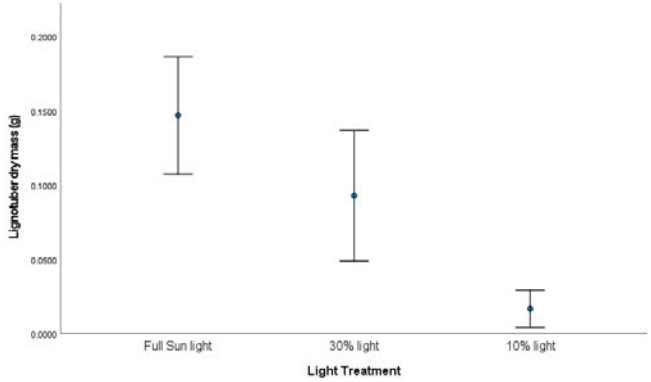

Figure 19: Lignotuber mass variation of *E. camaldulensis* seedlings grown under three different light conditions. Error bars represent the means ± standard error

Lignotuber biomass was not significantly different between different light conditions ($F_{2,61}$ = 2.68, p > 0.05, R^2_{adj} = 0.02) (Figure 19). Similarly, LT% was also not significantly different between three light levels ($F_{2,61}$ = 3.03, p > 0.05, R^2_{adj} = 0.06). Furthermore, the model of the LT suggested that there was no relationship between LT and TS among full sunlight seedlings ($F_{1,18}$ = 5.78, p < 0.05, R^2_{adj} = 0.02) and also seedling grown under 30% light level as well ($F_{1,34}$ = 0.13, p > 0.05, R^2_{adj} = -0.02). The model of the LT resulted no interaction between LT and TP under both full light ($F_{1,18}$ = 2.23, p > 0.05, R^2_{adj} = 0.06) and 30% light levels ($F_{1,34}$ = 0.0002, p < 0.05, R^2_{adj} = -0.02). According to the model outcomes, it was clear that there was no relationship between the LT variation in *E. camaldulensis* seedlings and foliar chemical concentration under various light conditions.

2.3.2.4 Root biomass

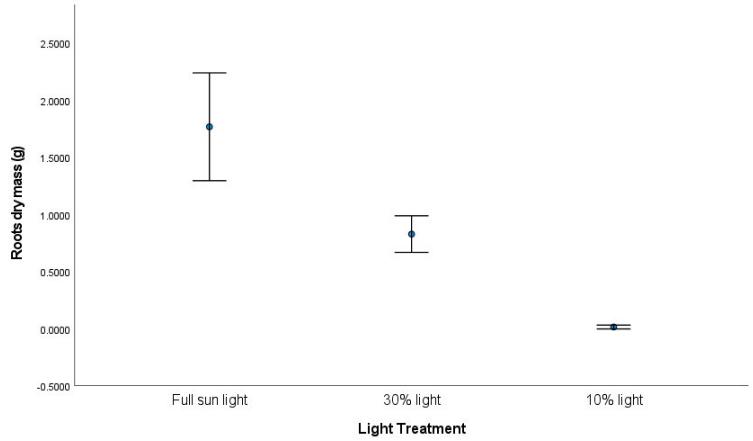

Figure 20: Root dry mass variation of *E. camaldulensis* seedlings grown under three different light conditions. Error bars represent the means ± standard error

RT biomasses were significantly varied under three different light conditions ($F_{1,54}$= 100.3, p < 0.0001, R^2_{adj} = 0.6) (Figure 20). The highest RT biomass was observed in full sunlight seedlings and the smallest RT biomass was observed in 10% light seedlings (Figure 20). The model of the RT mass suggested that there was no relationship between RT mass and TS in both full sunlight seedlings ($F_{1,18}$= 0.3, p > 0.05, R^2_{adj} = -0.03) and seedlings grown under 30% light conditions ($F_{1,34}$ = 0.3, p > 0.05, R^2_{adj} = -0.01). The model output suggested that foliar sideroxylonal concentration was not related to the RT mass of the seedlings under any light condition. The model of the RT mass and TP also resulted that there was no interaction between RT mass and

TP under both full light ($F_{1,18}$ = 0.26, p > 0.05, R^2_{adj} = -0.04) and 30% light levels ($F_{1,34}$ = 0.17, p > 0.05, R^2_{adj} = -0.02). According to the model analysis outcomes, it was clear that there was no relationship between the RT mass variation in *E. camaldulensis* seedlings and foliar chemical concentration under various light conditions.

2.3.2.5 Root: shoot Ratio

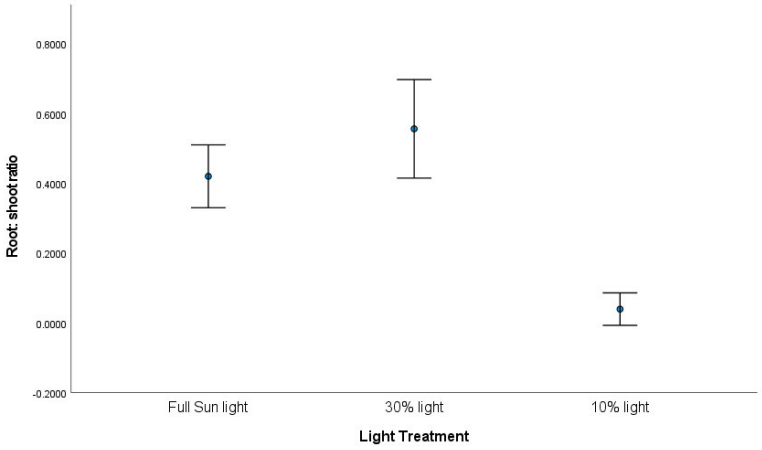

Figure 21: Root: shoot ratio of *E. camaldulensis* seedlings grown under three different light levels. Error bars represent the means ± standard error

Root: shoot ratio was not significantly different between three light levels ($F_{1,54}$ = 2.84, p > 0.05, R^2_{adj} = 0.03) (Figure 21). The model of the root: shoot ratio resulted that there was no relationship between TS among full sunlight seedlings ($F_{1,18}$ = 1.03, p > 0.05, R^2_{adj} = 0.002) and 30% light seedling ($F_{1,34}$ = 0.02, p > 0.05, R^2_{adj} = -0.02). The model of the root: shoot ratio resulted that no relationship was found between root: shoot ratio and TP in both full sunlight seedlings ($F_{1,18}$ = 0.009, p > 0.05, R^2_{adj} = -0.005) and 30% light seedlings ($F_{1,34}$ = 0.69, p > 0.05, R^2_{adj} = -0.008). The results suggested that there was no interaction between the concentrations of foliar secondary metabolites and root: shoot ratio of the seedling under different light conditions.

2.3.2.6 Specific leaf area

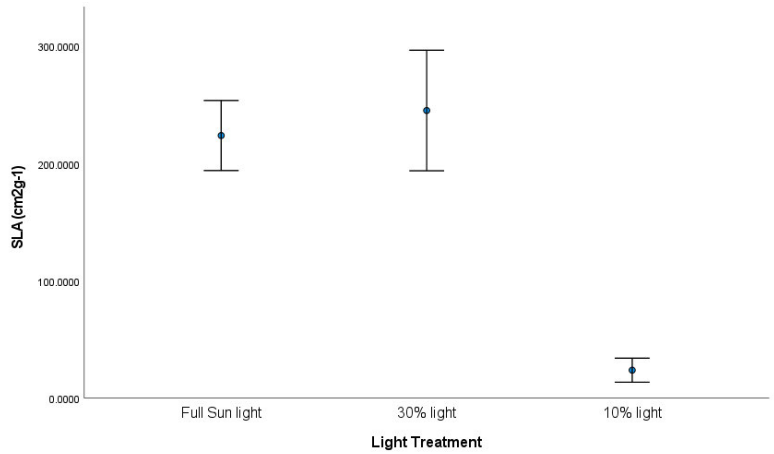

Figure 22: Specific leaf area (cm^2g^{-1}) variation of *E. camaldulensis* seedlings grown under three different light conditions.
Error bars represent the means ± standard error

SLA was not significantly different between the light treatments ($F_{1,54}$ = 0.34, p > 0.05, R^2_{adj} = -0.01) (Figure 22). The model of SLA resulted a weak significant negative relationship between SLA and TS for seedlings in full light level ($F_{1,18}$ = 10.1, p < 0.05, R^2_{adj} =0.32). Plants grown under 30% light condition did not resulted a relationship between SLA and TS ($F_{1,34}$ = 0.96, p > 0.05, R^2_{adj} =-0.001). The model of the SLA explained the 1% (R^2_{adj}) of the variation of SLA ($F_{2,53}$ = 177, p > 0.05) suggesting that SLA of *E. camaldulensis* seedlings did not show any relationship with TS under different light conditions. The model of SLA suggested that there was no significant interaction between SLA and TP in both full sunlight ($F_{1,18}$ = 0.38, p > 0.05, R^2_{adj} = -0.03) and 30% light seedlings as well ($F_{1,34}$ = 0.51, p > 0.05, R^2_{adj} = -0.01). Results suggested that SLA was not related to the foliar secondary chemical concentrations under different light conditions.

2.3.2.7 Mean Leaf mass

ML mass of *E. camaldulensis* seedlings was not significantly different among three light levels ($F_{2,61}$ = 0.77, p > 0.05, R^2_{adj} = -0.007). The model of ML mass suggested that ML mass was moderately, significantly and positively related to TS among full sun light seedlings ($F_{1,18}$ = 15.32, p < 0.05, R^2_{adj} = 0.42). But ML mass was not related to TS in 30% light seedlings ($F_{1,34}$ = 0.0001, p > 0.05, R^2_{adj} = -0.02). The model of ML mass identified that there was no significant interaction between ML mass and TS ($F_{2,53}$ = 0.27, p > 0.05, R^2_{adj} = -0.02) under different light conditions. ML mass was not related to TP in both full sun light seedlings ($F_{1,18}$ 0.33, p > 0.05,

R^2_{adj} = -0.03) and 30% light seedlings ($F_{1,18}$ = 1.56, p > 0.05, R^2_{adj} = 0.01). The full model of ML mass suggested that there was no significant interaction between TP and light level. Results of the model of ML mass suggested that there was no relationship between ML mass and foliar secondary chemical concentrations under different light conditions.

2.4 Discussion

It is a well-known fact that the light environment can strongly influence plant physiology and plant growth and development process (Pooter and Nagel; 2000; Burns, Gleadow and Woodrow, 2002). The growth of a successful individual plant in different light environments depends on various factors including plant photosynthetic rate, leaf structure, and biomass allocation for plant components which are also varied by other environmental conditions (Burns, Gleadow and Woodrow, 2002). Although it has been reported that the light condition can contribute to intraspecific variation in the development of defence traits, the impact of light on an intraspecific variation on the development of defence traits is less studied (Yamawo and Hada, 2010).

At the development stage of the experiment, the blister-like growth observed on leaves of the *Eucalyptus* seedlings might be occurred due to the environmental conditions at the growth chamber. Similar observations were recorded by Pinkard et al. (2006) in an experiment they have carried out on *E. globulus* leaves. Pinkard et al. (2006) proposed that non-pathogenic blister-like protuberant growth develops on leaves in such incidents where the interaction of high temperature and high humidity increase the water absorption rate more than transpiration rate which might be promoted by light condition as well.

According to the results, it is clear that the morphology of a plant is greatly affected by light levels. James and Bell (2000) observed higher plant height in full sunlight level and higher biomass allocation for leaves in 10% light seedlings of *E. globulus* in their experiment. They also observed that biomass of woody tissues was significantly reduced under 10% light level compared to other light levels. These results indicate that plants allocate more biomass to leaves under dark conditions and allocate more biomass to below ground when plants are under higher light levels.

Seedlings in full sunlight had an adequate carbon resource that can be allocated to different plant parts but seedlings in low light levels had to allocate the available C resources to leaves to acquire more sunlight for photosynthesis under shade conditions (James and Bell, 2000). Higher biomass allocation for roots and lignotuber in full sunlight will promote the regrowth of the seedlings and the establishment after an unfavorable condition such as higher light, lower nutrient, or after a fire (James and Bell, 2000). All the seedlings growing under three

different light levels have allocated the highest biomass for their leaves and the second-largest biomass allocation for the roots. A very small percentage was allocated to lignotubers compared to the other parts. Plants decrease the fraction of biomass allocated to leaves and increase allocation to roots as a whole plant level response to increase light level (Evans and Poorter, 2001; Poorter and Nagel, 2000; Ericsson, 1995). Under higher light levels photosynthetic rate per unit leaf mass is higher and therefore this allocation may facilitate the higher rate of water absorption from soil because of the increased transpiration and also supply the higher demand of nutrients from soil required by the stimulated growth (Evans and Poorter, 2001; Poorter and Nagel, 2000).

In an experiment by Cronin and Lodge (2003), a higher root: shoot ratio was observed in higher light treatment than low light level and it was 40% higher than shaded plants. Their results suggest that *Potamogeton amplifolius* plants allocate more resources to root when light and photosynthate are abundant. The results of my experiment also showed a similar pattern suggesting that full sunlight seedlings have allocated more resources to roots than low light seedlings. Higher light levels increase plants' growth rate through an increase in photosynthetic rate (Wilson, 1988). As a result, it is expected that allocation to leaves to decrease and allocation to root to increase. This results in a higher root: shoot ratio observed in higher light levels. At 10% light level plants allocate a higher fraction of biomass to leaves to maximize the photosynthetic rate and root biomass fraction is comparatively less. Therefore, a lower root: shoot ratio was observed in low light levels. However, Poorter and Nagel (2000) proposed that it is not only because of the decrease in leaves fraction. They proposed that in some instances it is because of a shift of the allocation of biomass from stem to roots in high light levels. Similar observations resulted in this experiment as the stem allocation percentage got comparatively reduced in full sunlight than 30% light level while the root percentage get increased. Under low light levels, longer stems might help to compete for sunlight in a forest environment.

Plants might need several defensive traits at once to protect the plant from a range of possible herbivores attacking plants. They are the most effective when they are acting together and rarely effective as a single trait (Agrawal, 2007). Agrawal (2007) suggests that there should be a trade-off between plant resistance traits and tolerance traits. Nunez-Farfan, Fornoni and Valverde (2007) did not find any trade-off between herbivore tolerance and resistance in

plants and suggested that both traits should work alternatively as defensive strategies and can perform independently. Following the trends proposed by Nunez-Farfan, Fornoni and Valverde (2007), no trade-off was observed between lignotuber mass and secondary metabolite concentration at any of the light levels. However, it is the ideal combination of having a large reservoir of dormant buds with higher storage of carbohydrates to give a rapid response by replacing the photosynthetic ability under serious damage caused by high light levels (Moore et al. 2015). Biomass allocation percentage for lignotubers was higher in high light levels and a less percentage was allocated to lignotubers under low light levels. Higher allocation for lignotuber under high light level will benefit the plant by resprouting after leaf damage. Lignotuber production cannot be taken as a good indication of whole plant survival (Moore et al. 2015). As shading directly affects the photosynthetic rate it is important to allocate more biomass to leaves to increase the photosynthetic capacity under shade conditions. Therefore, less percentage has been allocated to lignotubers under low light levels.

Specific leaf area (SLA) of *E. melliodora* seedlings were higher in 30% light plants compared to full sunlight seedlings but SLA was not significantly different in *E. camaldulensis* seedlings in different light levels. Mean leaf mass was higher in full sunlight and was lower in low light levels. James and Bell (2000) also observed increased specific leaf area with decreasing light availability and lower leaf weight in low light conditions. According to James and Bell (2000), higher SLA in low light levels increases the potential photosynthetic leaf area relative to leaf biomass by maximizing leaf display and light capture under low light conditions. James and Bell (2000) further described that low SLA in full sunlight level reduces the leaf temperature, potential water loss, and damage to photosystems. Increased SLA in low light levels increases the light capturing ability of leaves and also the cost of producing higher sized leaves in dark conditions will compensate for the increased light capturing and carbon gain by producing leaves with higher SLA under low light levels James and Bell (2000). Plants with higher SLA have low relative growth rates and low photosynthesis than leaves with low SLA (Dwyer et al. 2014). Plants growing in high light levels normally have thicker leaves with lower SLA (Evans and Pooter, 2001) and with some extra palisade cell layers. These extra cell layers increase the number of chloroplasts and a higher number of photosynthetic enzymes which then enhance the photosynthetic capacity per unit leaf area (Evans and Pooter, 2001).

According to the results, secondary chemical concentrations were also greatly affected by the light environment. For both *Eucalyptus* species, higher sideroxylonal and higher total phenolics were observed under full sunlight levels and lower concentrations of sideroxylonal and total phenolics were observed in 10% light level. Observations proposed that under high light levels genus *Eucalyptus* seedlings have higher biomasses along with higher concentrations of sideroxylonal and phenolics but under low light levels *Eucalyptus* seedlings have very little growth and fewer concentrations of sideroxylonals and phenolics. However, according to the trade-off's mechanisms, any environmental factor which reduces growth than reduces photosynthesis will increase the resource pool available for allocation for secondary chemicals (Herms and Mattson, 1992; Stamp, 2003). Coley and Barone (1996) suggested that according to C: nutrient balance hypothesis excess resources accumulate within the plant body than growth requirements should use for plant defence . Therefore, plants growing in high light conditions have a higher photosynthesis rate and thus a higher carbon accumulation within the plant body which would cause an increase in C-based secondary metabolites within the plant.

Similar observations were recorded for *Potamogeton amplifolius* in an experiment was done by Cronin and Lodge (2003) where light-stimulated plants showed 128% times higher shoot mass and 273% times higher root biomass than shaded plants. The root/shoot ratio is 40% higher in unshaded plants than shaded conditions which indicates these plants allocate more resources roots when light and photosynthate are present. Even the less shaded plants produce 25% phenolic concentration in their plant body which is sufficient to defend from herbivores. Plants phenolics concentration was high in light stimulated plant than shade growth plant. Plants growing under a stressful condition like limited light level have less ability to acquire resources and have to allocate resources to deal with the stress resulting in less C pool available to allocate to plant defence s making these plants a good food for herbivores because of the higher nutritional value as a result of low defensive compounds (Cronin and Lodge,2003).

In an experiment conducted by Dudt and Shure (1994) using *Liriodendron tulipifera* (tulip poplar) and *Cornus florida* (dogwood) in the USA, for both species of leaf, the dry matter was reduced under low light levels. Trees in deep shade inside the forest have significantly low leaf dry mass compared to the trees in the canopy gaps. Total leaf phenolics were positively

related to light intensity and resulted in higher total phenolics in open canopy gaps than the forest understory and open area than trees under shade cloth. Similarly, tannin concentration was highly sensitive to light levels for both species. Both hydrolyzable and condensed tannins were greatly reduced under forest understory than open canopy gaps. Herbivory damage was inversely related to light availability and plant phenolics concentrations. Herbivory is higher in highly shaded forest understory trees. A higher level of herbivory was associated with total phenolics reduction in low light in shaded trees. According to the results, it is clear that both species decreased photosynthetic allocation to phenolics as light become more limiting (Dudt and Shure, 1994).

Any morphological traits observed including biomasses of above ground and below ground, SLA, root: shoot ratio and mean leaf mass did not show any correlation with the sideroxylonal and total phenolics under light level gradient resulting in no trade-off was presented between the growth and defence light level variation. However, there was a positive trend between sideroxylonal of *E. melliodora* seedlings with plant height, AG, and RT biomass under different light conditions resulting in higher sideroxylonal concentrations in taller plants with higher above ground and root biomasses. Furthermore, the total phenolics of *E. melliodora* were negatively correlated with plant height and aboveground biomass. No such trend was observed in *E. camaldulensis* seedlings. In a mathematical model developed by DeAngelis et al. (2012) in their studies, they support the idea that there is a trade-off between plant growth and growth of leaves and root, defence , and reproduction processes. They observed that light intensity causes very little change in defence allocation but a decrease in allocation to leaves and an increase in allocation to fine roots. Furthermore, when shading increases C supply of the plant decreases and it shifts away from the defense to leaves. When plants increase the allocation to plant defence there is a shift of allocation from fine roots. This may be because higher defence reduces the nutrient loss caused by herbivores (DeAngelis et al. 2012).

Finally, sideroxylonal and phenolics concentration has created a great intraspecific variation under different light conditions. But did not show any trade-off between growth and defence. I have shown that the environment can strongly impact the phenotypic expression of defence traits. As Folgarait and Davidson (1995) recorded that there has been no consistent description developed about the effect of light on the development of defence traits.

According to them, this may be due to variation in the experimental condition, variation in light intensity levels, plant species among studies, and other environmental factors.

Chapter 3 – Impact of nutrients on resource allocation

3.1 Introduction

Plant secondary chemical concentration plays an important role in plant interactions with herbivores. The concentration of PSM changes the quality of leaves and thereby they change the palatability of leaves for herbivores (Close et al. 2005). Variation of environmental conditions such as the availability of nutrients to plants can alter the concentration of PSM produced within the plant (O'Reilly-Wapstra et al. 2005; Kainulainen et al. 1996; Lavola and Julkunen, 1994). For example, the availability of nitrogen to plants determines the plants' investment in PSM (Coley, 1986). Therefore, finding the interaction between leaf nitrogen and carbon-based secondary metabolites is important to identify how PSM concentration and leaf quality vary under different nitrogen levels (Kainulainen et al. 1996). Allocation of a definite amount of nutrient within a plant to a specific process is regulated by translocation to maximize the plant's success through photosynthesis gain and plant growth (Close et al. 2005). The balance between carbon and nutrient within an individual plant strongly affects their allocation of primary and secondary chemicals (Kainulainen et al. 1996).

According to the carbon: nutrient balance hypothesis (Bryant et al. 1983), under nutrient deficiencies plant growth is getting slower than photosynthesis. Therefore, Carbohydrate is accumulating within the plant in excess amounts than growth requirements and they will be allocated to C based secondary metabolites. In contrast, under fertile soil nutrient uptake from the plant is increased and the C: nutrient ratio within the plant is decreased. Plant growth receives the highest priority over plant defence. Therefore, C-based secondary metabolites getting decrease (Herms and Mattson, 1992). Therefore, according to the C: nutrient balance hypothesis C based PSM concentration is decreasing under high nutrients and getting increased under low nutrient conditions (Kainulainen et al. 1996).

The growth differentiation balance hypothesis (Tuomi et al. 1992) proposes that a low level of resource sink results from extreme environmental factors such as nutrient deficiency will result in accumulation of carbohydrates within the plant and thereby it increases the concentrations of C based secondary metabolites (Herms and Mattson, 1992). The growth is more sensitive to resource limitations than photosynthesis. Nutrient limitations can restrict

plant growth more than they reduce photosynthesis which will result in growth reduction while the plant increases its defence (Price, 1991; Lambers, 1993). This trade-off between the growth rate need for competition and plant defence is determined by the environment in which this plant is growing. If the importance of one is increases importance of the other decrease (Holopainen et al. 1995). According to Holopainen et al. (1995), under resource limiting environment importance of herbivory is higher compared to the competition and therefore more resources are allocated to defence. In contrast, fertilization with growth-limiting nutrients such as nitrogen will enhance the plant growth consequently decreases the carbohydrate reserves, and results in low concentrations of C-based PSM (Lavola and Julkunen, 1994).

Regulation of synthesis of phenolic compounds under environmental variation is explained by two modules (Lambers, 1993). Margna (1977), suggests that the production of some amino acids such as tyrosine and phenylalanine and phenolic compounds both depend on the shikimate pathway. As Margna (1977) proposed when protein production is high amino acids like tyrosine and phenylalanine will readily incorporate into proteins which will then limit amino acids available for the phenolic compound production. Under this situation, the plant tries its maximum to optimize the nitrogen supply to protein synthesis. At low nitrogen supply, protein synthesis is restricted and therefore less tyrosine and phenylalanine are incorporated into protein. But phenylpropanoid pathway is readily incorporating phenylalanine into phenolic production. According to this model, the demand for amino acids for protein production determines the incorporation of the C to PSM. There is another suggestion that sucrose level which is more than the amount required for protein synthesis enhances the C-based PSM production (Lambers, 1993). According to this module higher concentrations of sugar under nutrient limiting conditions regulate the incorporation of C to C-based PSM.

Competition between plants is believed to be largely responsible for the community structure, diversity, and density of plant community (DeAngelis et al. 2012). Although the secondary metabolites give the resistance against herbivory, the relative growth rate strongly affects the competitive ability of plants (McDonald, Agrell and Lindroth, 1999). Usually, plants are in regular competition for different resources (Ballhorn et al. 2014) including both above-ground resources such as light and below-ground resources such as nutrients (Kula et al. 2020). Therefore, the competition between two plants affects their plant growth and defence against

herbivores which will affect the performance of those plants in their environment (Chase et al. 2002). Higher plant density from the same species normally correlates with higher herbivores' attacks (Janzen, 1970). Broz et al. (2010) also suggest that individual plants from the same species growing together are more likely to trouble herbivores than plants growing in different communities. Therefore, the modification of the plant defence mechanism based on the plant's competitive neighbor would give an evolutionary advantage for plant species (Broz et al. 2010). Further, in a community of plants from the same species, a mechanism of accumulating PSM is more effective than prioritizing growth. In a community with plants of different species where the effect from herbivores is less the competition for resources such as light and nutrients is less because the requirements of different species are different from each other. Therefore, investing resources in growth even in the presence of herbivores is more effective than investing in defence in a diverse plant community (Broz et al. 2010).

Among different types of competition, the intraspecific competition which occurs between two individual plants from the same species strongly affects the plant fitness because both plants fight for the same resources and occupy the same special dimension (Broz et al. 2010). The competition may reduce the resources available for plants and therefore plant investment in defence also gets reduces (Agrawal, 2004). A study by Ballhorn et al. (2014) suggested that there is a negative correlation between constitutive defence and competitive ability of plants supporting the trade-off mechanism proposed by GDB hypothesis. According to (Ballhorn et al. 2014), when there are no herbivores present plants with the less constitutive defence have the highest competitive ability. But when there is herbivore pressure highly defensive plants are more successful than low defensive plants. This suggests that competition act as a selective force favoring the low expression of constitutive defence (Ballhorn et al. 2014). DeAngelis et al. (2012) observed that if a plant is in competition with a plant from the same species, the concentration of the phenolic was increased and the biomass accumulation was decreased. Plants can detect the presence of a competitor by detecting the changes in the quality of environments (Broz et al. 2010). Variation in nutrient level impact PSM accumulation in the presence of a competitor and lower the defence responses by reducing the expression of genes which result in less phenolic compounds (Broz et al. 2010).

However, the proposed trade-off was not observed in a meta-analysis done by Viola et al. (2010). Instead, they found a correlation between the competition and defence, and this

correlation behaves positively or negatively in a way to increase the diversity of the community. They proposed that strong competitors are more resistant to herbivores. According to Viola et al. (2010) trade-off between competition and defence is not very common in communities. Therefore, the behavior of the conspecific plants in the presence and absence of herbivores is still unclear and more experiments should be conducted to get a clear idea of how plants respond to intraspecific competition and herbivory.

This study is focused to identify a) how growth parameters of *Eucalyptus melliodora* seedlings changes under high and low nutrient concentrations b) how sideroxylonal and total phenolics concentrations varies under high and low nutrient concentrations c) to identify the relationships present between the growth parameter variation and sideroxylonal and total phenolics concentration variation under high and low nutrient conditions d) to identify how plant growth parameters and foliar chemicals concentrations vary at the presence of intraspecific competition under high and low nutrient levels. I have hypothesized that a) low nutrient *E. melliodora* seedlings produce higher sideroxylonal and higher phenolics concentrations than high nutrient seedlings and the trade-off between the growth and sideroxylonal is apparent in low nutrient seedlings b) In the presence of the competition plants with lower sideroxylonal and lower phenolics concentrations show the highest competitiveness resulting higher plant growth in both high nutrient and low nutrient seedlings because of the trade of present between the competition and plant defence. To test these hypotheses *E. melliodora* seedlings were grown in two different nutrient levels their morphological and physiological variations were recorded.

3.2 Materials and Methods

3.2.1 Experiment Set up

3.2.1.1 Planting seeds

Figure 23: Two different *E. melliodora* seedlings grown in the same pot for the nutrient experiment.

Eucalyptus melliodora seedlings were germinated in a commercial seed-raising mix "Seed and Cutting" (Evergreen Garden Care Australia Pty. Ltd., Bella Vista, NSW) in a controlled growth chamber with 25^0C temperature and 12 hours light-dark photoperiod. Seedlings were transferred from seed trays to plastic pots (65 x 65 x 150 mm) on the 16th of March 2020. The growing medium was prepared by mixing 50% perlite, 30% coarse sand, and 20% peat, along with a slow-release fertilizer. The intention for this experiment had been that two previously chemically characterized seedlings would be grown in competition in each pot, one with high FPC concentrations and one with low FPC concentrations. The sudden arrival of restrictions on laboratory and university access associated with the COVID-19 pandemic in early 2020 meant that screening small seedlings for constitutive chemical defence expression was not possible. Instead, two random *E. melliodora* seedlings were planted in each pot and the experiment was maintained at the private home of my supervisor, at Faulconbridge, NSW (Latitude -33.693 S, Longitude 150.548 E, Elevation 405 m) (Figure 23). The pots were arranged on an open north-east-facing balcony, from which vertebrate herbivores were excluded. From 1 May 2020, plants were irrigated daily with one of two different nutrient solutions, made with different concentrations (0.6 ml or 6.0 ml concentrate per 10 litters) of "Thrive (12.4: 2.7: 6.2 % w/v)" All-Purpose liquid plant food (Yates, Australia). In total, 176 pots, each with two

seedlings, were prepared for the nutrient experiment, and these were randomly allocated between two nutrient levels.

3.2.2 Data Collection

At the end of the growing period, harvest commenced on the 25th of September 2020. Plant height was recorded for both high and low nutrient seedlings from the soil surface to the highest point of the plant. The paired plants in the same pot were harvested separately. Leaves were harvested first, and fresh weight was recorded for each plant. Five leaves were selected randomly for leaf area measurements. Leaf area was measured using the LI-3100C leaf area meter (LI-COR Biosciences, Lincoln, USA). These leaves were then transferred into paper bags separately and put into the oven (70^0C) for dry weight measurements. The rest of the leaves of each plant were transferred into labeled ziplock bags and were stored in the freezer for future chemical analysis.

Stems were harvested and fresh weight was recorded. Then they were transferred into paper bags and kept in the oven (70^0C) for drying. Lignotubers were carefully separated from the below-ground root system and fresh weight was measured. Similarly, they were also put into the oven (70^0C) for drying. Root systems of both plants grown in the same pot were separated carefully with minimal disturbance of the root system. Extreme care was taken to separate two root systems from two separate plants as the roots from both plants were tangled together. Roots were washed carefully to remove soil debris and patted dry using paper towels. Fresh mass of roots was recorded, and they were also put into the oven (70^0C) for drying. Plant parts were removed from the oven when they resulted in constant weight and the dry weight of leaf area leaves, stems, lignotubers (LT), and roots (RT) were recorded.

3.2.3 Chemical Analysis

Extraction

Leaves saved for the chemical analysis were first freeze-dried and then ground to a fine powder using a ball mill (Mixer Mill MM200, Retsch GmBH, Germany). Extraction was carried out according to the rapid extraction method mentioned by Wallis and Foley (2005). First, 50 mg of leaf powder was weighed into a small glass vial containing 8 g of extraction solution and

three replicate samples were prepared from each plant. Following Wallis and Foley (2005), 7% water in acetonitrile containing 0.1% v/v trifluoracetic acid solution was used for the extractions. These samples were allowed to extract for 15 minutes and then sonicated for 5 minutes. Then they were filtered through a syringe filter into autosampler vials and analyzed using HPLC together with standard solutions.

HPLC Technique

Sideroxylonal Analysis – *E. melliodora*

Samples from the rapid extractions with 7% water in acetonitrile were retained in their autosampler vials, stored refrigerated (4°C), and analyzed again by HPLC on a Poroshell 120 EC-C18 column (4.6x75 mm 2.7 Micron) and the column temperature was 37^0C. The isocratic elution method was used with 7% water in acetonitrile, with 0.1% trifluoroacetic acid at a flow rate of 0.75 mL/min for optimal separation of Sideroxylonal compound. Sideroxylonal A, C, and B were detected at 3.72, 3.89, and 4.88 minutes respectively at 275 nm for all the *E. melliodora* samples. Total sideroxylonal concentration (TS) was calculated using a previously established standard curve.

3.2.4 Calculations

From the resulted dry weight values above-ground dry weight (AG) was calculated by adding dry weight values of leaves and stems together. Below ground, the dry weight value (BG) was calculated by adding LT and RT together. Lignotuber weight percentage was calculated (LT%) as lignotuber weight as a percentage of total plant dry weight [(LT/Total Plant weight) *100]. Root dry weight percentage was calculated the same way as a percentage of total plant weight [(RT/Total Plant weight) *100]. Specific leaf area (SLA) was calculated by dividing the leaf area value from the dry weight of leaf area leaves (Leaf area/Dry weight of leaf area leaves). Mean Leaf weight was calculated by dividing the weight of leaf area leaves by the total number of leaves used to get leaf area leaf measurements (Dry weight of leaf area leaves/ number of leaves). Sideroxylonal concentration difference was calculated for the plants grown in the same pot by subtracting the sideroxylonal concentration of one plant from the other. These two plants were then ranked as rank 1 and 2 based on their sideroxylonal concentration differences. These ranked plants were used to test the competition effect that arises for the plants grown in the same pot.

3.2.5 Statistical Analysis

Because sideroxylonal and total phenolic concentrations are traits subject to major gene effects (Andrew et al. 2007 and 2010) and are considered to be constitutive defences in *Eucalyptus*, I considered it to be an independent variable in models of seedling growth/morphological traits (e.g., plant height; aboveground biomass, etc). To test the effects of constitutive chemical defences and nutrient availability on these variables, I first constructed linear models (e.g. height ~ sideroxylonal) separately for each nutrient level. I then ran a model including both predictor variables (e.g. height ~nutrient level +sideroxylonal) including plants from both the high nutrient and low nutrient levels, and where both terms were retained, I ran a further model including a term for the interaction of light and chemical defence. To test the effect of competition sideroxylonal difference was used as the independent variable in models of seedling growth and morphological traits. To test the effect of sideroxylonal concentration differences and light availability on growth and morphological variables first I constructed a linear model (e.g. height ~ sideroxylonal difference) separately for each nutrient level. I then ran a model including both predictor variables (e.g. height ~nutrient level +sideroxylonal difference) including plants from both the high nutrient and low nutrient levels, and where both terms were retained, I ran a further model including a term for the interaction of light and chemical difference. Because both FPC and TP concentrations were influenced by nutrient level, I assessed the relationship between these variables separately for the high and low nutrient levels by calculating Pearson's product-moment correlations. All analyses were performed in R (Version 4.0.2).

3.3 Results

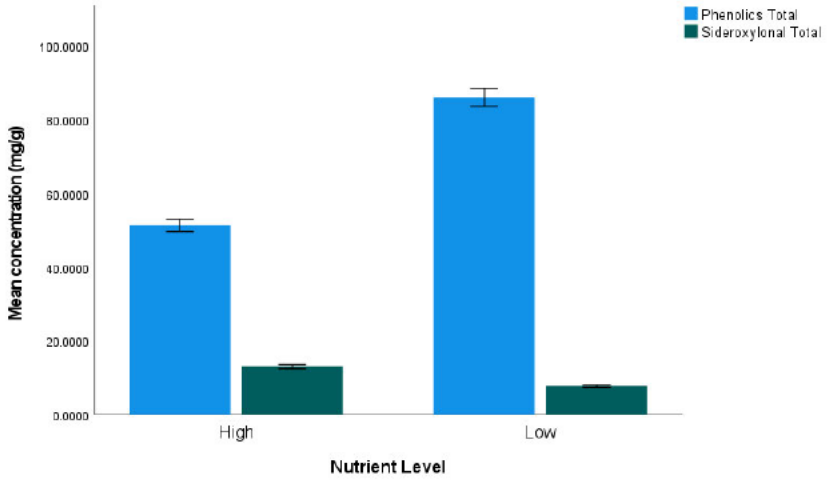

Figure 24: Mean sideroxylonal and phenolics concentration variation of *E. melliodora* seedlings under two different nutrient levels.
Error bars represent the means ± standard error

Both TS and TP differed strongly between the two nutrient treatments. Higher TS was observed in high nutrient plants ($F_{1,330}$ = 66.6, p < 0.0001, R^2_{adj} = 0.16) and higher TP was observed in low nutrient seedlings ($F_{1,330}$ = 119.9, p < 0.0001, R^2_{adj} = 0.26) (Figure 24). Further, correlation analysis resulted no relationship between TS and TP under different nutrient levels ($F_{2,329}$ = 33.64, p < 0.0001, R^2_{adj} = 0.26).

Figure 25: *E. camaldulensis* seedlings grown under two fertilization treatments at the end of the experiment. A) Growth of *E. camaldulensis* seedlings treated with high nutrient concentration B) Growth of *E. camaldulensis* seedlings treated with low nutrient concentration

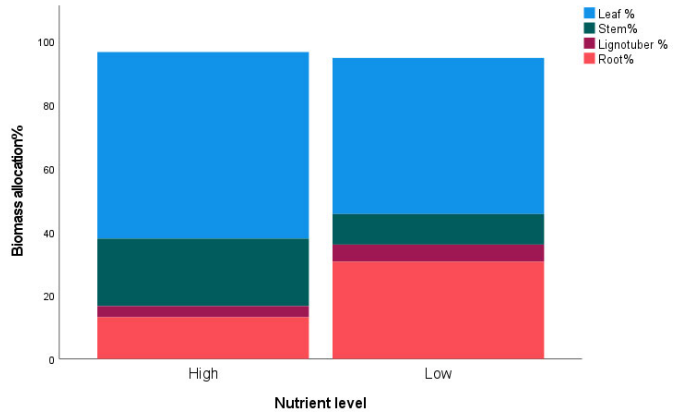

Figure 26: Biomass allocation percentages different plant parts of *E. melliodora* seedlings under two different nutrient levels.

High nutrient concentration has resulted in taller plants and higher plant biomasses where low nutrient levels have resulted in short plants with less biomass. However, high nutrient plants have allocated the highest biomass percentage for leaves than low nutrient plants. In contrast, low nutrient seedlings have allocated higher biomass percentages for roots compared to high nutrient seedlings. The second-largest biomass allocation in high nutrient seedlings was for stems. But under low light levels, biomass allocation percentage for stems has been decreased. Biomass allocation percentage for lignotubers had increased in low nutrient seedlings compared to high nutrient seedlings.

3.3.1 Plant height

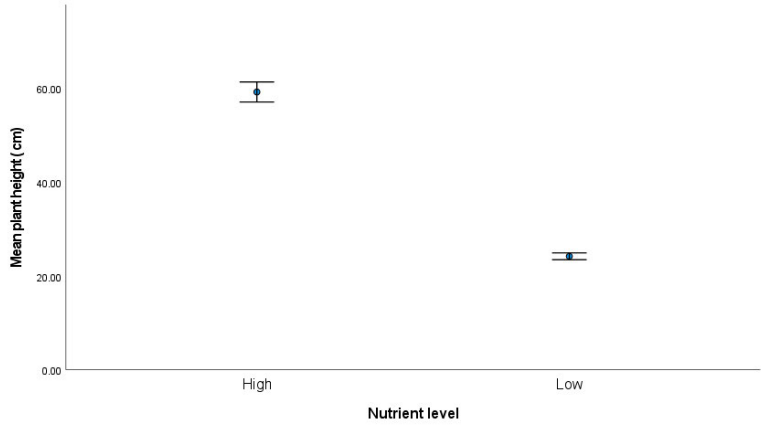

Figure 27: Mean plant height variation of *E. melliodora* seedlings grown under two different nutrient conditions.
Error bars represent the means ± standard error

Mean plant height were significantly different between two nutrient levels ($F_{1,200}$ = 1117, p < 0.0001, R^2_{adj} = 0.77) with taller plants in high nutrient level and shorter plants in low nutrients (Figure 27). Plant height was very weakly, but significantly, positively related to TS among low nutrient plants (slope = 0.37 ± 0.08, $F_{1,180}$ = 20.4, p < 0.0001, R^2_{adj} = 0.09) and but no relationship was found among high nutrient plants (slope = 0.37 ± 0.08, $F_{1,148}$ = 0.11, p > 0.05, R^2_{adj} =-0.005). A model of plant height suggested that there was no relationship between plant height and TS across both nutrition levels as TS dropped off out of the model ($F_{2,329}$ = 562.4, p < 0.0001, R^2_{adj} = 0.77). Model of plant height suggested that it was not related to TP in either high ($F_{1,148}$ = 1.583, p > 0.05, R^2_{adj} = 0.003) or low nutrient levels ($F_{1,180}$ = 1.702, p > 0.05, R^2_{adj} = 0.003).

3.3.2 Aboveground biomass

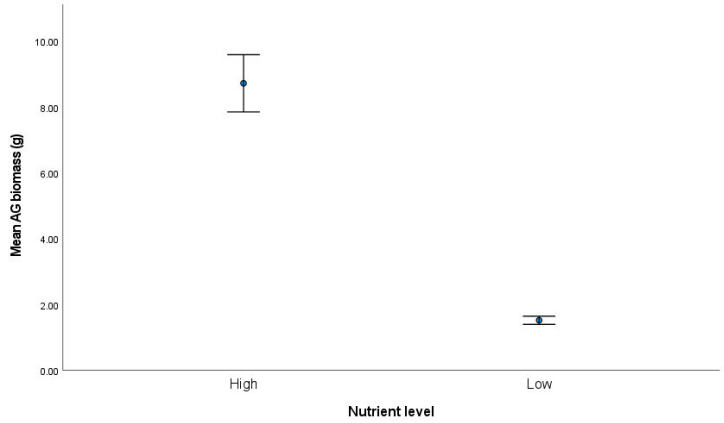

Figure 28: Mean aboveground mass variation of *E. melliodora* seedlings grown under two different nutrient levels.
-Error bars represent the means ± standard error

AG mass values were significantly different between two nutrient levels ($F_{1,330}$ = 490.2, p < 0.0001, R^2_{adj} = 0.59) with higher AG mass in high nutrient level and lower AG mass in low nutrients (Figure 28). AG mass was very weakly, significantly and positively related to TS in low nutrient plants (slope = 0.04 ± 0.01, $F_{1,180}$ = 7.56, p < 0.005, R^2_{adj} = 0.03) but no relation was found in high nutrient plants (slope = 0.05 ± 0.04, $F_{1,148}$ = 1.47, p > 0.05, R^2_{adj} =0.003). Model of AG mass suggested that there is no significant interaction between AG mass and TS across both nutrition levels as TS dropped out of the model ($F_{2,329}$ = 249, p < 0.0001, R^2_{adj} = 0.59). Model of AG mass suggested that AG mass was not related to TP in either high ($F_{1,148}$ = 0.317, p > 0.05, R^2_{adj} = -0.004) and low nutrient levels ($F_{1,180}$ = 0.10, p > 0.05, R^2_{adj} = -0.004).

3.3.3 Lignotuber mass

Table 9: Dry mass allocation variation of *E. melliodora* seedlings grown under two different nutrient conditions.

	High Nutrient (n=150)	Low Nutrient (n=182)
Lignotuber dry mass (g)	0.36±0.02[a]	0.12±0.008[b]
Dry mass allocation for lignotuber (%)	3.5±0.2[a]	5.3±0.2[b]

Within a row, different letters indicate significant differences (P < 0.05) from the Least significant difference test
(Mean concentration± 1 Standard error) of one-way ANOVA

LT mass was significantly different between two nutrient levels ($F_{1,330}$ = 117.2, p < 0.0001, R^2_{adj} = 0.25) with higher LT mass in high nutrient level and lower LT mass in low nutrients levels (Table 9). But LT% was significantly higher in low nutrient seedlings compared to high nutrient seedlings. This observation emphasized that plants had allocated a higher biomass percentage for lignotubers under nutrient limiting conditions. LT mass was strongly, significantly and positively related to TS among low nutrient plants (slope = 0.006 ± 0.001, $F_{1,180}$ = 12.6, p < 0.005, R^2_{adj} = 0.06) and but no relationship was found among high nutrient plants (slope = -0.002 ± 0.04, $F_{1,148}$ = 0.46, p > 0.05, R^2_{adj} = -0.003). A model of LT mass suggested that there was no significant interaction between LT mass with TS across both nutrition levels as TS dropped out of the model ($F_{2,329}$ = 55.6, p < 0.0001, R^2_{adj} = 0.24). Model of LT mass suggested that it was not related to TP in either high ($F_{1,148}$ = 1.2, p > 0.05, R^2_{adj} = 0.001) or low nutrient levels ($F_{1,180}$ = 0.05, p > 0.05, R^2_{adj} = -0.005).

3.3.4 Root mass

Table 10: Dry mass allocation of *E. melliodora* seedlings growing under two different nutrient conditions.

	High Nutrient (n=150)	Low Nutrient (n=182)
Root dry mass (g)	1.2±0.06[a]	0.7±0.03[b]
Dry mass allocation for Roots (%)	13.1±0.5[a]	30.5±1[b]

Within a row, different letters indicate significant differences (P < 0.05) from the Least significant difference test
(Mean concentration± 1 Standard error) of one-way ANOVA

RT mass was significantly different between two nutrient levels ($F_{1,330}$ = 55.3, p < 0.0001, R^2_{adj} = 0.14) with higher RT in high nutrient level and lower RT in low nutrients (Table 10). But RT% was significantly higher in low nutrient seedlings compared to high nutrient seedlings. RT mass was very weakly, significantly and positively related to TS among low nutrient plants (slope = 0.03 ± 0.008, $F_{1,180}$ = 19.46, p < 0.005, R^2_{adj} = 0.09) but no relation was observed among high

nutrient plants ($F_{1,148}$ < 0.0001, p > 0.05, R^2_{adj} =-0.006). A model of RT mass suggested that there was no significant relationship between RT mass and TS across two nutrition levels as TS dropped off from the model ($F_{2,329}$ = 55.6, p < 0.0001, R^2_{adj} = 0.24). Model of RT mass suggested that it was not related to TP in either high ($F_{1,148}$ = 0.40, p > 0.05, R^2_{adj} = -0.003) or low nutrient levels ($F_{1,180}$ = 1.59, p > 0.05, R^2_{adj} = -0.003).

3.3.5 Root: shoot ratio

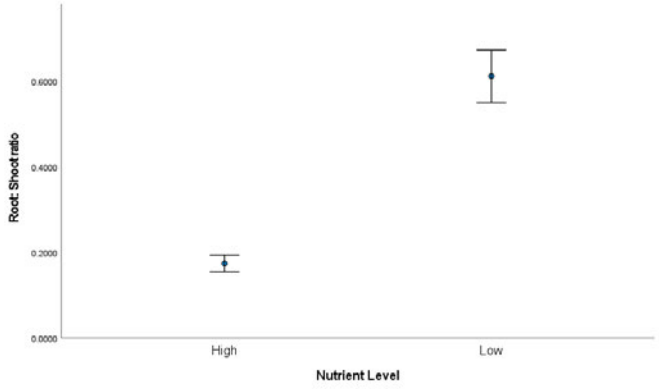

Figure 29: Root: shoot variation of *E. melliodora* seedlings grown under two different nutrient conditions. Error bars represent the means ± standard error

Root: shoot ratio was significantly different between two nutrient levels ($F_{1,330}$ = 178.3 , p < 0.0001, R^2_{adj} = 0.34) with higher root: shoot ratio in low nutrient level and lower root: shoot ratio in high nutrients seedlings (Figure 29). Root: shoot ratio was not related to TS among both low nutrient ($F_{1,180}$ = 2.0, p < 0.005, R^2_{adj} = 0.005) and high nutrient plants ($F_{1,148}$ = 0.15, p > 0.05, R^2_{adj} =-0.005). Furthermore, a model of root: shoot ratio suggested that root: shoot ratio was not related to TP in both high ($F_{1,148}$ = 0.40, p > 0.05, R^2_{adj} = -0.003) and low nutrient levels ($F_{1,180}$ = 1.59, p > 0.05, R^2_{adj} = 0.003).

3.3.6 Specific leaf area

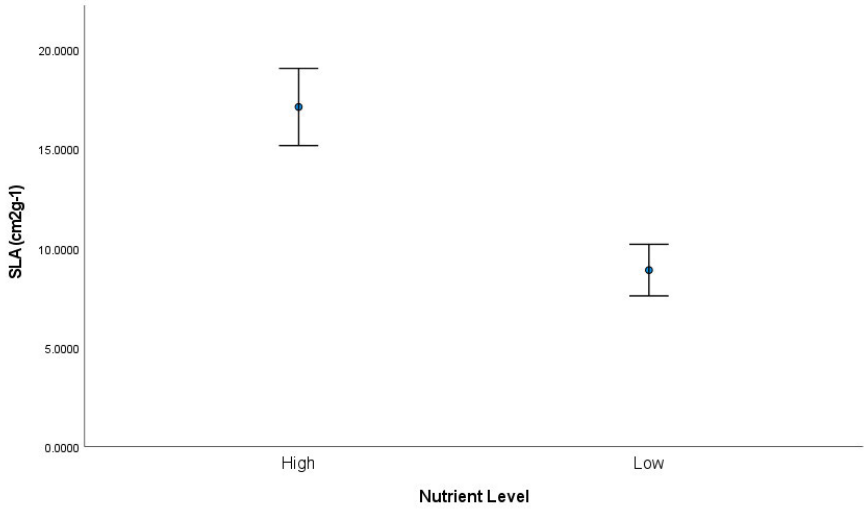

Figure 30: Specific leaf area (cm^2g^{-1}) measurement variation of *E. melliodora* seedlings grown under two different nutrient conditions. Error bars represent the means ± standard error

SLA values were significantly different between two nutrient levels ($F_{1,330}$ = 121.9 , p < 0.0001, R^2_{adj} = 0.26) with higher SLA in high nutrient level and lower SLA in lower nutrients (Figure 30). SLA was not related to TS among both low nutrient ($F_{1,180}$ = 0.69, p < 0.005, R^2_{adj} = -0.0001) and high nutrient plants ($F_{1,148}$ = 0.51, p > 0.05, R^2_{adj} =-0.005). Model of SLA suggested that SLA was not related to TP in both high ($F_{1,148}$ = 0.14, p > 0.05, R^2_{adj} = -0.003) and low nutrient levels ($F_{1,180}$ = 0.92, p > 0.05, R^2_{adj} = -0.005).

3.3.7 Mean leaf mass

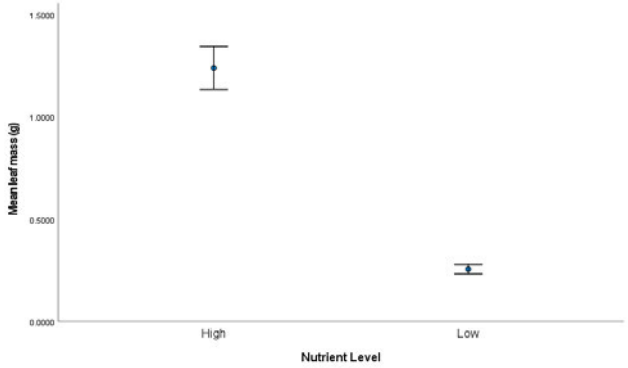

Figure 31: Mean leaf mass variation of *E. melliodora* seedlings grown under two different nutrient levels. Error bars represent the means ± standard error

ML masses were significantly different between two nutrient levels ($F_{1,330}$ = 391.4 , p < 0.0001, R^2_{adj} = 0.54) with higher ML mass in high nutrient level and lower ML mass in low nutrients (Figure 31). ML masses were not related to TS among both low nutrient ($F_{1,180}$ = 0.01, p < 0.005, R^2_{adj} = -0.005) and high nutrient plants ($F_{1,148}$ = 0.14, p > 0.05, R^2_{adj} =-0.005). Model of ML mass suggested that ML masses were not related to TP in both high ($F_{1,148}$ = 0.12, p > 0.05, R^2_{adj} = -0.005) and low nutrient levels ($F_{1,180}$ = 0.14, p > 0.05, R^2_{adj} = -0.005).

The model developed to test whether the difference in TS concentrations was related to the difference in growth parameters resulted in no significant interaction between sideroxylonal difference and the growth parameters resulting in plants do not get outcompete by plants with high or low sideroxylonal concentrations.

3.4 Discussion

Environmental variables such as nutrient availability directly affect plant's quality and growth (Barber et al. 2011). Plants' resistance to herbivory is determined by the intraspecific variation in plants' defence genotype (Hjalten, Ericson and Roininen, 2000; O'Reilly-Wapstra et al. 2002). Micro and macro-scale special and temporal variation in the environment such as nutrient content, water availability can affect the expression of plant defensive compounds and thereby plants susceptibility to herbivores as well (McArthur et al. 2003). It is important to predict how plants' defensive profile affects the plant growth and plant's interactions under nutritional variation (Cronin and Lodge, 2003).

Nutrient levels has significantly affected the growth parameters of *E. melliodora* seedlings. An increase in nitrogen(N) supply through fertilization usually increases photosynthesis by incorporating this N in photosynthesis enzymes, especially in Rubp carboxylase (Wilson, 1988). Resource limitation has a more negative effect on growth than photosynthesis and therefore, growth is considerably slow by the limited nutrient availability (Stamp, 2003). Both foliar sideroxylonal and total phenolics concentrations were significantly different between the two nutrient levels. The total sideroxylonal concentration of high nutrient seedlings is approximately two times higher than that of low nutrient seedlings. But in contrast, total phenolic concentrations in low nutrient seedlings were higher compared to high nutrient seedlings. But no relationship was identified between total sideroxylonal and total phenolics concentrations variation in either nutrient level.

According to the GDBH plants getting high nutrient availability have not limited their growth and photosynthesis and therefore allocate a larger proportion of photosynthates for their growth than differentiation traits (Stamp, 2003). But in nutrient-limited environments, as growth decreased carbohydrates accumulate more than growth demand are converted into C-based PSM with a low cost to the plant fitness (Stamp, 2003). If the trade-off presents as proposed by GDBH, plants in low nutrient environments produce a higher concentration of C-based secondary metabolites than high nutrient seedlings. According to CNBH factors that limit plant growth more than photosynthesis such as nutrient deficiency will increase the C pool available for allocation to secondary metabolites (Herms and Mattson, 1992). Although the concentration of the phenolic is high in low nutrient seedlings no trade-off has resulted

between any of the plant growth parameters and total phenolics. No trade-off was also observed between sideroxylonal concentration and any growth parameter as well.

There is a trade-off between the synthesis of phenolics and proteins (Haukioja et al. 1998). Biosynthesis of phenolics within tissues can be easily modified and varies according to N availability. Phenolics production reduces at high N availability and increases under N deficiency (Haukioja et al. 1998). According to this protein competition model hypothesis proposed (Haukioja et al.1998), demand for protein is higher during leaf expansion and allocation of phenylalanine to phenolics is decreased simultaneously (Riipi et al. 2002).

Low nutrient availability decreases both nutrient uptake per root area and root transpiration per unit leaf mass as well because of the reduced plant growth (Pooter and Nagel, 2000). But, under low nutrient levels, roots use relatively more available resources leaving fewer nutrients for leaves (Pooter and Nagel, 2000). Therefore, plants growing under nutrient-limited environments have a relatively low rate of photosynthesis per unit leaf mass. As a result, leaves growth of such environments are limited by the supply of nutrient and fewer photosynthates are allocated aboveground. Therefore, under low nutrient levels, there is a shift of biomass from shoots to roots is occurs (Pooter and Nagel, 2000). The root to shoot ratio was higher in low nutrient seedlings compared to high nutrient seedlings supporting the observation that higher belowground biomass than aboveground mass in low nutrient plants when compared to high nutrient seedlings.

As Brouwer (1962) proposed that the balance between carbohydrates and nutrients plays an important role in determining the plant growth towards the above ground or below ground. According to his theory shoots have the highest priority on carbohydrates and roots have the highest priority over nutrients and water. Therefore, when nutrients are grown focusing a large amount of the nutrient taken up to the plants will remain in the roots resulting in a higher root: shoot ratio (Brouwer, 1962; Eriscsson, 1995). Leaves with lower SLA are produced under a resource-limiting environment to maximize the photosynthetic efficiency (McArthur et al. 2003; Cunningham et al. 1999) and also to retain their competitive advantages (Liu et al. 2017). Low SLA occurred in the low nutrient environment is because nutrient conservation is important in low fertile environments and higher SLA can be observed in soils with higher N supply which allows plants to use the nutrients fast and rapid growth in fertile soil (Liu et al. 2017). When SLA is low it increases the number of chloroplasts and amount of photosynthetic

enzymes and thereby it increases the photosynthetic capacity per unit leaf area (Evans and Poorter, 2001).

As DeAngelis *et al.* (2012) proposed higher phenolics concentrations were observed when plants compete for nutrients under a low nutrient environment and the biomass of those plants also decreased. Similar patterns were observed with specific phenolics compounds in other experiments as well (Close and McArthur, 2002). For example, hydrolysable tannin levels were two times higher in nutrient-deficient *E. nitens* seedlings compared to nutrient-sufficient seedlings (Close et al. 2001). Close and McArthur (2002) proposed that photodamage is the main cause of variation in phenolics level within the plants in the same species and risk of photodamage rather than resource availability and risk of herbivory. Plants in nutrient limiting environments produces higher levels of phenolics when this low nutrient condition creates oxidative pressure within the plant body and production of reactive chemical species as a result of oxidative pressure (Close and McArthur, 2002). Therefore, it is clear that the defence against herbivory pressure is not the selective pressure that always decides the concentration of the phenolic within the plant body.

However, when plants are under high nutrient conditions plant growth is higher and the plant produces leaves with high nutrient quality which makes the susceptibility of these leaves to herbivores higher. Therefore, plants should produce more anti-herbivory compounds such as sideroxylonal which results in higher sideroxylonal in higher nutrient plants and lower sideroxylonal at lower nutrient levels. Higher sideroxylonal concentration in high nutrient seedlings might be because leaves with higher SLA are more likely to attack by herbivores than leaves with low SLA (Liu et al. 2017). A study by Moore et al. (2004) resulted in a similar observation with higher sideroxylonal concentrations at the site producing leaves with high mean N concentrations. They also found that there was a positive relationship between the foliar cineole and sideroxylonal concentration and site quality. Higher leaf mass per unit leaf area which is the inverse of specific leaf area in low-quality sites might have resulted in a dilution of sideroxylonal concentration in low nutrient seedlings (Moore et al. 2004).

Eucalyptus species possess various adaptations to soils they are growing, matching their nutrient demands to the nutrient availability of the site (Moore et al. 2014). Plants that grow in low-nutrient soil are more efficient in acquiring soil nutrients, recycling nutrients more efficiently, and have low growth rates (Holopainen et al. 1995). Therefore, the plants growing

under low-nutrient soils are not nutrient deficient. They might posse sideroxylonal which is enough for their plant defence. A similar observation was recorded by Moore et al. (2004). They observed that although the leaves of *E. microcorys* contained lower nutrient concentrations, nutrient content per unit leaf and unit leaf area differed very slightly from leaves growing in high-quality sites.

Two seedlings were planted together in this experiment to test the intraspecific competition between plants from the same species. However, no difference in any of the growth parameters was observed among plants. However, sideroxylonal concentration was significantly different between these two seedlings in both high and low nutrient seedlings. However, no tradeoff was observed between growth parameters of plant parts associated with this sideroxylonal difference between these seedlings. However, plants were not competing for nutrients as there was no difference in growth parameters between plants. Although the competition was not visible in biomass ratios there might be a competition occurring in below-ground root exudates produced by symbiotic microbes on a plant root. Therefore, root exudates must be taken into account when predicting tradeoffs occurring in competitive plants.

Chapter 4 – General Discussion

Plants allocate resources to maximize their fitness in the face of various abiotic challenges such as limited light and nutrient availability and biotic limitations such as herbivory and competition (Tuller et al. 2018). Resource limitation may create a conflicting demand for resources where plants allocate resources for growth, reproduction, and defence at the same time (Tuller et al. 2018). Understanding the patterns and processes determining how an allocation to defence and growth in a *Eucalyptus* species along under environmental gradient is important because of the domination of this genus in Australian ecosystems (Moore et al. 2004).

According to the results, it seems that the environmental conditions and plant size further modify PSM concentrations, but we expect that the rank order of defence among individuals remains the same. I have hypothesized that the constitutive defence profiles of the plants are pre-determined by genetics, and I was interested in the growth parameters of the plants under a variety of environmental conditions including light and nutrient limiting conditions with the expectation that the trade-off would be apparent under resource limiting conditions. However, this study highlights the effect of the environment on the phenotypic expression of genetically determined *Eucalyptus* resistance to herbivores. The pattern of allocation growth parameters and defence compounds such as phenolic compounds including sideroxylonal will varies based on the magnitude of the change in different abiotic environments (Abdala-Roberts et al. 2016).

Both sideroxylonal and total phenolics were higher in full sun light seedlings and lower in the light limiting environment. But in contrast under higher nutrient concentrations, sideroxylonal concentration was higher compared to the low nutrient seedlings and total phenolics were higher in low nutrient environments. Phenolics compounds are highly costly for the plant to produce and constraints are present in light limiting and nutrient limiting environments suggesting that these environmental constraints set limits to the growth and production of these compounds (Abdala-Roberts et al. 2016). Plants' phenotypic adjustment to increase light availability will result in increased production of total phenolics compounds which would in turn influence herbivores (Close et al. 2003). Therefore, it is clear that the observed higher concentration of total phenolics in high light availability could be because of photoprotection

from higher light intensity and nothing to do directly with plants' defence against herbivory (Abdala-Roberts et al. 2016).

Plants occurring in infertile soil and shade generally cannot accumulate sufficient resources to support their rapid growth as the evolutionary responses of plants to resource limitation is the slow growth and those plants have low capacity to photosynthesis and absorb nutrients (Bryant et al. 1983). The C: nutrient balance hypothesis and growth differentiation balance hypothesis simply suggests that resources in excess growth demand are shifted to plant defence (Coley and Barone, 1996). Therefore, high light and low nutrients should lead to high carbohydrate accumulation which should cause higher carbon-based secondary metabolites to accumulate in such conditions (Coley and Barone, 1996). According to the observations total phenolics concentrations were higher in full sunlight seedlings and low nutrient seedlings. In contrast, total sideroxylonal concentration was higher in full sunlight conditions as proposed by the trade-off hypothesis but resulted in lower sideroxylonal concentration under nutrient limiting environment and resulted in higher sideroxylonal under higher nutrient levels resulting opposite to proposed by trade-off mechanisms. However, no trade-off was apparent between plant growth parameters and defence in *Eucalyptus* seedlings under both light limiting and nutrient limiting conditions. The response of phenolics to nutrient limitation is similar to the responses of other antioxidant compounds (Close and McArthur, 2002). The higher concentration of phenolics in nutrient limiting seedlings only occurs when plants got photo inhibited and resulted in oxidative pressure has increased (Close and McArthur, 2002). They also suggested that this higher phenolics condition in nutrient limiting environment when plant experienced the periods of low temperature coupled with high light levels. Therefore, it is clear that a higher concentration of phenolics is reflecting the risk of photodamage as it’s the primary role and the second the risk of herbivory if presents (Close and McArthur, 2002).

It is important to consider that the measurements of plant defence in my experiment were based on total phenolics and total sideroxylonal concentrations. However, light and nutrient availability has various effects on other PSM compounds that have not been measured in this experiment including terpenes, and other phenolics compounds such as lignin, flavonoids (Abdala-Roberts et al. 2016). Furthermore, Moore et al. (2004) observed a correlation between the foliar FPC content and foliar terpene content in *Eucalyptus* species as well

(Moore et al. 2004). The trade-off might be present between the combination of these compounds and plant growth. Therefore, more future work is needed considering all the defensive compound varieties to get a more detailed and precise idea to understand how the genetically determined defense profile of *Eucalyptus* species varies in response to different light and nutrient availability.

The FPCs found in leaves such as macrocarpal G, jensenone, and sideroxylonal have been considered as antifeedant compounds impacting plant-herbivore interactions especially of marsupial folivores (Eyles, Davies and Mohammed, 2003). Lawler et al. (2000) observed that foliar sideroxylonal concentrations were greatly varied between plants from the same species even when the environmental conditions are almost identical. This observation suggests that sideroxylonal concentration within an individual plant is strongly determined by the genetic material. Lawler et al. (1998) suggested that *Eucalyptus* foliar sideroxylonal concentration was strongly correlated to cineole concentration. Further, an experiment conducted by Wallis, Watson, and Foley (2002), observed that the *E. melliodora* leaves refused by possums were similar in the concentration of sideroxylonal to the leaves consumed by possums which suggested that the sideroxylonal concentration is not alone acting as a herbivore deterrent compound but the strong correlation between the concentration of sideroxylonal and terpene specially cineole together acting against herbivores. Lawler et al. (2000) have hypothesized that the role of foliar terpenes is not to deter herbivory through toxicity but they act as a cue to the concentration of true different compound, sideroxylonal. All above observations suggest that experiments evaluating trade-off mechanisms considering sideroxylonal concentration should consider the terpene concentrations as well.

As a consequence of the higher plant growth rate, young leaves are rich in higher N and higher water content than mature leaves which makes these young leaves more susceptible to herbivores than mature leaves (Coley and Barone, 1996). When plants allocating resources to defence they always consider the cost: benefit ratio throughout the plant development and produce higher defensive chemicals concentrations only in life stages where the risk of herbivores is increased (Boege and Marquis, 2005). Therefore, it is believed that plant defence gets reduces when plants develop from a small sapling to a mature tree (Boege and Marquis, 2005). Therefore, it is clear that seedlings should intrinsically produce higher concentrations of defence compounds at their early stages to protect them from potent herbivores. So, there

might be no trade-off apparent in the early stages of life as plant defence is essential at that stage of life.

For both full sunlight and 30% light levels plant height was positively correlated with total sideroxylonal concentration but total phenolic concentration was negatively correlated with plant height. Similar trends were observed for the aboveground biomass as well. According to the observation under both full sunlight and low light levels plants with higher sideroxylonal concentrations had higher growth rates compared to the plants with lower sideroxylonal concentrations. But in contrast in both full sunlight and lower light levels plants with higher total phenolics concentration had a lower growth rate. In the nutrient experiment, above-ground biomass showed a positive trend with sideroxylonal under low nutrients conditions. Similarly, under low nutrient levels, lignotuber mass and root mass showed a positive relationship with sideroxylonal as well. These results emphasize that there are more positive trends between foliar chemical concentrations and growth parameters rather than a trade-off. Therefore, it is important to find out where this trade-off occurs.

Within each light and nutrient treatment level both sideroxylonal and total phenolics concentrations were greatly varied between different individual plants. Although the trade-off was not apparent in seedling levels there is a possibility that it will be visible in well-grown trees in forests and woodlands. There is another possibility that this delayed expression of defences in resource-limited trees, will catch up by resource-limited plants when they reach the same size as the high-resource plants. Furthermore, plants have mutualism and herbivory interactions below ground level as well. Therefore, they produce roots exudates as a result of these interactions where plant use their C resources to produces these root exudates. The trade-off might be apparent at below ground level as well. Therefore, further experiments in resource allocation should consider capturing the plants' root exudate production as well.

Due to the recent covid restriction, I was not able to analyze the foliar N and C concentration as I have planned earlier. If I was able to collect the foliar N and C concentration within each leaf I would be able to get more insight idea of the remaining C and N pool remaining within leaves. If I had been able to run the experiment as intended, I could also have confirmed whether or not the rank order of defense levels among trees stayed the same throughout growth. Below-ground root exudate production can also be taken into account when improving the experiment. A particular growth threshold level can be considered for both low

resource and high resource plants while harvesting to reduce the impact of delayed defense in low resource seedlings. Therefore, this experiment can be further improved in the future in many ways.

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