An investigation of the use of exogenous Glycine Betaine and Seaweed to ameliorate drought stress in cutting propagation of *Griselinia littoralis* and *Lavatera x Clementii*

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An investigation of the use of exogenous Glycine Betaine and Seaweed Extract to ameliorate drought stress in cutting propagation of *Griselinia littoralis* and *Lavatera x Clementii*

By Meili Duan

The exogenous application of Glycine Betaine (GB) and seaweed extract (SWE) has been suggested as a possibly effective approach for improving the drought tolerance of cuttings. The aim of this study were to test the feasibility of GB and GB+SWE helping cuttings fight drought stress during cutting propagation in glasshouses.

The research first examined whether exogenous spray application (GB and GB+SWE) alleviates the detrimental effect of drought stress on the root growth of *Griselinia littoralis* in fertilized and unfertilized potting mix. Then GB and GB+SWE were evaluated in terms of their agronomic values in fighting drought stress during cutting propagation was considered, through the use of a series of experiments.

From analyzing the data of all the experiments, the different times when the cuttings were taken, the frequency of the exogenous application and the environmental conditions were investigated to whether they may be factors affecting the cuttings' growth under drought stress. During cutting propagation, the drought conditions experienced by the cuttings were caused by high temperature and strong sunlight.

Experiments showed that GB and GB+SWE can be effective to fight drought stress. The use of GB gave better improvements in cutting growth, compared with the GB and fertilizer in the potting mix together, but these results were opposite to those of GB+SWE in the potting mix while this result was significantly different between cuttings in potting mix and in the soil with three month mix fertilizer. The effects of GB and GB+SWE were various in different treatments and inconclusive because of variations in high temperature, strong sunlight, and types of soil. However, further research is required to investigate ways of using GB and GB+SWE in the most efficient manner, while taking care to eliminate random variables which affect cutting growth.

Keywords: Glasshouses, drought stress, cutting propagation, Glycine Betaine, Seaweed extract, *Griselinia littoralis* and *Lavatera x clementii*

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Master's thesis editorial support statement

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I confirm that I have provided support for the production of this thesis. I have assisted re-writing the paragraph and sentence structure, grammar and spelling of text in the thesis. In some instances this involved clarifying the intended meaning of sections of a written draft through discussion with the student and suggesting wording that would ensure that information was presented clearly. I have also provided assistance addressing annotated comments made by a supervisor to earlier draft of a hard copy.

I provided English language support and I have not added to or amended the scientific content of the thesis. I have a bachelor's qualification in physics, but no professional expertise or high level qualification in the topic of the thesis.

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1.1 Introduction

Propagation by stem cuttings in a glasshouse is one of the most commonly used techniques in modern nurseries worldwide to propagate horticultural landscape plants. Cutting propagation tends to be easy in daily practice. Its development and use has had a profound impact on the large market of clonal plants by enabling fast growth and high productivity. This is evident in the production and distribution of millions of cutting-grown ornamental landscape plants a year by nurseries throughout the world to meet the growing demand of horticultural landscape plants (Blythe, Sibley, Tilt, & Ruter, 2007). Currently, this technique is widely applied to produce high demand plants like poinsettias and Christmas trees during the month of December (Lopez, 2008). Propagation of plants from stem cuttings has been used extensively in the horticulture industry for both herbaceous and woody species (Preece, 2003).

One of the challenges of cutting propagation has been with cuttings wilting and dying before they develop roots. Cutting propagation involves a considerable application of water for the control of humidity in soil and air to maintain good growth, promote the development of the cuttings' roots, and to minimize transpiration water loss (Santos, Fisher, & Argo, 2008). In this typical rooting environment for vegetative cuttings, water is the most important factor. Prior to root initiation, water uptake is limited and leafy stem cuttings are most vulnerable to wilting or death at this stage. Of the many causes of the cuttings wilting and dying in propagation, is the failure to take up sufficient water through the leaves and stems (Loach & Whalley, 1977). Environmental drought in a country or growing region will usually imply there will be drought stress in the glasshouse too because if there is no water outside the glasshouse there will be no water available to irrigate inside it as well. The reasons causing drought worldwide are different depending on the various environmental and human factors of the areas. Glasshouses have an extra factor for increased aridity, with strong sunlight and high temperatures causing further water loss that needs to be dealt with. Most glasshouses allow us to manipulate and control the temperature, light, and humidity, and different glasshouse cladding materials are different with respect to light transmission, heat transfer, and condensation behavior (Duarte-Galvan et al., 2012; Waaijenberg, 2004). Therefore, these various factors can alter the degree of drought stress in glasshouses as well.

Scientists and farmers have studied and tested various approaches to help crops and plants withstand drought stress. Among the various methods of fighting drought stress, the exogenous application of Glycine Betaine (GB) and seaweed extract (SWE) have been suggested as a possibly effective approach for improving the drought tolerance of cuttings (Aldesuquy, Abohamed, Abbas, & Elhakem, 2012; Ashraf & Foolad, 2007; Battacharyya, Babgohari, Rathor, & Prithiviraj, 2015). Recently, the application of GB and seaweed extract for combating drought stress has gained increasing research interest, and exogenous application of GB and seaweed extract has been used to improve the drought tolerance of most agricultural crops, as well as some horticultural plants, via foliar spray, irrigation and soaking. For example, exogenous application of GB and seaweed extract can improve and promote yield, plant height, and the quality of fruit and seeds of the plant (Battacharyya et al., 2015; Reader, 2015). As far as could be ascertained from preliminary readings, there have been no studies of the effects of GB and SWE together under drought stress during cutting propagation of landscape plants.

Therefore, this research aims to study the effects of GB and seaweed extract under drought stress during cutting propagation of *Griselinia littoralis and Lavatera x clementii.* The literature review explores the causes and effects and treatments of drought stress during cutting propagation. Drought can occur in glasshouses in areas without water deficit as well as in arid and drought-prone areas, and the methods of responding to drought stress, including successful application of GB and seaweed extract (SWE) are examined in detail.

1.2 Aim and research objectives

This study aims to investigate how using GB and SWE separately and together might constitute an effective exogenous application solution for the propagation of cuttings in conditions of drought stress. The study uses three common methods of exogenous application—foliar spray, irrigation, and soaking—of GB and SWE to improve the tolerance of cuttings of *Griselinia littoralis* and *Lavatera x clementii* to drought. To more effectively differentiate soil factors affecting the application and effectiveness of GB and SWE, different soil conditions have been considered. Root growth in cutting propagation is influenced by whether fertilized or unfertilized soil is used. Therefore, both fertilized and unfertilized soil have been used in the experiments.

The study has three objectives:

1. To investigate whether exogenous spray application (GB and GB+SWE) alleviates the detrimental effect of drought stress on the root growth of *Griselinia littoralis* in fertilized and unfertilized soil for cuttings taken in April (Experiment 1A and 1B), and for cuttings taken in October (Experiment 2A).
2. To investigate whether exogenous soaking application (GB and GB+SWE) alleviates the detrimental effect of drought stress on the root growth of *Griselinia littoralis* in fertilized and unfertilized soil for cuttings taken in October (experiment 2B).
3. To determine whether foliar spray application or soaking is the more effective application method for GB and GB+SWE for propagating cuttings in fertilized and unfertilized soil for *Lavatera x clementii* with 2 levels of drought stress (Experiment 3).

In this research, drought is always taken to mean the lack of sufficient available water to the cuttings inside the glasshouse. The cuttings drought tolerance is what the treatments of GB and seaweed extract were intended to improve. Ideally the treatments would enhance to ability of the cuttings to keep growing and stay alive even in the adverse conditions of drought within the glasshouse.

1.3 Background and Literature Review

In New Zealand, most horticultural production businesses grow landscape plants such as *Griselinia littoralis* and *Lavatera x clementii* by cutting propagation (Santos et al., 2008). However, drought commonly affects the growth of the cuttings, especially in the root development of the cuttings (Franco, Bañón, Vicente, Miralles, & Martínez-Sánchez, 2011; Santos et al., 2008). The cuttings are planted under high humidity to produce roots, and are subsequently grown to a rooted liner. Normally, the process takes 4 to 6 weeks. During this time, these cuttings are commonly affected by drought. Even light soil drought with 80% soil moisture and air drought caused by strong sunlight and high temperatures can be detrimental (Santos et al., 2008). However, not many studies focus on the effects of drought stress during cutting propagation.

There have been various studies done on various methods of protecting plants from drought, with a lot of information available on genetic engineering and irrigation, but these are both methods which require a lot of capital investment to make them work, and so therefore they are not so efficient or attractive for plant propagation nurseries. However, these previous studies can give good insights to what may be an effective line of enquiry, and provide clues as to what may be more effective ways of protecting cuttings from drought stress during cutting propagation, cost effectively.

From recent research, GB and SWE have been reported to effectively improve drought tolerance in the process of plant growth (Aldesuquy et al., 2012; Craigie, 2011). The use of GB and SWE, therefore may significantly contribute to increased crop production in stress environments. However, their research lacks evidences as to its effectiveness for cutting propagation. This is why I am interested in investigating this further to see how GB and Seaweed extract can benefit cutting propagation with *Griselinia littoralis* and *Lavatera x Clementii*.

1.3.1 The main factors causing drought stress in the glasshouse

Many studies presented research on water deficit caused by the growing scarcity of freshwater, and showed that the limitations on irrigation were not confined only to the fields but had a significant impact on the drought conditions inside the glasshouses of these regions as well (Pereira, Oweis, & Zairi, 2002; Postel, 2000; Wang et al., 2017). This research focused on countries surrounding the Nile River and found that high use of freshwater external to the glasshouses for irrigation combined with stronger sunlight and hotter temperatures from climate change, has contributed to a water deficit inside glasshouses.

Other studies such Duarte-Galvan et al. (2012) and Waaijenberg (2004)have also reported that many uncontrollable factors in glasshouses can cause cuttings to face drought stress. Most glasshouses allow us to manipulate and control the temperature, light, and humidity; and different glasshouse cladding materials are different with respect to light transmission, heat transfer, and condensation behavior (Duarte-Galvan et al., 2012; Waaijenberg, 2004). Therefore, these various factors can alter the degree of drought stress in glasshouses as well. It was found in my experiments as well, that these uncontrollable factors could cause significant variations in cutting growth.

Clearly, we cannot ignore the external environmental factors and some uncontrollable factors when assessing the drought risks to cutting propagation. While it may seem possible to irrigate the cuttings inside the glasshouse, provide cover for shading from strong sunlight, and maintain temperature by using a cover or air conditioner; in reality the availability of freshwater may be limited, and stronger sunlight with higher temperatures means that overall there is less water to be had for the cuttings inside the glasshouse. The reality of the conditions from the external environment may be different to what we imagine so it is important to fully assess the situation.

1.3.1.1 Drought in the glasshouses caused by high temperature and strong sunlight

Drought in glasshouses is increasingly seen not only in arid and drought prone areas but also in regions without water deficits like New Zealand (Pereira et al., 2002). This is because the factors leading to drought stress in glasshouses are not only irrigation restrictions caused by a scarcity of freshwater, but also a lack of water caused by high temperature and strong sunlight. Wang, Caradus, and Chu (1996) and Hetherington and Woodward (2003) reported that strong sunlight and high temperature lead to both soil drought and air drought. Strong sunlight often coincides with periods of drought that affects root development.

Vicente-Serrano et al. (2014) reported that air temperature and soil moisture content is directly related, the soil will lose 8% of its moisture per 1 °C rise in temperature. Such a lack of moisture in the soil and atmosphere causes temporary or sometimes permanent water deficits that limit the growth as well as the productivity of agricultural crops and horticultural plants and cuttings (Shao et al., 2009).

The necessarily higher temperature that is ideal for cutting propagation is one of the biggest risk factors causing drought stress in the glasshouses because while cutting propagation requires a higher temperature to encourage rooting, the temperature cannot be so high that it makes the leaves become stressed. The air temperature is maintained at 20-22 °C to promote root development but this increase in temperature is exposing the soil and air to a higher potential moisture loss which needs to be managed properly for the healthy growth of the cuttings. Excessive heat causes drought stress from overly dry soil and air in the glasshouses, and both may be the most significant factors causing drought leading to growth, rooting, damage and mortality problems with cutting propagation in glasshouses (Clemson, 2008).

1.3.2 The effects of drought stress in the glasshouse

Farooq, Wahid, Kobayashi, Fujita, and Basra (2009a) reported that the effects of drought range from morphological to molecular levels, and these effects are evident at all phenological stages of plant growth at whatever stage the water deficit takes place. At the stage of cutting propagation, the effects of drought stress are mainly on the growth of the shoot, leaf area, water relations, nutrient relations, and photosynthesis. Drought stress during cutting propagation may be the main factor leading to wilting and dying of cuttings before root development.

1.3.2.1 Drought stress limits growth of shoots, roots and leaves

More and more studies have shown that drought stress affects plants' growth (Manickavelu, Nadarajan, Ganesh, Gnanamalar, & Babu, 2006; Tripathy, Zhang, Robin, Nguyen, & Nguyen, 2000). Drought stress negatively affects shoot height (Figure 1), leaf area, root growth and crop yield (Table 1) of plants in general (Hussain, Malik, Farooq, Ashraf, & Cheema, 2008; Kaya, Okçu, Atak, Cıkılı, & Kolsarıcı, 2006; Nonami, 1998). This means that drought stress will likely affect the growth of leaves and shoots and root development and growth during cutting propagation as well.

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Figure 1: The effects of drought stress on shoot in rice.

Sourced from Tripathy et al., 2000 and Manikavelu et al., 2006. The two plants were grown in a well-watered environment for 20 days to emergence. Then one continued to be grown in the well-watered condition, but the other one was grown in soil naturally drying out (drought stress). This picture showed the effects of drought stress after a drought cycle of 20 days.

Table 1: The effects of drought stress on different crops in different growth stages - comparing the yield reduction from drought stress by stage and that stress at different stages produces different yield reductions. Adapted from (Farooq, Wahid, et al., 2009a).

Growth stage	Crop	Yield reduction	References
Seed filling	Barley	49-57%	Samarah (2005)
Reproductive	Canola	30%	Masoud (2007)
Reproductive	Chickpea	45-69%	Nayyar, Kaur, Singh, and Upadhyaya (2006)
Grain filling	Maize	79-81%	Monneveux, Sanchez, Beck, and Edmeades (2006)
Reproductive	Maize	63-87%	Kamara, Menkir, Badu-Apraku, and Ibikunle (2003)
Vegetative	Maize	25-60%	Atteya (2003)
Flowering	Potato	13%	Kawakami, Iwama, and Jitsuyama (2006)

1.3.2.2 Drought stress affects water relations

Farooq, Wahid, et al. (2009a) and Farooq, Wahid, Lee, Ito, and Siddique (2009) reported that plants and cuttings take up water as an important physiological process for their growth and development. Water potential, stomatal resistance, and transpiration are significant factors influencing plant water relations. Drought stress directly affects water relations which limits plant growth, and this may be an important factor leading to cuttings wilting and dying under drought stress.

The effects of drought stress from the soil on water potential

Matrix and pressure, osmotic activity and gravity are three components comprising water potential. Water potential is measured in megapascals (Mpa) to reflect the energy level of water in the plant tissues and the soil (Brodribb & Hill, 2000). Steudle and Peterson (1998)reported that pressure potential is separated into zero, negative and positive, increasing pressure increases water potential. Normally, water moves from an area of high (less negative) to low water potential. Water potential varies with the prevailing weather conditions and soil attributes like texture, drainage, organic matter content and structure (Farquhar & Sharkey, 1982). Therefore, these external abiotic and biotic factors can affect the water potential of plants. For example, under drought stress or wilting, living plant cells have a pressure potential near zero. The soil is dry, which means that the soil has a more negative water potential than the plant, so the movement of water is away from the plant into the soil (Gallego, Rico, Moreno, & Regina, 1994). This means that the plant's roots, shoots and leaves will wilt and die.

The effects of drought stress from the air on stomatal behavior and functioning

With drought stress from strong sunlight, and hot and dry conditions, the balance of bringing in enough carbon dioxide for efficient photosynthesis and controlling the water loss in this process is particularly critical (Hetherington & Woodward, 2003). Effective balance requires optimal stomatal behavior to control the flow of gases and water vapour in and out of plant. In all of the processes controlling plants' growth and development, stomatal behavior is the most complex. Stomata are small pores on the surfaces of leaves and stems, controlling the exchange of water vapor and carbon dioxide between the leaves and the atmosphere (Hetherington & Woodward, 2003).

Drought stress from air and soil affects the stomata's behavior and functioning (Schulze, Turner, Gollan, & Shackel, 1987). Stomatal movement is where plants close their stomata, when they responds to drought stress (Mott & Parkhurst, 1991). Stomatal movement is a response to the atmospheric and rhizospheric environment. High temperature, drought stress from the air, and strong light directly limits stomatal movement. When the amount of available soil water or air humidity is moderately or severely limited, the first option for plants is to close their stomata to conserve water (Mott & Parkhurst, 1991).

1.3.2.3 The effects of drought stress on transpiration and photosynthesis

Alem (2010) showed virtually all plants fight the water deficit (drought stress) by closing their stomata to prevent water loss through transpiration and to limit. Under drought stress, the stomata close to protect the plant and to limit damage as much as possible, but the limiting of these vital processes affects growth and roots development as well.

1.3.2.4 Summary

Drought will affect plants more or less strongly in the different stages of their growth (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009b). For example, during cutting propagation in the glasshouse, drought stress affects the growth of the shoot, leaf area, water relations, stomatal behavior and hence transpiration and photosynthesis, thus limiting water movement which leads to wilting and leaf curling and death before the roots develop (Farooq, Wahid, et al., 2009b; Gallego et al., 1994). Availability of water is a significant factor for activating root development, but stomatal closure caused by drought stress limits water transport, resulting in cuttings wilting and dying (Loach, 1988). Therefore, the most effective approach to help the plant grow must be focused on the types of drought that are most likely to seriously affect the plant at each particular stage of growth.

Despite the fact that agricultural growers have made efforts to fight drought, and these methods have effectively improved drought tolerance of crops in the field,, the drought effects on cutting propagation in glasshouses still have not been effectively addressed (Burrows, 1995). Therefore, there is an urgent need to improve drought tolerance of cuttings to meet the requirements of the large market for cutting propagation. Hence, more studies are needed for the effects of drought stress during cutting propagation in glasshouses.

1.3.3 Methods of addressing drought

Under drought stress, plants are more vulnerable than animals (Xu, Zhou, & Shimizu, 2010). Plants, unlike animals, cannot easily move and select a more comfortable environment (Thomas, 1997), so when water shortages occur, higher plants always respond and adapt to the drought stress by escape, avoidance, and phenotypic flexibility strategies (Farooq, Wahid, et al., 2009b). For example, plants can deal with drought stress through a shortened life cycle or growing season; or reducing water loss by methods such as glaucousness or waxy bloom on leaves; and limiting the number and area of leaves or enhancing root density (Farooq, Wahid, Lee, et al., 2009). However, even though plants have strategies for surviving water shortage, drought stress still affects plants' growth and fruit or harvest yield, water relations, nutrient relations, and photosynthesis.

Scientists and farmers have studied and tested various approaches to help crops and plants withstand drought stress. These methods can be broadly separated into; genetic engineering, agroecological or agronomic approaches such as altering farming methods or the growing conditions of crops and plants to reduce their susceptibility to drought (Gurian-Sherman, 2012). Ecological farming practices includes methods such as improving the water-holding capacity of the soil through utilizing crop or crop residues to cover the soil surface, using broad canopies to shade the soil to reduce water evaporation and soil temperature, or using supplemental irrigation (Strelcová et al., 2008).

Among the studied and tested methods, genetic engineering is treated cautiously by certain groups such as the Union of Concerned Scientists (UCS) but touted as being a positive solution by practitioners and proponents of genetic engineering. The biotech industry has suggested that improving crops through genetic engineering would be a good solution because it can reduce water use overall and prevent losses under drought stress. Through the insertion of genetic material of unrelated organisms such as bacteria into crops, or by in-vitro manipulation of the genes of the crops (Gurian-Sherman, 2012) the plants can be made more resilient to drought stress. However, Genetic engineering (GE) is still facing a challenge in helping plants deal with drought stress, because there are many genes which respond to drought stress, and the details of these are still relatively unknown. In addition, the cost of GE is much higher than classical forms of breeding, while agroecological or agronomic approaches and other technologies such as exogenous application of treatments are more effective and have a lower cost (Gurian-Sherman, 2012). This makes GE a less attractive choice and exogenous application more attractive at this time.

From recent research, GB and SWE have been reported to effectively improve drought tolerance in the process of plant growth (Aldesuquy et al., 2012; Craigie, 2011). This approach, which may significantly contribute to increased crop production in stress environments but has not received sufficient consideration to be able to state that it is beneficial for cutting propagation. This is why I am interested in investigating this further to see how GB and Seaweed extract can benefit cutting propagation with *Griselinia littoralis* and *Lavatera x Clementii*.

1.3.4 Glycine Betaine (GB) and seaweed extract (SWE)

GB is a small amino acid which is found in sugar beet, spinach, barley, wheat, and sorghum (McCue & Hanson, 1990; Rhodes & Hanson, 1993; Weimberg, Lerner, & Poljakoff-Mayber, 1984; Yang et al., 2003). It is a water-soluble organic molecule, a fully N-methyl-substituted derivative of glycine, and a major organic osmolyte and osmo-protectant that fights the biological effects of drought on plants by protecting cellular components from dehydration-related injury (Ashraf & Foolad, 2007; Lambou et al., 2013). SWE can be used as a plant biostimulant, source of nutrients, and components of organic manure and fertilizers to promote plant growth (Battacharyya et al., 2015).

1.3.4.1 Importance of Glycine Betaine (GB)

Most GB product is purified from sugar beet now and most of the commercially available product is in powder form (Mäkelä et al., 1996). Natural GB is a plant metabolite produced in normal cultivation environments. Beiß (1994) reported that the concentration of GB in sugar beet depends on the variety, drought condition and crop stand density of sugar beet. Older varieties of sugar beet are able to accumulate more GB than newer varieties, drought stress during the growing period increases the concentration of GB and denser plant populations can accumulate more GB than plants which are more widely spread out.

GB can easily be collected relatively inexpensively from high-yield plants such as sugar beets (Rhodes & Hanson, 1993). Cultor Finnsugar Bioproducts is a leading producer of natural GB and uses the following method for GB extraction. Molasses from the beet sugar production process is used as the raw material for GB production in a chromatographic separation process carried out in large columns filled with separation resin where water is used to elute the molasses through the column system. Then pure natural GB can be collected at the outlet points of the column system (Mäkelä et al., 1998).

GB combats drought stress via osmotic adjustment by controlling the loss of water to the environment (Lambou et al., 2013). It might be useful for root growth during cutting propagation because it can protect plants from stress at different stages of growth via different mechanisms. These include production of detoxifying reactive oxygen molecules, contributing to cellular osmotic adjustment, production of stabilizing enzymes and proteins, and by protecting membrane integrity (Mäkelä et al., 1998). GB can also protect the plant's reproductive organs, accumulating more in these organs than in other parts of the plant (Park et al., 2004). In flowers, siliques, and inflorescence apices, levels of GB are approximately five times more than in leaves when plants are fully matured (Chen & Murata, 2008).

GB is abundant mainly in chloroplasts to adjust and protect the thylakoid membrane, thereby maintaining photosynthetic efficiency (Aldesuquy et al., 2012; Ashraf & Foolad, 2007). In higher plants, serine normally synthesizes GB through ethanolamine, choline, and betaine aldehyde in chloroplasts. Choline monooxygenase (CMO) makes choline convert to betaine aldehyde, then it is converted to GB by betaine aldehyde dehydrogenase (BADH) (Figure 2). GB is naturally accumulated in some plants but most cannot naturally accumulate a sufficient amount of GB to alleviate the adverse effects of dehydration caused by environmental drought stress. So exogenous application of GB may help reduce the adverse effects of drought for low-accumulating or non-accumulating plants (Aldesuquy et al., 2012)

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Figure 2: Biosynthetic pathway of glycine betaine in higher plants. Source from Ashraf and Foolad (2007)

1.3.4.2 Seaweed extract (SWE) and its Importance

SWE can improve crop performance and yield, elevating resistance to biotic and abiotic stress (Figure 3). There are 9000 species of seaweeds which are an integral part of marine coastal ecosystems, they are separated into three main groups; Phaeophyta, Rhodophyta, and Chlorophyta; or the brown, red, and green algae, respectively (Khan et al., 2009). SWE has been shown to enhance growth and crop yield via cellular metabolism. SWE are made up of macro- and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid (ABA)-like growth substances (Khan et al., 2009).

In the present market, the seaweed extracts are aqueous preparations ranging in color from almost colorless to an intense dark brownish-black. Likewise, there are wide varies in odors, viscosities, and particulate matter contents. Significantly, the methods of manufacture are held as proprietary information, being almost rarely published. In general, seaweed extracts are made by processes using alkalis or acids, water, or by physically disrupting the seaweed by low-temperature milling to give a micronized seaweed suspension which are mildly acidic and are greenish to greenish-brown in color (Craigie, 2011; Hervé & Percehais, 1983; Stirk & van Staden, 2006).

Heating the seaweed in alkaline sodium or potassium solutions is the most common methods to extract SWE, pressurizing the vessel as in the high temperature process used by Maxicrop (Milton, 1952) and similar processes developed later by Algea and others. Then the final product in liquid form is dried or prepared in the pH 7-10 range. Depending on the intended application of the seaweed extract they are frequently mixed with fertilizers and micronutrients depending on the needs of the specific crop they are intended for. This is done to take advantage of the natural chelating properties of the seaweed extracts which prevent trace metal ions from precipitating (Milton, 1962).

Numerous studies have shown that SWE is effective as fertilizer nutrients and sources of organic matter that can be used as soil conditioners. There are 15 million metric tonnes of seaweed derived products used to increase plant growth and yield in horticulture and agriculture per year (Craigie, 2011). Significantly, SWE are bioactive at low concentrations, the extracts commonly are diluted as 1: 1000 or more. Much commercial seaweed concentrates (SWCs) is used for plant growth stimulant

(Table 2) because rich auxins and auxin-like compounds such as cytokinins (Brain, Chalopin, Turner, Blunden, & Wildgoose, 1973), IAA (indole acetic acid), and ABA (Applied behavior analysis) (Crouch, Smith, Van Staden, Lewis, & Hoad, 1992; Kingman & Moore, 1982) have been detected in seaweed extracts which can enhance soil health by improving moisture-holding capacity and promoting the growth of beneficial soil microbes (Khan et al., 2009). It can also promote root growth and development (Khan et al., 2009). In a study by Rayorath et al. (2008)SWE increased shoot height and leaf number and could help the plant fight drought stress to aid the plants' growth.

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Figure 3: Schematic representation of physiological effects elicited by seaweed extracts and possible mechanism(s) of bioactivity. Source from Khan et al., 2009. This picture illustrates the effects and possible mechanisms of the effects from application of seaweed extracts by seeding dip, foliar spray, and seed treatment, and also by soil application such as incorporation of marine bio-products, soil drenching, and addition of extracts to hydroponics on the growth of plants, response to biotic and abiotic stress, and nutritional quality.

Table 2: Seaweed extracts by product name, manufacturer and usage. Most products are plant growth stimulants.

Company	Product name	Application
Acadian Agritech	Acadian	Plant growth stimulant
BASF	Kelpak	Plant growth stimulant
BASF	Profert	Plant growth stimulant
China Ocean University product development Co., Ltd	Sea Winner	Plant growth stimulant
Dolphin Sea Vegetable Company	Emerald RMA	Plant growth stimulant
Farmura Ltd	Seanure	Plant growth stimulant
Green Air Peoducts, Inc.	Bio-Genesis™ High Tide™	Plant growth stimulant

In summary, numerous studies have reported that both GB and SWE separately can improve drought tolerance of plants, including cuttings, by the various properties they possess. Exogenous application of GB may be an economically-feasible approach to increase the plant's GB levels and promote resilience to drought stress in the root and whole-plant growth stage, via foliar spray, irrigation or soaking. This is because GB is a relatively inexpensive product that can be extracted from easily grown, high-yield crop plants such as sugar beet (Ashraf & Foolad, 2007). Khan et al. (2009) reported that seaweed extract is an important role in response to drought stress for plants as well.

1.3.5 Exogenous application of GB and seaweed extract (SWE) in crops and plants

As GB synthesis and seaweed extract usage are linked to several metabolic pathways in the plant cell (Khan et al., 2009; Mäkelä et al., 1998), the approach to improve stress tolerance of crops by GB has focused on exogenous applications. Several studies have shown that exogenous application of GB and exogenous application of seaweed extract has been successful in improving drought stress in agricultural crops (Khan et al., 2009; Lipoma, Gurvich, Urcelay, & Díaz, 2016). Applications of GB and of seaweed extract enabled the plants to fight drought stress in the process of root growth and whole plant growth through foliar spray, irrigation and soaking. Because studies about drought stress during cutting propagation are not so numerous, some methods from agricultural cultivation may be relevant to investigate what may help cuttings fight drought stress (Lipoma et al., 2016).

1.3.5.1 Methods of Exogenous application of GB

Foliar Spray

Foliar spray is spraying the GB solution directly on the plant's leaves. Ashraf and Foolad (2007) did a study of foliar spray in 2007 which showed that foliar spray may be an effective way to improve the drought tolerance of plant cuttings. Since GB always accumulates in the chloroplasts, it remains stable in the leaves and thereby provides a long-term protective capability (Rezaei, Kaviani, & Masouleh, 2012). Foliar spray can be used for cuttings and whole plant growth because the application falls on the leaves which provide nutrition and energy by absorbing the nutrients and water into the cuttings (Drahn, 2007). Additionally foliar spray with GB has been studied in propagating *Griselinia littoralis* by using cuttings, however the optimal concentration and application times still remains unclear. Exogenous application of GB has been one of the most common ways to successfully fight drought in agricultural crops and horticultural plants (Ashraf & Foolad, 2007).

A study by Agboma in 1997 showed that exogenous GB application had positive effects in grapevines (Vitis), sorghum (Sorghum bicolor) and maize (Zea mays). The study showed that foliar application of aqueous GB marginally enhanced biomass production (Agboma, Jones, Peltonen-Sainio, Rita, & Pehu, 1997). Mickelbart, Chapman, and Collier-Christian (2006) showed that GB is an effective compound to protect against osmotic stress and that the application of GB to grapevines in particular increased foliar concentration. More recently, another study showed that the foliar application of aqueous GB significantly increased the grain yield of sorghum, and residual tissue GB levels remained high 3 weeks after the application (Miri & Armin, 2013). Another study showed that, in horticultural systems in particular, exogenous application of GB via foliar sprays can reduce the adverse effects of drought on sunflower achenes and oil content (Iqbal, Ashraf, & Ashraf, 2005).

GB has different effects on different crops and for the same crop, the effects of GB foliar spray also depend on the concentration of GB. For instance, Reddy et al. (2013) reported that 4 kg ha^{-1} GB enabled maize to continue growing and overcome water limitations, thereby leading to more biomass than in the untreated mildly stressed plants. 4 kg ha^{-1} GB was used in three drought levels (100%, 60%, and 40% irrigation with full-strength Hoagland nutrient solution) during maize growth. Another study found that maize grain yield increased from 3.2×10^{-6} kg ha^{-1} to 4.2×10^{-6} kg ha^{-1} in drought conditions when the exogenous application of GB was increased from 4 to 6 kg ha^{-1} (Agboma et al., 1997).

When spraying with different levels of GB on two occasions; plant height, yield, chlorophyll, and seed weight all increased when the GB concentration was at 100 millimoles/L, whereas production quality was highest at 150 millimoles/L (Mickelbart et al., 2006). When the concentration of exogenous GB was changed in the same way for sorghum, spraying GB with concentration values at the optimum range of maize caused some negative effects on sorghum. When the exogenous foliar spray GB concentration was at 50 millimoles/L, the leaf area of sorghum decreased, and at 100 millimoles/L of GB or more, severe phytotoxicity and the cessation of growth ensued (Mickelbart et al., 2006).

Clearly, the different concentrations of exogenous GB in spraying can enhance yields of plants under drought stress, but the same concentrations will not always work on different plants. Just from observing the effects of GB on maize and sorghum, the positive and negative effects of GB clearly depend not only on the concentration of exogenous GB but also the plant type, and we can infer that this will apply to other plants as well.

In addition, the concentration of GB can affect how long GB remains active in crops. A concentration of GB at 4 kg ha^{-1}, would mean that GB would remain in unmetabolized maize tissues for up to 17 days after application (Reddy et al., 2013), In comparison at a concentration of GB of 6 kg ha^{-1}, residual GB levels in maize tissue remained high 3 weeks after application (Agboma et al., 1997). This means that if the concentration of GB increased, the time that GB remains in crops increases as well. However, this relationship needs more studies to verify it clearly as it has not been studied in a wide variety of crops and since GB reacts quite differently depending on plant species, it is hard to draw definitive conclusions so easily.

Studies of foliar spray of GB have only investigated its use for agricultural crops or cuttings in well-watered conditions, but no studies have investigated GB being used for cutting propagation under drought conditions. GB should be useful for fighting drought stress during cutting propagation because of the positive effects of GB on crops and importance of GB for plant growth. I feel foliar spray may be a useful method of applying GB for cutting propagation under drought stress because foliar sprays have been applied for rooting solutions have been successfully used to propagate leafy cuttings taken in the growing state, producing roots at the basal end of the cuttings (Kroin, 2014). Foliar Applied Rooting Solutions for Plant Propagation from Cuttings: Historical Background and Utility), and GB has been used for cutting propagation of *Griselinia littoralis* and *Lavatera x clementii* successfully before (Reader, 2015).

Overall, since the positive and negative effects of the GB foliar spray depend on the plant type, concentration and times of GB spraying, more studies on the exogenous application of GB for propagation with cuttings are necessary.

Soaking treatment of GB

Soaking cuttings in a solution of GB may be another effective way to alleviate the adverse effects of drought stress in *Griselinia littoralis* and *Lavatera x clementii* cutting propagation. It is logical the leaves of the cuttings and the whole plant may be able to absorb GB before planting to fight drought stress during the subsequent plant growth. Studies have shown that seeds soaked in GB can fight drought stress. For example, the exogenous application of GB as a pre-sowing seed treatment by soaking in 50, and 100 millimoles/L (mM) of GB improved wheat biomass, although wheat growth was best following treatment with 50 mM of GB (Mahmood, Ashraf, & Shahbaz, 2009). *Griselinia littoralis* and *Lavatera x clementii* can be grown from cuttings and seeds, although plants grown from cuttings tend to do better, so treatment by soaking cuttings may be effective for improving their drought tolerance.

Irrigation with GB solution

Irrigation of the soil may be yet another effective method of improving root growth and whole-plant development of *Griselinia littoralis* and *Lavatera x clementii*, where exogenous GB is indirectly transferred to the leaves after being absorbed by the roots. Irrigation with GB has been shown to be a useful way to combat drought stress and improve the development of plants and crops. For example, Agboma et al (1997) reported that in Maize, application rates of exogenous GB via irrigation of 6 kg ha-1 increased grain yield by 34% under drought stress, while application rates of exogenous GB via irrigation of 4 kg ha^{-1} increased grain yield by 12% under drought stress. For wheat, application rates of exogenous GB via irrigation of 6 kg ha^{-1} and 4 kg ha^{-1} increased grain yields by 18% and 12% respectively under drought stress. GB has a different effect on different crops, even with the same concentrations of GB, the results are quite different.

In summary, the 3 different ways of applying exogenous GB all have their advantages, but may be better suited to different stages in cutting development. Soaking seems to be more suited to seed propagation and irrigation has good results for crops which are already established. Cuttings don't have enough root growth to absorb the GB through the roots, but they do have leaves which can absorb the GB when applied through foliar spray. Therefore for cutting propagation foliar spray may be the most effective application method. These differences deserve more attention in further study. This study proposes to determine the optimal concentration of exogenous GB for G*riselinia littoralis* and *Lavatera x clementii* during cutting propagation, and also the optimal times of application through foliar spray and soaking only (See objectives 1.2).

1.3.5.2 Methods of exogenous application of seaweed extracts (SWE)

SWE may work in the same way as exogenous application of GB in fighting drought stress during cutting propagation because SWE contain betaines, including gamma-aminobutyric acid betaine, 6-aminovaleric acid betaine, and GB (Battacharyya et al., 2015; Fornes, Sanchez-Perales, & Guardiola, 2002; Haider et al., 2012; Khan et al., 2009). SWE are the most common exogenously applied seaweed in agriculture and horticulture.

SWE may help cuttings to fight drought stress, because they have beneficial effects on soil structure, moisture retention, rhizosphere microbes, root development, plant growth and yield, during cutting propagation. SWE has been used in cutting propagation for many crops to improve the rooting capability of cuttings in certain species which are difficult to take root (Atteya, 2003; Crouch & Van Staden, 1992; Kowalski, Jäger, & Van Staden, 1999). Crouch and Van Staden (1992) reported that 10% SWE increased the number of and dry weight of marigold (Tagetus patula) roots. Similarly, SWE improved the vigor of the roots in difficult-to-root cuttings of Pinus pinea and increased the number of rooted cuttings (Atzmon & Van Staden, 1994). In another study, Leclerc, Caldwell, Lada, and Norrie (2006) observed that foliar application of extract from Ascophyllum nodosum seaweed, produced by Acadian Seaplants Limited, increased the number of propagules per plant in the ornamental herbaceous perennial Hemerocallis Sp by adding additional BA and IBA to the plants. The desired effects or the part of the plant to be targeted may influence the best method of application of seaweed during cutting propagation.

SWE for irrigation

The Bioefficacy of seaweed extract was first found from observations made in field and glasshouse trials using Maxicrop product as a soil additive (Craigie, 2011). Studies suggest that seaweed extracts enhance abiotic stress tolerance (drought, salt, high temperature) in plants and that enhances plant performance (Khan et al., 2009). For example, Zhang, Ervin, and Schmidt (2003) conducted experiments to confirm the action of seaweed extracts (Ascophyllum nodosum) with 16 mg·m^{-2} used for irrigation on drought tolerance in creeping bentgrass. Drought-stressed plants treated with a combination of humic acid and seaweed extract had root mass enhanced by 21–68%. Xu and Leskovar (2015) reported seaweed extract increased leaf area by 21%, with seaweed extract drench.

Foliar Spray

SWE application through foliar spray is used to fight drought stress in other crops and plants, as it can be used as a plant biostimulant, nutrient, and a component of organic manure and fertilizer to promote horticultural plant growth under drought stress (Battacharyya et al. 2015). Exogenous application of seaweed has been used in foliar sprays to enhance tomato, plum, cherry, and orange crop yields under drought stress. Different studies showed very good results for SWE by foliar spray application. One showed a total fruit flesh weight increase of 17% and the number of harvested fruit increased by 10% under drought stress with SWE applied by foliar spray (Crouch & Van Staden, 1992). Another showed that SWE increased orange yields by 15% under drought stress; and foliar spray with boric acid and SWE on eggplant plants increased growth and the final yield (Osman, 2014).

SWE applied by foliar spray seems to have provided good results over a wide range of cutting propagation conditions and so this looks to be a logical avenue to follow for cutting propagation of *Griselinia littoralis* and *Lavatera x clementii* as well.

Combined application of biostimulants

Many biostimulants (as indole-3-butyric acid (IBA), abscisic acid (ABA), Shikimic acid and so on) and GB have been used in combination to fight drought stress, so it should follow that seaweed and GB may also be used effectively together to combat drought more effectively. Indeed, other plant biostimulants such as indole-3-butyric acid (IBA) and abscisic acid (ABA) with GB have been used to promote drought tolerance in plants. For example, shikimic acid as a precursor of L-tyrosine, Lphenylalanine and aromatic amino acids can regulate plant growth and metabolism. Combined with GB, shikimic acid increased levels of health, photosynthesis, and leaf growth to benefit the growth of sorghum (Ibrahim & Aldesuquy, 2003). Reader (2015) applied IBA to cuttings to promote plant growth for results that parallel those of genetic engineering. ABA, a sesquiterpenoid, is a key plant stress-signaling hormone that regulates plant growth and development.

When plants are under drought stress, a metabolic response associated with an ABA-dependent pathway and an ABA-independent pathway coincides with the plant's perception of the signal (Agarwal, Agarwal, Reddy, & Sopory, 2006). The ABA-dependent pathway has been widely studied, and the results of this study indicate that it seems likely that exogenous GB and ABA may be used together to be an effective method to combat drought stress. Accordingly, GB and fertilizers or plant-biostimulants used together may combat that stress better than the application of GB alone (Aldesuquy et al., 2012; Ibrahim & Aldesuquy, 2003; Mahmood et al., 2009).

The ABA and GB effects the working of the ABA dependent pathway in some way which enables the plant to perceive the signal of stress more effectively and promote gene expression which then provides the plant with faster physiological changes to conserve water or otherwise adapt to increase stress tolerance. Hence the GB and ABA application helps the plant to optimize its growth functions for the lower water availability.

It is from these results that using GB and seaweed together through foliar spray, irrigation and soaking in cutting propagation, is hypothesized to have a significant effect in helping plants fight drought stress.

1.3.6 Summary of literature review

Numerous studies have indicated that exogenous application of GB and seaweed independently are effective methods of promoting plant growth and development under drought stress. The positive and negative effects of application methods such as foliar spray, irrigation, and soaking depend greatly upon the concentration of GB and the timing of application. Consequently, in this study, it was assumed that the most appropriate timing and method of application of both exogenous GB and seaweed via foliar spray, irrigation, and soaking, depending on the stage in the cutting's development, should provide the plant with the means to combat drought stress and benefit the growth of roots, shoots. These three application methods will be used to investigate the effects of exogenous GB and GB+SWE on the growth of *Griselinia littoralis* and *Lavatera x clementii* cuttings under drought stress and find out which combinations and timings are most beneficial to the plant overall in its growth.

Chapter 2: General methods in Experiments

2.1 Introduction

Cutting propagation is the most common method to propagate many landscape or woody ornamental plants. Stem cuttings of many shrubs are quite easy to take root, but the selection of cuttings depends on the species of plant. Typically, there are four main types of stem cuttings categorised as herbaceous, softwood, semi-hardwood, and hardwood depending on the hardness and age of the cutting (Davies, Davis, & Kester, 1994). Most plants do better as semi-hardwood cuttings as they can easily take root and the rate of survival is higher. In general cuttings of tree species are more difficult to get to take root than flower species for example. One of my test plants, *Griselinia littoralis* is a native evergreen tree (Terrain, 2018), another of my test plants, *Lavatera x clementii* is a weak shrub but it tends to like the same conditions and behaves similarly to herbaceous plants(Terrain, 2018). Logically, *Griselinia littoralis* should be more difficult to take root than *Lavatera x clementii*. However, the survival rate of *Griselinia littoralis* is close to 100% under well-watered conditions, which is same as *Lavatera x clementii*. Under drought stress, whether the survival rate of these two plants is still the same or not is unknown, which may have had unpredictable effects on the results. In the experiments, semi-hardwood cuttings of *Griselinia littoralis* and *Lavatera x clementii* were used for cutting propagation and treated with GB and SWE to test their effects for alleviating drought stress.

2.2 Materials and methods

The study uses two common types of exogenous application—foliar spray, and soaking—of GB and GB+SWE to improve the tolerance of the test plant cuttings to drought. To effectively quantify the results of exogenous application of GB and SWE, soil factors that can influence root and whole plant growth have been considered. Specifically soil with or without fertilizer can starkly affect the propagation of cuttings, so both fertilized and unfertilized soil have been used in the experiments.

2.2.1 Test cuttings

Griselinia littoralis and *Lavatera x Clementii* which are endemic to New Zealand are most popular native landscape plants here (White & Lovell, 1984). There are three main reasons why *Griselinia littoralis* and *Lavatera x Clementii* were selected. Firstly, *Griselinia littoralis* is one of the hardiest native garden plants of New Zealand. It grows between sea level and an altitude of 1km, and can tolerate a wide range of salinity conditions (Hall, 1977). Secondly, it is widely-grown as a hedge or screen plant in New Zealand, especially in lowland to subalpine forests with deep shade, and in gardens (Burrows, 1995). Finally, the market demand for both *Griselinia littoralis* and *Lavatera x Clementii* is significant. *Lavatera x Clementii* is widely grown and selected for the landscape in New Zealand. Meanwhile, *Griselinia littoralis* is abundant in the South Island, but cannot be found in the wild in the North Island (Burrows, 1995). Therefore, Cutting propagation is the main method of propagation for *Griselinia littoralis* and *Lavatera x Clementii* to meet the large market demand. However, cutting propagation has limited effectiveness because of drought stress (Tron, Bodner, Laio, Ridolfi, & Leitner, 2015). All aspects of the droughts the cuttings may experience during cutting propagation should be well understood for growers to help the cuttings thrive during different drought conditions.

2.2.2 Glasshouse experiment design

All experiments were conducted in a glasshouse at the nursery at Lincoln University from April in 2017 to February 2018. In all trial, the heels of the cuttings were cut laterally with a knife at a length of approximately 5 mm to increase the contact area for root growth (Figure 4), following a 3 second dip into LIBA 10,000 root promotant (Figure 5) to the base and wounded area, then each cutting was stuck into a pot 5cm wide and 12cm deep. All the pots were then put into trays (25x50 cm), and there was no bottom heat applied. Additional watering was done as required by hand. The GB used was provided by Norman Phillips PhD from Adva Ltd Nelson, and the GB+SWE solution (FruitGuard Figure 6) is a new product that combines the biostimulant qualities of Undaria Pinnatifida Brown Kelp, with a greater level (35%) of GB than is naturally found in seaweed. One drop of sprayfix (Figure 7) was also added to the solution. There were 2 types of exogenously applied solutions used. The concentration of GB was selected at 2 kg/ha (0.2 g of GB, 200 mL of water, 1 drop of sprayfix) because previous studies in the effects of GB for plants reported that there were many negative effects of 3.4 kg/ha GB on *Griselinia littoralis* and *Lavatera x Clementii* cuttings in well-watered conditions, so a lower concentration of GB may be better during cutting propagation than a higher concentration of GB (Reader, 2015). Significantly, lower concentrations of GB have been reported to increase the yield of crops and promote the growth of plants under drought stress. The concentration of GB was set at 6 kg/ha (0.6 g of GB+SWE, 200 mL of water, 1 drop of sprayfix), because lower concentrations of SWE have also been shown to be better for increasing crop growth and development, and having many other positive effects as well.

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Figure 4: Preparing the Heel of the cutting. The heels of the cuttings were cut laterally with a knife at a length for approximately 5 mm to increase the contact area for root growth. Source from Green Plant Swap (2018) https://www.greenplantswap.co.uk/grower_tips/heel-cuttings

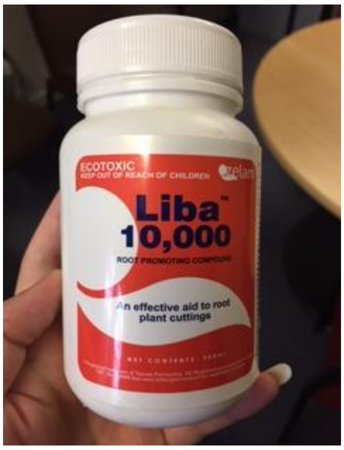

Figure 5: Root promotant (Liba Root Promotant 10,000 200ml) used in the experiments. Liba Root Promotant 10,000 is produced by Zelam a New Zealand company for promoting root development when propagating plant cuttings. The active ingredient is 10g/l of Indolebutyric acid.

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Figure 6: FruitGuard (GB+seaweed) used in the experiments. FruitGuard is a new product with a greater level of GB than is naturally found in seaweed to help reduce abiotic stress in plants. It can effectively reduce fruit splitting due to rain events prior to harvest, reducing cold weather stress in early season, reducing heat and drought stress.

Figure 7: Yates Sprayfix 200ml improves the efficiency and effectiveness of the spray. Yates Sprayfix is produced by Yates to improve the efficiency and effectiveness of garden sprays. It can be mixed with all kinds of treatments to assist spray coverage, adhesion and penetration into leaf tissue. Sprayfix added to the GB and SWE should improve the effectiveness of the applications.

2.2.3 Plant growth conditions

The experiments used two sources of plants; *Griselinia littoralis*, taken from Lincoln University grounds and the Lincoln University Nursery, and *Lavatera x clementii* which was taken from a garden in Springston, Canterbury. These cuttings were planted in pots 5 cm wide and 12 cm deep then put into trays (25x50 cm) using two kinds of soil, potting mix (Figure 8) and soil with three month mix fertilizer (Figure 9) in different experiments. The cuttings were grown under natural light conditions in covered areas within the glasshouse.

Inside the glasshouse all cuttings were then covered with a sheet of clear polyethylene (Figure 10) to maintain humidity, and a black cloth for shade on top (Figure 10) to stop strong sunlight. Unrooted cuttings require indirect and diffused sunlight to provide cuttings with sufficient sunlight for supporting photosynthesis without being too harsh. The light should be varied throughout the root initiation and development process and intensity should be maintained at 500-1,000 foot-candles from stick to callus, and increased to 1,000-2,000 foot-candles at the stage of root initiation (Roland Leatherwood, 2006). During cutting propagation, a light intensity below or above the optimum range reduces or inhibits rooting. If the light intensity is too low, the cuttings are unable to get enough light for adequate photosynthesis, thus delaying rooting. If the light intensity is too high, transpiration rates are increased and drought stress can inhibit rooting. Therefore, the black shade cloth was taken off on cloudy days, and put back on sunny days. After 6 weeks the black shade cloth was removed and the clear plastic was gradually opened little by little to gradually acclimatize the cuttings to the glasshouse environment. Any additional watering was done by hand to maintain a specific level of drought in the soil, and there was no bottom heat.

Figure 8: The two types of potting mix with fertilizer and without fertilizer used in the study. The potting mix in both bags is 60% peat and 40% polite. The fertilizer is Osmocote exact Min (16-3.5-10) which was put into the potting mix on the left.

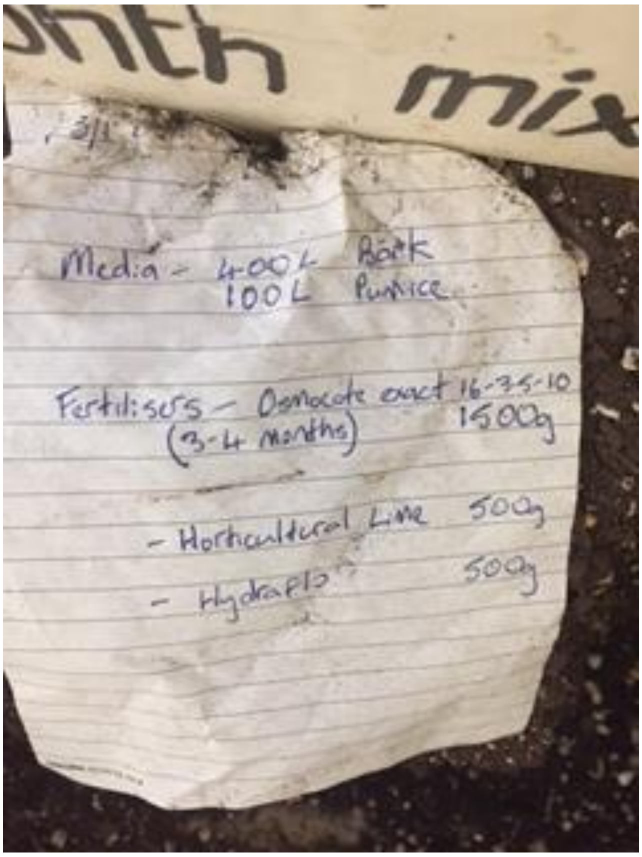


Figure 9: The soil with three month mix fertilizer used in Experiment 1 B. The soil is made up from is 400L bark and 100L pumice with fertilizer Osmocote exact 16-3.5-10 for 1500g, 500g horticultural lime, 500g hydraplo. The glasshouse workers at Lincoln University mixed this to my specifications.

Figure 10: The plants and polyethylene structures inside the glasshouse. All cuttings were covered by polyethylene plastic sheet. Then black cloth was also used for shade on top to stop strong sunlight.

2.2.4 Water treatments

There were two irrigation treatments applied in the experiments, and the treatments were different in different experiments to keep a consistent level of drought. In all the experiments, the drought environment was set by controlled irrigation. The cuttings were watered by watering can once every 7 days in Experiment 1A, the soil moisture was maintained at around 80%, measured by weighing the soil and calculating the water content (see 2.2.5). A second irrigation treatment, used only for Experiment 3, consisted of weighing the pots every four days over the drought cycle to determine the soil moisture content and calculating the amount of water to add to ensure that the moisture content remained at around 80% for half of the experiment and at around 93% for the other half.

In experiments 1A, 1B, 2A and 2B all cuttings needed to experience the same drought stress conditions so water was applied evenly to all cuttings. However in experiment 3, half of the cuttings needed to have 18% less water than the other half, so it was necessary to weigh and calculate this difference.

2.2.5 Soil moisture

Soil moisture was used as a measure of drought level and to quantify the effectiveness of water treatments. Initially, determining soil moisture was to be done using a TDR (Time Domain Reflectometer), where the connecter head with two metal prongs would be inserted into the soil to a depth of 17cm. However, the pots are only 12 cm deep and 5 cm wide and there were too many pots to measure with a limited number of TDRs, so this method was abandoned.

Instead the soil moisture percentage was calculated by determining the weight of the soil at its fresh weight (FW) and comparing that to the dry weight (DW). FW was the weight of the potting Mix naturally. DW is the weight of soil which was put into a drying oven for 3 days at 105 °C.

Equation 3-1; calculation of soil moisture percentage was done as follows:

Soil moisture= (1- (B-C) / (A-C))*100% E.g. (1- 8g/10g)*100%=20%, so soil moisture is 20%

A: Fresh soil of 10g; B: Dry weight of 10g fresh soil; C: The weight of aluminum box

Equation 3-1; calculating weight of adding water to maintain soil moisture was done as follows:

E.g. 80% (soil moisture) = 1- 8g/ (10g + weight of water)

So, adding 30g water to maintain 80% soil moisture.

2.2.6 Cuttings' growth parameters

The weights, lengths and areas of the cuttings were compared to give meaningful quantification of a cutting's resistance to drought stress

2.2.6.1 Fresh weight of leaves, shoots and roots

The fresh weight of the leaves, shoots and roots were measured immediately after collection to minimize errors from postharvest water loss. To measure the fresh weight of the leaves or shoots, any obvious soil contamination or surface moisture was removed before weighing. The soil was washed away from the roots carefully in a root washing facility and gently blotted using paper towels and, then the roots were left at room temperature for about four hours before weighing so they would lose only the water from washing.

2.2.6.2 Dry weight of leaves, shoots and roots

After measuring the fresh weight, the leaves, shoots and roots were dried and weighed. These samples were labelled and then oven dried at 65°C for at least 48 hours before weighing on scales. Samples were then placed in a dry environment for about 10-15 minutes to cool before weighing to make them easier to handle.

2.2.6.3 Cuttings' length

The shoot length of the dried cutting was measured from the apex of the main stem to the base of the cutting, and the root length of the cutting was measured from the base of the cutting to the apex of the longest root.

2.2.6.5 Cuttings development

Number of leaves

The leaf number were measured by counting the number of emerged leaves excluding cotyledons.

Leaf area and leaf area ratio

The leaf area was calculated using the method of Pandey and Singh (2011). Leaves were cut off and spread over millimeter graph paper to draw an outline of the leaves, then the outline of the leaves were cut out and that paper was weighed. The original sheet of millimeter graph paper was weighed ten times, calculating the average weight of the original sheet of paper.
The leaf area can be calculated by using the equation:
Leaf area (cm2) Z= X/Y (X: the weight of outline of the leaves in millimeter graph paper; Y: weight of 1 sheet of millimeter graph paper)

However, at the end of the experiments, the leaf area was measured by LiCor LI-3100 Leaf area meter as well (Figure 11). For reducing error between original leaf area before planting and leaf area after collecting the cuttings, original leaf area before planting was measured by LiCor LI-3100 Leaf area meter as well. The leaf area ratio was measured using the formula below according to (Lambers, Chapin, & Pons, 2008).
Leaf area ratio (LAR) = A/W
* A is the leaf area; W is the dry mass of leaves.

Figure 11: LiCor LI-3100 Leaf area meter. It can precisely and rapidly measurements of large or small leaves, with an area resolution of either 1 mm^2 or 0.1 mm^2 is available without having to change optics. Samples are placed between fixed guides on the lower transparent belt and allowed to pass through the LI-3100C. As a sample travels under the fluorescent light source, the projected object is reflected by a system of three mirrors to a linear array camera within the rear housing.

Root/shoot ratio

The root /shoot ratio was calculated using the weight of the dried roots and dried shoots, with the root/shoot ratio (R/S ratio) = Dry weight of roots/ Dry weight of shoots

2.2.7 Plant survival rate

Plant survival rate was calculated as the ratio of the number of cuttings with roots (Figure 14) at the end of the experiment compared with the total number of cuttings planted in the experiment.

Figure 12: Cutting with roots. The roots occurred from the fourth week after planting.

2.2.8 Statistical analysis

Analysis of the main parameters and determining significance were performed with the Origin pro (8.5), and Microsoft Excel by Analysis of Variance (ANOVA) in Origin pro (8.5), comparisons between the interactions of the means were based on Tukey's 95% confidence intervals calculated in Origin pro (8.5). Where appropriate, the LSD $P<0.05$ was used to further explore the relationships.

In all analysis, for viewing the effects of different treatments on cuttings' growth, different treatments as the x-axis, while the y-axis is variety parameters of cuttings' growth such as survival number, dry weight of leaves, shoots, and roots, length of cuttings' shoot and root, leaf number, leaves' area. All cuttings were separated into blocks, the number of blocks depended on the cuttings' number. For example, cuttings in Experiment 1B were separated into six blocks and there was ten replication per blocks.

All data were imported into Origin pro (8.5) for ANOVA, and comparisons between the interactions of the means were based on Tukey's 95% confidence. The significant difference of cuttings' parameters in the effects of different treatments can be viewed by Tukey's 95% confidence as well.

Chapter 3: Experiments

3.1 Experiment 1

3.1.1 Experimental aim and design

The aim of Experiment 1 was to conduct an investigation as to whether exogenous spray application (GB and GB+SWE) alleviates the detrimental effect of drought stress on the root growth of *Griselinia littoralis* in fertilized and unfertilized potting mix and 3 month mix fertilizer for cuttings taken in April.

The experiment was separated into two sub-experiments; Experiment 1A which tested fertilized and unfertilized potting mix, and Experiment 1B which tested fertilized and unfertilized potting mix against 3 month mix fertilizer. The experiments ran for 15 weeks, from April to August. During this period, the average daily temperature in the glasshouse dropped from 24°C to 15°C, with a mean over the whole period of 15°C. The humidity was about 52% over the whole period. The cuttings were further covered with plastic, creating two covered blocks (Figure 13). Inside these blocks the average temperature also ranged from 24°C to 15°C, with a mean of 17°C, and the humidity was about 90%. Within the covered block at noon the humidity reduced to about 60% because of strong sunlight. The experiment used potting mix and three month mix soil (see 3.2.3) with soil moisture of 80%.

The variables measured in Experiment 1A and 1B, were:

Survival number of cuttings

Root length

Please note, leaf area, dry weight of leaves, shoots, and roots, leaf area ratio and root/shoot ratio was not measured, as these cuttings were used for another experiment (unpublished) about the effects of GB and GB+SWE on the whole plant. This study has been left out this thesis as my focus was the effects of GB and GB+SWE on cutting propagation. However, these variables have been included in my later experiments in this thesis. If this research were to be repeated, I recommend all six variables being studied.

3.1.2 Methods

3.1.2.1 Experiment 1A

Experiment 1 A uses the same soil type to eliminate any possible variables within the soil that may contribute to different levels of soil drought or growth performance. Cuttings were tested to see whether exogenous spray application (GB and GB+SWE) alleviates the detrimental effect of drought stress on the root growth of *Griselinia littoralis* in fertilized and unfertilized potting mix, with soil type not being a factor.

All cuttings were planted into potting mix with and without fertilizer, and separated into categories receiving 6 different treatments. There were a total of 320 cuttings with 20 cuttings arranged evenly in each tray. Each tray received a particular treatment and the trays were arranged randomly within a larger block of trays to randomize outside influences such as different levels of sunlight (Table 3). There were 2 larger blocks of trays making up the total 320 cuttings. For the first four weeks the experiment black cloth was used to cover the cuttings in order to shelter them from sunlight.

The growth of the cuttings was measured daily. After 4 weeks, the black cloth was taken off on cloudy days, and put back on again on sunny days to make sure there was always enough sunlight, but not so much as to damage the young cuttings. The cuttings were harvested after 15 weeks to measure survival number and cuttings' growth parameters including shoot length and root length.

Table 3: The map of *Griselinia littoralis'* cuttings by treatment type.

Block 1:

No fertilizer control	Fertilizer 2xGB	No fertilizer 1xGB	No fertilizer 2xGB
Fertilizer 1xGB	No fertilizer GB+SWE	Fertilizer control	Fertilizer GB+SWE

Block 2:

Fertilizer 2xGB	No fertilizer 1xGB	Fertilizer GB+SWE	Fertilizer control
No fertilizer GB+SWE	No fertilizer 2xGB	No fertilizer control	Fertilizer 1xGB

Experiment 1A was arranged in two blocks with the same temperature, humidity, and soil moisture. The six treatments were the same as well. 1xGB: Spray GB on Day 1; 2xGB: Spray GB on Day 1 and 14; GB+SWE: Spray the solution on Day 1. F control, F 1xGB; F 2xGB, F GB+SWE: cuttings were planted in potting mix with fertilizer.

Figure 13: The covered growing areas within the glasshouse.

The cuttings were all covered, and the temperature of the covered block was different from the temperature in the glasshouse.

Results of the Experiment 1A

The results for both blocks were quite different and with different patterns in growth for each different type of application. Outside environmental factors such as exposure to sunlight may be an explanation for this.

After the period of initial root growth, there was the first collection of data on July 3. The cuttings with exogenous application of GB and GB+SWE showed very different results for growth under drought stress in the two blocks (Figure 14). In block 1, the effects of fertilizer in the potting mix were significant on applications of GB and GB+SWE (Figure 14 A). Cuttings with 1XGB with no fertilizer and with fertilizer had the highest and lowest survival number and ratio respectively, while 2XGB without fertilizer was only a little better than the 2XGB with fertilizer. In essence the effects of the GB were less prominent when applied more times in the potting mix without fertilizer, but the results were opposite in the potting mix with fertilizer. GB+SWE however had a worse result without fertilizer, with more cuttings dying than surviving, but with fertilizer, the results were only slightly worse than the control with fertilizer. It seems that the GB+SWE combination was not as good as expected.

In block 2, the survival numbers and ratios had much less variation than in block 1, and all treatments, including the control had good survival rates. Surprisingly, 1XGB actually had the worst results comparatively (Figure 14 C). It seems that because the overall results of all the cuttings and treatments for the experiment for block 2 were very good (Figure 14), then this would imply that the growing conditions in this block were significantly better than in block 1. Therefore the benefits for fighting drought stress were less needed and this is perhaps why we see that GB was not useful.

In the experiment, there were many cuttings without roots, but that were still alive (Figure 14 B and D). In order to know these whether cuttings would continue to grow enough to get roots after the defined period of initial root growth (between the 4th and 6th weeks), the final date of collection was extended to the 5th of August to give enough time to be certain.

At the end of this experiment, after checking the growth of all the cuttings, it was found that almost all of the cuttings without roots in July were dead at the final check in August (Figure 15 and 16). Only cuttings with the 2xGB and GB+SWE treatments in block 1 and 1xGB and F GB+SWE treatments in block 2 went on to grow roots and survive. This affected the resultant survival rate of the cuttings (Figure 17). The survival number under GB+SWE with and without fertilizer was the only group of cuttings unchanged, the number surviving under the other treatments were all slightly decreased.

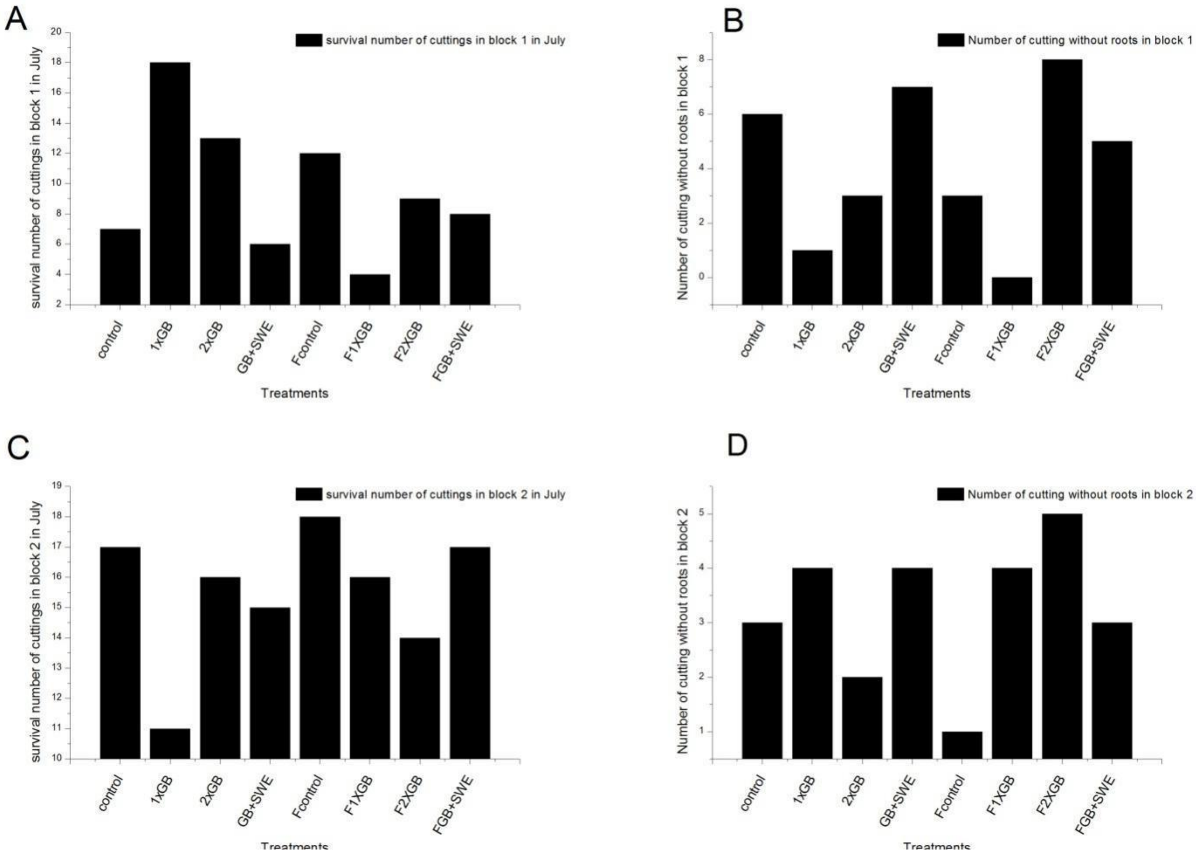


Figure 14: Comparing *Griselinia littoralis* cuttings' survival number and number of cuttings without roots in different treatments between block 1 and block 2 respectively.

A: The effects of six treatments on survival number of cuttings in block 1 in July. B: The effects of six treatments on number of cuttings without roots in block 1 in July. C: The effects of six treatments on survival number of cuttings in block 2 in July. D: The effects of six treatments on number of cuttings without roots in block 2 in July.

- 1XGB: Spray GB on the cuttings only on Day 1; 2XGB: Spray GB on Day 1 and Day 14; GB+SWE: Spray the solution on Day 1.
- F control, F 1XGB, F 2XGB, F GB+SWE: cuttings were planted in potting mix with fertilizer.

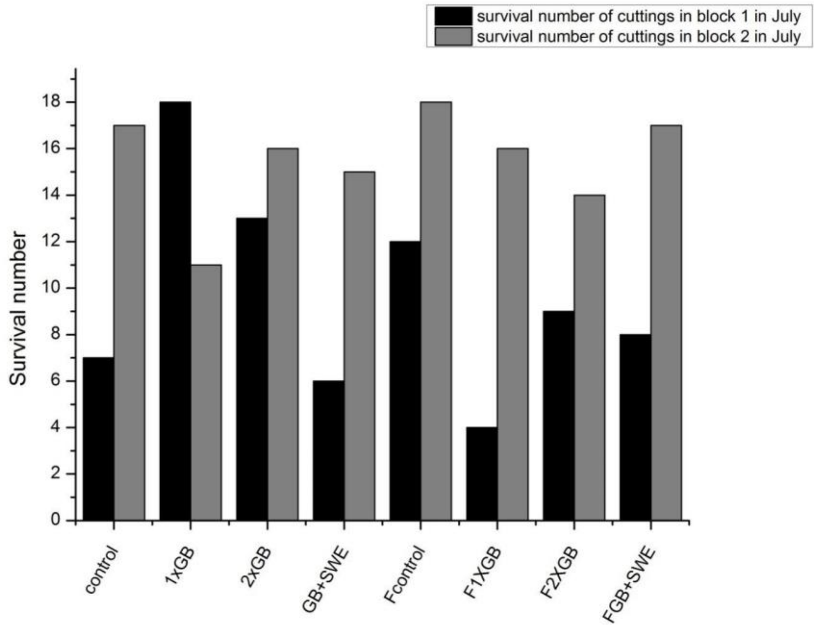


Figure 15: Comparing *Griselinia littoralis* cuttings' survival number between block 1 and block 2 in different treatments in July. The graph shows the survival number for the following treatments for block 1 and block 2. The treatments were applied as follows: 1XGB: Spray GB on Day 1; 2XGB: Spray GB on Day 1 and 14; GB+SWE: Spray the solution on Day 1. F control, F 1XGB, F 2XGB, F GB+SWE: cuttings were planted in potting mix with fertilizer.

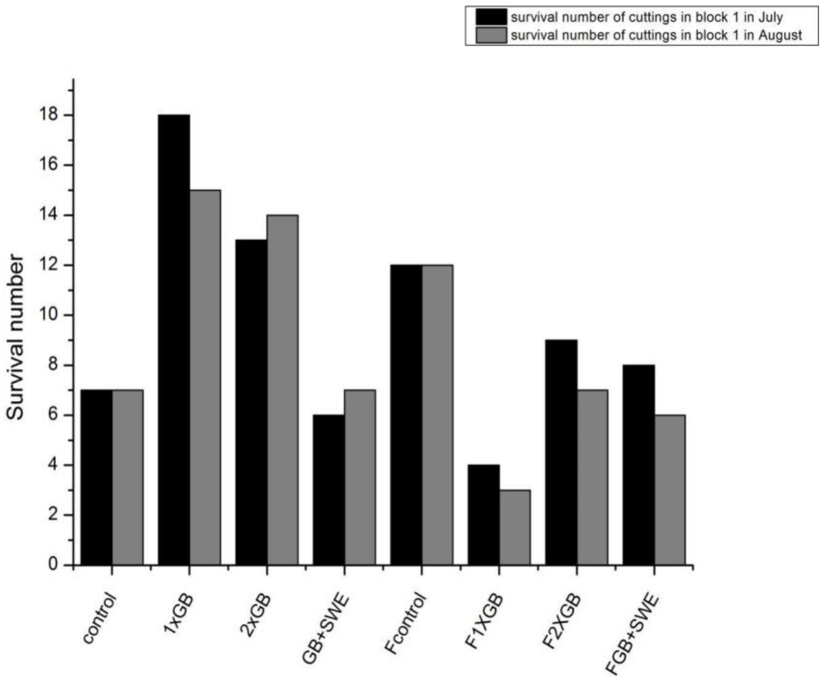


Figure 16: Comparing *Griselinia littoralis* cuttings' survival number in different treatments, in block 1 between July 3 and August 5. The graph shows the survival number for the following treatments for block 1 in July and August. The treatments were applied as follows: 1XGB: Spray GB on Day 1; 2XGB: Spray GB on Day 1 and 14; GB+SWE: Spray the solution on Day 1. F control, F 1XGB, F 2XGB, F GB+SWE: cuttings were planted in potting mix with fertilizer.

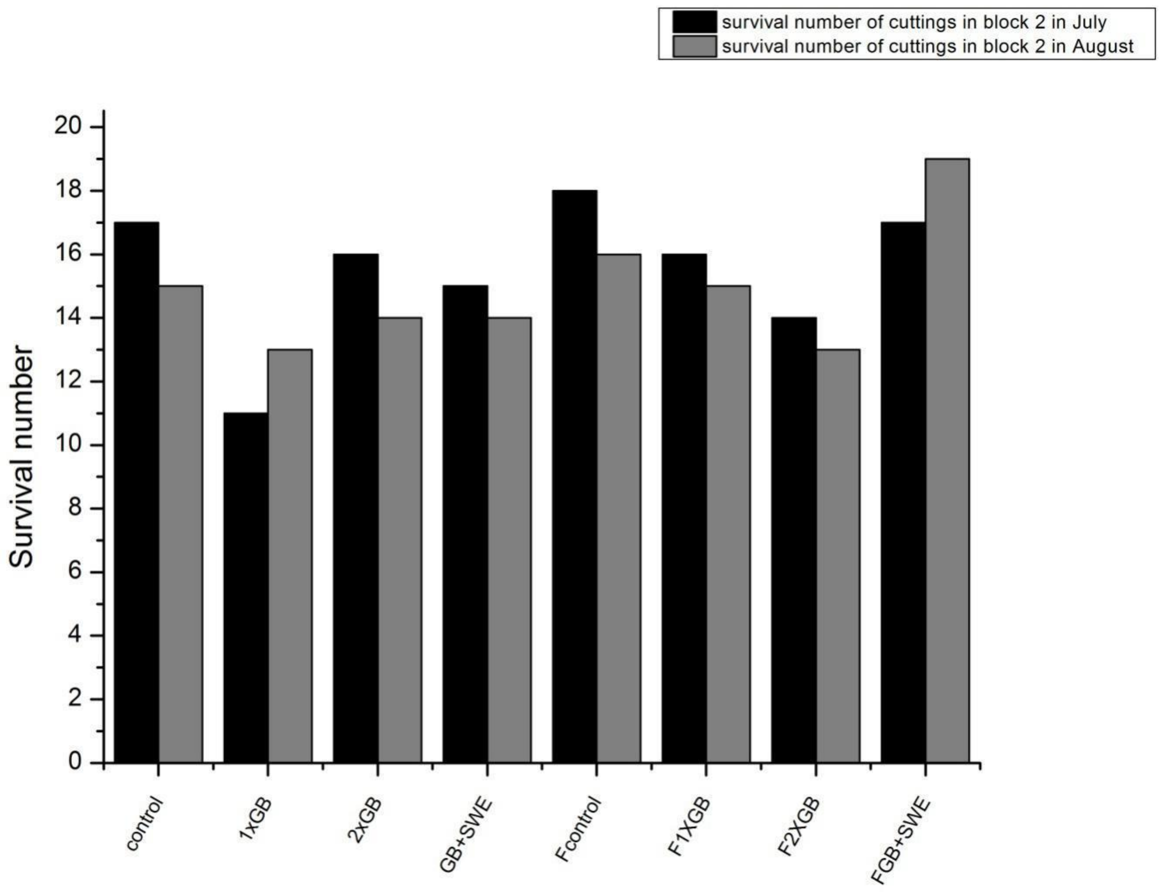


Figure 17: Compering *Griselinia littoralis* cuttings' survival number in different treatments, in block 2 between July 3 and August 5. The graph shows the survival number for cuttings in block 2 in July and August for the following treatments: 1XGB: Spray GB on Day 1; 2XGB: Spray GB on Day 1 and 14; GB+SWE: Spray the solution on Day 1. F control, F 1XGB, F 2XGB, F GB+SWE: cuttings were planted in potting mix with fertilizer.

Mean root length

The root length in different treatments is shown in Figure 18. It can be seen that the best exogenous application treatment for the development of the root system was 1xGB, with an average root length of 9.86 cm. Importantly, the length of the root decreased as the number of applications of GB increased, While the treatment of GB and no fertilizer was beneficial, the results were opposite for the treatment of GB with fertilizer.

With the exogenous application of GB and GB+SWE, growing the cutting in potting mix without fertilizer was better for the cuttings' root growth than growing in potting mix with fertilizer. GB+SWE was not an effective exogenous application in the soil with fertilizer.

Overall, the results from block 2 were significantly better than block 1, showing better growth overall for almost all treatments (Figure 18). Because the treatments and potting mix were all the same, this would mean that block 1 and 2 were in different drought environments with block 2 enjoying better growing conditions. Interestingly, the only exception was that the average root length was higher only for the treatments of 2xGB with fertilizer in block 1 than in block 2(Figure 18).

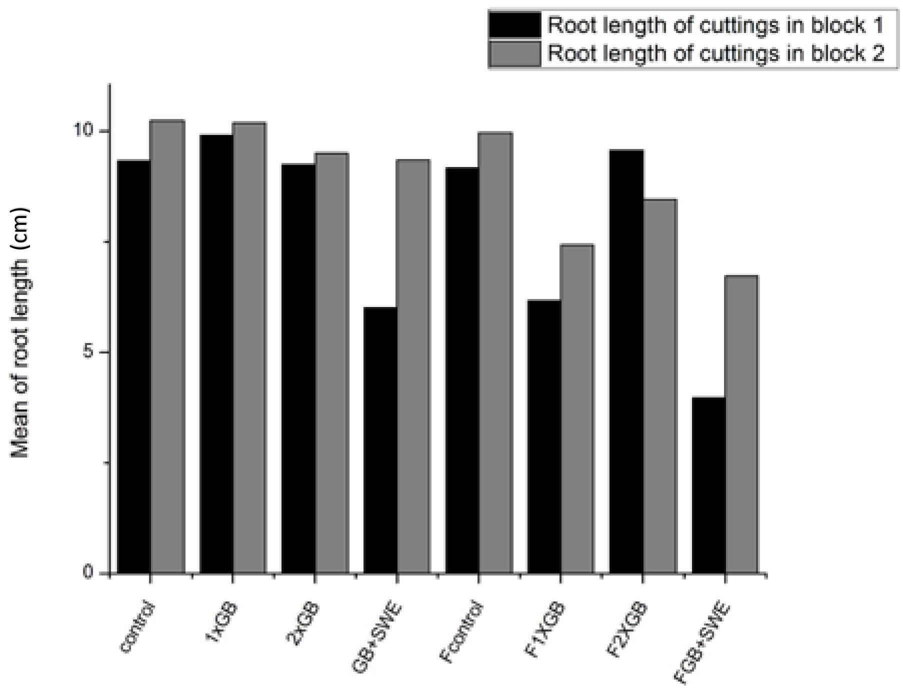


Figure 18:**Comparison of *Griselinia littoralis* mean root length (cm) in different treatments between block 1 and block 2.**The graph shows root length for cuttings in block 1 and 2 for the following treatments: 1XGB: Spray GB on Day 1; 2XGB: Spray GB on Day 1 and 14; GB+SWE: Spray the solution on Day 1. F control, F 1XGB, F 2XGB, F GB+SWE: cuttings were planted in potting mix with fertilizer.

Discussion of Experiment 1 A

Whether these treatments were beneficial for fighting drought stress during cutting propagation is dependent on the level of drought. In this experiment, the drought environment was different between block 1 and block 2 because of their relative locations within the glasshouse. The drought level and effects of sunlight in block 1 was stronger than block 2, because the sunlight was shining more directly on the area where block 1 was located (Figure 19) making the air drier and giving a longer time in sunlight than for block 2. The stronger sunlight caused a stronger drought conditions and also affected the stomata of the cuttings by causing them to close which then led to a limitation of water relations, transpiration and photosynthesis, more strongly in block 1 than in block 2. Even within the same block, the soil was observed to be drier in areas that spent longer in sunlight than others. This was confirmed by checking soil color, weighing the soil and calculating the soil moisture.

In block 1 and 2, some cuttings were observed to have yellowing leaves after a period of 14 days. This effect was observed first on the cuttings with GB and GB+SWE treatments and later on some other cuttings. By Day 20, not only were there yellowed leaves on many cuttings but dark patches on a few leaves as well. The shoots of some cuttings also started to turn black from the bottom then spreading up the stem by day 20 as well. On Day 30, the shoots of many cuttings became black, and this effect was more pronounced in block 1 than in block 2. There were no signs of disease, and this appeared to be physiological or possibly the effects of drought stress.

In the stronger drought conditions in block 1, the exogenous application of GB was more beneficial than GB+SWE, and the best treatment overall was 1xGB. At the same time, due to the differences of GB with and without fertilizer, the survival number was higher with continued application of GB in potting mix with fertilizer. When the cuttings were in potting mix without fertilizer, the survival number was less with continued applications of exogenous GB.

Under the lighter drought conditions of block 2, the exogenous application of GB+SWE was more beneficial than GB only, and the best treatment was fertilizer with GB+SWE. The results for cuttings grown in potting mix and fertilizer, with GB+SWE were always better than for just GB+SWE.

For root length, GB always gave better results than for GB+SWE, no matter whether the drought was stronger or weaker. GB tends to react differently for cuttings with and without fertilizer. If cuttings are grown with fertilizer, a double application of GB (F 2xGB) was better, but without fertilizer a single application of GB (1xGB) was better. This trend is seen on both root length and survival number.

In conclusion, exogenous application of GB is more effective than GB + SWE for fighting drought stress. Additionally, fewer applications of GB without fertilizer, or more applications of GB with fertilizer are both good for fighting drought stress. However, high concentrations of GB without fertilizer, and GB + SWE and fertilizer were both actually damaging, having worse results than just plain soil during drought stress.

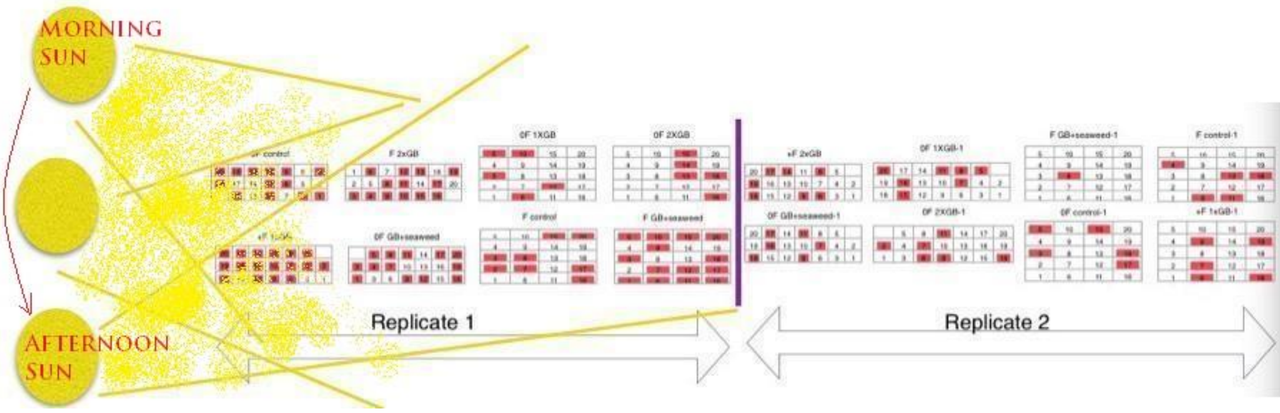


Figure 19: The glasshouse layout and how sunlight affects cuttings growth.

Dead cuttings are highlighted in red. The picture shows the different effects of sunlight on the level of drought. The left block, block 1 faced a longer time in the sunlight, leading to air drought which limited cuttings' growth and root development. The strong sunlight directly result in closing stomata, then limiting cuttings' water relations, transpiration, and photosynthesis.

3.1.2.2 Experiment 1B

Experiment 1B replicates the investigation of Experiment 1A but with an introduced variable of another soil type. The different soil types were used because of their different moisture retention qualities. Since fertilized and unfertilized potting mix were shown to affect the treatments' effectiveness differently, the aim was to investigate further how effective the treatments (GB and GB+SWE) are in fertilized potting mix, unfertilized potting mix and soil with three month mix fertilizer. This experiment also focused on root growth of *Griselinia littoralis* cuttings taken in April.

In Experiment 1B, there was a total of 378 cuttings, arranged in 6 sets of 63 cuttings each, with 6 different treatments (2 exogenous GB or GB + SWE treatments combined with 3 different soil types), arranged so that in each set 7 cuttings each were applied with the same treatment, with

3 being the control. The growth of the cuttings was measured daily. In Experiment 1 B, the environmental conditions of cuttings' growth including the cover, shade cloth, irrigation and the time the cover and shade cloth was on or off for. These conditions were the same as Experiment 1A.

Results of Experiment 1B

The effects of the six treatments were significantly different on the cuttings' growth and development (Fig 20, Table 4). All the treatments had significant benefit to the cuttings when compared to the control in fighting drought stress during cutting propagation. The survival number of cuttings were the highest with GB without the fertilized potting mix (GB (PM)) in all treatments. However, the effects of the other treatments in two types of soil on survival number were different.

In the potting mix, GB and GB+SWE gave a better result than the control, not only in the fertilized potting mix but also in the unfertilized potting mix (Figure 20). However, GB without fertilizer gave a higher survival number than GB with fertilizer, and the result was opposite from the results using GB+SWE. This clearly means that the fertilizer added to the potting mix affects the ability of GB to help plants fight drought stress, but somehow the SWE neutralizes the fertilizers' negative effect on GB and allows the GB to work well in both fertilized and unfertilized soil.

In the soil with three months mix fertilizer, exogenous application of GB and GB+SWE were both an effective way to fight drought stress during cutting propagation. The treatment of GB+SWE gave the highest survival number.

In terms of shoot and root length and number of leaves, all cuttings regardless of the treatment, showed an increase in these growth parameters (Table 4). The number of leaves and root and shoot length both increased well. However the increase in the number of leaves was similar for all treatments, while root and shoot length had significant differences depending on the treatment. GB gave the most significant increase in root length, with a difference of 1.15 cm compared to the control. The most significant improvement in shoot height was when cuttings used GB+SWE, planted in potting mix which gave a difference of 1.80 cm in shoot height. In the potting mix with fertilizer, the effects of GB were similar to the control, and GB+SWE giving significant growth benefits over the control. There were the familiar differences among control, GB, and GB+SWE in the unfertilized potting mix as well, with GB being beneficial and GB+SWE not helping at all. In the soil with three month mix fertilizer, the average shoot height was slightly higher for GB+SWE than the control, with a difference of 0.09 cm more than the control. This was however better than a single application of GB, which while had positive results, the difference in average height was only 0.05 cm.

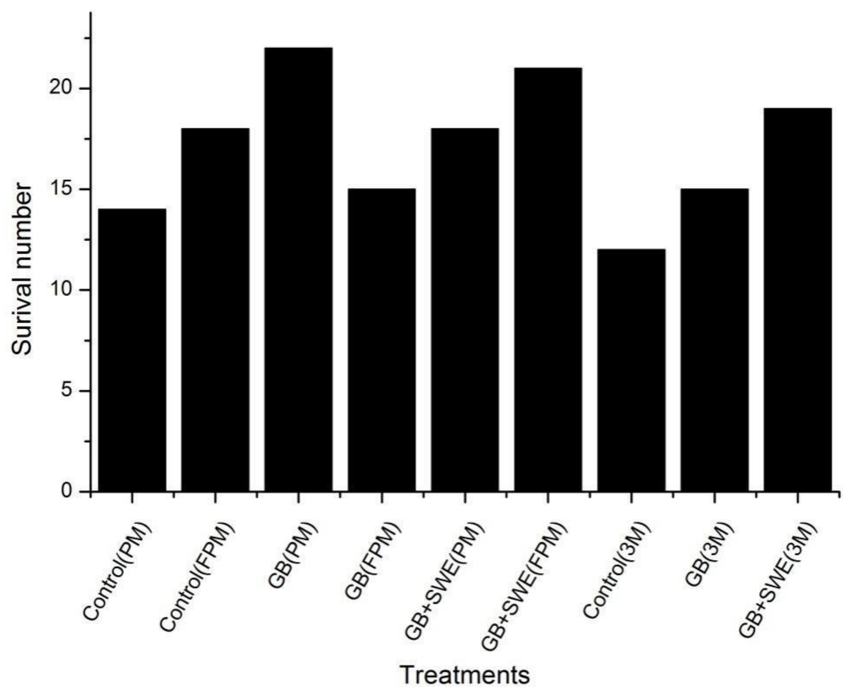


Figure 20: the effects of different treatments on the growth and development of *Griselinia littoralis* in various growth parameters.

The treatments were applied as follows:

GB: spray GB on Day 1; GB+SWE: spray GB+SWE (mix solution with GB and Seaweed extract) on Day 1.

GB (PM), GB+SWE (PM): cuttings were planted in the unfertilized potting mix after spraying GB and GB+SWE.

GB (FPM), GB+SWE (FPM): cuttings were planted in the fertilized potting mix after spraying GB and GB+SWE.

GB (3M), GB+SWE (3M): cuttings were planted in the soil with three month mix fertilizer after spraying GB and GB+SWE.

Table 4: The effects of different treatments on *Griselinia littoralis* shoot height, leaf number, and root length.

Treatments	Increased height of shoots (cm)	Increased number of leaves	Mean of root length (cm)
Control(PM)	1.302±0.014 d	1±0 b	8.118±0.239 b
Control(FPM)	1.37±0.016 bc	1±0 b	9.206±0.060 a
GB(PM)	1.168±0.057 f	1.8±0.447 a	9.26±0.080 a
GB(FPM)	1.382±0.022 b	1.2±0.447 ab	6.224±0.107 d
GB+SWE(PM)	1.798±0.029 a	1.4±0.547 ab	4.9±0.122 f
GB+SWE(FPM)	0.9±0.022 g	1±0 b	4.166±0.262 g
Control(3M)	1.238±0.013 e	1±0 b	4.706±0.046 f
GB(3M)	1.29±0.016 de	1±0 b	6.498±0.030 d
GB+SWE(3M)	1.326±0.019 cd	1.6±0.548 ab	7.76±0.093 c

- All values are in Means ± Std Deviations.
- Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Tukey's HSD-test), and indicated by different letters.
- Key to Table 3: The treatments were applied as follows: GB: spray GB on Day 1; GB+SWE: spray GB+SWE (mix solution with GB and Seaweed extract) on Day 1. GB (PM), GB+SWE (PM): cuttings were planted in the unfertilized potting mix after spraying GB and GB+SWE. GB (FPM), GB+SWE (FPM): cuttings were planted in the fertilized potting mix after spraying GB and GB+SWE. GB (3M), GB+SWE (3M): cuttings were planted in the soil with three month mix fertilizer after spraying GB and GB+SWE.

Discussion of Experiment 1 B

In Experiment 1B, some results were the same as for Experiment 1A, because the environmental conditions were the same in both experiments. The leaves of the cuttings turned yellow after 14 days, and started to turn black from Day 20. From Day 30, shoots of some cuttings turned black from the bottom of the cuttings as well. As with experiment 1A, there were no signs of disease, and this appeared to be physiological or possibly from the effects of drought stress.

The mechanics of how the different soil types affect GB and GB+SWE's efficiency to fight drought stress during cutting propagation is unknown. However, there seem to be consistent and clear patterns that can be observed. The chemical makeup of the soil seems to be part of a complex interaction between the soil, fertilizer, GB, GB+SWE and the plants themselves. The overall pattern usually observed is that GB by itself is the most beneficial treatment but fertilizer inhibits its effectiveness, and then GB+SWE with fertilizer gives similar results to GB without fertilizer. Somehow the SWE seems to neutralize fertilizer's negative effects on GB.

However in Experiment 1B some results were quite unexpected. GB without fertilizer had worse results than the control for shoot length, but better results for root length. It didn't seem to give a clear benefit over the control, but increased root strength is a benefit. GB+SWE usually does well in fertilizer but in this case the GB + SWE in fertilized potting mix gave quite bad results on both shoot and root length. The 3 month mix fertilizer showed very minimal differences to the control so it seems that in the makeup of this soil, both treatments were neither especially effective nor damaging. There was increased growth but it was not significantly more that the control, and much smaller than the benefits for growth for cuttings grown in potting mix.

These results may have been due to different soil types causing various soil droughts and the higher nutrient concentration caused by fertilizer in the soil. Further research is required.

In conclusion, different types of soil are important factors affecting how we should use GB and GB+SWE in fighting drought stress during cutting propagation. We need to think about how we apply treatments depending on the soil type or how to apply fertilizers depending on the treatments we intend to use. In general for unfertilized soil, exogenous application of GB is more effective than GB+SWE for fighting drought stress, but for fertilized potting mix and 3 month mix, GB+SWE gave better results. GB without fertilizer and GB + SWE with fertilizer were both effective, having better results than just the control under drought stress, so there isn't a lot of difference if the appropriate treatment is selected for the soil type. The different treatments have different results on different growth parameters so depending on what part of the plant is intended to benefit from the treatment, different combinations of soil and treatments can target specific areas.

3.2 Experiment 2

3.2.1 Treatments and experimental design

The aim of Experiment 2 was to conduct an investigation as to whether exogenous spray or soaking application methods with GB and GB+SWE are more effective to alleviate the detrimental effects of drought stress on the root growth of *Griselinia littoralis* in fertilized and unfertilized potting mix for cuttings taken in October (Figure 21).

The experiment was separated into three sub-experiments: Experiment 2A which tested the effects of spraying GB and GB+SWE in fertilized and unfertilized potting mix with soil moisture at 80%, and Experiment 2B which tested soaking the cuttings' stems in GB and GB+SWE, and Experiment 2C which tested both methods, spraying and soaking, in a much lower drought level with soil moisture at 20%.

The experiment ran for nine weeks, from October 4 to December 6. During this period, the average temperature in the glasshouse increased from 24°C to 35°C, with a mean of 29.5°C, over the whole period. The temperature in the covered block where the cuttings were grown, with the same layout as in Experiment 1, also increased from 21°C to 28°C, with a mean over the whole nine weeks of 26°C. The humidity averaged 95%, but did fall to about 40% around noon each day.

Figure 21: Example of a single tray of *Griselinia littoralis* cuttings containing cuttings with each type of treatment as well as control cuttings.

3.2.1.1 Experiment 2A

The aim of Experiment 2A was to investigate whether exogenous spray (GB and GB+SWE) alleviates the detrimental effect of drought stress (soil moisture 80%) on the root growth of *Griselinia littoralis* in fertilized and unfertilized potting mix for cuttings taken in October. The layout was different to Experiment 1 in that the cuttings with each treatment were spread more evenly to minimise local drought effects and provide much more meaningful comparisons for comparing the different application methods.

In Experiment 2A, there were 300 cuttings randomly planted in six trays arranged in six blocks with 10 replications per block with the cuttings subject to 8 different treatments. All cuttings were taken in October (spring), all treatments were applied by spraying the cuttings. Experiment 2A used 8 treatments instead of the 6 used in Experiment 1A. The treatments were: 1x GB with fertilizer, 1x GB without fertilizer, 2xGB, which was 1xGB with fertilizer on Day 1 and then again on day 14, 2xGB without fertilizer done the same way, 1x GB+SWE with fertilizer, 1xGB+SWE without fertilizer, and 2xGB+SWE with and without fertilizer also applied 1 application on day 1 with another 1 application on day 14.

The cuttings were collected after nine weeks to measure plant growth parameters including shoot length, root length, number of leaves and plant dry weight. The survival number of cuttings and the number of deaths in each tray was also counted at the end of the experiment.

Results of Experiment 2A

The results for this experiment were not quite as expected. A significant factor for almost all growth and survival parameters was that the unfertilized control was consistently one of the best performing cutting samples. This would indicate that there was not significant drought stress to overcome by the cuttings. In this environment the treatments were often more damaging than beneficial, with some exceptions.

For survival rate, exogenous application of 1xGB without fertilizer and Single application of GB+SWE without fertilizer and 2xGB all had similarly good results, though they were also all similar to the control (Figure 22). Of the 8 treatments, the most effective treatment was 2xGB. The other treatments, including the fertilized control all gave poor results and were damaging in terms of survival number of the cuttings. The worst result (apart from the control) was 1XGB+SWE in fertilized potting mix, with other treatments with fertilizer also faring badly. In this experiment it seems that the less added treatments there were, the better the results. The survival number was always higher when using GB and GB+SWE without fertilizer than GB and GB+SWE with fertilizer, except for the case of 2xGB+SWE.

The poor performance of GB+SWE in fertilizer was surprising as it usually does well, but the fact the unfertilized control was one of the best performers hints at something else being a factor.

For the other growth parameters such as shoot and root length and so on, the treatments didn't have such a negative effect and in fact were demonstrably useful in some cases. All treatments were effective to improve leaf number, root length, and shoot height, but there were no significant differences with all the treatments for the number of leaves (Figure 23).

However, different treatments had a variety of effects on shoot and root length. The best treatment for the development of the root system through exogenous application was 2xGB+SWE with fertilizer, with an average root length of 6.01 cm for this treatment (Figure 24). This treatment gave significantly better results than other treatments, since the average root length of other treatments was only 3.20 cm. 2xGB was the worst treatment for root growth at only 0.85 cm. Increased applications of GB reduced the root growth resulting in a shorter root length, while the results for GB+SWE were opposite.

While fertilizer decreased the effectiveness of a single application of GB, interestingly it increased the effectiveness of a 2xGB treatment. Both single and double applications of GB were less effective than the control, but it appears that if it is necessary to apply a double application of GB for some other reason, then combining it with fertilizer will lessen the negative effects on root growth. Conversely if using a single application of GB, then comparatively better results will be obtained by not combining it with fertilizer.

For shoot height, the effects 1xGB+SWE and 2xGB + SWE with and without fertilizer were all positive, with clear differences to the control (Figure 25). GB+SWE gave a significant increase with the greatest increase of shoot height at 3.4cm seen from the 2xGB+SWE with fertilizer (F2xGB+SWE) treatment. F2xGB+SWE was similar in performance to 2xGB+SWE and 1xGB+SWE, and the root length of these treatments was significantly higher than other treatments as well, indicating that these may be effective over a wider range of growth parameters. The worst treatment was F1xGB, with an increase in shoot height of just 1.7cm, 0.7 cm less than the control. In all treatments, the double application of GB and GB+SWE had a better effect on shoot height than the single application of GB and GB+SWE, except GB+SWE without fertilizer. Fertilizer was an effective factor for shoot height if the applications of GB+SWE were increased.

For leaf area, the results were not significantly different for all treatments (Figure 26). The only applications in which an increase in leaf area was observed were: 1xGB, 1xGB+SWE, and F2xGB+SWE, with the biggest increase being for F2xGB+SWE. The amount of increase for 1xGB+SWE and 1xGB were almost the same as the increase seen in the control, so their effects would appear to me minimal, however F2xGB + SWE gave an increase of 3 times the area of the control. 1xGB only gave an increase of 1.5 times the area increase of the control.

The control cutting's leaf areas both increased while the leaf areas for all the other treatments apart from 1xGB, 1xGB+SWE, and F2xGB+SWE decreased.

The changes in Dry weight of cuttings' leaves, shoots and roots, leaf area ratio (LAR) and root/shoot ratio in different treatments didn't have much meaningful variation, with quite similar results (Table 5). The effects of all treatments were close on the dry weight of leaves and shoots, leaf area ratio (LAR) and root/shoot ratio, and were not significantly different, but there was a slight variation, both positive and negative on the dry weight of roots. 2xGB+SWE with fertilizer had a consistent promoting effect on all growth parameters. The dry weight of roots was least with 2xGB with very poor growth results compared to the control.

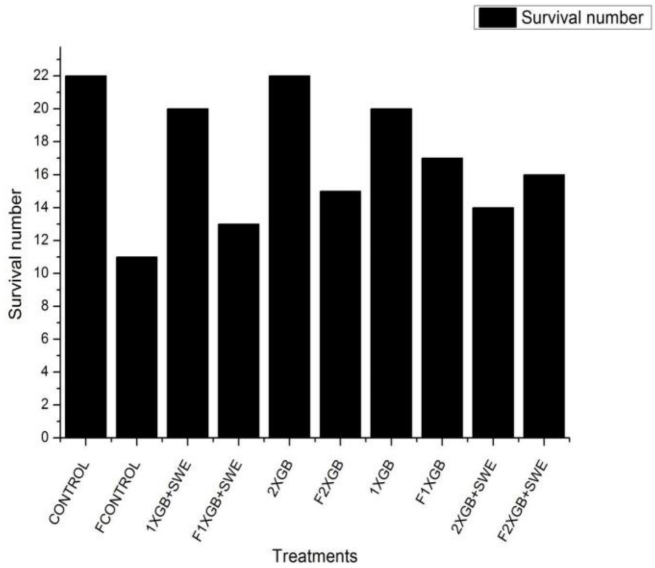


Figure 22: Survival number of *Griselinia littoralis* cuttings by different treatments.

The graph shows the survival number for the following treatments:

F 1xGB: GB with fertilizer, 1XGB: GB without fertilizer, F 2XGB: GB with fertilizer, 2XGB: without fertilizer, F 1XGB+seaweed: GB+SWE with fertilizer, 1XGB+seaweed: GB+SWE without fertilizer, F 2XGB+seaweed: GB+SWE with fertilizer, 2XGB+seaweed: GB+SWE without fertilizer.

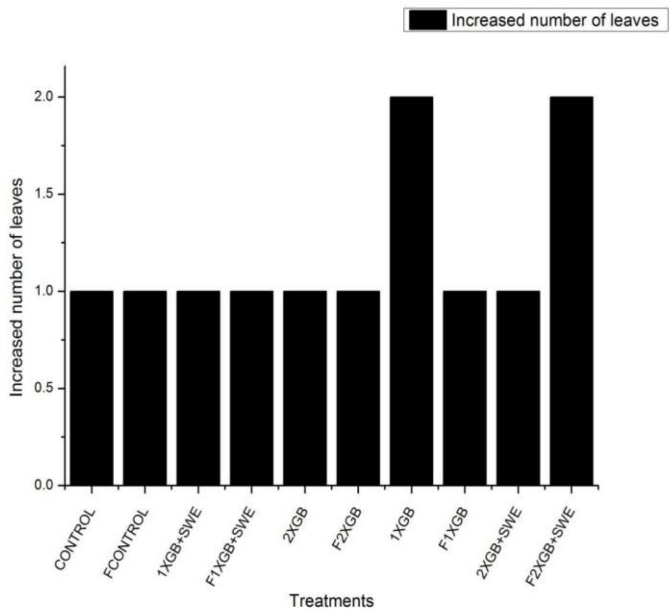


Figure 23: Leaves' number of *Griselinia littoralis* original cuttings and cuttings by different treatments.

The graph shows the leaf number for the following treatments:

F 1xGB: GB with fertilizer, 1XGB: GB without fertilizer, F 2XGB: GB with fertilizer, 2XGB: without fertilizer, F 1XGB+seaweed: GB+SWE with fertilizer, 1XGB+seaweed: GB+SWE without fertilizer, F 2XGB+seaweed: GB+SWE with fertilizer, 2XGB+seaweed: GB+SWE without fertilizer.

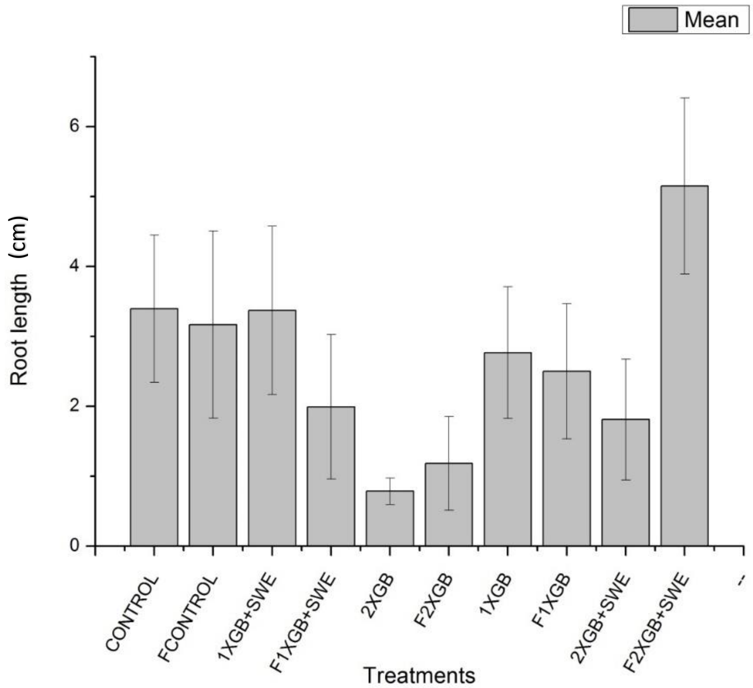


Figure 24: Mean of *Griselinia littoralis* root length by different treatments.

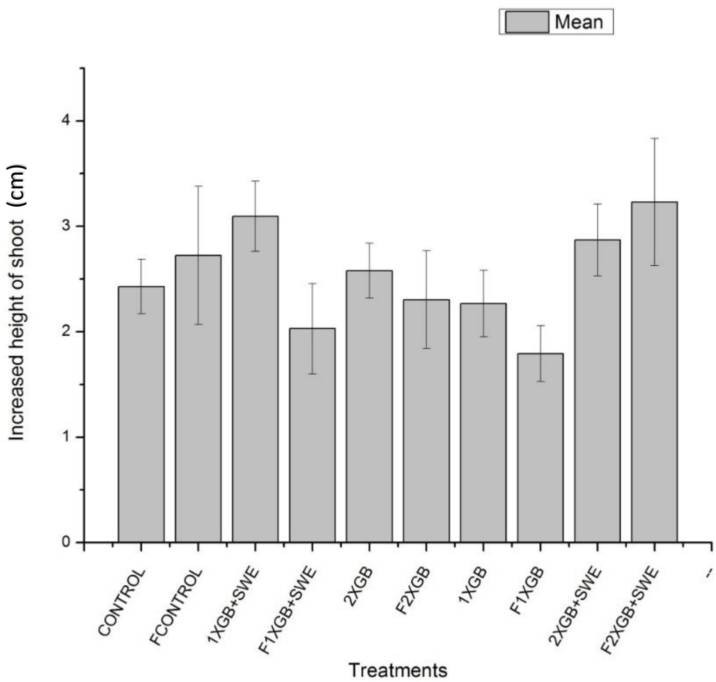


Figure 25: Mean of *Griselinia littoralis* shoot height by different treatments.

Key to Figure 24 and 25: The graph shows the root length and increased shoot height for the following treatments: F 1xGB: GB with fertilizer, 1XGB: GB without fertilizer, F 2XGB: GB with fertilizer, 2XGB: without fertilizer, F 1XGB+seaweed: GB+SWE with fertilizer, 1XGB+seaweed: GB+SWE without fertilizer, F 2XGB+seaweed: GB+SWE with fertilizer, 2XGB+seaweed: GB+SWE without fertilizer.

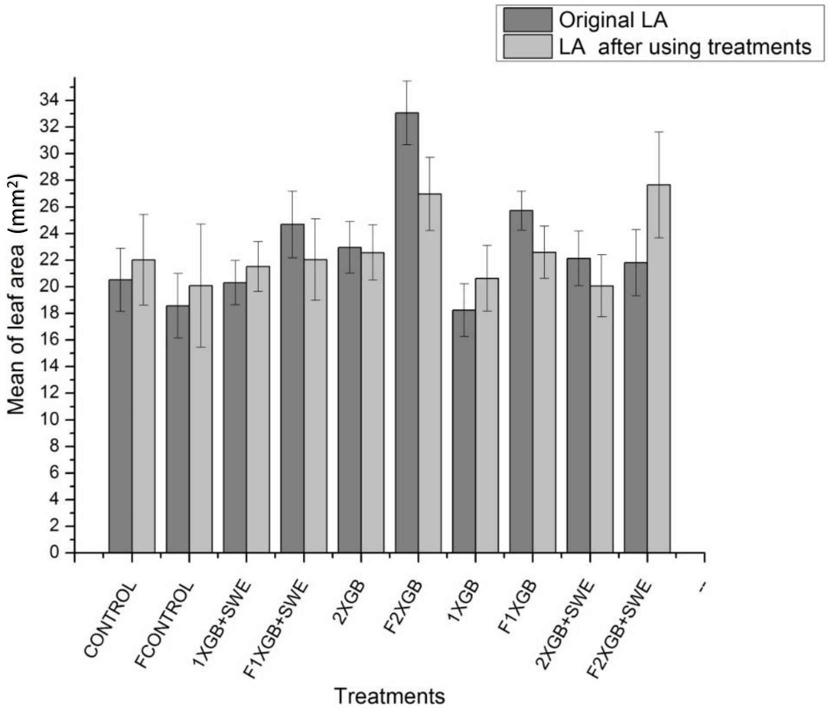


Figure 26: Mean of *Griselinia littoralis* leaf area by different treatments.

The graph shows the mean of leaf area for the following treatments: F 1xGB: GB with fertilizer, 1XGB: GB without fertilizer, F 2XGB: GB with fertilizer, 2XGB: without fertilizer, F 1XGB+SWE: GB+SWE with fertilizer, 1XGB+SWE: GB+SWE without fertilizer, F 2XGB+SWE: GB+SWE with fertilizer, 2XGB+SWE: GB+SWE without fertilizer.

Table 5: The effects of different treatments on *Griselinia littoralis* Dry weights and ratios.

Treatments	LDW (mg)	SDW (mg)	RDW (mg)	LAR (mm^2 / mg)	Root/Shoot ratio
CONTROL	0.33±0.19 a	0.49±0.25a	0.08±0.10 ab	61.07±25.33a	0.0011±0.0021 a
FCONTROL	0.27±0.12 a	0.41±0.18a	0.10±0.13 ab	56.97±40.12a	0.0027±0.0050 a
1XGB+SWE	0.29±0.11 a	0.47±0.17a	0.06±0.09 ab	70.49±19.90a	0.0013±0.0034 a
F1XGB+SWE	0.31±0.13 a	0.45±0.19a	0.07±0.15 ab	64.64±23.07a	0.00169±0.041 a
2XGB	0.31±0.12 a	0.49±0.20a	0.01±0.02 b	68.12±18.73a	0.0001±0.0001 a
F2XGB	0.38±0.11 a	0.53±0.15a	0.06±0.13 ab	71.19±20.13a	0.00219±0.0041a
1XGB	0.29±0.15 a	0.44±0.22a	0.07±0.10 ab	63.53±23.28a	0.00167±0.0031a
F1XGB	0.33±0.08 a	0.46±0.12a	0.07±0.13 ab	67.20±15.50a	0.00168±0.0035a
2XGB+SWE	0.28±0.12 a	0.46±0.18a	0.03±0.06 ab	64.46±20.13a	0.0003±0.0008 a
F2XGB+SWE	0.33±0.17 a	0.49±0.25a	0.15±0.18 a	70.66±30.87a	0.00257±0.0052a

- All values are in Means ± Std Deviations. The number of replicates (n) is six.
- Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Tukey's HSD-test), and indicated by different letters.
- Key to Table 4: The treatments were applied as follows: 1xGB: spray GB on Day 1; 1x GB+SWE: spray GB+SWE (mix solution with GB and Seaweed extract) on Day 1. 2xGB: spray GB on Day 1 and 14; 2x GB+SWE: spray GB+SWE on Day 1 and 14. F control, F1xGB, F2xGB, F 1XGB+SWE, F 2XGB+SWE: the method of spraying were same as 1xGB, 2XGB, 1XGB+SWE, and 2x GB+SWE, the only difference was cuttings were planted in the potting mix with fertilizer.

Discussion of Experiment 2A

Overall, not all treatments can effectively help cuttings fight drought stress, but some treatments are good at promoting growth in specific areas of the plant. In Experiment 2A, the temperature was higher than in experiment 1, and so the air drought level was higher as well. Some results of experiment 2A were the same as for Experiment 1. For example, leaves turned yellow then black and the shoots turned black from the bottom of the cuttings but there were no signs of disease. This was consistent right throughout all of the cuttings regardless of the treatment, including the controls. This would indicate that it was a result of general drought stress overall or some consistent outside influence.

Experiment 2A showed that some treatments have good effects overall for a wide range of growth parameters, with the best overall being F2xGB +SWE, except for survival number. However the survival number data was difficult to draw strong conclusions from because the control (unfertilized) was the best performing sample. For survival number all fertilized cuttings except F2xGB + SWE did worse than the unfertilized treatments. There seems to be another factor in play which is making the treatments less effective for the overall survival of the cuttings, while still enhancing individual growth parameters.

Assuming that there is another factor affecting overall survival number, GB+SWE was the best treatment combination for cuttings' growth parameters overall, and especially 2xGB+SWE with fertilizer showed very good results. At the same time, 1xGB was also useful for the cuttings growth, and the effects of a single application of GB without fertilizer were better on cuttings' growth parameters than a double application without fertilizer.

Overall, GB produces better results without fertilizer, but when combining GB+SWE, it produces better results with fertilizer. We need to think carefully whether we need fertilizer in the propagation of the cuttings and then if so the combination of SWE, GB and fertilizer in different amounts all need to be considered for optimum growth, or when targeting specific growth parameters.

3.2.1.2 Experiment 2B

The aim of Experiment 2B was to investigate whether exogenous soaking (GB and GB+SWE) alleviates the detrimental effect of drought stress at soil moisture of 80% and make comparisons to exogenous spraying as an application method on the root growth of *Griselinia littoralis* in fertilized and unfertilized potting mix for cuttings taken in October.

Experiment 2B ran for nine weeks, from October 04 to December 06. During this period, the average temperature in the glasshouse increased from 24°C to 35°C, with a mean of 29.5°C, over the whole period. The temperature in the covered block where the cuttings were grown, as in Experiment 1, also increased from 21°C to 28°C, with a mean of over the whole nine weeks 26°C. The humidity averaged 95%, but did fall to about 40% around noon each day. In this Experiment, there were 288 cuttings randomly planted in six trays arranged in six blocks with 12 replications per block (Figure 27) with the cuttings subject to 6 different treatments. All treatments were applied by soaking the cuttings for 3 hours, and there were 6 treatments. The treatments were: GB with fertilizer, GB without fertilizer, GB+SWE with fertilizer, water with fertilizer, and water without fertilizer.

The cuttings were collected after nine weeks to measure plant growth parameters including shoot length, root length, number of leaves and plant's dry weight. The survival number of cuttings and the number of deaths in each tray was also counted at the end of the experiment.

Figure 27: The cuttings were randomly planted into trays, after soaking.

Results of Experiment 2B

For survival rate, the control (which had no treatment at all) and soaking in water (SW) were better than other treatments. This means that soaking in GB and GB+SWE was not a significantly effective method for fighting drought stress during cutting propagation. However, the results of Experiment 2B still provide some information for future study.

All treatments without fertilizer had a better result than all treatments with fertilizer. Of the 6 treatments, the most effective treatment was SW, the survival number was 15 cuttings out of 36 (Figure 28). SW and the control without fertilizer were not significantly different, but they both gave a significantly higher survival number than other treatments. The survival number was higher when using GB+SWE without fertilizer than GB without fertilizer. In the potting Mix with fertilizer, the results of all treatments were negative, giving significantly worse results than the control.

Overall, all treatments without fertilizer were better than they were with fertilizer. This may be analogous to the same result when over fertilizing a plant as too much fertilizer can be very detrimental to a plant's growth. This suggests the combination of treatments and fertilizer may overstress the plant, which shows in poor growth in the cuttings.

In growth parameters, all treatments were not significantly different in leaf number, leaf area, mean of the shoot and root length, and dry weight of leaves, shoots, and roots (Figure 29-32 and Table 6). However, in these insignificant differences, there were still changes of growth parameters in different treatments.

For leaf parameters, cuttings receiving all treatments experienced an increase in leaf number, with 1 extra leaf for most plants. All plants and treatments equally shared this increase and the number of extra leaves was the same regardless of the treatment. However, the leaf area in all treatments was reduced, even though the number of leaves had increased.

For root length, almost all treatments gave a better result than the control, except soaking in GB+SWE. It can be clearly seen in Figure 32 that GB was better than GB+SWE for root growth, the best treatment for the development of the root system is through soaking in GB without fertilizer, and GB+SWE without fertilizer had the worst result.

Figure 32 and Table 6 illustrates that shoot height, dry weight of leaves, shoots, and roots, leaf area ratio (LAR), and Root/ Shoot Ratio were not significantly different in treatments. Shoot height increased almost to the same height by using treatments, giving a resultant growth of around 1.5cm. The dry weight had slight differences. The dry weight of leaves and shoots was best in the control cuttings, with soaking in GB giving the second best result. For root dry weight, SGB and SW with fertilizer and SGB without fertilizer also had a consistent promoting effect.

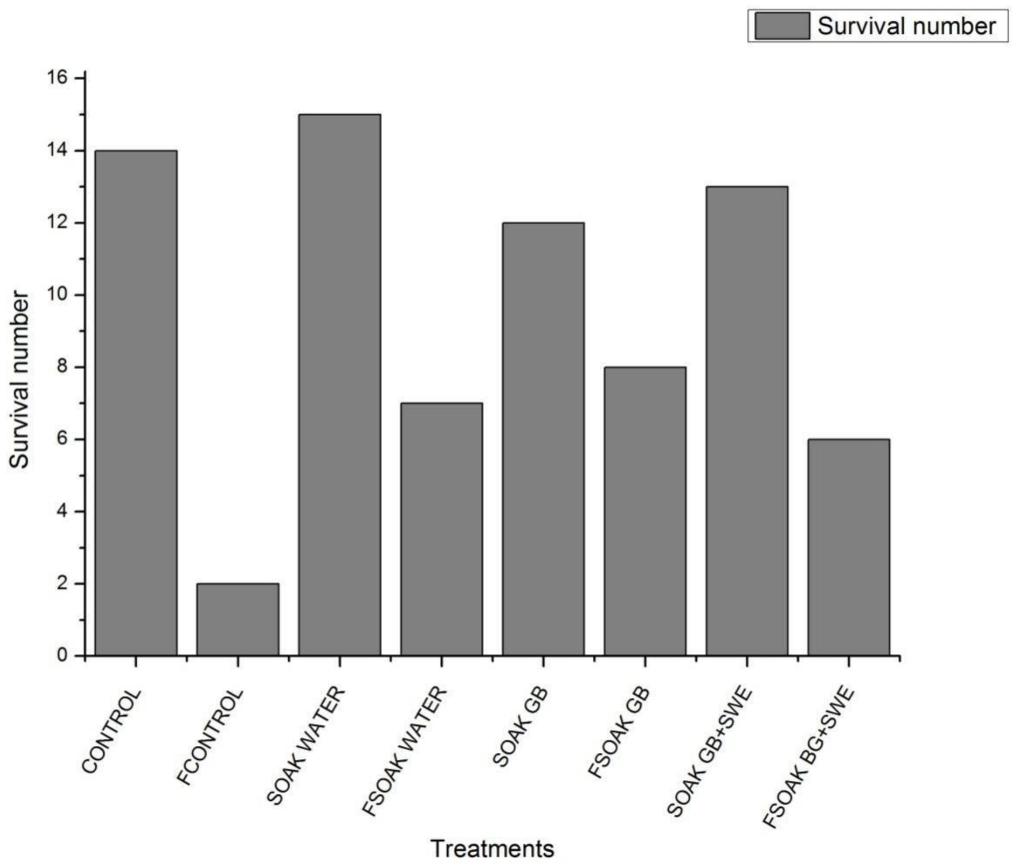


Figure 28: Survival number of *Griselinia littoralis* cuttings by different treatments.

SGB: cuttings soaking in GB, then be planted in potting mix without fertilizer, F SGB: cuttings soaking in GB, then be planted in potting mix with fertilizer, SGB+SWE: cuttings soaking in GB+SWE, then be planted in potting mix without fertilizer, F SGB+SWE: cuttings soaking in GB+SWE, then be planted in potting mix with fertilizer, SW: cuttings soaking in water, then be planted in potting mix without fertilizer, F SW: cuttings soaking in water, then be planted in potting mix with fertilizer.

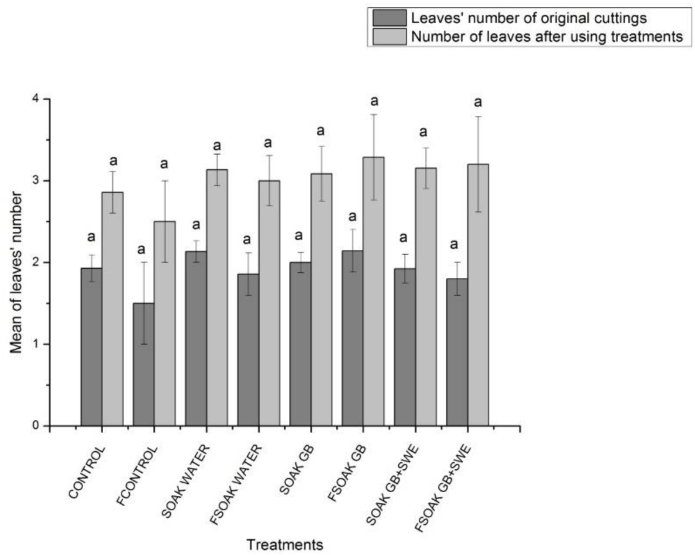


Figure 29: *Griselinia littoralis* leaf number at the beginning of the experiment compared with leaf number at the end of the experiment.

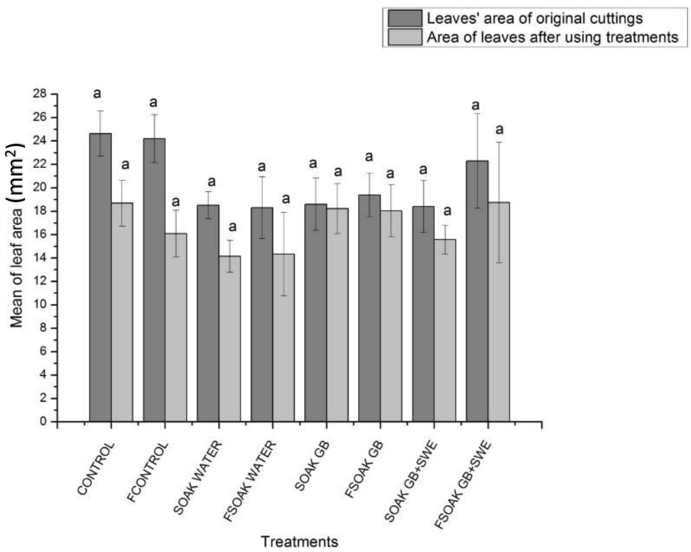


Figure 30: The effects of different treatments on *Griselinia littoralis* leaf area.

- All values are in Means ± SE.
- Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Tukey's HSD-test), and indicated by different letters.
- Key to figure 29 and 30: SGB: cuttings soaking in GB, then be planted in potting mix without fertilizer, F SGB: cuttings soaking in GB, then be planted in potting mix with fertilizer, SGB+SWE: cuttings soaking in GB+SWE, then be planted in potting mix without fertilizer, F SGB+SWE: cuttings soaking in GB+SWE, then be planted in potting mix with fertilizer, SW: cuttings soaking in water, then be planted in potting mix without fertilizer, F SW: cuttings soaking in water, then be planted in potting mix with fertilizer.

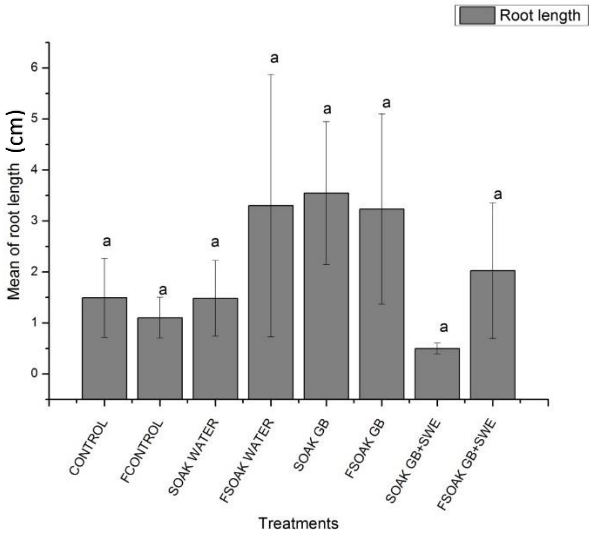


Figure 31: Mean of *Griselinia littoralis* root length by different treatments.

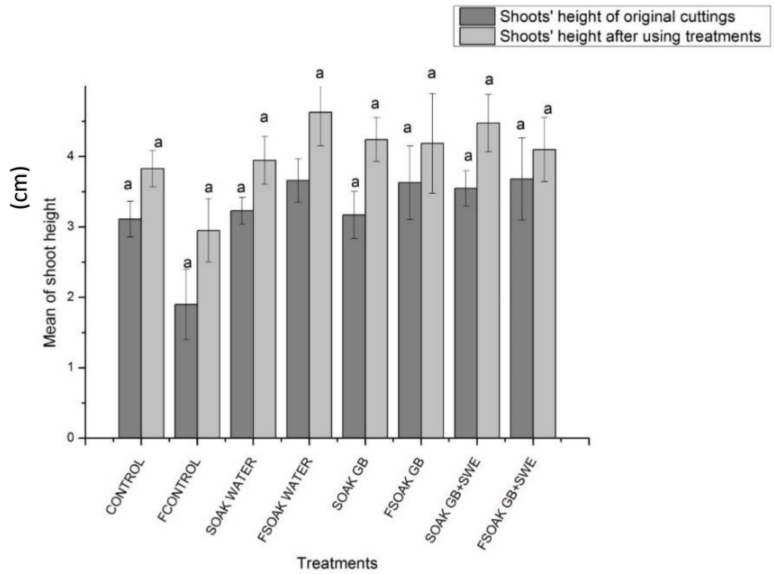


Figure 32: Mean of *Griselinia littoralis* shoot height by different treatments.

- All values are in Means ± SE.
- Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Tukey's HSD-test), and indicated by different letters.
- Key to figure 31 and 32: SGB: cuttings soaking in GB, then be planted in potting mix without fertilizer, F SGB: cuttings soaking in GB, then be planted in potting mix with fertilizer, SGB+SWE: cuttings soaking in GB+SWE, then be planted in potting mix without fertilizer, F SGB+SWE: cuttings soaking in GB+SWE, then be planted in potting mix with fertilizer, SW: cuttings soaking in water, then be planted in potting mix without fertilizer, F SW: cuttings soaking in water, then be planted in potting mix with fertilizer.

Table 6: The effects of different treatments on *Griselinia littoralis* dry weight of leaves, shoots, and roots, LAR, and root/shoot ratio.

Treatments	LDW (mg)	SDW(mg)	RDW(mg)	LAR (mm^2/ mg)	R/S Ratio
Control	0.31±0.13a	0.42±0.15a	0.02±0.03a	62.47±11.32a	0.03±0.009a
F Control	0.26±0.03a	0.33±0.04a	0.01±0.002a	62.93±2.31a	0.02±0.006a
SW	0.23±0.06a	0.33±0.09a	0.03±0.06a	61.39±15.17a	0.04±0.02a
FSW	0.22±0.10a	0.33±0.11a	0.10±0.1a	62.30±15.17a	0.09±0.07a
SGB	0.25±0.10a	0.36±0.13a	0.05±0.02a	76.04±27.88a	0.10±0.03a
FSGB	0.27±0.07a	0.39±0.11a	0.08±0.05a	66.22±10.19a	0.11±0.07a
SGB+SWE	0.19±0.05a	0.29±0.06a	0.009±0.003a	80.94±19.27a	0.03±0.02a
FSGB+SWE	0.25±0.05a	0.35±0.05a	0.03±0.02a	69.40±28.64a	0.06±0.09a

- All values are in Means ± Std Deviations. The number of replicates (n) is six.
- Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Tukey's HSD-test), and indicated by different letters.
- SGB: cuttings soaking in GB, then be planted in potting mix without fertilizer, F SGB: cuttings soaking in GB, then be planted in potting mix with fertilizer, SGB+SWE: cuttings soaking in GB+SWE, then be planted in potting mix without fertilizer, F SGB+SWE: cuttings soaking in GB+SWE, then be planted in potting mix with fertilizer, SW: cuttings soaking in water, then be planted in potting mix without fertilizer, F SW: cuttings soaking in water, then be planted in potting mix with fertilizer.

Discussion of Experiment 2B

Overall, soaking treatments of GB, and GB + SWE have little positive effect on helping cuttings fight drought stress. In this experiment, the environment including temperature, air and soil moisture was the same as in experiment 2A. For survival rate, the control and soaking in water were better than the other treatments, Soaking in GB was the best treatment for fighting drought stress in terms of leaf number, leaf area, mean of shoot and root length, and dry weight of leaves, shoots and roots.

All the potting mix was well watered before the cuttings were planted. The control experiment had no treatments at all, but the cuttings were just planted in the wet potting mix. The treatments of just water and the control, gave the best results, possibly because they provided the cuttings most directly with the water they needed. Treatments including GB and GB +SWE may have affected how the plants absorb water, making the cutting actually receive less water than the other treatments when there was plenty of water available. In severe drought stress the GB helps to retain the little water the cutting has available to it, but when there is ample water or nutrients available to the plant, the GB seems to act as a barrier for absorption and so the growth of the cuttings was not as good as in the treatments with water or fertilizer and without GB

3.2.1.3 Experiment 2C

The aim of Experiment 2C was to investigate whether exogenous spray (GB and GB+SWE) alleviates the detrimental effect of drought stress with a lower soil moisture of 20%, on the root growth of *Griselinia littoralis* in fertilized and unfertilized potting mix for cuttings taken in October.

The experiment ran for nine weeks, from October 04 to December 06. During this period, the average temperature in the glasshouse increased from 24°C to 35°C, with a mean of 29.5°C, over the whole period. The temperature in the covered block where the cuttings were grown, as in Experiment 1, also increased from 21°C to 28°C, with a mean over whole nine weeks of 26°C. The humidity averaged 95%, but did fall to about 40% around noon each day. The period and environmental conditions were the same as for Experiment 2A and 2B.

In Experiment 2C, there were 200 cuttings randomly planted in five trays arranged in five blocks with 5 replications per block with the cuttings subject to 8 different treatments. All cuttings were taken on October, 4 (spring), and all treatments were applied by spraying and soaking the cuttings. The treatments were: spraying 1x GB with fertilizer on Day 1, Spraying 1x GB without fertilizer on Day 1, Spraying 1xGB+SWE with fertilizer, Spraying 1xGB+SWE without fertilizer, and Spraying; soaking the cuttings for 3 hours GB with fertilizer, GB without fertilizer, GB+SWE with fertilizer.

The cuttings were collected after 9 weeks to measure plant growth parameters including shoot length, root length, number of leaves and plant dry weight. The survival number of cuttings and the number of deaths in each tray was also counted at the end of the experiment.

Results of Experiment 2C

Experiment 2C was unsuccessful, almost all of the cuttings died within 8 days. On Day 5, some leaves turned yellow, and the number of yellow leaves increased by 40% on Day 6. On Day 7 in the morning, 80% of the leaves had turned yellow. Almost all of the cuttings died on the morning of Day 8.

The most likely reasons why Experiment 2C failed are very strong drought stress and the treatment applications not being suitable in concentration or application timing, or just the simple fact that 20% soil moisture is too low for any treatment to be beneficial, however it is applied. The temperature was the highest and the air moisture content was the lowest, in these three days during the whole period of the experiment. Therefore, with the concentration of GB and GB+SWE the same as for Experiment 1, 2A and 2B, the treatments may not have been effective to protect the cuttings in such harsh drought conditions. The drought level in Experiments 1A, 1B, 2A and 2B was not so strong, so the level of treatments demonstrably helped the cuttings, but at 20% soil moisture this level of treatment simply seemed to be overwhelmed by the lack of water available to the cuttings.

There is likely a workable range for when GB and GB+SWE can be helpful for cuttings growth, and this experiment showed that the low soil moisture of 20% is outside the effective range for the concentrations of treatments used.

3.2.1.4 Discussion and contrast of Experiment 1A and 1B, and Experiment 2A and 2B

In all of the experiments, the cuttings used were *Griselinia littoralis*. There were so many variables in these experiments which affected the results, for example the season of taking cuttings, the type of cuttings, the methods of exogenous application, and the growth environment. It was sometimes not possible to control all the variables perfectly and therefore the results may well contain anomalous results. Care was taken to minimise random effects that may have influenced the cuttings growth unfairly, but nevertheless, some patterns were still seen, such as the stronger sunlight in particular areas having adverse effects.

It was clear that spray application is a better way than soaking for treatment application. By comparing Experiment 2A and 2B, nearly all growth parameters that were measured showed better results for spraying than for soaking. Normally, the optimal season to take cuttings is spring, in October. However the data from experiment 1 shows that early autumn is also suitable for cutting propagation for *Griselinia littoralis*. Nurseries almost exclusively take cuttings to grow *Griselinia littoralis* in Spring, but if my data is correct then it would provide a substantial growing opportunity for nurseries if they could plant, with consistently positive growth results in autumn as well, By controlling the light and temperature of the growth environment, there should be no reason why Autumn should not be used for cutting propagation as well as spring.

In experiment 1A, the soil drought level was light drought, and the soil moisture was consistently around 80%. After analysing the data there was more pronounced drought conditions caused by sunlight, which I hadn’t accounted for. This air drought was the main reason for the negative effects on cuttings’ survival number and growth of leaves, shoots and roots. During cutting propagation, the survival number and root growth of cuttings can be increased and be promoted by effectively using shade cloth and exogenous application of GB and GB+SWE. There were different results obtained because of different levels of more pronounced drought conditions caused by sunlight. In the stronger drought conditions of block

1, the exogenous application of GB was more beneficial than GB+SWE, and the best treatment overall was a single application GB. There were however differences related to how the treatments worked in cuttings grown with or without fertilizer. In these cases a double application of GB with fertilizer and a single application of GB without fertilizer were both optimal treatments. In Experiment 1B, the results were same as Experiment 1A in the potting mix. However, the soil with three month mix fertilizer gave different results with the potting mix. The different types of soil may be an important factor affecting GB and GB+SWE to fight drought stress during cutting propagation.

That GB is more effective than GB + SWE is seen in both experiment 1 and in experiment 2. In experiment 2, the drought was stronger than in even the more exposed cuttings of replication 1 of experiment 1. Because the sunlight was more intense and the temperature was higher overall in experiment 2 than in experiment 1, this created stronger drought conditions in experiment 2. It is highly likely the stronger sunlight was the main factor affecting the survival number and cuttings' growth in experiment 1, and still had an effect in experiment 2 as well. At around noon, the direct sunlight exposure was the strongest of the day, so the air moisture was lowest during that time, at about 40%. Conversely, the growth parameters of GB + SWE showed better results than just GB, even though in this case there was a higher mortality with this treatment. I will discuss this in more depth in chapter 5.

The strength of the sunlight on different hardness by growth stage of cuttings, semi-hard or soft, also affected survival number and cutting growth. The cuttings used in experiment 2 were the very soft growth of early October, but spring growth (middle of October) is the optimal time to take the cuttings because they are slightly harder and in a better condition to withstand the stresses of propagation. These semi-hard cuttings are hardier and can more effectively fight drought stress, aided by exogenous application of treatments.

Overall, it was clear that these treatments can effectively help cuttings fight drought stress, but are not significantly helpful when there is no drought stress. In experiment 1 for example some cuttings of the control showed good growth, but these were areas that had lower sunlight exposure and hence less drought stress. The growth parameters of the control cuttings that were not experiencing significant drought stress were similar to the cuttings with exogenous GB application that were experiencing drought stress.

3.3 Experiment 3-Effects of GB and GB + Seaweed extract (GB+SWE) on cuttings of Lavatera x Clementii

3.3.1 Treatments and experimental design

The aim of Experiment 3 was to determine whether foliar spray application or soaking is the more effective application of GB and GB plus seaweed for propagating cuttings in fertilized and unfertilized soil in *Lavatera x clementii* with 2 levels of drought stress.

Along the same lines as experiments 1 and 2, Experiment 3 was conducted to investigate how effective the treatments applied by spraying would be for the growth of the *Lavatera x Clementii* cuttings' roots under different levels of drought stress. All the cuttings were first planted in well-watered potting mix (soil moisture 93%) with half of the cuttings then given light drought conditions (soil moisture of 80%) for the duration of the experiment, with the other half remaining at well-watered conditions. The experiment ran for 9 weeks, from November 26, 2017 to January 20, 2018. During this period, the average temperature in the glasshouse increased from 24°C to 35°C, with a mean of 29.5°C, over the whole period. In the covered block where the cuttings were grown, as in Experiment 1, the average temperature also increased from 21°C to 28°C, with a mean over the whole nine weeks of 26°C. The humidity averaged 95%, but was lowest, at about 40%, at noon. In this Experiment, there were 200 cuttings randomly planted in five trays arranged in five blocks with 8 replications per block (Figure 33), with the cuttings subject to 4 different treatments. The cuttings in well-watered soil faced drought from air, while the other, less watered cuttings faced drought from the soil as well as from air. The experimental procedure and assessments were same as experiment 2.

Figure 33: The example of *Lavatera x clementii*. The cuttings of using treatments and control were randomly planted into the tray.

3.3.2 Results of Experiment 3

3.3.2.1 The effects of GB and GB+SWE on survival number of *Lavatera x clementii*

In this experiment, it was clear that GB and GB+SWE can both effectively help cuttings fight drought stress and keep them growing well under drought conditions (soil moisture of 80%), but using GB and GB+SWE on well-watered plants did not give a good result (Figure 34).
Comparing the treatments to the control cuttings at 93% soil moisture, all of the treatments had a negative effect on survival number, clearly the plants had enough water already and the treatments were interfering in their growth somehow. However comparing the control cuttings to the treatments at 80% soil moisture, the results of all the treatments were better than the control. Clearly at this drought level, there treatments helped the cuttings fight drought stress.

Under drought stress (soil moisture at 80%), the effects of GB and GB+SWE were close on survival number, however, GB with fertilizer was a slightly better treatment for fighting drought.

While for the well-watered plants, no treatment is best for survival number, treatments can have positive effects on other growth parameters so it is still worth knowing which treatments don't damage survival number significantly. For the well-watered cuttings survival number was higher for GB+SWE than for just using GB. GB without fertilizer was the worst treatment for well-watered cuttings. 93% soil moisture clearly isn't a drought level, so the control cuttings weren't experiencing drought stress, which is likely why the treatments had little effect.

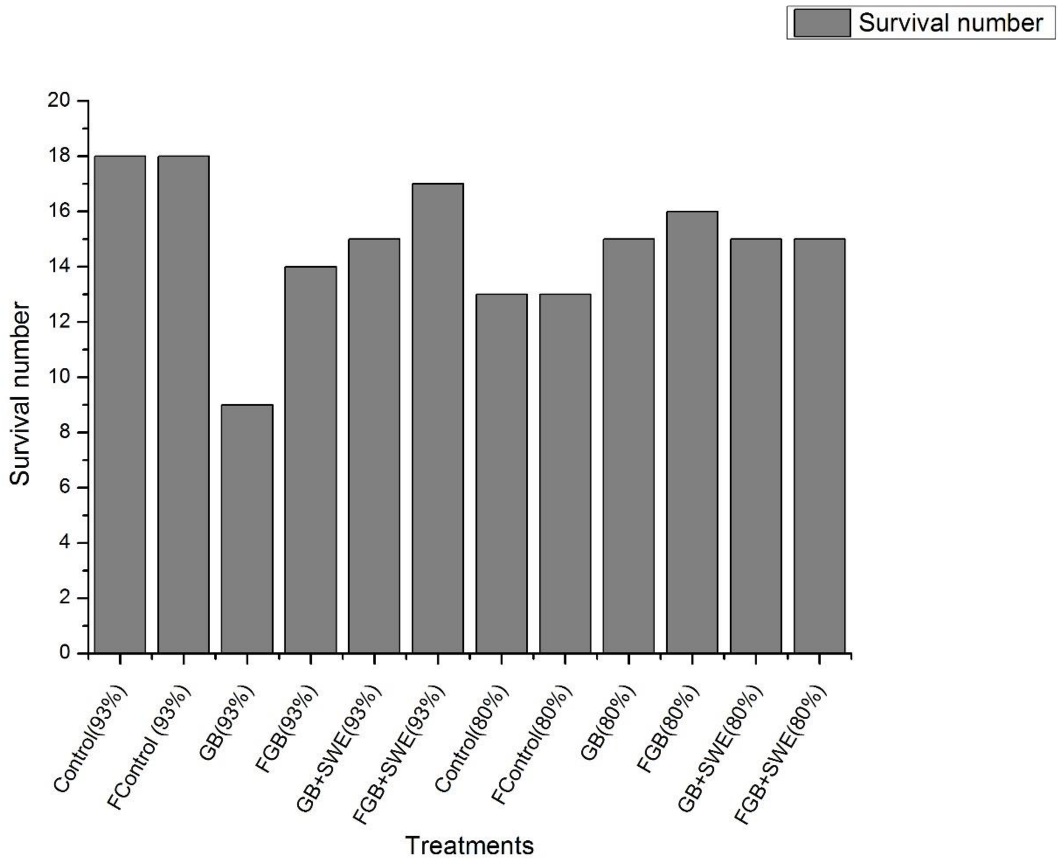


Figure 34: Comparing survival number of *Lavatera x clementii* cuttings under well-watered conditions and drought stress.

Key: F GB: GB with fertilizer, GB: GB without fertilizer, F GB+SWE: GB+SWE with fertilizer, GB+SWE: GB+SWE without fertilizer.

80%: soil moisture is 80% (light drought); 93%: soil moisture is 93% (well-watered condition)

3.3.2.2 Growth parameters

Under both well-watered and drought stress conditions, the shoot height always increased, but there were some significant differences depending on the treatments. Under drought conditions shoot growth still experienced significant increases, for example, the shoot height increased to 8.7 cm from 4.5 cm by using exogenous application of GB with fertilizer under drought stress with soil moisture at 80% (Figure 35). For each level of soil moisture, the effect of GB with fertilizer was better than GB without fertilizer on shoot length, but GB+SWE with fertilizer only did well in the well-watered soil and was the worst performing treatment at 80% soil moisture. It seems that this combination of 3 additives tends to suppress the growth benefits of this treatment, but with enough water the negative effects are not apparent. GB+SWE was more beneficial for shoot growth than just GB under well-watered conditions, and the fertilizer was more effective for shoot growth with GB and GB+SWE, with both giving better results than the controls (Figure 35).

For root length, there were significant differences in the various treatments (Figure 36). GB and GB+SWE showed a more significant benefit for root growth under drought conditions than under well-watered conditions. Among these treatments, GB with fertilizer was the best treatment under drought stress conditions, having the most significant difference to all treatments and the controls. GB with fertilizer was far better than just GB under drought stress as well as GB and GB+SWE in well-watered conditions. GB+SWE with fertilizer was the best treatment under well-watered conditions, and it was significantly better than other treatments in the same well-watered condition (Figure 40).

Leaf area, leaf number, and leaf area ratio, all showed significant differences among various treatments with only root/shoot ratio not showing any measurable change (Figure 37-39). At the beginning of the experiment every cutting had 2 leaves, by the end of the experiment the number of leaves had significantly increased but the average leaf area had significantly decreased through the use of GB and GB+SWE. FGB at a soil moisture of 80% was consistently better than all other treatments overall growth parameters at 80% and 93% soil moisture.

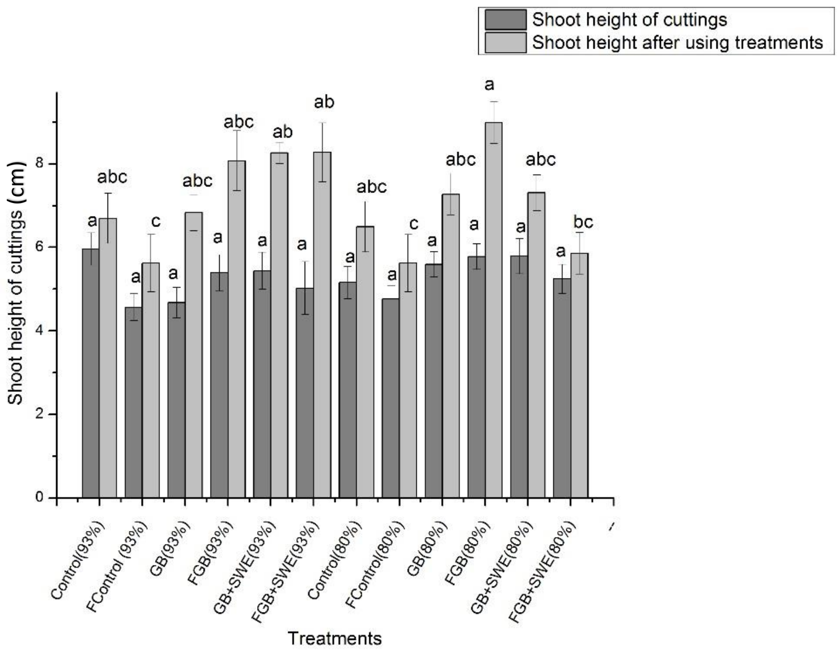


Figure 35: Mean of *Lavatera x clementii* shoot length under well water and drought stress.

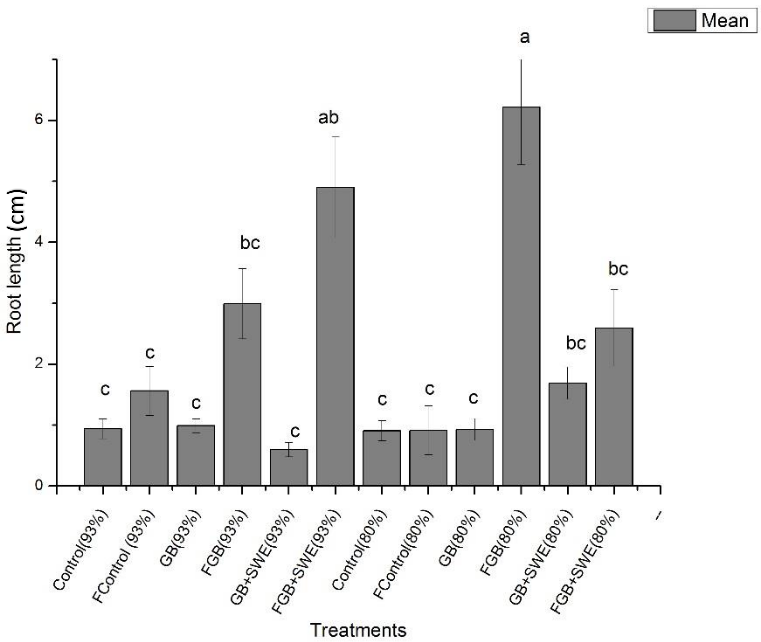


Figure 36: Mean of *Lavatera x clementii* root length under well water and drought stress.

- All values are in Means ± SE.
- Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Tukey's HSD-test), and indicated by different letters.
- Key to Figure 35 and 36: FGB: GB with fertilizer, GB: GB without fertilizer, F GB+SWE: GB+SWE with fertilizer, GB+SWE: GB+SWE without fertilizer. 80%: the soil moisture is 80%, cuttings are under drought stress; 93%: the soil moisture is 93%, the cuttings are in well-watered conditions.

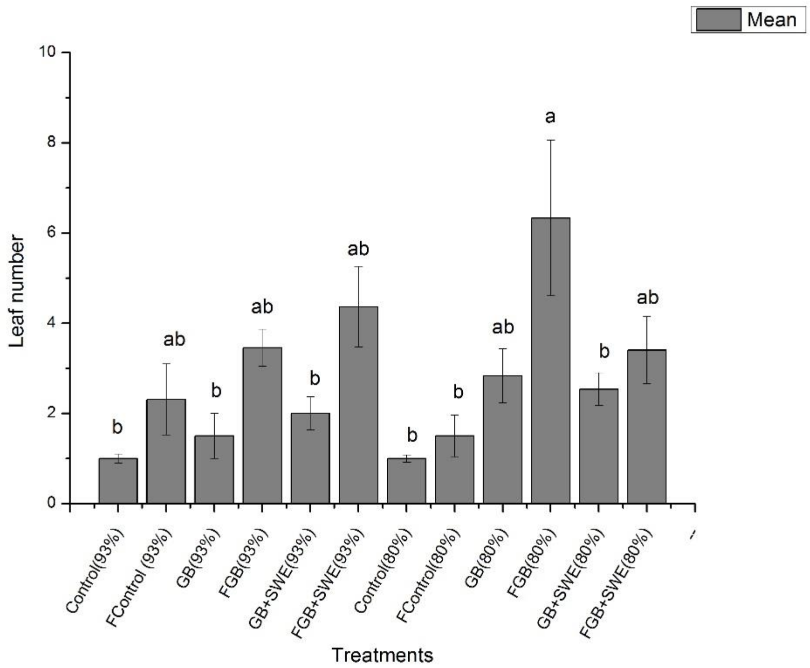


Figure 37: The changes of *Lavatera x clementii* leaf number in different treatments.

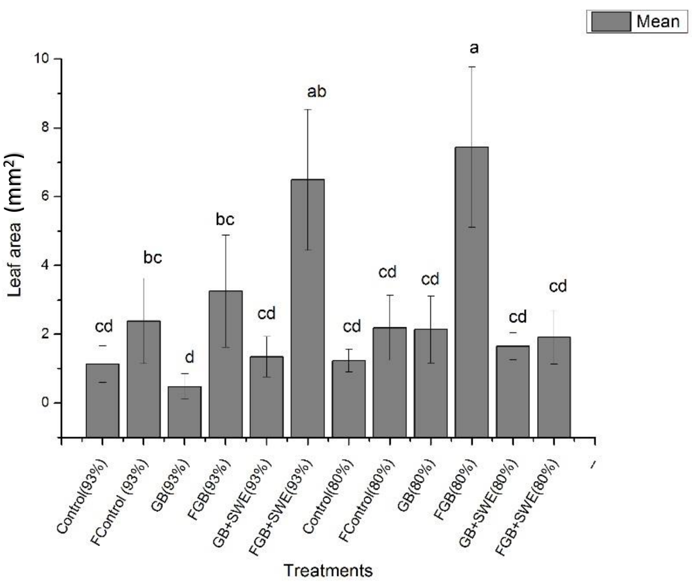
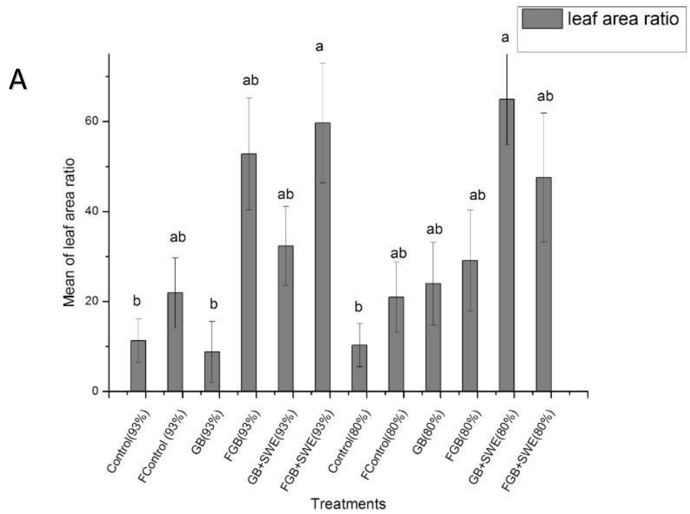


Figure 38: The effects of different treatments on *Lavatera x clementii* leaf area (mm^2).

- All values are in Means ± SE.
- Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Tukey's HSD-test), and indicated by different letters.
- Key to Figure 37 and 38: FGB: GB with fertilizer, GB: GB without fertilizer, F GB+SWE: GB+SWE with fertilizer, GB+SWE: GB+SWE without fertilizer. 80%: the soil moisture is 80%, cuttings are under drought stress; 93%: the soil moisture is 93%, the cuttings are in well-watered conditions.



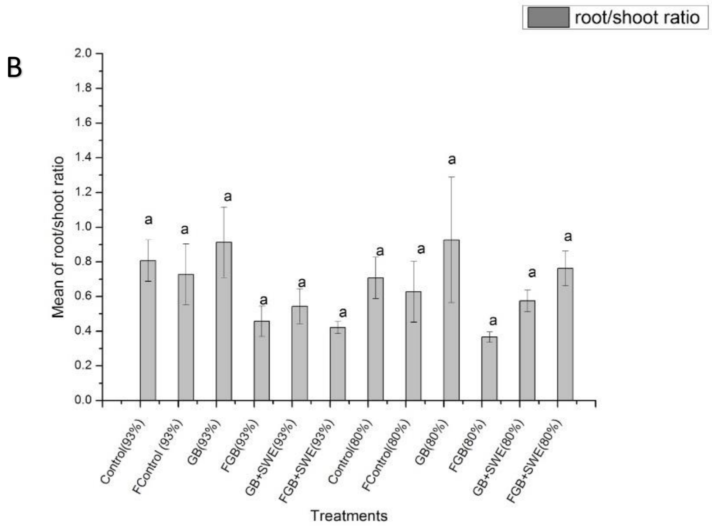


Figure 39: The effects of different treatments on *Lavatera x clementii* leaf area ratio (A) and Root/Shoot ratio (B).

- All values are in Means ± SE.
- Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Tukey's HSD-test), and indicated by different letters.
- Key to Figure 39: FGB: GB with fertilizer, GB: GB without fertilizer, F GB+SWE: GB+SWE with fertilizer, GB+SWE: GB+SWE without fertilizer. 80%: the soil moisture is 80%, cuttings are under drought stress; 93%: the soil moisture is 93%, the cuttings are in well-watered conditions.

Figure 40: The effects of GB and GB+SWE on *Lavatera x clementii* root growth under drought conditions than under well-watered conditions.

3.3.2.3 Dry weight

For the dry weight of cuttings' leaves and shoots, there were significant differences in the 8 treatments but the dry weight of roots was quite similar for all treatments (Figure 41). Under drought stress, GB was more beneficial than GB+SWE for cutting growth and the same result was seen in dry weight as well. The dry weight of leaves and shoots was higher from using just GB than it was from using GB+SWE, and highest of all when applying GB with fertilizer. GB+SWE with fertilizer gave the best result for the dry weight of leaves and shoot under well-watered conditions, where the other treatments were not much different or slightly worse than the controls.

GB without fertilizer gave the highest root dry weight under drought stress as well as in well-watered conditions and was the only treatment that did better than the controls. Exogenous application of GB consistently gave the best result on cuttings' dry weight over the range of growth parameters.

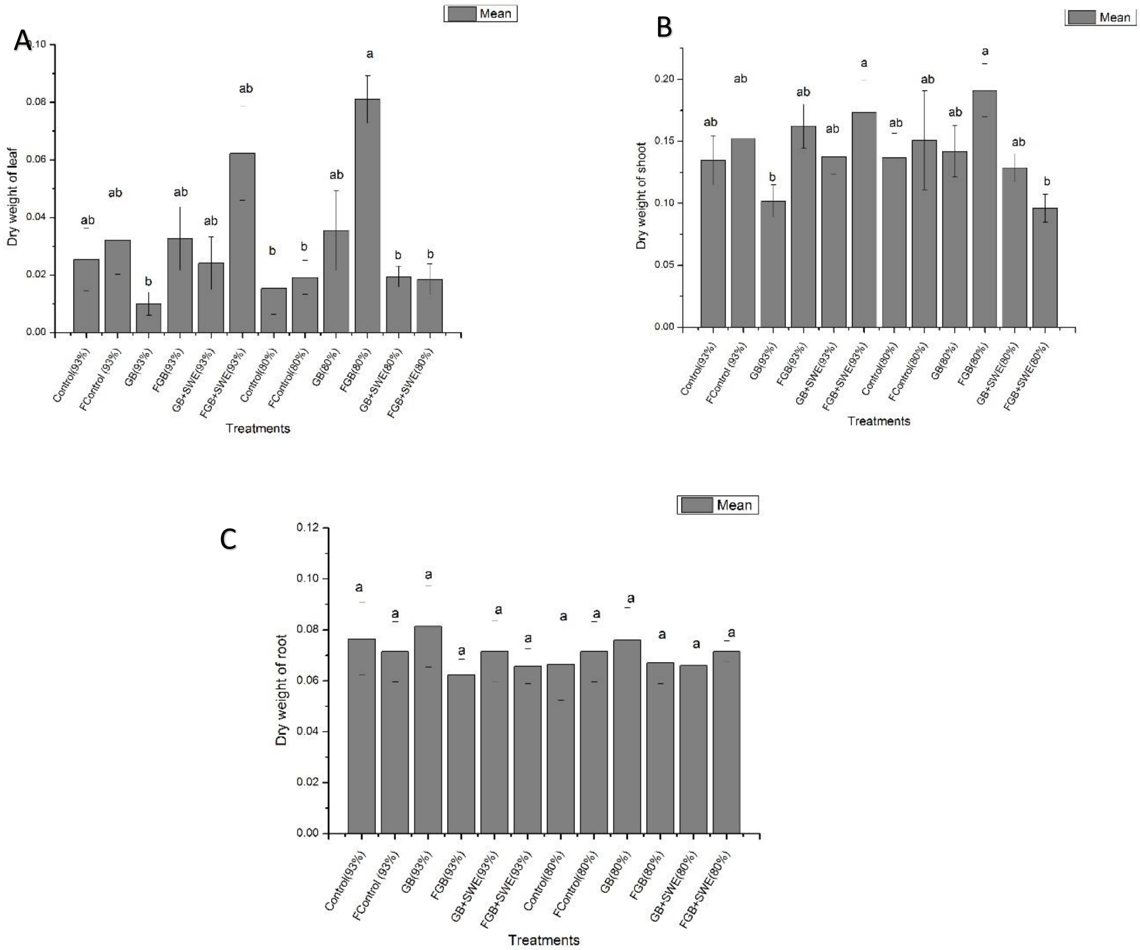


Figure 41: Mean *Lavatera x clementii* dry weight of leaves (A), shoots (B), and roots (C).

- All values are in Means (mg) ± SE.
- Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Tukey's HSD-test), and indicated by different letters.
- Key to Figure 41: FGB: GB with fertilizer, GB: GB without fertilizer, F GB+SWE: GB+SWE with fertilizer, GB+SWE: GB+SWE without fertilizer. 80%: the soil moisture is 80%, cuttings are under drought stress; 93%: the soil moisture is 93%, the cuttings are in well-watered conditions.

3.3.3 Discussion of Experiment 3

It was clear from the data, treatments are mainly effective when there is less water available to the plant and the plant needs support. Although applying treatments on plants which were well watered generally damaging for their survival, treatments on plants in well-watered conditions could positively affect some growth parameters. This could be useful when some growth parameter needs to be specifically targeted for some reason. In real life the amount of water that plants receive will vary, some days they may be well watered and some days they may be experiencing drought conditions. In this situation if we know that certain treatments are significantly damaging to well-watered plants, we can choose to avoid these treatments. Even though it is true that a 98% soil moisture content should be defined as well watered rather than under drought stress, this situation is still important to assess the effects of GB and SWE for cutting propagation.

GB was shown to help cuttings fight drought stress, and GB with fertilizer had a very good result over a wide range of parameters, and was much better than GB+SWE under drought stress. The effect of GB+SWE was only greater than GB under well-watered conditions, where it is unlikely you need exogenous treatments in the first place, so while the SWE is possibly neutralising the positive effects of GB, the plant doesn't need the extra help if it is getting enough water in the first place. GB actually limited the cuttings' growth under well-watered conditions, and only helped cuttings grow under drought stress, so it is important to judge when the application of GB is needed and not to just apply it randomly. In between 80% and 93% there will be a soil moisture level where GB will be neither useful nor damaging, and this would be an interesting topic for further study. It would be beneficial if the experiment was repeated in order to be confident of consistent of data.

In the experiment, all the cuttings died from the top. So the cuttings could continue to grow, if they had roots after the rooting period. This is one reason why the survival number of *Lavatera x clementii* was higher than the survival number of *Griselinia littoralis*. With *Griselinia littoralis* once the root dies, there is little that can be done to save the cutting, whatever treatments are applied, whereas with a still healthy root system, *Lavatera x clementii* can be prompted to grow with treatment applications.

Chapter 4: Overall Observations, Discussion, Conclusion and future research

4.1 Discussion of main comparisons made in entire study

Although short summaries have been made separately for each experiment, some findings that are consistently seen across several experiments have not been covered. These will be discussed in this chapter.

From analyzing the data of all the experiments, certain patterns could be seen. The different times when the cuttings were taken, the frequency of the exogenous application and the environmental conditions the cuttings were grown in, were shown to be factors affecting the cuttings' growth under drought stress. During cutting propagation, the types of drought experienced by the cuttings in my study were air drought caused by high temperatures and strong sunlight, and soil drought also caused by high temperatures, strong sunlight and lack of watering.

Normally, there are three levels of drought; strong drought, middle drought, and light drought. During cutting propagation, the cuttings were always in light drought conditions, from the high temperature and strong sunlight in the glasshouse. Drought from the soil was well controlled by the amount of watering given to each tray of cuttings, but drought from air was less manageable because there were localized drought from air around certain trays, and this drought wasn't experienced by the other cuttings due to their location in the greenhouse and how much exposure to the sun they had. The effect of these localized droughts turned up in the results and gave some anomalous data that had to be addressed. If repeating the experiments this anomaly would be addressed by either moving the cuttings' locations within the glasshouse regularly or by arranging the experiment set up in a way to minimize this effect.

4.1.1 Comparison between cuttings taken from autumn and spring

In experiments 1A, 1B and 2A, cuttings were taken in autumn and spring and the results were very similar with both experiments experiencing the same issues, especially with the environmental factors such as sunlight which affected how the cuttings grew. If these factors are taken into account and treatments used well, then the overall results showed that growing from cuttings taken in either season is very similar.

Cuttings in both experiments (as well as in the other experiments) suffered a similar dying process. The leaves of around half of the cuttings turned yellow then black, leading to the death of cuttings from drought stress, but if the cuttings were facing a longer time in the sunlight, the death of cuttings started with the stem turning black first. The reasons leading to these effects and death of the cuttings were drought stress and some other unknown factors. Reader (2015) also reported the blackening of the stems and leaves, but did not attribute this to a disease; this is a further area for future study.

In considering the overall research in this study, direct sunlight has clearly had a major influence. Unfortunately direct sunlight was not measured. Therefore, if this study was to be repeated, sunlight should be recorded, and growth results measured against this variable, to determine its effect.

Sunlight seemed to be the main factor causing uneven drought stress which showed in different and seemingly random results in the two blocks. If the sunlight is controlled and the cuttings are protected from overexposure then drought will not affect them so strongly and growth will be better. This meant that as long as these factors are considered, then growing in either season is similar. The sunlight angles and exposure times are different depending on the season so this needs to be considered for any given growing time. For survival number and root growth, GB was an effective treatment, but GB+SWE was more effective for shoot growth than GB.

In the summer experiment, there were also more cuttings without roots that were alive at the initial 2-week check, which however died in the end. In the autumn experiment this result wasn't seen at all. Cuttings dying from the stem first wouldn't be able to put down roots, so this is most likely the cause of this result.

4.1.2 Comparison between spraying and soaking

In Experiment 2A and 2B it was shown that soaking in GB and GB+SWE (Experiment 2B) had less of an effect on helping cuttings fight drought stress than spraying GB and GB+SWE (Experiment 2A). Soaking did not seem to be the best way to get the benefits of GB for the cuttings, and spraying gave much better results. Since GB is abundant mainly in chloroplasts to adjust and protect the thylakoid membrane, (Aldesuquy et al., 2012; Ashraf & Foolad, 2007). Perhaps the direct application of GB to leaves encourages the plant to be able to synthesize GB if it is given a boost from the start. Whereas soaking in GB doesn't give the same boost, but rather the plant just uses the GB it accumulates from soaking and GB synthesizing is not promoted perhaps.

Since all the experiments show consistently that plants only get the benefits of GB when they need it, i.e. when they are lacking in water, soaking a fresh, green, cutting with a high moisture content in GB may not be effective as the plant may not absorb the GB if it doesn't need it. When the fresh cutting is soaked in GB it seems the positive effects of GB are not manifest in the growth of the plant as the results of cuttings soaked in water were the same as for cuttings soaked in GB. However, cuttings that had an exogenous spray application of GB did much better because they were getting the GB when they needed it, i.e. when they were more dehydrated and the GB could work more effectively for the plants water relations.

In the hotter summer conditions of this experiment the cuttings were periodically sprayed to reduce the adverse effects of the heat. This means that even the soaked cuttings were sprayed a little (though not with exogenous treatment). In a nursery environment this would be the same, so for cutting propagation comparing soaking and spraying would likely yield the same results. It was clear that soaking by itself is overall not effective in this type of cutting propagation, especially when it was seen that the control gave better results in survival number than soaking in treatments of GB and GB+SWE.

4.1.3 Comparison between *Griselinia littoralis* and *Lavatera x Clementii*

One of the most significant differences found between the two species was that *Lavatera x Clementii* tends to die from the top of the cuttings (Figure 43), whereas *Griselinia littoralis* dies from the bottom (Figure 42). Due to this factor, new leaves of *Lavatera x Clementii* can grow after old leaves die and fall if the cuttings still have roots, but *Griselinia littoralis* is much harder to recover. Both species showed yellowing leaves and blackening stems equally however.

The effects of GB and GB+SWE with or without fertilizer on *Griselinia littoralis* and *Lavatera x Clementii* were different for each plant and did not give consistent results between the plant types. Since the root condition of *Griselinia littoralis* will determine the health and hardiness of the cutting much more critically, perhaps it was more sensitive to treatments and had less chance to recover once damage was done.

For *Griselinia littoralis*, GB without fertilizer or GB+SWE with fertilizer were both beneficial, but GB with fertilizer was actually damaging. However for *Lavatera x Clementii* both GB and GB+SWE with fertilizer were beneficial. This would indicate that depending on the type of plant and the amount of water available the treatment needs to be adjusted and not applied equally to all types of plants. Each different species of plant will have different growth characteristics so a one application system fits all approach clearly will not be reliable.

Comparing these 2 plant species was to give an insight into how different plants react to treatments, and even with just 2 comparisons it is clear that there are differences so comparisons between more different types of plants would be beneficial for further study.

Figure 42: shoots of *Griselinia littoralis* also started to turn black from the bottom then spreading up the stem.

Figure 43: *Lavatera x Clementii* tends to die from the top of the cuttings

4.1.4 Combinations of treatments

Certain combinations of treatments were consistent throughout the experiments in how they worked well or very poorly together. There were some exceptions depending on the plant species, but consistent patterns were identified.

GB works well by itself in reasonable drought stress for *Griselinia littoralis*, but not for *Lavatera x Clementii* which did better with GB and fertilizer together. For *Griselinia littoralis*, the effects of GB without fertilizer were always better than GB with fertilizer on survival number, and the more severe the drought the more often GB should be applied (within the drought level variations within this study). This means that whether GB works well by itself or in combination with fertilizer depends on the species of plant. The reasons may be due to some plants needing more fertilizer nutrients to help GB fight drought stress during their cutting propagation and some plants not needing it at all.

GB+SWE was generally not a good combination for survival number, similar to GB with fertilizer also being not very effective, indicating that perhaps the fertilizer nutrients are somehow neutralizing the effects of GB. As SWE can also act as a fertilizer, this seems to be a common factor in this observation. GB+SWE with fertilizer consistently gave a lower survival number, which seemed to have similar characteristics to how plants die due to over-fertilization.

Overall, GB with or without fertilizer can both be used to fight drought stress and maintain survival number, but how it is used must depend on the species of plants. GB is also only effective when there are drought conditions and will be damaging for plants when there is enough water. In general when the plants have enough water, treatments tended to inhibit growth.

4.2: Conclusion

This study was fundamentally aimed at investigating the effects of exogenous application of GB and GB+SWE during cutting propagation under drought stress to consider the value of GB and GB+SWE in horticultural production. The results showed that overall GB alone was beneficial in most situations, but GB combined with SWE was only beneficial in some limited circumstances. The most significant results to draw from this study was that treatments may work well or work badly in combination with each other and in combination with fertilizer or on different plant species, and that treatments are only beneficial when there is actually drought stress. It would be a mistake to think that since GB is beneficial and SWE is beneficial then they will both be beneficial together in all circumstances, and it would also be a mistake to think of these types of treatments as a one size fits all option for fighting drought stress for any plant species, applied at any time .

In New Zealand, droughts are principally caused by high temperatures and strong sunlight. In most studies, spraying, soaking, and irrigation were widely used to fight drought stress in plant growth. However, irrigation is not a good way to fight drought during cutting propagation, because cuttings are without roots for a long time during this process, and so they cannot effectively draw moisture from the soil. Other studies focusing of crop growth by irrigation with certain treatments showed good results because plants with roots can take advantage of this method, but for cuttings of *Griselinia littoralis* and *Lavatera x clementii*, and probably similar types of plants, it is clear that spray treatments work much better. Since nearly all cuttings don't have roots, but will have leaves, then soaking or irrigation methods of exogenously applying treatments may have similar results to *Griselinia littoralis* and *Lavatera x clementii*.

While some treatments don't work well together, some do, and some work well in specific circumstances. For example GB+SWE generally doesn't give great results during drought stress, with overall results being a little worse than GB by itself. However, GB+SWE wasn't detrimental to the cutting when it was well watered, while GB by itself was. This attribute of the GB+SWE combination would be useful in areas that had variable rainfall or strong sunlight leading to quickly changing times of drought and non-drought. Plants treated with GB in these conditions would do well when there was drought stress but do badly when there was enough water. However with the GB+SWE combination the benefit would be slightly less during periods of drought, but there would be no damage during the well-watered times, so the overall result may be better. Finding the situation that suits the characteristics of the treatment combination is more important than saying certain treatments just don't work.

An interesting result seen consistently throughout this study was that while some treatments were detrimental to the overall survival of the cuttings, they were sometimes beneficial for individual growth parameters. This attribute could be useful if it was important to promote growth of a particular area of a cutting for a specific purpose, and then control the treatment so it would not harm the survival of the plant overall. If the treatment schedule is planned so only GB is used at the start of cutting growth, and then once the cutting becomes established, combinations of treatments could be used to get faster shoot or root growth for example. More detailed study of exactly how this works would be necessary, but it looks like an interesting effect that could be utilized in the cutting propagation industry. If it were possible to determine how much treatment or combination of treatments, applied for a specific period of time would give certain results without harming the overall chances of survival then this could be of immense value in nurseries.

Treatments should only be considered where there is definite drought stress because in some cases the treatments can be damaging. Throughout the study the controls were better than the treatments when there was less drought stress. It was a consistent theme through all experiments that GB and GB+SWE negatively affected cuttings' growth under well-watered conditions so care should be taken to apply these treatments only when necessary and restrict the application during times of less drought. When there is less drought the application of treatments needs to be carefully considered and applied in different amounts and different combinations for different plants, or even stopped completely.

GB and SWE both alone and in combination can be useful but is not a miracle growth formula to be used indiscriminately for all cuttings in all conditions grown in any soil type. This study highlighted some important results and also showed some interesting possibilities for further research.

4.3: Recommendations for future research

This study investigated the effects of GB and GB+SWE on cuttings' growth during cutting propagation under drought stress. However, there were some problems encountered during the course of the study and further research in the following areas may be beneficial to be able to determine precisely how exogenous application of GB and GB+SWE should be applied in practice.

- The experiment set up in all experiments was not ideal in that variables were not controlled to the extent necessary to minimize anomalous results from varying levels of drought in soil or air, sunlight levels and localised exposure and non-consistent levels of soil moisture in the control cuttings. Some cuttings were not actually under drought stress when they should have been so more care needs to be taken with this aspect.

- The types of drought. In the glasshouse, the air and soil drought from sunlight should be properly controlled as well. Depending on the different types of drought, cuttings need different treatments of GB or GB+SWE. Further research in this area is needed.

- The results of the experiments indicated a complex interaction between soil type, concentration of GB and GB+SWE, application method, processing method and chemical fertilizer addition. The possible mechanisms behind these interactions may be associated with the changes in the physical, chemical and biological properties of the growth medium. Nevertheless, it is still not known how these changes interact with each other to result in the growth promoting effects observed. Further studies are required in this area.

- Concentration of GB in GB+SWE. If the concentration of GB in the GB+SWE treatment can be changed, the solution not only can fight drought stress, but also can promote individual cutting growth parameters. However, in these experiments, the cuttings of *Griselinia littoralis* died from the bottom of the cuttings. The reason of death may be soil drought or potting Mix or other unknown reasons. This problem requires future study.

- As treatments can be beneficial for certain growth parameters but detrimental to overall survival, more detailed study on how this could be utilised would be useful. If we can understand how much treatment of which combinations at which timing and when to stop treatment to ensure cuttings survival is possible, then it may be possible to target specific growth parameters without the cutting dying.

While there are some inconsistencies introduced by variations caused by a less than ideal experimental set up, this research still has established several interesting results and findings which can be of benefit to cutting propagation activities. To obtain clearer and more substantial relationships and conclusions I would redo the experiments with more care to reducing random variables.

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