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Evaluating alternatives to peat based growing substrates for use in ornamental horticulture

By

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Abstract

The horticultural industry urgently needs alternatives to peat based growing media as harvesting peat depletes a huge carbon sink. Although peat is an incredibly effective growing medium, the environmental costs are incredibly concerning. Factorial experiments involving spring barley (*Hordeum vulgare*), rye (*secale cereale*), dianthus (*dianthus plumaris*) and petunia (*petunia F1* prism sunshine) were carried out in four substrates: a high peat content potting and bedding mix, a peat and wood fibre mix, a peat free coir and wood mix, and a peat free sand and topsoil mix. The research aims to address the following hypotheses: (I)The addition of surfactants improves plant growth in potted ornamental species; (II) Ornamental species grown in peat reduced and peat free media produce a lesser biomass and overall plant health than those grown in a peat-based medium; (III) Peat reduced and peat free substrates are not able to sustain potted plants and tolerate drought stress under a deficit irrigation regime to the same extent as peat-based substrates. The addition of surfactants increased above ground dry biomass in the substrates while the high peat content mix produced the greatest biomass compared to the other substrates in the majority of instances. However, there were few instances in which the peat and wood fibre mix produced plants with a greater biomass. It was determined through measurements of leaf water potential, stomatal conductance and biomass measurements that the plants grown in the high peat content substrate performed better than those grown in other substrates under deficit irrigated conditions.

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Author’s declaration

I, Olivia Sophia Haines, declare that this thesis is my own work, in my own words, and has not been submitted for the award of a higher degree elsewhere. Many of the ideas in this thesis were the product of discussion with my supervisor Professor Ian Dodd, Sarah Fairhurst of Arden Lea Nurseries Ltd. and Richard Collins of ICL.

Chapter 1

Introduction and literature review

1. Introduction and literature review

1.1. Introduction

1.1.1. Aims

While peat has traditionally been used as an ideal growing medium for horticultural plant production, draining and destruction of peat bogs for use in growing media depletes a huge carbon sink. The horticultural industry urgently needs alternatives to peat based growing media, not only to exercise corporate social responsibility but increasingly to adhere to government guidance and/or legislation. To evaluate the viability of alternative growing media, factorial experiments were set up with grasses (spring barley and rye) and ornamental species (*dianthus plumaris* and *petunia F1 prism sunshine*) and media with different peat contents (both with and without surfactants).

The research aims to address the following hypotheses:

I. The addition of surfactants improves plant growth in potted ornamental species
II. Ornamental species grown in peat reduced and peat free media produce a lesser biomass and overall plant health than those grown in a peat-based medium
III. Peat reduced and peat free substrates are not able to sustain potted plants and tolerate drought stress under a deficit irrigation regime to the same extent as peat-based substrates

1.1.2. Peat formation

Peat used in growing media forms under anaerobic conditions from the decomposition of mosses due to the low pH and wet conditions inhibiting microbial activity, allowing biomass to accumulate (Maher *et al*., 2007). Basin peat bogs were initially formed from post glacial lakes and therefore were initially made up of aquatic plants. The lack of decomposition and the build up of plant debris caused water tables to rise. The displacement of this water then allowed for the development of peat forming plants beyond the original lake margins (Hammond, 1975). If nutrient supply is sufficient, the basin bogs transition to raised bogs where species such as sphagnum moss become the primary source of organic matter. Peat is classified by The International Peat Society (Kivinen, 1980) according to the organic matter it is made up of, its degree of decomposition and its nutrient status (Table 1).

Table 1. Classifications of peat based on botanical composition, degree of decomposition and trophic status. Adapted from The International Peat Society (Kivinen, 1980).

Botanical composition	**Degree of decomposition (H)**	**Trophic status**
moss peat (predominantly sphagnum and other mosses) sedge peat (sedges, grasses, herbs) wood peat (remains of trees and woody shrubs)	weakly decomposed (H1– H3) medium decomposed (H4– H6) strongly decomposed (H7– H10)	oligotrophic (low in nutrients) mesotrophic eutrophic (high in nutrients)

1.1.3. The problem with peat

Between 14 and 20% of harvested peat is used for growing media (IPS, 2008) with much of this being used in the horticultural sector for cuttings and potted plants in nurseries. The remainder is used predominantly for fuel and heating purposes. The environmental impacts of harvesting peat are incredibly detrimental, reducing a huge carbon sink and releasing greenhouse gasses, as well as damaging ecosystems. There is a limited supply if not harvested responsibly, therefore peat is not a renewable resource, so effort is being made to move away from peat-based media and towards more sustainable alternatives.

1.1.4. Peat as a growing substrate

Peat was used as one of the key components in the first standardised soilless growing media for use with potted plants, accounting for a quarter of the standardised mix, alongside loam and sand (Lawrence & Newell, 1939). Work carried out in the 1960's then concluded that peat was able to

be used as a growing media without the addition of other components (e.g. Penningsfeld & Kurzmann, 1966; Woods & Kenny, 1968).

Peat is now the most widely used soilless growing medium in horticulture due to its desirable physical properties for example, high porosity, good aeration and high water holding capacity (Maher *et al.*, 2007). The most widely used peat variety for use as soilless growing media is sphagnum peat with more than 27 million m^3 a year harvested for use in growing media in the European Union (Schmilewski, 2009).

Table 2. Largest global peatland areas by country with the volume used for horticulture in 1977. ([a]Lappalainen, 1966; [b]Hood and Sopo 2000; [c]Peatland area over 70 cm deep; [d]Excluding Alaska).

Country	**Area of peatland (km^2) over 30 cm deep[a]**	**Production for horticulture (000' m^3) in 1977[b]**
Canada	1,113,270	7,250
Russia	568,000[c]	2,540
USA[d]	105,000	2,201
Finland	89,210	1,626
Sweden	45,940	1,203
Belarus	23,967	272
Norway	23,700	140
UK	17,549	2,500
Germany	14,205	9,000
Ireland	13,570	1,616
Poland	12,050	680
Estonia	10,091	3,497
Ukraine	6,932	85
Latvia	6,691	650
Lithuania	4,826	1,250

As a growing medium, peat also has desirable chemical properties such as high cation exchange capacity. Due to the low pH and low nutrient availability when in its raw form, use of peat as a soilless growing media allows for easily obtained optimal growing conditions through the addition of lime and fertilisers (Maher *et al.*, 2007). Penningsfeld and Kurzman (1966), suggested base levels for lime and fertiliser that should be added to peat for petunia and dianthus species,

such as those used in this study, shown in table 3, as well as dosages for species with higher and lower salt tolerances and nutrient requirement.

Table 3. Suggested nutrient concentrations for addition to peat for growing dianthus and petunia plants (Penningsfeld, 1962).

Dolomitic lime (kg m^{-3})	N (g m^{-3})	P (g m^{-3})	K (g m^{-3})
2.0 – 5.0	180	80	200

1.1.5. Alternatives to peat

The effectiveness and environmental costs of these alternatives are largely unknown in a horticultural context. The primary alternatives to peat include wood fibre, coir and waste products from various industries such as paper production and olive oil production, although wood fibre and coir are more popular than other alternatives in the UK market. These alternatives have the potential to replace peat-based media when combined with appropriate surfactants and fertilisers. When combined with peat or as a standalone substrate, these alternatives can make excellent growing media which in some cases, can be a more effective growing media than solely peat-based media (e.g. Smith, 1995; de Kreij and van Leeuwen, 2001).

1.1.5.1. Coir

Coir is derived from the mesocarp of the coconut fruit (*Cocos nucifera*) and is a waste product from the extraction of long fibres from the husk which are used to form textiles and fabrics. Shorter fibres and coir dust are separated from the fibres and soaked in brackish water to soften it.

Coir often has high levels of sodium, chloride and potassium and therefore, these have to be leached from the substrate before it can be used for horticultural purposes. This is often done using calcium nitrate (Maher *et al.*, 2007). The treated product is referred to as treated coil and is dried and compressed into bricks or blocks in order to facilitate export. The treated, dried coil is then rehydrated and fertilised before use as a growing substrate. Due to the different treatment methods used in coir, its chemical properties can vary greatly. Therefore, not all types of treated coir are suitable for different plant species.

Table 4. Summary of studies where coir was used as a peat reduced or peat free growing substrate.

Citation	Crop	Key finding
Raviv *et al.*, 2001	Roses	19% more rose flowers were produced on plants grown in coir than in a standard UC mix

Islam *et al.*, 2002	Tomatoes	Plants performed better in high temperatures when grown in coir compared to those grown in stone wool
Smith, 1995; de Kreij and van Leeuwen, 2001	Various potted ornamental plant species	Plants performed as well or better in coir than those grown in peat
Offord *et al.*, 1998	Australian native plants such as Grevillea	Plants performed as well in coir as they did in peat

1.1.5.2. Wood fibre

Wood fibre can be produced from waste wood such as pallets, or fresh wood - usually pine or spruce. As a substrate, it is produced by shredding wood at high temperature and high pressure. Due to the high temperatures of approximately 80 ^{o}C to 90 ^{o}C, plant pathogens are killed off. Much like coir, the pH levels in wood fibre can vary greatly. However, both electrical conductivity and nitrogen concentrations are generally very low. Because of this, wood fibre needs to be treated with fertilisers. In general, wood fibre as a substrate has high levels of total porosity and air filled porosity, as well as low levels of easily available water. Due to the mechanical compression involved in production of wood fibre, its physical properties can vary greatly. Seedlings such as lettuce (*Lactuca sativa L.*) and cabbage (*Brassics oleracea*) have been successfully grown in 100% wood fibre up to the point of transplant. While the likes of peppers (*Capiscum annuum*) and cucumbers (*Cucumis sativus*) have been successfully grown to maturity in wood fibre (Gerber *et al.*, 1999).

1.2. Literature review

1.2.1. Water

1.2.1.1. Drainage

When using soilless substrates with potted plants, a common issue is the “container effect”- a form of waterlogging, resulting from insufficient drainage due to the gravitational head being too small to overcome matric tensions. This results in water being stored in the pore spaces of substrates (Fields *et al*. 2020) which can lead to inhibited root and plant growth, as well as an increase in pathogenic risk (*Bradford et al*., 1982).

1.2.1.2. Irrigation

Due to the small volume of growing media used in potted plants, substrates experience significant fluctuations in moisture between wetting cycles. These fluctuations in gas, water and nutrient availability can lead to severe plant stress (Kerloch and Michael, 2015). These fluctuations caused by frequent wetting/drying cycles have drastic impacts on water and air flow, as well as the physical properties of the substrate such as wettability, due to swelling and shrinkage phenomena (da Silva et al, 1993). These factors can lead to variations in air and water distribution throughout the pot, and therefore cause hinderances in water and nutrient uptake, as well as airflow.

1.2.2. Substrates

1.2.2.1. Performance of peat alternatives

Table 5 summarises a number of studies carried out with less commonly used peat alternatives.

Table 5. Previous studies carried out using alternatives to peat based growing media.

Citation	**Substrate**	**Crop**	**Experimental design**	**Results**
Dalias *et al.*, 2018	Hydrochar (from wheat)	Cress (*Lepidium sativum*)	Cress seeds were germinated in the substrates to measure the dry biomass of the plants and the germination rate. Seedlings were harvested 10 days after	After 10 days, 1 seed had germinated in biochar, 15 in hydrochar and 10 in peat, with mean dry masses of 0.4 mg, 1.19 mg and 1,74 mg respectively
	Sphagnum peat			
	Biochar from maize			
Chrysargyris *et al.*, 2018	Peat	Marigold (*Calendula officinalis L.*), petunia (*Petunia x hybrita L.*) and matthiola (*Matthiola incana L.*)	Plants were grown for 1.5 months under greenhouse conditions	Peat and olive stone mixes increased plant height in marigolds compared to 100% peat but there were no changes in plant biomass. In matthiola, adding olive stone waste decreased biomass in comparison to peat. Plant height was increased in petunias with the addition of olive stone waste. The addition of paper waste decreased chlorophyl concentration in all species.
	Peat and olive stone waste (90%:10%)			
	Peat and olive stone waste (70%:30%)			
	Peat, olive stone waste and paper waste (60%:20%:20%)			

Chrysargyris *et al.*, 2019	Peat	Marigold (*Calendula officinalis L.*), petunia (*Petunia x hybrita L.*) and matthiola (*Matthiola incana L.*)	Plants were grown in peat with the addition of 0%, 10%, 30% and 50% of paper waste, as well as in 100% paper waste.	Plant fresh weight decreased with the addition of paper waste when compared to 100% peat
	Paper waste			

1.2.2.2. Rhizosheath

Although there is no single method of determining what proportion of the rhizosphere makes up the rhizosheath, it is widely accepted that the rhizosheath is the mass of soil which remains adhered to the root following gentle shaking (Pang *et al.,* 2017). Rhizosheath is thought to aid plant growth and function in a number of ways through assisting in nutrient uptake and water uptake. This is due to the soil in the rhizosheath having a higher water content than that of the bulk soil (Pang *et al.,* 2017). Drought tolerant plants such as barley and rye tend to form a greater rhizosheath in comparison to non-drought tolerant plants whilst also forming a more porous rhizosheath (Rabbi *et al*., 2018). It is suggested in the literature that rhizosheath formation could prove to be a key factor in determining a solution to the global decrease in water availability in agriculture (e.g. Rabbi *et al.,* 2018).

1.2.2.3. Air space porosity and aeration

It is widely accepted that root growth mostly occurs in macropores (e.g. Cannavo and Michaael, 2013; Caron *et al.,* 2010) causing these spaces to be filled, reducing air space porosity and aeration within the substrate. The impact that this has on gas exchange within the substrate is largely disputed among scientists with largely contradicting findings with some reporting an increase in gas exchange when pore spaces are filled with roots (e.g Allaire-Leung *et al*. 1999), while Caron et al (2010) reported a decrease in gas exchange in these conditions. A possible explanation for these differences is differences in particle size within the peat, with Cannavo and Michael (2013) reporting an increase in relative gas diffusivity in coarse peat and a decrease in fine peat under the same irrigation regimes.

1.2.3. Plants

1.2.3.1. Leaf water potential

Leaf water potential is the pressure of water within the leaf at the time of sample collection. It is a useful indicator of the level of water stress experienced by the plant. When water availability is low, plants engage mechanisms that reduce leaf water potential such as stomatal opening and closing (e.g. Diatta *et al.*, 2021). Measurement techniques to determine leaf water potential were developed in the 1950s and 1960s (Turner, 1981), such as the thermocouple psychrometer used in this study.

1.2.3.2. Chlorophyll

Leaf chlorophyll concentrations are a relatively accurate indication of measuring leaf nitrogen content in a non destructive way. Nitrogen content is an indication of the availability and uptake

of nutrients from the growing substrate. Nutrient deficiency is a key hinderance in plant growth, particularly in peat reduced and peat free growing media.

1.2.3.3. Roots

As the first organ to emerge from a germinated seed, the roots of plants are essential throughout the life cycle. The root system is key to healthy plant development not only because root growth allows for exploration of the soil for the purpose of water and nutrient uptake but also since it acts as a physical support and anchor for the above ground organs. Because of this, restricted root growth can lead to insufficient nutrient uptake, water uptake and an unstable plant, all of which can contribute to poor plant development (Kafkafi, 2007). It is therefore essential when investigating a potential new substrate, that root growth is supported in this substrate. Due to the limited volume of substrate in potted plants, there is less potential for root elongation to counteract water and nutrient deficiencies, therefore water and nutrient availability are more important in soilless media than in soil. Root tip growth is known to be heavily dependent on touch and gravity, with both factors being closely related in their influence on root elongation. (Massa and Gilroy, 2003 for further explanation). Several characteristics (Table 6) of a substrate are known to impair root growth and therefore, identifying substrates in which these factors are not an issue is essential when moving away from peat based substrates.

Table 6. Physical and chemical properties of growing substrates known to inhibit root growth (Kafkafi, 2007).

Physical properties	**Chemical properties**
Compaction, porosity, water shortage, poor aeration, extreme temperature	Salinity and sodium content, low pH (causing excess exchangeable aluminium), plant macronutrient availability, heavy metal availability, oxygen availability

Plants grown in potted conditions are more sensitive to these environmental changes due to the roots being exposed to the entirety of the subsurface environment, as opposed to in field plants, where deeper roots detect changes in factors such as atmospheric temperature and moisture to a lesser extent than shallower roots.

It is not uncommon for the roots systems of plants grown in containers to grow predominantly around the container walls. This is due to the more anoxic conditions in the centre resulting from compaction of the medium (Asady *et al*., 1985). This is often accompanied by a root mat developing at the bottom of a container where downward growth has been restricted. This root mat can lead to oxygen deficiency and root death due to competition between the roots for oxygen in the layer of water which often settles at the bottom of the container. These anoxic conditions are often more severe in organic substrates where matter is decomposed by oxygen dependent microorganisms (Kafkafi, 2007).

1.2.3.4. Stomatal conductance

Stomata are pores in the leaves through which carbon dioxide enters the leaf through diffusion, and water vapour escapes through transpiration. When substrates dry, the moisture tension rises towards a point at which the plants begin to lose the ability to adequately control water loss. At this point visible appearances of the plant change as the moisture content of the leaves begins to drop and the leaves wilt. When this happens, plants are not able to regulate their temperature and therefore the temperature rises - especially in conditions with high levels of light. The stomata of a plant are essential in the process of photosynthesis. When a plant begins to lose moisture, the stomata close in order to mitigate the negative impacts from the increase in temperature and reduce water loss to avoid wilting resulting from the decrease in pressure and turgor in the leaves (Leith and Oki, 2007).

1.2.4. Surfactants

Water repellence is a common issue in horticultural growing media, particularly when used for longer growth periods (Abad *et al.*, 2004). The addition of surfactants can aid with uniform penetration of water (Blodgett *et al*., 1993) within growing media by creating a hydrophilic surface of the substrate. In a study by Bilderback and Lorscheider (1997) there was a linear increase in dry shoot biomass when surfactants were utilised alongside a decrease in irrigation volume. However, Urrestarazu *et al.* (2008) found that both the germination index and germination percentage decreased in a linear fashion as surfactant dosage increased in a trial with tomatoes (*Lycopersicon esculentum* Mill. cv. Daniela), while in lettuce (*Lactuca sativus* L. cv. Elvira), there was little change in germination percentage with the increase of surfactant dosage but the germination index was significantly higher when the dosage of surfactant exceeded 100 mg L^{-1}.

Chapter 2

Experimental Setups

2. Experimental setups

2.1. Standardised equipment and growing conditions

2.1.1. Substrates

A total of four different growing substrates were used in the trials with varying compositions and peat contents. As a high peat content substrate, Levington® M3 Advanced Pot and Bedding media (ICL, Tel-Aviv, Israel) was used. For a 50% peat content substrate, ICL produced a bespoke mix of peat and wood fibre for use in trials of this nature. Finally, two peat free mixes were utilised – one organic and one inorganic. The organic substrate was a peat free mix produced by Jiffy® (Jiffy® Products International BV, Zwijndrecht, Netherlands) composed of coir, wood fibre and wood bark. The inorganic substrate was a mix of turf dressing sand (Boughton, Kettering, Northamptonshire, UK) and Norfolk top soil (Bailey's of Norfolk Ltd., Hevingham, Norfolk). The main constituents of each substrate can be seen in Table 7.

Table 7. Peat content, main components and surfactant presence of Levington® M3 Advanced Pot and Bedding media, ICL bespoke peat reduced ornamentals mix, Jiffy® Peat Free Growing Media and Sand and Norfolk topsoil peat free mix.

Substrate	**Referred to in paper as:**	**Peat content (%)**	**Main components**	**Surfactant added during production?**

Levington® M3 Advanced Pot and Bedding media	Levington®	80	Peat, wood fibre	Yes
ICL bespoke peat reduced ornamentals mix	Peat reduced	50	Peat, wood fibre	Produced both with and without
Jiffy® Peat Free Growing Media	Jiffy®	0	Coir, wood fibre, bark	Yes
Sand and Norfolk topsoil peat free mix	Sand	0	Turf sand, topsoil	No

2.1.2. Standard pots

Seeds were all either germinated in seedling trays or damp tissue paper. Seedling trays were 37 cm * 22.9 cm * 12.7 cm and each contained 40 cells each. Trays were placed in a base tray in order to replicate an ebb and flow irrigation regime where the base trays were filled with water and then drained after 30 minutes. They were also covered by a humidity lid during the germination phase in order to maintain substrate moisture.

Following germination, all seedlings (see methodology chapters for treatments and number of seedlings) were transplanted into 2 L round plastic pots filled with the relevant substrate. Each pot had a base diameter or 12 cm and a diameter or 17 cm at the top of the pot. Each pot had nine drainage holes in the base.

All pots and seedling trays were cleaned with bleach prior to use in order to minimise contamination from previous uses.

2.1.3. Growing Space

All trials, following the initial germination phase, were carried out on a bench under glasshouse conditions of photosynthetically active radiation (PAR) ~350 between the hours of 0700 and 2100 daily. During germination, some seedlings were grown in a controlled environment room where temperatures did not exceed 21 ^{o}C, in order to reduce water loss from evaporation and reduce heat stress.

2.1.4. Statistical analysis

All data were recorded using Microsoft Excel while statistical analyses were carried out using SPSS. All graphs were produced and edited using Microsoft Excel.

2.2. Specific experimental designs

2.2.1. Barley and rye

For this trial, 2 L plastic pots were filled with substrates (as mentioned in section 2.1). Due to the fine grained nature of the sand mix, the base of the pots were lined with a fine mesh to minimise substrate loss through the drainage holes without hindering drainage.

Spring barley (*hordeum vulgare*) seeds and rye (*secale cereale*) seeds were used in the trial due to an understanding of rhizosheath formation in these species grown in sand and topsoil mixes, derived from the existing literature. In order to obtain the 36 seedlings necessary for the trial, 80 seeds were germinated in order to ensure sufficient successful germinations.

For barley seeds, this was done by lining aluminium foil trays with two layers of tissue paper and wetting this with deionized water. The seeds were then placed on top of the paper and the tray covered in aluminium foil to maintain humidity and darkness. Rewetting of the tissue paper occurred daily for 5 days, at which point the seeds had sprouted and were able to be transplanted into pots. Each seedling was sown at a depth of 2 cm in a 2 L pot of each substrate (12 pots of Levington® M3, 24 pots of sand mix) and then covered with substrate and watered. After 7 days, H2Gro® surfactant was added to half of the sand and topsoil mixes at a dose of 60 ml of surfactant per 100 L of substrate. This was done by diluting the surfactant with water to a volume of 2.4 L and thoroughly mixed, at which point, 100 ml of the solution was applied to each of the 12 pots. H2Gro® was the surfactant of choice to ensure a fair trial as it is the same surfactant used in Levington® M3. The same substrates and surfactant treatment were employed in the rye trial.

The sowing rate of the rye seed was 18.5 g per square metre which equated to 0.4255 g of seed per 2 L pot. This was sown evenly across the area of the pot at 1 cm depth. Once germination had occurred, additional seedlings were removed from the pots to leave each pot with 3 plants.

The 72 pots were arranged randomly on the bench in order to ensure that light availability wasn't favouring one treatment over another. During the first two weeks of growth, watering occurred every two days in order to ensure wetting/drying cycles were supporting rhizosheath development. At the end of the second week, watering frequency was increased to daily in order to meet the water requirements of the plants as they grew. An average mass of three pots of each treatment was calculated in order to estimate the water requirement of each plant. Plants were then watered to 90% WHC. In addition to watering, the sand/topsoil mixes were supplemented

with 100 mL of Miracle-Gro® (Evergreen Garden Care (UK) Ltd., Frimley, Surrey) every four days in order to ensure that nutrient stress was not a factor in plant development.

During the early stages of plant growth, which accounted for the first two harvests, two replicates of each treatment were harvested due to the ease of harvesting these small plants and root systems. For the remainder of harvests, a single replicate was harvested for time conservation purposes. All harvests were carried out prior to plants' daily watering. Selection of plants for harvesting was carried out randomly.

2.2.2. Dianthus

The trial involved using dianthus (*dianthus plumaris*) grown in Levington®, peat reduced and sand substrates. Five treatments were used with 16 pots of each treatment. The positive control was Levington® M3. As this is a commercially available substrate with wetting agents added, it was not available with no wetting agents for a direct comparison against the peat reduced mix. Alongside this, the sand mix in a 3:1 ratio of sand to topsoil was used as a peat free control. This mix was used both with and without surfactants applied, at a rate of 60 mL per 100 L of soil. The surfactant applied to this mix was H2Gro® (AmegA Sciences, Daventry, Northamptonshire) using the drenching method. This involved diluting the surfactant to a volume of 200 mL and saturating the pot. The sand mixes, both with and without surfactant, were supplemented with Miracle-Gro® at the recommended dosage of 15 ml of all purpose soluble plant food per 4.5 L of water every four days, incorporated into the irrigation regime. The peat reduced bespoke ornamentals mix of 50% peat and 50% wood fibre was also used. Much like the sand and top soil mix, treatments of this mix were used both with and without the addition of H2Gro® however, the surfactant was incorporated into this substrate during the mixing phase of production, prior to shipment.

Seedlings were sown directly onto the surface of each substrate in seedling trays, with more seedlings than necessary being sown in order to account for failed germination, with the intention to transplant seedlings to two litre pots two weeks after sowing. However, the germination phase took longer than anticipated, therefore transplant occurred after four weeks. During the germination phase daily watering occurred due to the low volume of substrate and therefore the low volume of water retained. This decreased the risk of water stress during the initial germination phase, ensuring the highest possible rate of germination success.

During the germination phase, seedlings were unable to thrive in the sand substrate due to their fragile root systems not being able to penetrate the high-density substrate. It was therefore decided to terminate the trial in the sand mix as it was not considered a viable option for this particular plant species.

In order to determine the Gravimetric Water Holding Capacity (WHC), and therefore the weight to which each substrate needed to be watered too in order to reach 90% of its WHC, two litre pots were filled with each substrate with four replicates of each. This ensured that the same mass of substrate used in the plant trials was used to determine WHC. These pots of substrate were saturated with tap water and allowed to drain until water no longer dripped from the base of the pots. These pots were then weighed in order to determine their saturated wet mass, at which point substrates were transferred into foil trays and placed in an oven to dry for a week at 60 ^{o}C. The substrates were then weighed and the dry mass subtracted from the wet mass in order to determine WHC. 90% of the water holding capacity value was then added to the dry mass value for each substrate to determine the weight to which each plant needed to be watered as shown in Table 8.

Table 8.Gravimetric Water Holding Capacities (WHC) Levington, peat reduced and sand substrates.

Substrate	**Wet mass (g)**	**Dry mass (g)**	**Tray mass (g)**	**Water holding capacity (ml)**	**Mass to water to at 90% WHC**
Sand and topsoil	1539	1349.3	22.7	167	1499.6
Ornamental mix	583	133.9	22.6	426.5	517.75
Levington® M3	660	134.9	22.6	502.5	587.15

Once the mass of the pot at 90% of water holding capacity had been determined, each pot was watered to this mass. Watering then continued every two days at which point, the mass of each pot prior to watering would be recorded to measure evapotranspiration before each pot was rewatered 90% of its WHC. This practise continued throughout the growing cycle.

Following transplant to pots, plants were harvested at two-week intervals, with four replicates of each treatment harvested each time. This enabled measurements to be taken throughout the growing cycle, enabling analysis of the performance of each substrate and treatment at various stages of the plant's life cycle. The destructive measurements taken were fresh and dry aboveground biomass, dry root mass, root length and root area. Alongside this, regular measurements of water loss from each plant were carried out by weighing in order to evaluate each substrates water retention.

2.2.3. Petunia

This trial involved investigating the effects of deficit irrigation and water stress on petunia (*Petunia F1 prism sunshine*). Seven treatments were used. These seven treatments included three

different substrates - Levington®, a peat reduced and Jiffy®. All three of these substrates contained wetting agents to aid in water retention. For each substrate a deficit irrigation regime was implemented on 10 plants, while a further 10 plants were watered to 90% WHC. An additional 10 plants grown in Jiffy® were supplemented with Miracle-Gro® in the same dosage as in previous trials to determine whether nutrient deficiency was a factor in the lower biomass in plants grown in this substrate than in other substrates in previous trials.

For the germination phase, pelleted seeds were used in order to aid visibility when ensuring each chamber of the seedling trays contained a singular plant. In order to reduce water loss, transparent lids were placed on seeding trays. All seeds were sown on the surface of the substrate and germinated in Levington® in order to ensure that seedlings were uniform at the point of transplant into pots and to minimise stress on the plants prior to transplant. Levington® was chosen for germination as it had the highest germination rate off all substrates used in previous trials. During the germination phase, all seedlings were well watered daily to eliminate water stress within the early stages of the plants' life cycles. Once seedlings had reached a height of approximately 4 centimetres, seedlings were transplanted as plugs into individual 2 L round pots, as previously described, filled to capacity with each substrate. This occurred approximately three weeks following sowing of the seeds.

There were ten pots of each treatment. Well-watered samples were watered every two days to 90% of the substrates water holding capacity. Those samples under a deficit irrigation regime were watered to 50% of the average water loss of the well-watered samples in the same substrate, again, every two days. The purpose of this was to induce drought stress in the plants and to determine how well each substrate combated this.

In order to measure the effects of the deficit irrigation regime a range of destructive and non-destructive measurements were taken. Substrate moisture as well as evapotranspiration were measured throughout the growing cycle. Whilst stomatal conductance and leaf water potential were measured once the plants had flowered. Alongside this, measurements of leaf chlorophyll and aboveground biomass were taken.

2.2.4. Substrates

In order to determine what proportion of water loss in each substrate was the result of evaporation and what proportion was the result of transpiration, a trial was carried out using only the substrates. Eight pots with a 2 L capacity as described in chapter 2 were filled with each substrate and the water loss through evaporation measured over a time period of 34 hours. The filled pots were saturated to their maximum WHC and allowed to dry for 34 hours under the greenhouse

conditions mentioned in chapter 2. During this time both morning and evening measurements of substrate moisture and pot mass were recorded. Substrate moisture was measured in %vol using a ML3 ThetaProbe (Delta-T, Cambridge, UK) by inserting the probe approximately 10 cm into the substrate surface, ensuring the probe was wiped between measurements to remove any residual moisture from previous samples. The ML3 ThetaProbe was calibrated to the WHC of each substrate and was therefore recalibrated between substrates. Water loss from evaporation was measured by weighing the pots and calculating the change in mass. Measurements were taken at 8000 and 1600 GMT on both days in order to determine how much water was lost during daylight hours and during the night.

Chapter 3

Impacts of surfactant use

3. Impacts of surfactant use

3.1. Techniques

3.1.1. Barley and rye rhizosheath

In order to determine the rhizosheath mass of each plant, the contents of the pot were emptied into a large metal tray. The root system was carefully excavated and gently lifted from the bulk soil. In order to remove any excess substrate from the wider rhizosphere, the root systems were gently shaken until the root systems no longer shed excess substrate. Due to the wet, sticky nature of the substrate, this proved problematic in Levington® as larger pieces of matter, such as wood chips, were entangled within the root system and had to be removed manually. Once excess substrate had been removed, the root systems were placed in aluminium foil trays and rinsed with deionised water inside the tray in order to remove the rhizosheath from the roots. The sand mix was easily removed by this simple rinsing however due to the stickiness of Levington®, initial rinsing was not sufficient to remove all of the substrate. These roots were transferred into a larger foil tray with approximately 10ml of deionised water. The root system was then gently brushed with a paintbrush to remove any further substrate. The substrate removed by brushing was then added to the contents of the tray from the initial rinsing. The mix of deionised water and rhizosheath was then placed in an oven at 80°C until constant mass was achieved with daily weighing, in order to evaporate any water, leaving only the rhizosheath mass. The tray and substrates were then weighed, and the mass of the tray subtracted in order to determine the dry

mass of rhizosheath. For the rye samples, this mass was divided by three to determine an average rhizosheath mass for each of the three plants per pot.

3.1.2. Biomass

As an additional measure, biomass data were collected for each plant. This included both above and below ground biomass. For the purpose of this trial, above ground biomass was defined as any plant mater above the surface of the substrate, as opposed to all plant matter above where the root system started, in order to be consistent with the methods used to determine leaf area. Only the roots were used to determine below ground biomass, in order to best quantify the relationship between rhizosheath and the root system. Once leaf area had been measured and the root systems were clean following rhizosheath removal, samples were placed in paper bags and dried in the oven at 80°C until constant mass (approximately five days). Once dry, samples were removed from the bags and weighed. The rye measurements were once again divided by three in order to determine mean biomass values for each of the plants in a pot.

3.1.3. Leaf area

In order to determine the leaf area, all plants were cut at the point where the plant met the surface of the substrate. The leaves were separated and lain flat with no overlaps, and the LI-COR model 3100 area meter was used to measure leaf area for each plant. For rye, the leaf area of all three plants was measured and the mean calculated.

3.1.4. Roots

Following harvesting the plants, the root systems were delicately separated from the substrate in a process of washing and re-washing in deionised water. The brush was then used on the roots to remove any remaining substrate and ensure the routes were completely cleaned and free of debris. These samples were then stored in a 50/50 mix of ethanol and ionised water in 50 mL tubes and stored in a fridge until root analysis. In order to scan the root systems, roots were placed in a transparent Perspex tray filled with deionised water and delicately separated to ensure that no roots overlapped. These roots were then scanned using an Epsom scanner along with WinRhizo root analysis software in order to determine the length, diameter and area of the root systems and gain and image of the roots in their entirety.

3.2. Results

3.2.1. Barley and rye rhizosheath

The greatest mass of rhizosheath developed in the peat reduced mix with surfactant. This was followed by the bespoke mix without surfactant and finally Levington®. In both rye and barley, there was a distinct spike in rhizosheath mass in plants harvested at 30 days in the mix with surfactant (Figure 1). Although the general trend is that a great day massive rise as she was formed with surfactant, this is very variable.

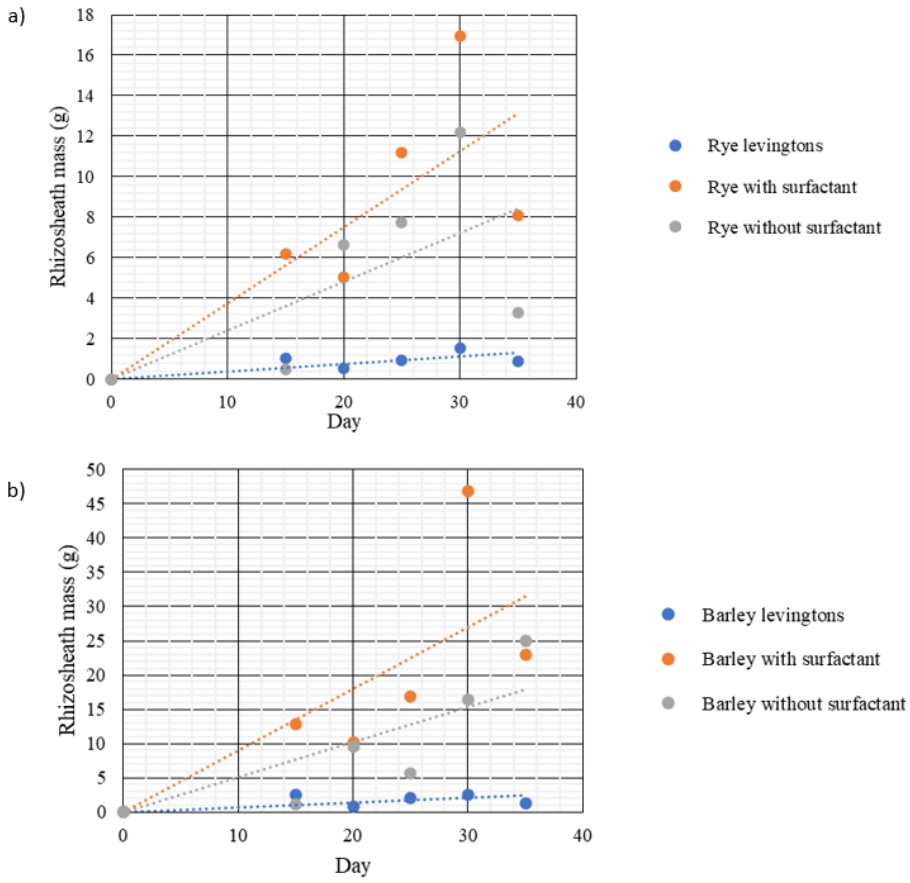

Figure 1. Rye (a) and barley (b) rhizosheath mass over time grown in Levington® and sand mix with and without surfactant.

When analysed a degree of variance of 218.25 and 88.45 in barley with and without surfactant respectively. While a degree of variance of 22.93 and 19.85 in rye with and without surfactant respectively. However, for the majority of harvests, the mix with surfactant did in fact produce a greater mass of rhizosheath than the mix without. When a paired t test was carried out on plants grown in a sand mix with and without surfactant, barley returned a P value of 0.072 while rye returned a P value of 0.03. While the difference in rhizosheath mass with and without surfactant was not deemed statistically significant in barley plants, as the P value for right was lower than

0.05 there is a statistically significant difference in rhizosheath mass in rye plants grown in sand with and without surfactant.

3.2.2. Barley and rye leaf area

When leaf area was investigated it was found that plants grown in Levington® had the greatest leaf area. The addition of surfactant to the sand and top soil mix produced a greater leaf area than without surfactant. However, plants grown in Levington® had a weaker correlation, with and R^2 value of 0.871 in barley, compared to 0.917 for barley grown in the sand and top soil mix both with and without surfactant (Figure 2). There was a similar trend in rye plants (Figure 3), with plants grown in Levington® producing a greater leaf area, followed by the sand and top soil mix with surfactant and finally the sand and top soil mix without. As far as variability is concerned Levington® produced the greatest degree of variability in rye plants, much like barley. However, the difference in variability between Levington® and the mixes of sand and top soil was less significant in rye plants. A paired T test carried out on both rye and barley plants grown in the sand mix with and without surfactant revealed that in both barley and rye, the difference in leaf area with and without surfactant was statistically significant.

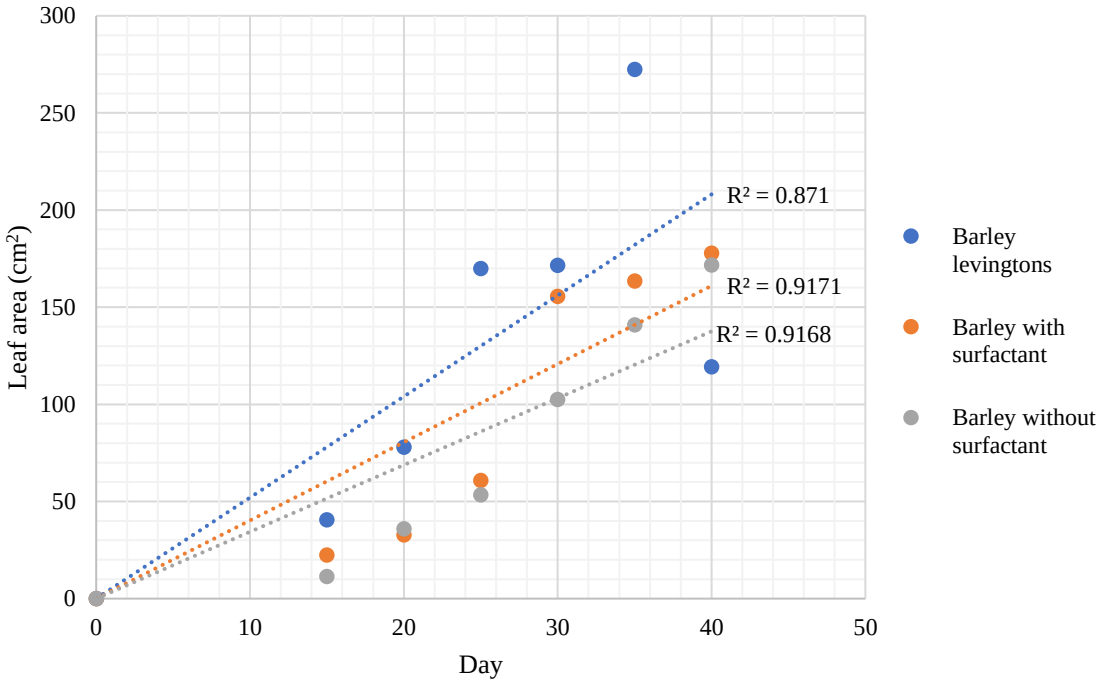

Figure 2. Leaf area in barley plants grown in Levington® and a sand mix with and without surfactant.

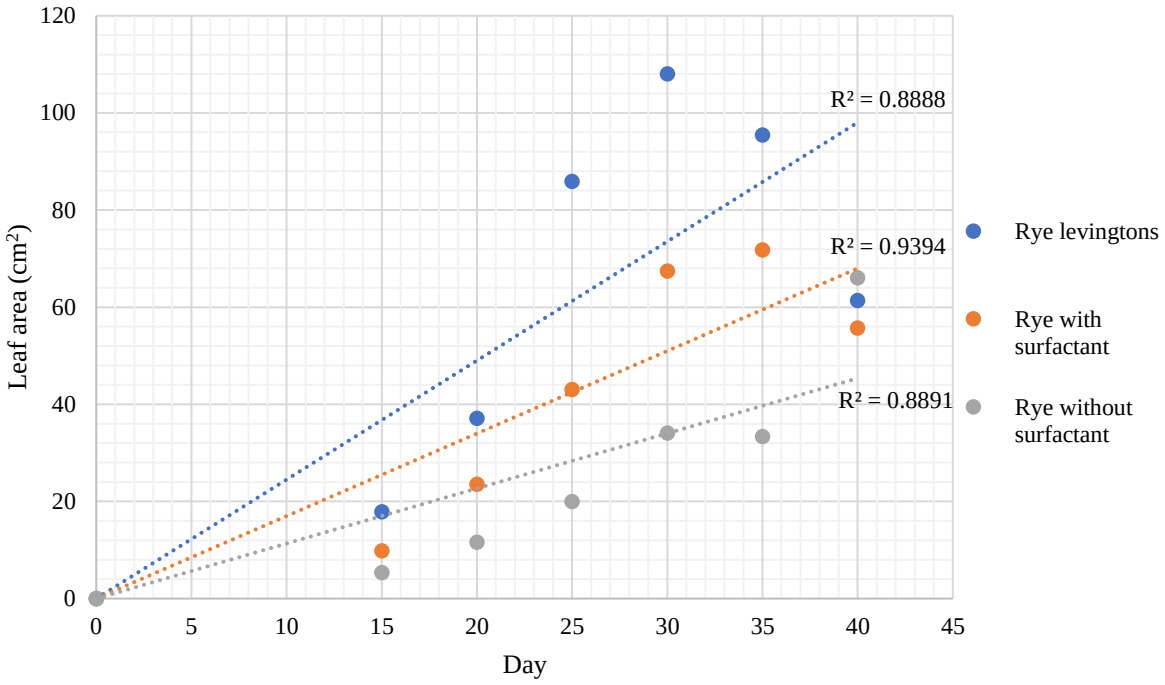

Figure 3. Leaf area in rye plants grown in Levington® and a sand mix with and without surfactant.

3.2.3. Barley and rye biomass

In all treatments, there was an increase in dry biomass over time (Figures 4 and 5).

Plants grown in sand with surfactant had a higher biomass than plants grown without surfactant. But in almost all harvests the biomass with surfactant was greater than without surfactant. However, at 25 days following transplant to the pots, visual observations suggested that plants grown without surfactant were beginning to experience stress. Leaves were wilting and fading but this was not reflected in dry biomass measurements.

When a paired T test was carried out assuming equal variances, a P value of 0.344 was returned for barley with and without surfactant and a P value of 0.134 was returned for rye with and without surfactant. For this test, an alpha value of 0.05 was used. As these P values are both greater than 0.05, the difference in dry above ground biomass in both barley and rye with and without surfactant was deemed to be not statistically significant.

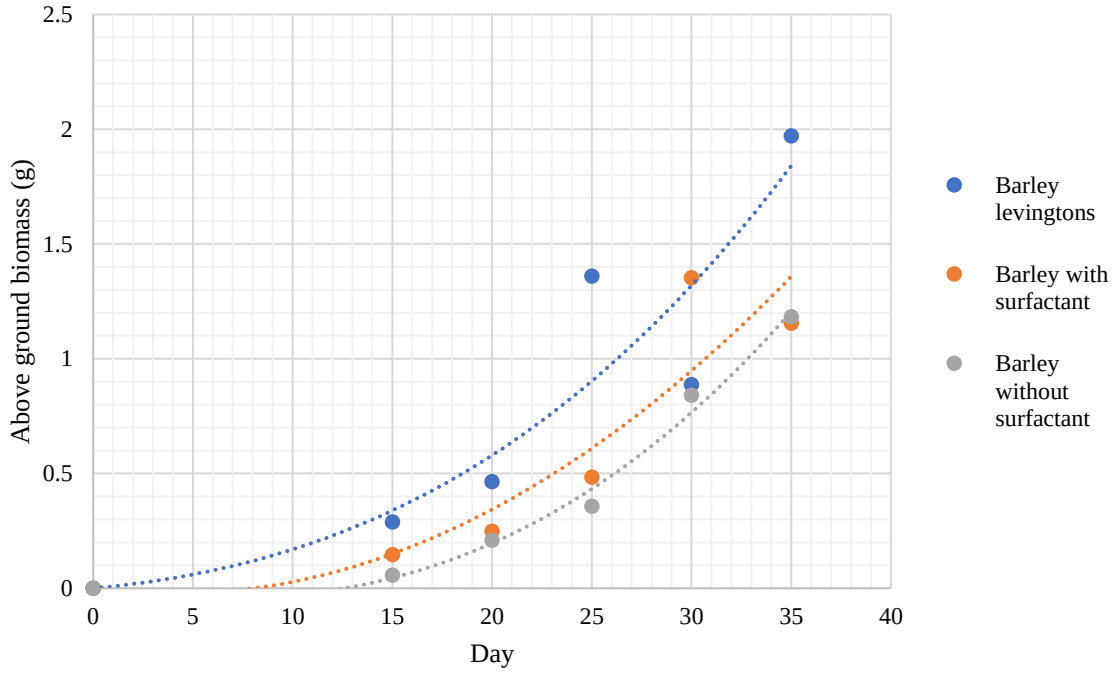

Figure 4. Dry above ground biomass in barley plants grown in Levington® and a sand mix with and without surfactant.

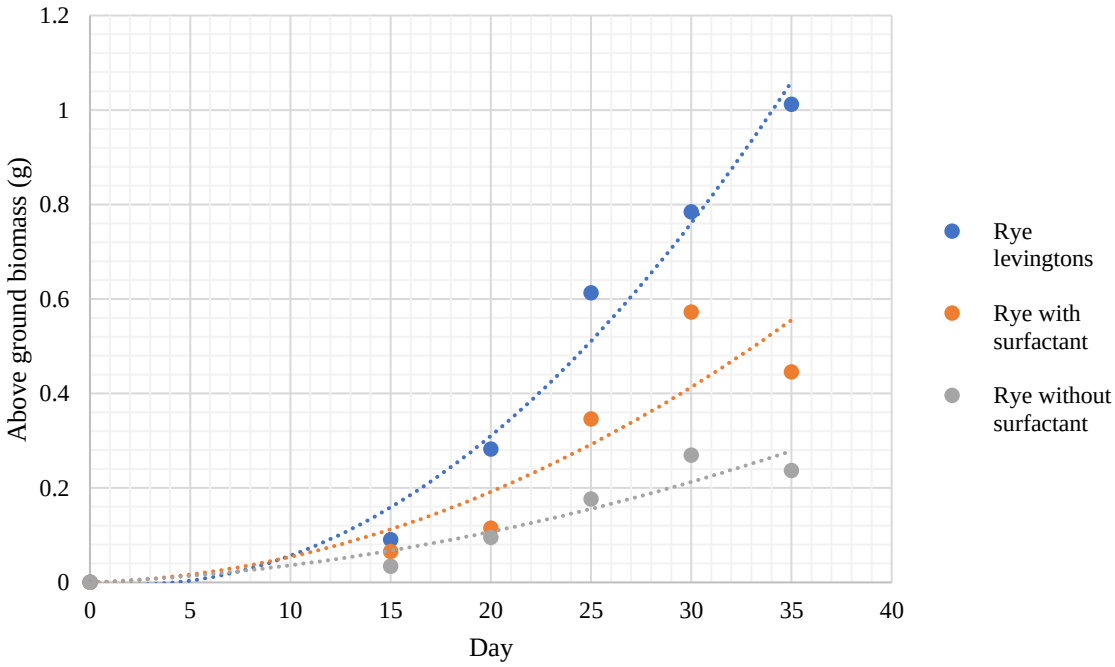

Figure 5. Above ground dry biomass in plants grown in Levington® and a sand mix with and without surfactant.

3.2.4. Dianthus biomass

When dry above ground biomass was measured in dianthus, it was determined that plants grown in the mix with surfactant had a lower biomass than those grown in the same mix without surfactant. Plants grown in Levington® hard a higher biomass than those grown in the peat

reduced mix. The mean mass of dry above ground biomass was 0.53 g in Levington®, 0.27 g in the peat reduced mix with surfactant and 0.40 g in the mix without surfactant. There was, however a much greater range within those grown in Levington® than in the peat reduced mixes. There was a range of 0.49 g in Levington®, 0.18 g in the mix with surfactant and 0.26 g in the mix without surfactant. This is supported by the values for variance within all mixes, with Levington® having a variance of 0.05, the mix with surfactant a variance of 0.007 and the mix without surfactant a variance of 0.015. When a one way ANOVA test was carried out on the above ground dry biomass of all three substrates, a P value of 0.117 was returned suggesting that although there were differences between the masses of biomass when grown in each substrate, this difference is not statistically significant.

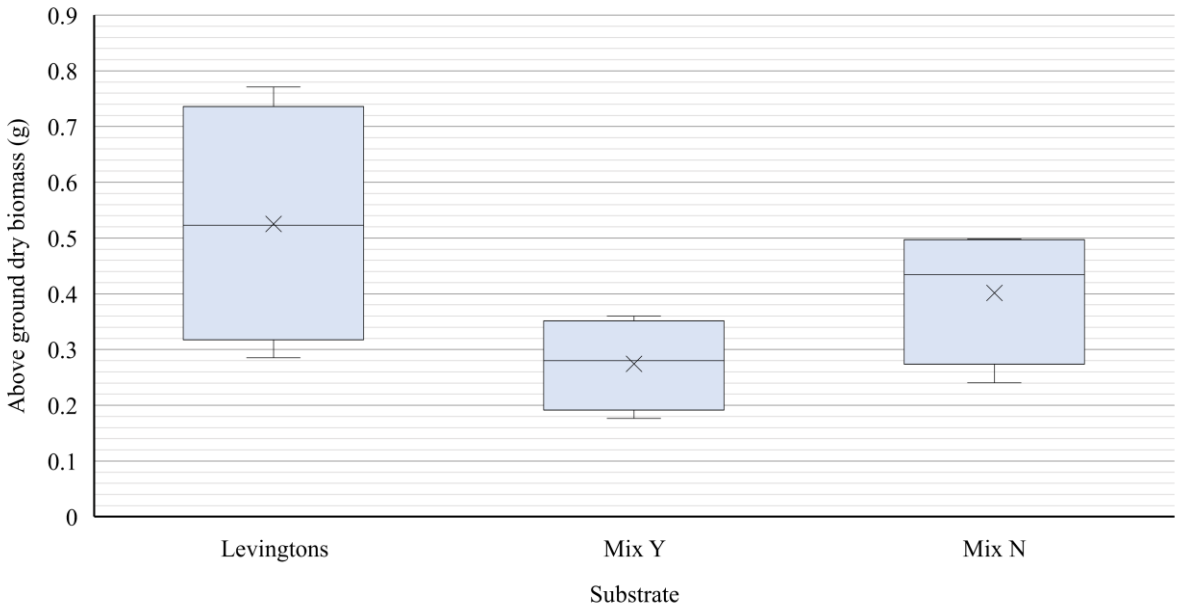

Figure 6. Above ground dry biomasses of dianthus plants grown in Levington®, peat reduced mix with surfactant (Mix Y) and peat reduced mix without surfactant (Mix N).

3.2.5. Dianthus evapotranspiration

When water use was measured in the dianthus trial, it was found that the peat reduced mix performed well. There were no large disparities between evapotranspiration of plants in the peat reduced mix with or without surfactant. There was a value of 127.2 ml of water loss returned for one of the Levington® samples but after statistical analysis this was determined to be an outlier and was therefore excluded from further statistical analysis. For measurements recorded at approximately ¾ of the way through the trial, it was determined that the maximum water loss for plants grown in Levington® M3 was 105.8 ml, the peat reduced mix with surfactant 127.2 ml and the mix without surfactant 124.6 ml. Additionally, the minimum values for water loss at this point

in the trial in Levington® was 87.5 ml, in the mix with surfactant 89.6 ml and the mix without surfactant 83.5 ml.

Although the mean values for each mix were similar with Levington® returning a mean of 98.4 ml the mix with surfactant 104.19 and the mix without surfactant 96.81 ml, there was a significantly larger range in the data set for the peat reduced mixes both with and without surfactant, than in the Levington®. The range in Levington® was 18.3 ml, while the mix with surfactant returned a greater range of 37.5 ml and the mix without surfactant producing an even higher range of 41.1 ml. There was also a much higher variance within the peat reduced mixes, with the mix with surfactant giving a variance of 143.7 ml and the mix without surfactant 137.7 ml, compared to 38.4 ml in the Levington®. There was also significantly higher standard error in peat reduced mixes with the mix with surfactant giving a standard error of 3.46, the mix without surfactant, 3.54 while the Levington® produced a standard error of 1.87.

Although there were discrepancies between water loss within the substrates, when a one way ANOVA test was ran, a P value of 0.213 was returned suggesting no statistical significance in this difference.

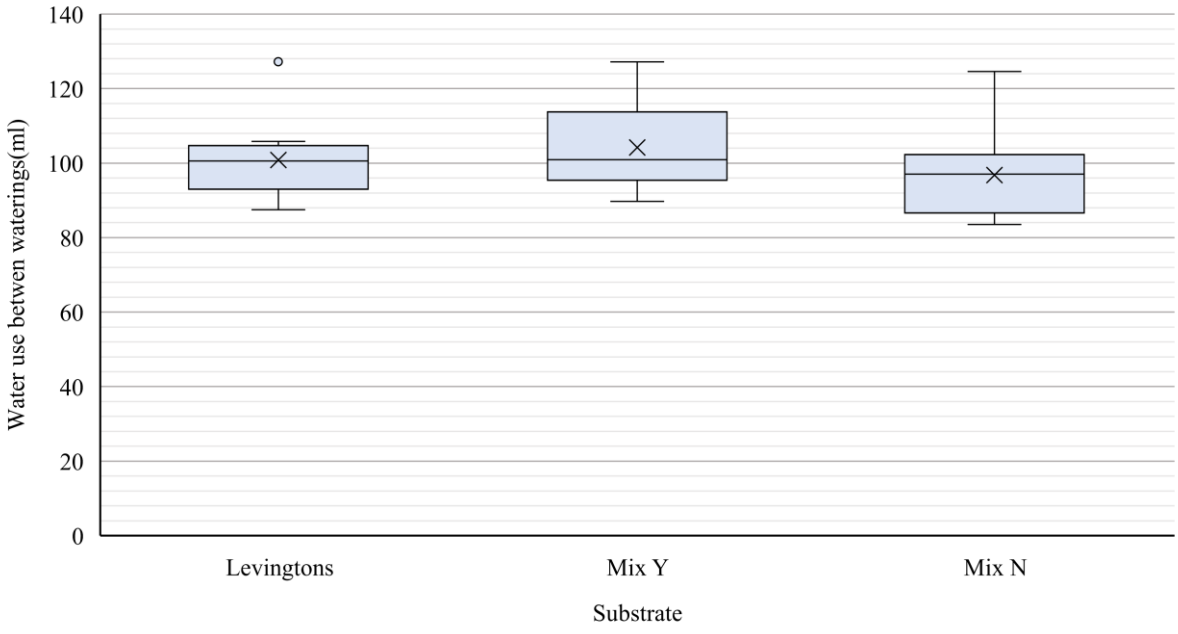

Figure 7. Evapotranspiration of dianthus plants grown in Levington®, peat reduced mix with surfactant (Mix Y) and peat reduced mix without surfactant (Mix N).

3.2.6. Dianthus roots

Roots were analysed using an Epsom scanner and WinRhizo software at weekly intervals during the last 4 weeks of growth. Root length was found to be greatest in plants grown in the peat reduced mix with surfactant, with a mean length of 1104.2 cm, followed by the peat reduced mix

without surfactant, which gave a mean length of 980.15 cm. Finally, plants grown in Levington® M3 produced the shortest mean root length of 878.73 cm. When a one way ANOVA test was ran on root length across plants grown in the three substrates, a P value of 0.5225 was returned, suggesting a statistically significant difference in root length between the substrates. However, when a paired t-test was carried out on the plants grown in the peat reduced mix with and without surfactant, a P value of 0.6612 was returned, suggesting that the difference in root length between the mixes with and without surfactant was more statistically significant than those within all three substrate treatments. The range in root length was fairly uniform between substrates, with Levington® M3 producing a range of 633.57 cm, peat reduced mix with surfactant producing 616.42 cm and the peat reduced mix without surfactant producing a range of 644.83 cm. Both the minimum and maximum values for root length within each substrate follow the same trend as the means, with Levington® M3 producing the shortest roots, followed by the peat reduced mix without surfactant, and finally the peat reduced mix with surfactant producing the longest roots.

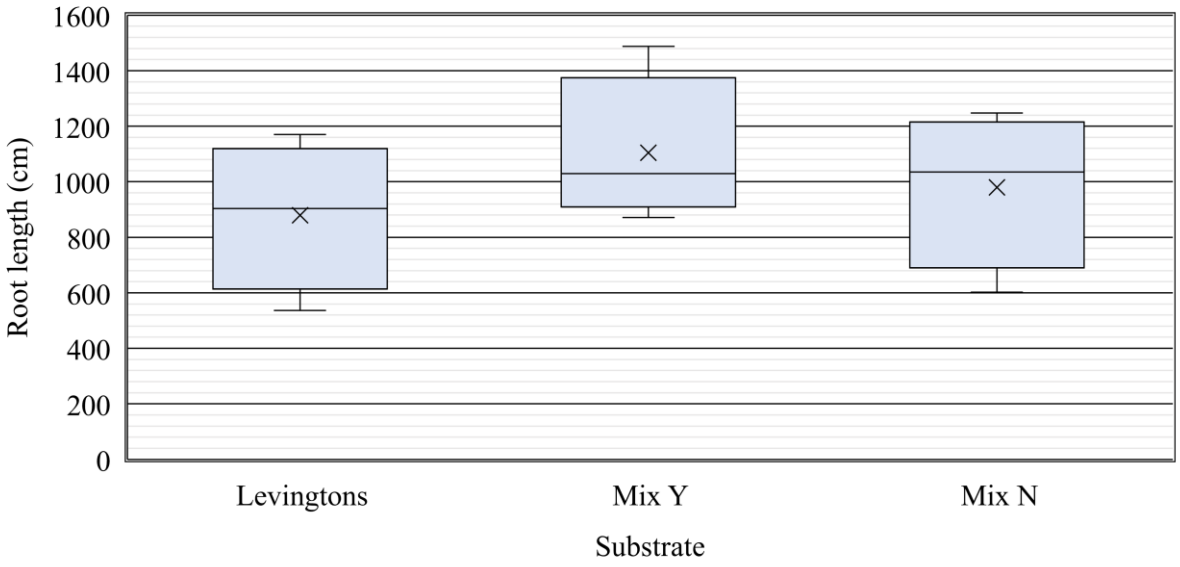

Figure 8. Root lengths of dianthus plants grown in Levington®, peat reduced mix with surfactant (Mix Y) and peat reduced mix without surfactant (Mix N).

3.3. Discussion

3.3.1. Barley and rye rhizosheath

The reason for the greater rhizosheath mass in the mix with surfactant than the mix without surfactant is likely due to the adhesive properties of surfactants. This would result in a greater mass of substrate becoming adhered to the root system whilst also contributing to additional substrate becoming adhered to the substrate which is already attached to the roots. This suggests that although a greater mass of rhizosheath was measured, not all of this substrate is necessarily rhizosheath. In order to determine what proportion of this substrate is in fact rhizosheath, detailed

analysis of water and nutrient content of the substrate adhered to the roots would be necessary. This would allow the moisture content of the substrate to be measured while still adhered to the roots in order to determine what proportion of this substrate had a higher moisture content and was therefore able to be classed as rhizosheath. However, the scope of this project did not allow for such measurements to be made, therefore further investigation would be necessary. There was a significantly greater mass of rhizosheath in the sand and topsoil mix than in Levington®. This is likely, in part at least, a result of Levington® being a lighter substrate when dried than the mix. Therefore, when comparing rhizosheath between Levington® mix and the sand mix, rhizosheath mass is not an accurate parameter. It is therefore recommended that volumetric measurements be taken under the same conditions in order to gain a clearer understanding. Furthermore, due to the nonuniform properties of organic growing substrates in relation to their particle shape and size, large clods of the Levington® became entangled in the root systems making it difficult to determine what proportion was actually adhered to the roots and what proportion was simply entangled. It was determined that rhizosheath formation in organic substrates could not be measured accurately at this point with current techniques and rhizosheath measurements in further trials with organic substrates would not be taken. The extensive research into rhizosheath formation in inorganic substrates suggests that although rhizosheath formation appears to increase with the addition off surfactants, these results could not be accurately replicated in organic substrates. Due to the physical manipulation off the potted mixes when harvesting the rhizosheath, it is also difficult to determine if any of the substrate had been removed during harvesting prior to root rinsing. This could account for the variability in rhizosheath mass results and the lack of a clear trend even within substrates. Since many of the root systems grew around the edges and base of the pots, rhizosheath formation that was hindered in these roots and therefore rhizosheath measurements were not consistent throughout the root system, with roots towards the centre of the pot – which were not limited by being pressed up against the side of the pot – appeared to form a greater mass of rhizosheath.

3.3.2. Barley and rye biomass

Although the addition of surfactant to the sand mix did result in an increase in biomass, this increase was not significant enough to merit the additional cost of adding surfactant from a commercial perspective. The increase in biomass was likely the result of more water being retained within the substrate which is not necessarily available for uptake by the plant. Therefore, the plant is required to extend its root system, as supported by the increased root mass in the mix with surfactant. This results in a greater root area, enabling higher nutrient uptake by the plant. It is therefore assumed that although adding surfactant does increase plant biomass, this is not

necessarily due to more water being available to the plant and it's more likely a result of the plant having to extend its root system. In potted plants with limited substrate, this could prove problematic in the later stages of the plants development as the lower capacity of the pots means that plants are only able to extend their root systems a limited amount. This could then result in additional strain on the plant and subsequently have a negative impact on the later stages of the plants life cycle. This is particularly true in smaller pots as they are more likely to suffer from the container effect, whereby the root system forms a matt at the base of the pot, resulting in greater competition for oxygen and nutrients and ultimately, the death of roots. Conversely, the addition of surfactant could prove beneficial in bedding environments as there are less limitations on the plants ability to extend its root system. The addition of surfactants could encourage greater root growth in bedded plants, resulting in greater nutrient uptake, as well as a greater anchoring and stability for security against weather conditions in an outdoor environment. As far as water consumption is concerned, the exclusion of surfactants could potentially save water in both potted plants and those grown in a bedding setting as the addition of surfactants resulted in an increase in water loss and plants grown with surfactant required more irrigation than those without in order to reach the same water holding capacity.

3.3.3. Dianthus biomass

It is clear from the results that the addition of surfactant with dianthus plants does increase biomass. This is similar to the result obtained from barley and rye in the sand mix. This is further evidence that surfactant does increase biomass by a small proportion but again, this is likely due to the extended root system. This is supported by the data on root length in dianthus plants grown in the peat reduced mix with and without surfactant. The increased root length enables greater nutrient uptake and prevents limiting the root system to taking up nutrients from a limited volume of substrate within the pot which would become depleted over time. In order to confirm this theory further work would be necessary to investigate the nutrient concentrations at the end of the growing cycle in the substrates both with and without surfactant. It is theorised that nutrient concentrations in the rhizosphere of the plants grown with surfactant would be greater than the nutrient concentrations in the rhizosphere of plants grown without. This is because the plants with longer root systems in the mix with surfactant up taking up nutrients from a greater proportion of the substrate and therefore not depleting a small proportion of substrate of its nutrient supply, as in the mix is without surfactant.

3.3.4. Dianthus evapotranspiration

The results suggest that evapotranspiration rates were much the same between mixes with and without surfactant. This is likely due to plants taking up water from a greater proportion of the substrate in the mix with surfactant due to the limited volume of water readily available for uptake. Therefore, while the same volume of water is taken up by the plants both with and without surfactant, it is likely that the volume of water taken up per mm^2 of root area is greater in the mix without surfactant.

3.3.5. Dianthus root growth

Since the most significant root growth was found in the sand mix with surfactant, this suggests that although a larger volume of water was retained in the mix with surfactant, a lower proportion of this water was readily available for uptake by the plant root system. This triggers a response in the plant whereby it extends its root system further in order to seek more readily available water sources. It was also found that plant roots growth occurred mostly around the edges and base of the pot as referenced in Chapter 1.

Chapter 4

Substrates

4. Performance of plants in different substrates

4.1. Methodology

4.1.1. Barley and rye

A comparison between substrate performance was carried out with barley, rye, dianthus and petunia. For barley and rye, the measurements analysed were dry biomass, leaf area and rhizosheath mass in plants grown in Levington® and sand substrates – both with surfactant to ensure that it was a fair comparison as Levington® is produced with surfactant already incorporated. Measurements were taking at harvests throughout the growing cycle. These measurements were taken under the experimental conditions from the Barley and Rye trial described in Chapter 2, using the same measurement techniques outlined in Chapter 3.

4.1.2. Dianthus

The purpose of the trial using dianthus was to carry out a basic initial comparison the performance of ICL's reduced peat mix to that of the Levington® substrate in an ornamental horticultural context. The dry biomass of dianthus plants grown in Levington® and peat reduced mixes, both with surfactant for the same reason mentioned above. The technique used for dry biomass measurements is described in chapter 3. These measurements were taken at harvests throughout the growing cycle, with four replicates harvested each time.

4.1.3. Petunia

In order to gain an understanding of the performance of a wider range of peat contents in substrates, the peat free mix by Jiffy® was incorporated into the trial with petunias. Ten plants were grown in each of the three substrates used in the trial, with surfactant incorporated into all of them. Each of these 30 plants were watered to 90% WHC every two days with tap water. In order to evaluate performance, leaf chlorophyl concentrations were measured during the growing cycle and the dry biomass measured at harvest.

Biomass was measured in the same manner as in previous trials.

In order to determine leaf chlorophyll concentrations, the Apogee MC-100 chlorophyl concentration meter was used. The chlorophyll meter was calibrated to measure in units of µmol of chlorophyll per m^{-2} of leaf surface area, as opposed to in CCI or SPAD as in other meters, to a degree of reliability of ± 1%. The metre works by emitting light from its LED within the red and near infrared ranges (653 nm and 931 nm respectively). The ratio of the two wavelengths is then analysed to determine the chlorophyl concentration index (CCI) as shown in the following equation, where *%T at 931 nm* is the percentage of transmittance due to chlorophyll and *%T at 653 nm* is the percentage of transmittance due to leaf thickness.

$$CCI = \frac{\%\ T\ at\ 931\ nm}{\%\ T\ at\ 653\ nm}$$

This index is then converted to actual chlorophyll concentration using a series of generic equations (Utah State university). The metre was calibrated to the pre-programmed generic equation (made up of averages from the 22 pre-programmed species-specific equations) due to the absence of a petunia-specific setting. The metre measurements were taken by applying the 63.9mm^2 chamber to each plants largest leaf, measuring 3 replicates and determining the mean.

4.2. Results

4.2.1. Barley and rye biomass

Dry biomass of Barley plants grown in both Levington® and sand with surfactant increased over time. There was a greater increase in dry biomass in plants grown in Levington® than in the sand mix with all harvest's bar 1 producing a higher biomass in Levington®. The same trend applies to Rye plants grown in these substrates. However, the increase in biomass for one grown in Levington® is greater than the increase in Barley grown in Levington®. Unlike the results of the comparison between the sand mix with and without surfactant, plants grown in Levington® did not show a peak in dry biomass at 30 days.

When a paired T test was carried out assuming equal variances on the dry above ground biomass, the Barley and Rye plants grown in Levington® and sand, Barley plants resulted in a P value of

0.2503 while Rye had a P value of 0.1509. As both P values are greater than 0.5, the null hypothesis that there is no statistically significant difference between dry above ground biomass in plants grown in Levington® and plants grown in a sand mix is accepted.

Similar to the results from dry above ground biomass, in both Barley and Rye a greater aboveground biomass was produced in plants grown in Levington® than in plants grown in the sand mix. In comparison to dry aboveground biomass results, the correlation coefficient of plants grown in Levington® was significantly lower than the correlation of above ground biomass in Levington®.

When a paired T test assuming equal variances was carried out on leaf area, Barley grown in Levington® and the sand mix returned a P value of 0.2315 while Rye grown in the same substrates returned a P value of 0.1641. As both P values are greater than 0.05, the null hypothesis that there is still a statistically significant difference in leaf area between plants grown in Levington® and a sand mix should be accepted.

4.2.2. Dianthus biomass

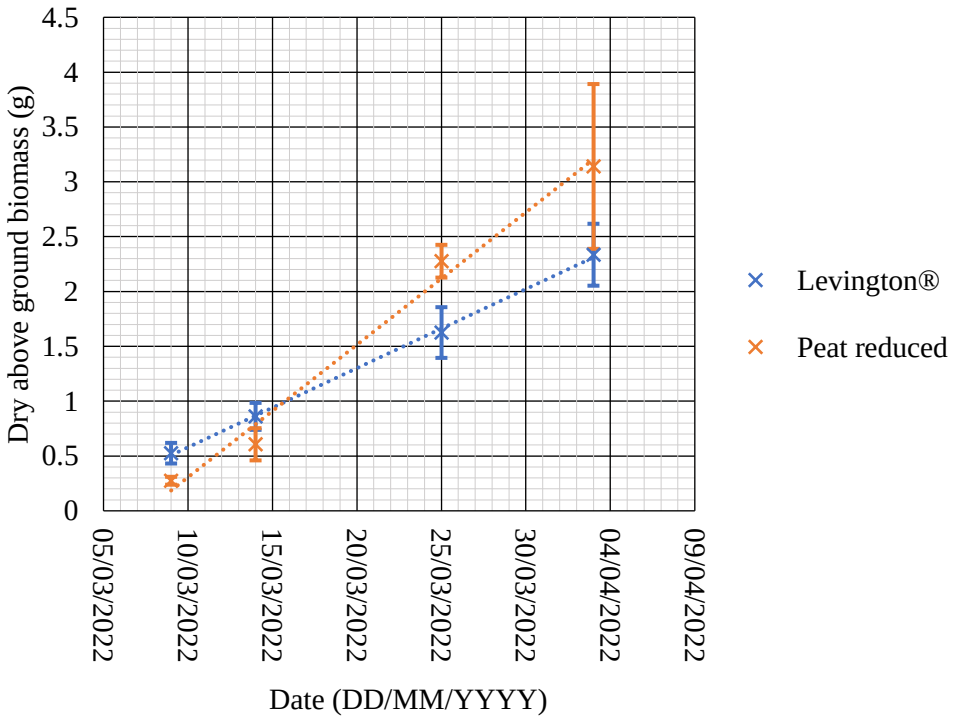

Figure 9. Dianthus dry above ground biomass grown in Levington® and peat reduced mixes.

Dry above ground biomass increased over time in both Levington and the peat reduced substrate (Figure 9). Although towards the beginning of the plants' life cycle, biomass was greater in Levington®. At the final harvest, the peat reduced substrate had a greater biomass. Although the

mean dry biomass from each harvest has an almost perfect correlation coefficient, the correlation coefficient for the whole data set of plants grown in Levington® was 0.8696 while the correlation coefficient for all plants grown in the peat reduced substrate was 0.9105, suggesting that overall, although from the mean values Levington® produced a more prominent trend from the whole data set the peat reduced substrate resulted in a stronger correlation. When a paired T test was carried out on plants grown in Levington® and a peat reduced substrate, with the data set analysed in their entirety, a P value of 0.3473 was produced, suggesting no statistically significant difference in above ground biomass of plants grown in Levington® and the peat reduced substrate.

4.2.3. Petunia biomass

When petunia was grown in Levington®, peat reduced and Jiffy® substrates, there was little difference in dry above ground biomass between the substrates as shown in Figure 10. These initial observations were confirmed when a one way ANOVA test was run on the data. A P value of 0.523 was returned confirming that there is no statistically significant difference in aboveground biomass between petunia plants grown in Levington®, peat reduced and Jiffy® substrates.

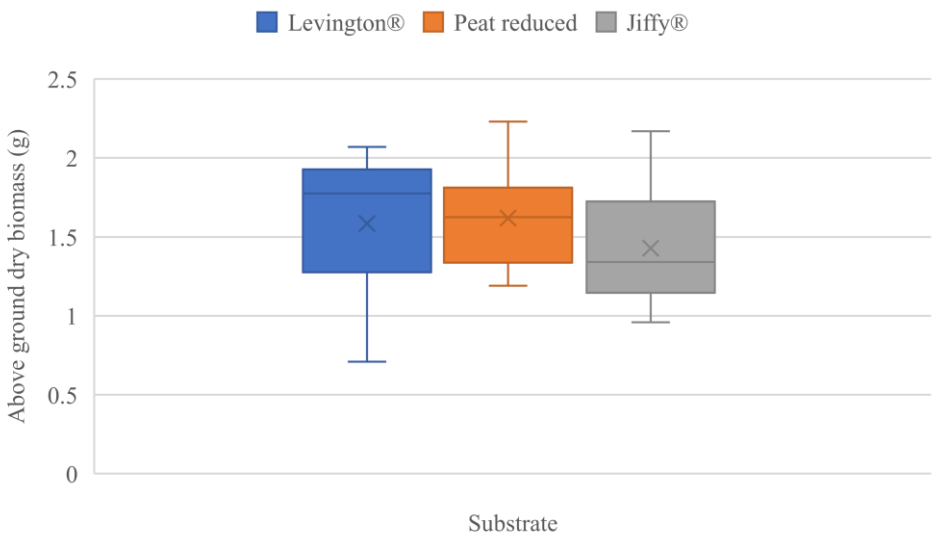

Figure 10. Petunia above ground biomass grown in Levington®, peat reduced and Jiffy® mixes.

4.2.4. Petunia chlorophyl

Mean chlorophyll concentrations were very similar between plants grown in each substrate, with Levington® producing a mean chlorophyll concentration of 250.55 µmol m^{-2}, peat reduced substrate producing a mean chlorophyll concentration of 251.87 µmol m^{-2} and Jiffy® producing a mean chlorophyll concentration of 243.69 µmol m^{-2}. However, there was a much greater range

in peat reduced substrate than in the other two substrates. The degree of variance was greatest in peat reduced substrate with a variance of 1498.289, followed by Jiffy® with a variance of 535.211 with Levington® producing the lowest variance of 301.7983. When a single factor ANOVA test was carried out on the data, a P value of 0.782 was returned. As this P value is greater than 0.05, the difference between concentrations in petunia plants grown in Levington® s, peat free and Pete reduced substrate is not deemed statistically significant.

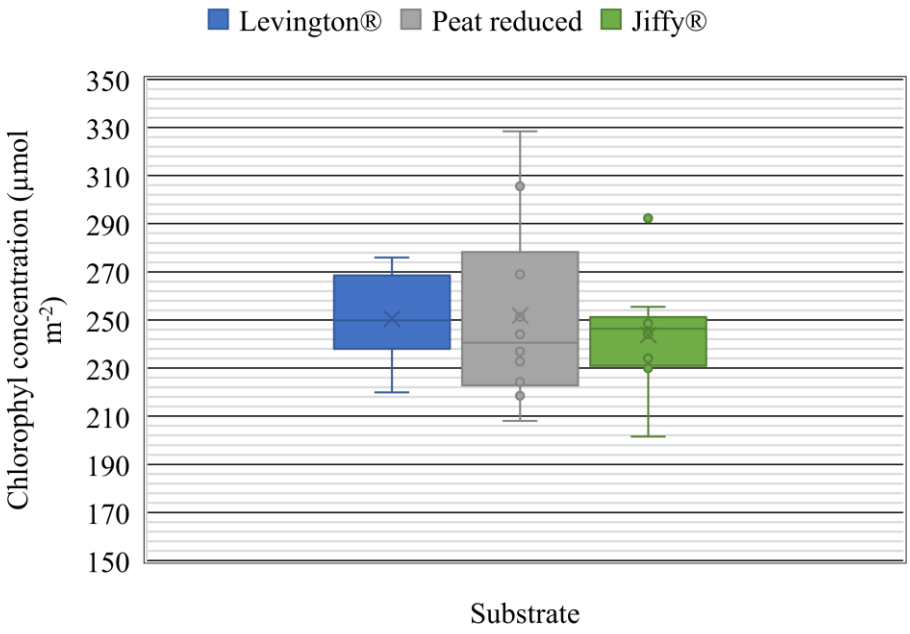

Figure 11. Chlorophyl concentration in the leaves of petunia plants grown in Levington® s, peat reduced and Jiffy® substrates.

4.3. Discussion

4.3.1. Biomass

In Barley and Rye, Levington® produced the greatest mass of dry biomass and leaf area. This is likely due to Levington® superior water retention ability compared to the peat reduced and Jiffy® substrates. This is supported by the rapid transportation measurements. It's also likely that Levington® has a greater nutrient availability than other substrates. The greater water retention and nature and availability reduces stress on the plant, which in other substrates inhibits plant development and therefore results in a smaller, above ground, biomass. The increased biomass in Levington® compared to the sand mix is probably dependant on the density of the sand mix inhibiting root development. This was indicated in the germination stage of the Dianthus trial whereby Dianthus plants, germinated in the sand mix, did not survive as the root systems were too delicate to penetrate the high density substrate. Since root growth occurs predominantly in micropores within the substrate insert reference, it is likely that in the sand mix, the limited

volume of micropores was the cause of inhibited root growth and therefore inhibited above ground plant development.

Unlike the results in Barley and Rye, Levington® did not produce plants with the greatest biomass. Overall biomass was greater in the peat reduced substrate than in Levington®, particularly in the later stages of the plants' development. It is likely that the lower density of the peat reduced mix, compared to both Levington® and the sand mix, enabled greater root growth in the micropores and therefore greater above-ground biomass.

There is very little difference in above ground dried biomass between plants grown in Levington® and the peat reduced substrate in the Petunia trial. As this trial was carried out at a later date than the Barley, Rye and the Dianthus trial, from the same bag of substrate, it is possible that over time the substrate became slightly more compacted. This would reduce the micropore space in which root growth could occur and could therefore be a possible explanation for the decrease in the difference of above ground biomass between plants grown in Levington® and the peat reduced mix from the Dianthus trial to the Petunia trial. The Jiffy® substrate produced the slightly lower biomass than both the Levington® and the peat reduced substrates. This aligns with the findings of Di Lonardo *et al*. (2021) in which a peat based control produced a mean dry biomass of 8.7 g pt-1 and a similarly composed mix of coir and wood fibre produced a mean mass of 7.4 g pt-1 when *L. vulgare Lam. Cv. 'Filigram'* were grown in the substrates. This is likely due to poor nutrient availability in the Jiffy® substrate, causing nutrient stress on specimens grown in this substrate.

In order to further investigate this, future work should incorporate substrate density and micropore volume into the research. This should be carried out throughout the plants life cycle in order to determine whether root length grows at a steady rate throughout the life cycle in each substrate.

Chapter 5

Plant water relations

5. Plant water relations

5.1. Methodology

5.1.1. Petunia leaf water potential

In order to determine leaf water potential 8 replicates of each treatment were collected from the largest leaf of the plant. Measurements were taken over a period of two days, with two samples of each treatment collected for each batch of measurements. One sample in the morning and one in the afternoon on both days resulting in measurements of each treatment being taken at four different times. This was due to the limited number of C-52 chambers available and also to allow for comparison between leaf water potential in the mornings and the afternoons. A small disc of the oldest leaf of each plant was collected using a cork borer using gloves and clean tweezers to prevent contamination of the samples. These leaf discs were then placed in individual sample holders and immediately wrapped in aluminium foil to prevent drying and water loss. Once samples had been collected, the sample holders were placed in to C-52 chambers and the chambers sealed. Samples were then left for three hours to calibrate. The chambers were then connected to a Wescor HR33T microvoltmeter and the voltage measured using the dew point method (see Wescor). The voltmeter was calibrated to zero for each chamber. Samples were then cooled to reach their dew point at which point the samples were allowed to reach equilibrium and the voltage recorded. Voltage values were then converted to leaf water potential values.

5.1.2. Stomatal conductance

Stomatal conductance was measured using an AP4 porometer by Delta T devices. The device works by measuring the rate of humidification in the chamber against readings from the calibration plate. The device is calibrated to six different diffusion settings by placing moist filter paper on the calibration plate and taking readings at the six different placements on the plate. The device times how long it takes the relative humidity within the chamber to rise by 2.3%, with leaf stomatal conductance then calculated automatically. Multiple readings are taken during cycles of humidification and desiccation until a value is produced when conditions within the chamber have stabilised. Dry air is blown into the chamber to lower the relative humidity 5% below ambient relative humidity and the time taken for relative humidity to increase by 2.3% of the original value is measured. This cycle is repeated until stabilisation. Based on previous studies, it was determined that the optimum time of day for taking measurements was as close to midday as possible and only on days when atmospheric weather conditions would not cause the stomata to close, therefore measurements were only taken on overcast days. These measurements were taken on the largest leaf that was not shaded by other leaves, in order to avoid the shading effect.

5.2. Results

5.2.1. Leaf water potential

When analysing leaf water potential in petunias in relation to deficit irrigation, it was determined that in Levington® and peat reduced mixes, leaf water potential was higher overall in well watered samples than in those under the deficit irrigation regime (Figure 11). The exception to this trend is the Jiffy® substrate in which plants which underwent deficit irrigation had a higher leaf water potential than those that were well watered. There was a smaller degree of variance in leaf water potential values in plants grown under a well watering irrigation regime in comparison to those under deficit irrigation. The greatest range in values was produced by the Jiffy® mix which underwent a well watered regime in addition to supplementation with Miracle-Gro®.

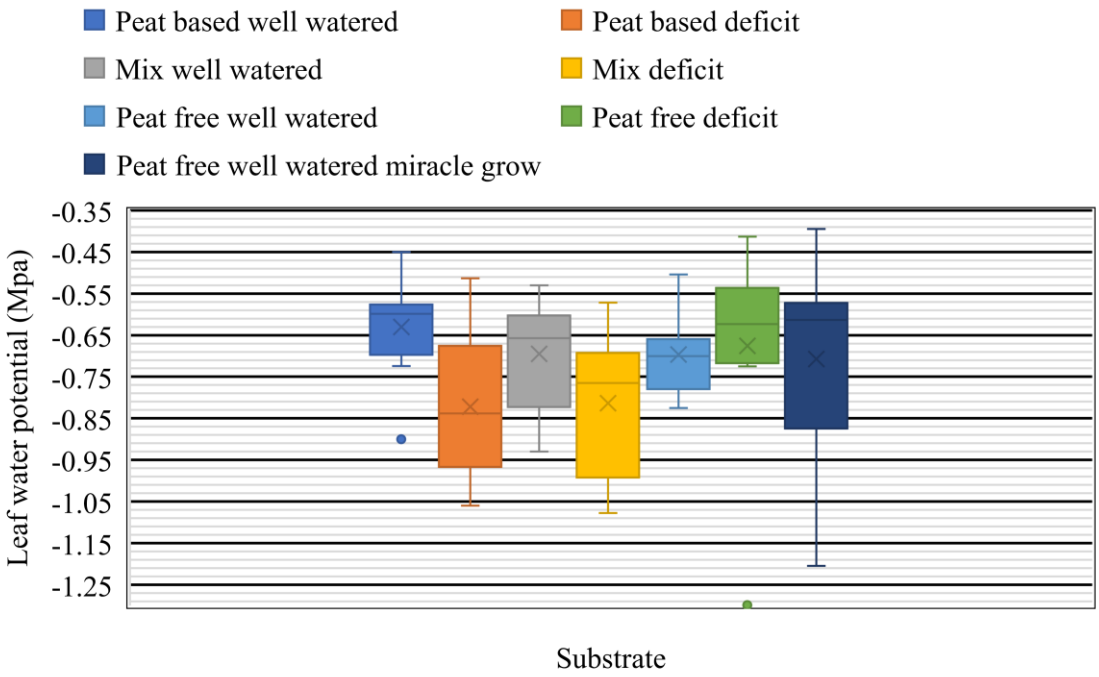

Figure 12. Leaf water potential of petunia plants grown in Levington®, peat reduced and Jiffy® substrates under two irrigation regimes (replacing 100% of lost water and 50% of lost water). These data are based on 8 replicates of each treatment.

An unpaired T test assuming equal variances was carried out on all substrates to analyse the difference between leaf water potential in petunia plants grown under deficit irrigation regimes and the well watered irrigation regime in those substrates. When the test was carried out on plants grown in Levington®, a P value of 0.026 was returned, indicating that there is a statistically significant difference in leaf water potential of petunia plants grown under the two irrigation regimes, with the well watered specimens having a higher leaf water potential. However, in both repeat reduced mix and Jiffy® substrate, a P value greater than 0.05 was returned. It was therefore deemed that the difference in leaf water potential of specimens grown in these substrates was not statistically significant.

5.2.2. Stomatal conductance

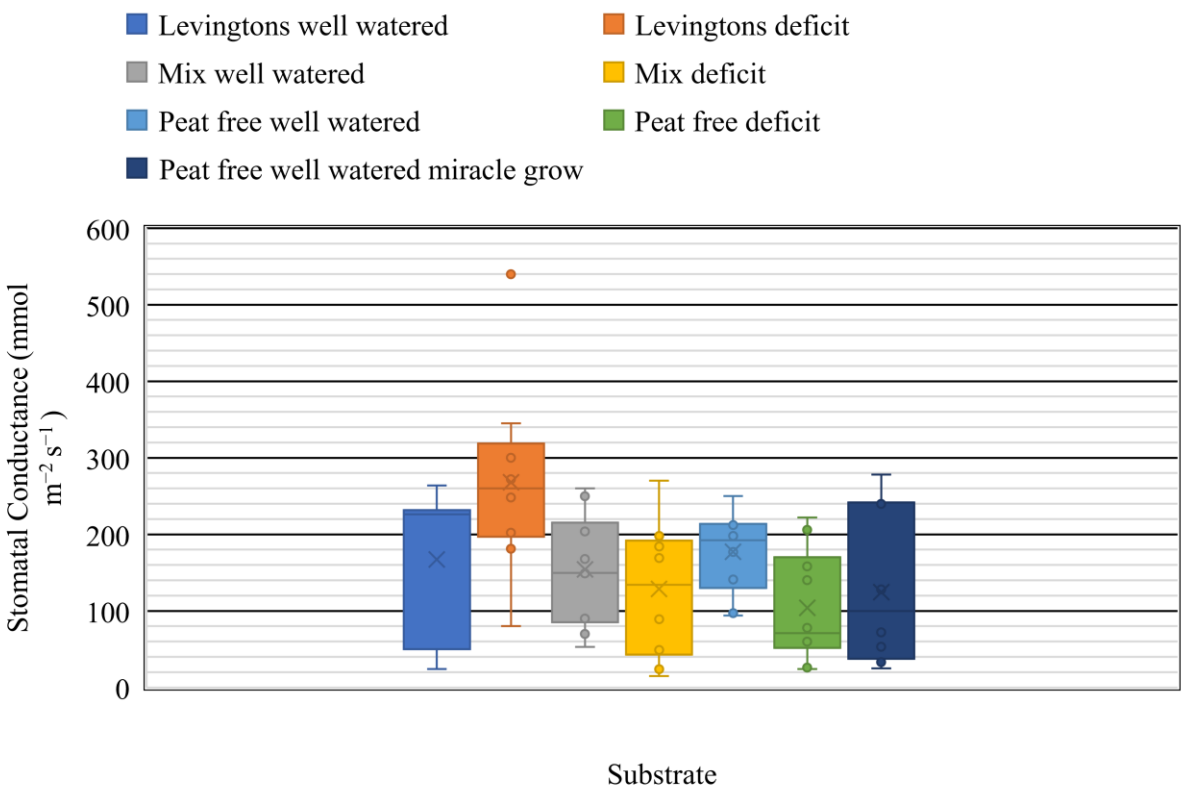

Figure 13. Stomatal conductance of petunia plants grown in Levington®, peat reduced and Jiffy® substrates under two irrigation regimes.

In Jiffy® and the peat reduced substrate, stomatal conductance was slightly higher in well watered samples than in those which had undergone a deficit irrigation regime. However, this was not the case in plants grown in Levington®. Plants grown in Levington® under deficit irrigation regime had a mean stomatal conductance of 268.2 mmol m^{-2} s^{-1} while well watered specimens had a means stomatal conductance of 167.2 mmol m^{-2} s^{-1}. In both Jiffy® on the peat reduced substrate there was a greater range in stomatal conductance in plants grown under deficit irrigation treatments. These samples also had a smaller interquartile range than well watered samples. Conversely, the samples grown in Levington® under deficit irrigation conditions had a smaller interquartile range than those well watered specimens grown in Levington®. Well watered specimens in Levington® were also not normally distributed, unlike the majority of other treatments. An unpaired T test assuming equal variances was carried out on all substrates to investigate the impact of the different irrigation regimes on stomatal conductance. In Levington® s a P value of 0.53 was returned, suggesting no statistically significant difference in stomatal conductance under the two irrigation regimes. The same applied to plants grown in the peat reduced mix, whereby a P value of 0.47 was returned. However, in Jiffy®, a P value of 0.018 was returned suggesting that in the Jiffy® substrate, there is a statistically significant difference in stomatal conductance under the two irrigation regimes.

5.2.3. Petunia biomass

Plants grown under a well watering irrigation regime had a greater dry above ground biomass than those grown under a deficit irrigation regime in the same substrate as shown in Figure 14.

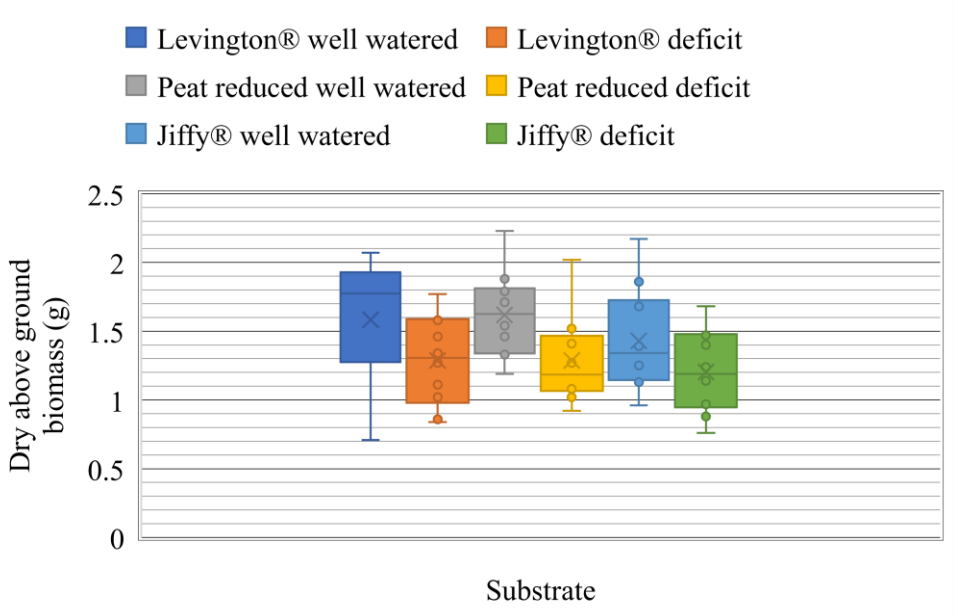

Figure 14. Dry above ground biomass of petunia plants grown in Levington®, peat reduced and Jiffy® substrates under two irrigation regimes.

When a paired T test was carried out between all samples grown under deficit irrigation regimes and all samples which were well watered, they pee value of 0.012 was returned. This suggests that there is a statistically significant difference in dry above ground biomass between samples grown under the two different irrigation regimes. As the P value is lower than 0.05, the null hypothesis that there is no statistically significant difference in above ground dry biomass between plants grown under a deficit irrigation regime and plants grown under a well watered irrigation regime should be rejected. When an unpaired T test was carried out on each of the substrates there was some variance in the results. Plants grown in the peat reduced substrate were the only ones that produced a statistically significant difference in above ground biomass when grown under a deficit irrigation regime and a well watered irrigation regime, with a P value of 0.032 being returned. In contrast, plants grown in Levington® and Jiffy® substrates displayed no statistically significant difference, with Levington® returning a P value of 0.12 and Jiffy® returning a P value of 0.15.

5.2.4. Substrate moisture

Over the 46 hour period, in all substrates pot mass decreased in a linear trend with a very similar rate of decrease in each substrate (Figure 15). The trend line for Levington® had a gradient of -2.68, the trend line for peat reduced had a gradient of -2.16 while the gradient for the Jiffy® trend line was -2.38. This suggests that Levington® was losing water at a faster rate than the other two substrates by evaporation.

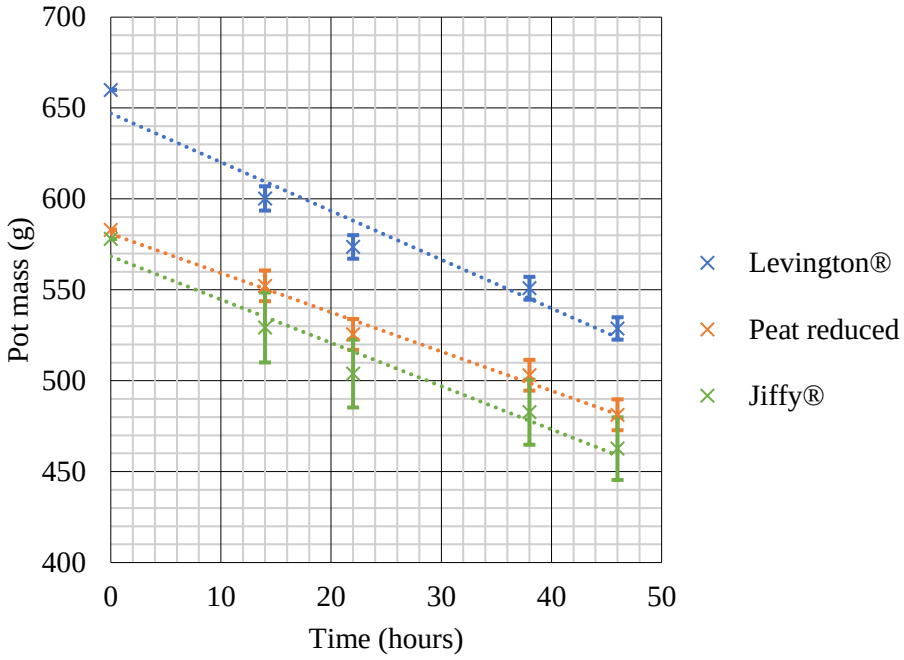

Figure 15. Pot mass due to evaporation in Levington®, peat reduced and Jiffy® substrates.

Similar to pot mass, substrate moisture decreased in a linear fashion in all substrates (Figure 16). However, while Jiffy® had the slowest rate of water loss in terms of pot mass, it had the fastest decline in substrate moisture of all substrates, with the trend line for Jiffy® having a gradient of -0.29. There is a strong correlation between pot mass and substrate moisture, with little variation between substrates.

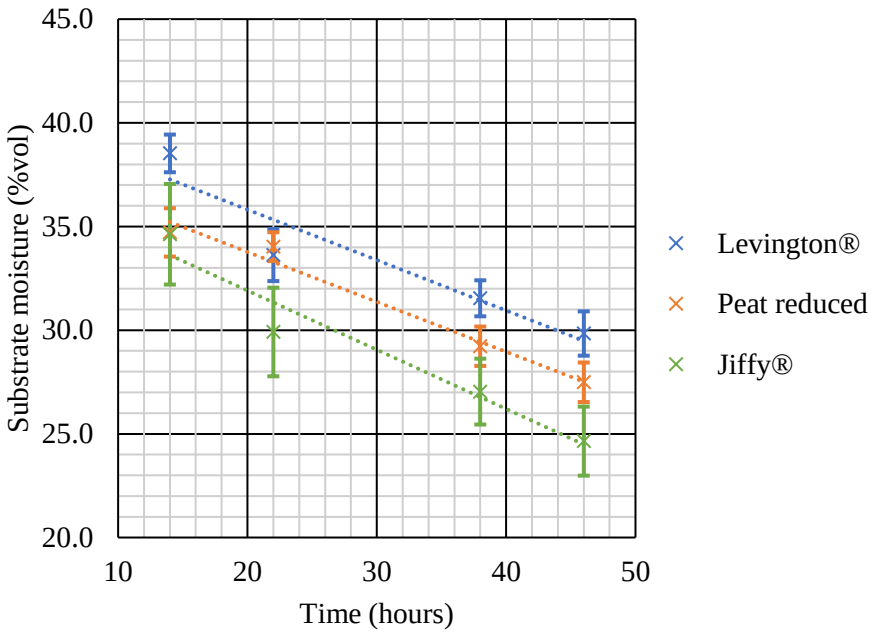

Figure 16. Substrate moisture of Levington®, peat reduced and Jiffy® substrates.

5.3. Discussion

5.3.1. Leaf water potential

The lack of increased leaf water potential in the well watered peat free plants suggests that this particular substrate may perform better under drought conditions than other substrates. However, the similarities in leaf water potential between the well watered and the deficit irrigated samples in this substrate could also be a result of the substrate already having low water availability for plants and therefore the proportion of water which is available to plants in this substrate may be lower than the volume of water added under the deficit regime. Therefore the additional water given to the plants under the well watered regime was in excess of that which is actually available to the plants.

5.3.2. Petunia biomass

Plants grown under well watered conditions had higher biomasses than their deficit irrigated counterparts. This is likely a result of water stress on the deficit irrigated plants inhibiting growth and redirecting nutrients and water to the plant organs which required them most. The greatest difference in biomass was within the peat reduced substrate suggesting that the substrate has little tolerance towards drought stress.

5.3.3. Stomatal conductance

Plants grown in Levington® under deficit irrigation conditions had a greater stomatal conductance than those which were well watered. Levington® was the only substrate with this trend, likely resulting from the increased biomass in these plants due to nutrient and water uptake. Therefore, the larger plants in Levington® were more developed and could adapt to changing moisture conditions and drought stress better than the less developed plants grown in other substrates.

Chapter 6

Conclusions

6. Conclusions

6.1. Surfactants

I. The addition of surfactants improves plant growth in potted ornamental species

When rhizosheath mass was compared with and without surfactant in the sand mix it was determined that there is a greater rhizosheath mass with the addition of surfactant. However, it was not determined whether this increased mass was a result of increased rhizosheath development with surfactant or if it was a result of the adhesive properties of surfactants. It is unclear whether the trends found in this trial could be replicated, due to limited time only enabling one or two replicates but each harvest.

From biomass measurements, evidence suggests that adding surfactant to the substrate does increase biomass. This is likely due to surfactant retaining water within the substrate making it unavailable for uptake by the plant root systems. This causes plants to extend their root systems further into the substrate, which could result in higher nutrient uptake by the plant due to the increased surface area of the roots. It was deemed that this could be particularly beneficial in bedded plants as it would enable water uptake and nutrient uptake from deeper within the soil or substrate, resulting in less frequent irrigation. However, this could potentially have the opposite impact on potted plants as there is only limited substrate within which they can elongate their roots, at which point there is a risk of the container effect.

The addition of surfactants did not have a significant impact on evapotranspiration within the substrates and plants, therefore further research is necessary to determine what proportion of water stored in the substrate, as a result of the addition of surfactant, is actually available for uptake by the plant.

6.2. Substrates

II. Peat reduced and peat free alternatives do not compare to peat-based substrates in sustaining potted plants

In barley and rye, above ground biomass, measured by dry above ground biomass and leaf area, was greater in Levington® than in the sand mix. This is likely due to the higher nutrient availability in Levington®, as well as the lower density compared to sand, allowing root growth in the micropores. It is also likely that the density of sand inhibited root systems from penetrating deep into the substrate.

In comparison, in the dianthus trial the peat reduced substrate produced a greater biomass than Levington®, particularly in the later stages of the plants' development. Due to the lower density of the peat reduced mix, it is likely that roots were able to extend into the substrate more easily and occupy a greater volume of the substrate to maximise water and nutrient uptake.

The peat free Jiffy® mix produced a lower biomass in petunias than the Levington® and the peat reduced mix. This is likely due to poor nutrient availability within the peat free Jiffy® mix resulting in nutrient stress on specimens grown in that substrate.

6.3. Plant water relations

III. Peat reduced and peat free substrates are not able to sustain potted plants and tolerate drought stress under a deficit irrigation regime to the same extent as peat-based substrates

The results from biomass and leaf water potential, as well as the stomatal conductance data suggest that plants grown in Levington® are able to tolerate water stress to a greater extent than those grown in other substrates with lower peat contents. This is the result of the increased biomass of plants grown in Levington® producing more resilient plants than the less developed plants grown in other substrates.

6.4. Further work

The results from these experiments create a strong base of understanding of the effectiveness peat reduced and peat free media. However, further investigation should be carried out into the

mechanisms which cause the differences in plant growth between these substrates. This should include investigations into the density and pore space in each medium and the role that these play in root development. Investigations should also be carried out into how these variables effect water retention and uptake by plants. It is suggested that respiration of both the substrates and the plants grown in them should be carried out under various watering regimes and surfactant applications. It may also be beneficial to carry out nutrient analyses in the same manner. In future studies, a wider variety of ornamental species should be used in order to identify any trends in ornamental species growth in differing peat concentrations and alternative substrates. Atmospheric conditions in greenhouses should be considered as a variable in future work. Following these studies, further trials of a similar nature should be carried out in an outdoor setting, such as in a garden, where conditions are more variable in order to determine how effective the varying peat concentrations in substrates are in a commercial setting. Furthermore, work should be carried out to further investigate whether a reduction in irrigation alongside surfactants increases plant growth when compared with increased watering alongside surfactants.

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