2 Literature Review

2.1 Introduction

Growth in environmental concern, awareness and evidential anthropogenic effects on our planet have spurred huge changes in the processes and products of our everyday lives. These changes can be seen in the emergence of sustainability certifications on our food, strict emission standards on factories and cars, and solar panels on our homes.
As awareness grows, so does the reach of change. Governments across the world have ratified various climate protocols, the most notable and engaging one of late being the Paris Agreement wherein governments worldwide approved and committed to the goals of limiting global warming. These agreements result in individual governments proactively legislating industry to limit greenhouse gases (GHG's) and other pollutants. The result of these legislative efforts has also given rise to a shift in practices in the horticultural sector in the U.K; Nutrient capture to prevent environmental contamination (E.G: eutrophication) and removal of harmful active ingredients (AI's) in various pesticides. One element however has been slower to reign in on its acceptance; the use of Peat.

2.1.1 Peat

Peat is a broad term used to describe several different types of partially decomposed vegetation that occurs in wetlands. Peat has been used as an engine for economic growth for thousands of years. As with any abundant resource of value, it has been exploited for its potential for creating wealth (exportation), energy generation (fuel), a valuable source of organic products such as waxes, medicinal products such as antibiotics, and as a growth media.
Peat is difficult to quantify due to the changing definitions between authors, governments and NGO's. Typically, a thickness of 30cm or more is mutually agreed upon as a loose definition of a peat bog. The plant material occurring within the bog should have a high organic matter content of around 30 to 100% (Lindsay 2010), with the peat being formed through the anaerobic degradation of water saturated acid heath plants, most notably sphagnum moss. They are incredibly efficient tools in the sequestration of GHG's and climate change mitigation as the rate of CO_2 intake is higher than its output through organic matter degradation

(Frolking et al. 2001).

Peat bog extraction is a practice that has taken place for millennia. Traditional techniques of extraction involved hand cutting individual blocks, known as 'Sods', typically used as a combustive material for fuel. Modernisation has developed a significantly mechanised process for peat extraction. The peat bog area is drained, the upper layer known as the Acrotelm (Ivanov 1981) , where the live plant material is still functioning, is removed. This then exposes the dense peat underneath; Catoelm (Ivanov 1981), which once uncovered begins releasing CO2 into the atmosphere, (Lindsay 2010) this then extracted via milling through a rotovator system. Once milled, this granular form of peat is placed into ridges and left to dry (effectively windrows) and then collected. Other techniques for extraction depend on the conditions of the bog and the desired product (i.e. granular or sods). These systems include mechanised sod cutting and hydraulic mining which essentially washes away the peat to avoid collection of other detritus such as dead trees (Carncross 1983; Bunt 1988).

The extraction of peat from peatbogs is a contentious topic due to several factors. Lowland acid peat bogs occur across ca. 2-3% of the worlds terrestrial surface (Parish et al. 2008) or about 400-500 x10^7 ha (Bather and Miller 1991) and store ca. 500-600 Gt of Carbon (Whittle and Gallego-Sala 2016; Page and Baird 2016) , and is one of the largest terrestrial carbon stores on the planet (Robroek et al. 2017). The sequestration rate of CO_2 from the atmosphere into peatbogs via assimilation by vegetative matter is suggested as around 100 x10^7 tonnes per annum (Strack 2008). The extent of peat bogs within the U.K. is difficult to properly quantify due to varying definitions of peat and the components that create such conditions. The estimated area is somewhere between 1.4 x10^7 ha to 5 x10^7 (Lindsay, 2010), with the WER estimating it be 2.75 x10^7 ha (WER 2013).

Peat bogs are unique areas of special scientific interest as they represent niche biodiversity and ecological functions, as well as being a large part of the local culturalural landscape (Barkham 1993). The rare and pristine habitats peat bogs are also able to mitigate against changes in our climate on a more localised scale. Water regulation is a significant factor in reducing flooding, an important issue in the U.K. which has been attributed to the occurrence of peat bogs, as well as water purification, reduction in soil erosion and pollutant filtration (Bragg and Lindsay 2003).

2.1.2 Peat in Horticulture

Although Peat bogs have been traditionally used as a fuel source, in the 20th century they were being more commonly exploited as a source of substrate (Zhang et al. 2013). This popularity as a growth media is due to Peats unique characteristics; water holding capacity, ability to transfer nutrients and structure (Bunt 1988; Ángeles-Argáiz et al. 2016). However, as awareness of peat extraction has become more public, producers and horticulture are moving away from peat-based products (fuelled by retailer demand). More recently alternative growth medias have made an emergence as suitable replacement (Perez-Murcia et al. 2005) for peat in the horticultural market at both the professional and amateur end of the scale.

2.1.3 Policy and funding

The issues of peat use, and peat bog degradation in the U.K. has been relatively common knowledge in the public and government domain for many years now. The issue was predominantly in the public realm in the early 90's due to 'The Peatlands Campaign Consortium' (PCC), an assemblage of conservation originations that aggressively called for the complete halt of peat use (Barkham 1993; Alexander et al. 2008) . The PCC organised itself by addressing the main issues with peat exploitation; that the current practices of extraction were outstripping the self-renewing capacity of peatbogs, thereby eliminating any potential recovery (Barkham 1993). The tactics of the PCC nevertheless drove a definitive line between industry and conservationists and may have hindered the pursuit of peat bog conservation due to enmity felt by peat extraction companies, however the results of this campaigned did result in over 1,000ha of peat bogs donated to English Nature (Alexander et al. 2008). Further development of Peat conservation saw the government buy out peat holdings to prevent further extraction and develop management schemes to restore partially extracted areas (Alexander et al. 2008). More recently, funding was released by the U.K. government for the restoration of peatlands within England worth £10 million as part of the goverments 25 year environmental plan (Her Majesty's Government 2019) , a sign that regardless of current geopolitical upsets, peat bog conservation is nevertheless still of concern. Peat bog restoration is a long-term endeavour; however, the effects of restoration can bring many benefits to localised areas and the U.K. on a more holistic scale. The main benefit of restoration is the stabilisation of water table levels and sequestration of CO_2. Another GHG has significance regarding Peat bogs; Methane is released by peat bogs at relatively low levels once rewetted due to the degradation of plant materials. The level of methane emissions compared to the long term benefits of restoring peat bogs and the carbon sequestration associated with it is however negligible (Lindsay 2010). This however highlights the critical point that pristine peat bogs should not be disturbed. Previously a consultation and subsequent white paper (2011) lead by DEFRA was formed for the phasing out peat use in the U.K. by 2020 for amateur users and 0% peat usage in the professional sector by 2030. This is in line with various other government lead incentives to reduce the United Kingdom's carbon footprint and waste outputs, notably; the 'Zero Waste' Wales and Scotland programmes which aim to eliminate waste sent to landfill by 2050. These programmes will produce more effective and efficient waste streams, thereby providing more resources for creating alternate growth media ingredients such as green compost, wood chippings and loam.

2.1.4 Substrates and demand

Substrate has two main pathways of distribution in the U.K.; Retail sector for amateur use and professional glass houses to produce ornamental and food crops. The use of substrates in this instance is primarily concerned with potted plants, rather than as a soil amendment. Since the inception of specific growth media as early as the 1930's (Bunt 1988), the growth of the amateur and professional horticultural sector has provided an uptake in the role of growth media providers as well as the variety of options in media composition. The demand for these substrates has also grown in part, due to the secondary effects of a renewed awareness of healthy eating, a focus on organic and home grown food (Jones, Comfort and Hillier, 2004), and the continued success of garden centres (Alexander et al. 2008). The recent introduction and development of a responsible sourcing scheme (Alexander, Stuart, and Bragg 2017) address's the benefits and costs of producing, transporting and using materials for substrate. The scheme is supported by the use of a free calculator tool that allows retailers and producers to assess the components of a substrate for overall sustainability through a scoring function. This encompasses both environmental and societal effects drawn out from the use of any components.

2.1.5 Alternative Substrate

The growth of alternative medias has been steady for many years now. Initial mixtures however struggled to gain traction due to lack of homogeneity and effectiveness/specificity (Alexander and Bragg 2014). Further development of alternative growth medias has however in recent years proved more effective and there are now several components that typically constitute as an effective replacement for peat. However, the major contributor to most potting mixtures is still peat at 63.9%. Following this is coir (coconut husks) at 20.7%, and woody materials at 8.3% (3.6% for bark), (Barrett et al. 2016). Other components are also present in mixtures, tweaked to suit conditions. These materials can be agents to add structure such as minerals, or a significant replacement carbon source (instead of peat) such as green compost. These materials have traditionally been less favourable due to issues of sourcing and consistency, especially when compared to the relative cheapness and reliability of peat. The Waste and Resources Action Programme (WRAP) has dedicated incentives to provide alternate methodologies for increasing the reliability of various peat-free growth medias, specifically green compost by proposing suitable guidelines for acceptable compost products (WRAP 2014). The AHDB (Agriculture and Horticulture Development Board) and BHTA (British Herb Traders Association) have focused their attention on several initiatives going forward in regards production, namely peat-free production for all crops. Some companies have already converted into largely peat-free production or entirely peat freeproduction methods across their business. This study will aim to provide evidence to support this transition of horticultural practices towards a peat free future. This will be achieved by addressing current peat-free mixtures and improving their ability to provide quality, homogenous crops.

2.1.6 Common Growing Media Components

2.1.6.1 Peat Also referred to as "Black Gold" (Robertson 1993) (Peter Seabrook, BBC Gardeners' Question Time, 22 April 2008: (Alexander et al. 2008), peat (see Figure 1) is the most common and prolific growth substrate currently used in the U.K. and across the horticultural sector globally. The, availability and low costs, mailability (the ability to change chemical properties (pH, EC), incorporate other components with ease etc.), and effectiveness of peat across a variety of crops has resulted in its retentive use by horticulture. The issues associated with peat extraction have done relatively little to dampen its use as considerations of profit, food security and quality control are at the forefront of concern for many horticultural enterprises. That being said, mobilisation towards changing horticultural practices to a peat-free environment is gathering momentum (AHDB, 2016). The are many viable components able to replicate peat in a variety of different practices and crops, although none are without drawbacks.

Figure 1: Peat.

2.1.6.2 Coir Coir is essentially a waste product derived from coconut cultivation in tropical climates. The husk is removed, washed and ground to form a pith or retained as fibre, most of this processing is aimed at producing components for horticultural use, the resultant product looks remarkably simililar to peat (see Figure 2). The properties of coir are well suited for use as a growth media due to its excellent water-retaining abilities, structure and texture which produces excellent aeration properties; critical for healthy root growth. However, due to the cultivation practices and questionable quality-assurance of coir/coconut products, there are large issues of homogeneity within batches and problems associated with washing of the product in salt water. This washing in salt-water and the general halophytic nature of coconut trees results in the coir having higher EC than mediums such as peat, even after washing (Arenas et al. 2002). Another issue of coir is the potential for nitrogen deficiencies in the substrate due to biological activity (Vavrina et al. 1996). Questionable socio-economic/ethical quandaries are also associated with the production of coir. The logistical costs of bringing coir product from the tropics increases price and may potentially offset the benefits of using a 'sustainable' product due to CO_2 emissions related to transportation. There are also issues surrounding the use of child-labour, and the potential socio-economic implications derived from this. Issues such as these can be addressed by the use of life cycle analysis tools (Torrellas, Antón, and Montero 2013) and the newly developed decision making tool created in partnership with various interest groups associated with growth medias and their impacts (Alexander, Stuart, and Bragg 2017). Systems like LCA and the sustainability calculator can allow for organisations to assess the sustainability and ethical value of substrate choice.

Coir itself is not only used as a substrate for growing plants. Other uses of its unique chemical and physical properties include use as an bio-absorbent for heavy metal (Namasivayam and Sureshkumar 2008) and reactive dyes in wastewater (Namasivayam et al. 2001).

Figure 2: Coir.

2.1.6.3 Green Compost The notion of utilising large quantities of organic waste like sewage or food waste for food production has been a common place practice in agriculture for a long time. The waste material degrades on the land, thereby releasing valuable nutrients and improving soil structure, which is consistently degraded with cultivations and harvests. Green compost is an attractive proposition as a peat alternative.

Green compost is used as a component of potting/bedding mixtures rather than as a sole ingredient due to the factors such as its typically high nutrient content, high EC and lack of aeration when saturated (Spiers and Fietje 2000). The benefit of green compost and its high nutrient value translates into a potential cost saving measure regarding fertilizer inputs (Hartz, Costa, and Schrader 1996). Green compost is composed of various waste streams; domestic food stuffs, garden waste, forestry etc. It is the issue of these various waste streams that makes green compost particularly troublesome to work with. Maintaining consistent chemical and physical properties are still anecdotally referenced as the primary concerns regarding incorporating green compost into commercial operations.

The composition of green waste often varies from batch to batch, often as a result of the changing nature and source of waste stream used to create it. Recent developments with increased quality control measures (WRAP 2014) have resulted in increased uptake of green composts as a potentially reliable substrate.

2.1.6.4 Bark Pine bark (see Figure 3)has been used within the horticultural sector for decades. Often seen as a weed suppressor on the surface of bedding areas, pine bark has also been used as a component of specific substrate mixes. The costeffective nature of sourcing pine bark coupled with its physical characteristics have lent itself as a common component within many potting mixes. The ability to easily sort bark into varying degrees of particle size allows for easy determination of suitability for certain tasks, such as the demonstration of certain sizes allowing higher porosity and nutrient storage, while other sizes lend themselves more easily to increasing substrate aeration (Airhart, Natarella, and Pokorny 1978). However, there are some issues found within bark, namely nitrogen drawdown (Handreck 1992); this processes is often induced by biological activity within the mix itself wherein said biologicals will metabolise nutrients within the substrate mixes, thereby limiting plant nutrient availability. This biological activity is predominantly associated with the presence of white-rot-fungi and their ability to excrete extracellular enzymes such as lignin and manganese peroxidase (Sinsabaugh et al. 2005). Another issue is the presence of toxins such as heavy metals and phenols, which tend to accumulate in higher concentrations in older wood stands, characteristically of a harvestable age (Bunt 1988).

Figure 3: Bark.

2.1.6.5 Wood Fibre Wood fibre (see Figure 4) is a created through an energy intensive process of steam extruding wood chippings. The product created through this high-pressure heat treatment is a very fibrous and fluffy material with potentially very dynamic capabilities as a substrate amendment (Bohne 2001). Wood fibre is a product derived from wood chippings, and as such represents a sustainable, locally available resource. These attributes in themselves lean towards the sustainability drive occurring in the horticultural and food sector.

An issue with wood fibre, as with other organic, woody or high lignin content components listed in this section, is the issue of nitrogen drawdown occurring with the presence of biological elements. The impact of nitrogen drawdown has the potential to be more limited in steam extruded wood fibre as the processes of producing this component (pressure + heat) results in a sterilisation processes, thereby eliminating microbiological elements already established within the material.

Figure 4: Wood.

2.2 Herbs

This study will address the cultivation of two globally cultivated herbs; Coriander (*Coriandrum sativum*) and Basil (*Ocimum basilicum*). These herbs are among the most commonly cultivated globally and are used throughout different cultures and nations in a variety of ways. Coriander and Basil are typically grown in peat mixtures in the U.K. and are therefore a significant candidate for observing the opportunities presented by the use of peat free media and biological amendments. The data for herb production worth and volume produced is difficult to quantify due to a lack of incentive in displaying this data (BHTA 2018), resulting in estimates rather than specific data: Herb sales in the U.K. alone are worth *ca.* £78million, this amount is estimated to continue growing year on year by around 18% (Kantar). The growth in this market can be largely attributed to the increasing awareness and benefits surrounding healthy eating and living. IRi (Information Resources, incorporated) data from a 2017 European Shopper Survey indicates that 72% of British consumers consider healthy food as a relevant and typical part of their diet, with general health and weight loss being the main contributors to buying preferences in store. This trend in healthy eating compliments other data from within the U.K. such as the rise of electronic cigarettes and decrease of people smoking (Robinson and Bugler 2010), and growth in the organic foods market (DEFRA 2016). Large herb producers such as Vitacress are pro-actively seeking new retailing opportunities riding on the trend of healthy living by PR campaigns such as 'Herbilicious'; a promotion which purports the health benefits of using fresh herbs and offers suggestions of dishes for how to use them via a website. This website alone is bringing in 40,000 visitors a week (Kantar). The rise in the awareness and popularity of fresh herbs is a good indicator of the need to develop new, more ecologically sound methods of meeting this demand in a sustainable and responsible manner. It is interesting to note that the findings of a recent 2016 agri-food report (Horticulture 2016), ethical or ecological properties of foodstuffs was the least concerning factor influencing customer choice when purchasing products (such as sustainably sourced substrate for potted plants). This report from DEFRA compliments a study into food consumption and buying behaviour (Joshi and Rahman 2015), which observed that although the demand from consumers for 'green' products has increased, the actual purchasing of these products does not correlate to the increase in demand. The disproportion of demanding ethically sourced goods and not buying them is a quandary that may be resolved at the point of retail. The driving force behind change is the retailers themselves; demanding responsible sourcing of materials to maximise their projection as a sustainable company in the public domain. This demand for sustainable and ethical sourced goods is beneficial from an economic point as the early adopters of such practices will develop products and services that will provide a significant edge as the global economy shifts towards such ideals (Nidumolu, Prahalad, and Rangaswami 2009). Basil is a culinary herb grown globally due to its delicate, distinctive taste and unique aroma. Basil is not strictly used for cooking, its essential oil (typically extracted through distillation) is widely used in various medical, cosmetic and culinary endeavours.

The production of anti-oxidants and phenolic compounds within basil is therefore of significant interest to consumers and producers alike. Changing dynamics in management regimes, cultivars and other factors have the potential to alter the production of these compound (Nguyen, Kwee, and Niemeyer 2010). Studies have shown shifts in nutrient availability in substrates modifying phytochemical production in basil, as well as changes in the componentry of substrates. Burdina and Priss (2016) demonstrated that 100% Peat mixtures performed poorly when compared to some mixed substrates (most notably 60% peat, 40% perlite) in regard to phenolic production in Basil. The use of alternative growth medias for basil has been widely investigated, with methods utilising spent coffee grounds (Ronga et al. 2016), compost (as an agent to reduce soil-borne diseases) (Reuveni et al. 2002) bio-char (Pandey, Patel, and Patra 2016) and many others besides with varying degrees of success.

2.2.1 Coriander

Coriander is an annual, flowering, leafy plant used widely across the world in cooking. Coriander is most commonly used as a spice (ground up seeds), its leaves are also widely used in a variety of dishes across several cultures. The importance of Coriander has been apparent for millennia as it has been found present in archaeological sites from the Neolithic era, and is now used across all continents in the present day (Vaughan and Geissler 2009) . Its value is well observed and is considered a significant cash crop in certain countries; India for example, and so its continued cultivation in both an amateur and professional setting proliferates. Similar abiotic factors have been shown effect the quality and yield of both biomass and essential oil concentrations of coriander in parallel to basil (Farahani et al. 2008), although the body of research is somewhat less extensive.

2.2.2 Essential Oils and Volatile Compounds

The secondary metabolic function of various organisms is a continued point of interest, and this is the case with herbs too. A huge variety of secondary metabolites are produced by herbaceous plants such as Coriander and Basil. The most vital of which are found within the plant essential oils (EO's). EO's are significant sources of revenue for many as they are favoured in many developing countries as a cheap alternative to contemporary medicines (Farnsworth et al. (1985)). This is in part due to traditional values but also significantly due to their demonstrated ability to alleviate certain symptoms. Modern medicine, although somewhat reluctant to the idea of using herbal remedies due to their potential for toxicity in regards to overdosing or lack of effectiveness, has grown traction in a movement known as 'phytotherapy' (Schulz, Hänsel, and Tyler 2001). Phytotherapy heavily relies on the concentrated extracts from plant material, notably EO's for the treatment of a variety of health complaints. Several EO's have shown the ability to alleviate gastronomic complaints, headaches, and aid the treatment of mild infections among others.

Other uses of EO's and secondary metabolic compounds within plants are far reaching. Phytochemical compounds are widely used in cosmetic and perfume products (Burdina and Priss 2016), either as an aroma or an specific agent to aid the effects of the cosmetic product in question. U.K. retailers such as 'The Body Shop' and 'Lush' base their products heavily on plant-based materials and have a loyal customer base because of this. EO's have also been established as potential preservative agents in foodstuffs such as cheese (Smith-Palmer, Stewart, and Fyfe 2001) and as natural pesticides (Batish et al. (2008)). The market value therefore, although difficult to quantify due to the quantity of small-scale production centres in the developing world, is significant. Consequently, the ability to generate increased essential oil content from the same crop space is very desirable. However, crops grown for EO production are almost exclusively field crops due to the logistics, costs and other benefits such as mechanised harvesting. EO's in the case for potted herbs are a reflection of the flavour profile, which may be altered depending on factors such as substrate and microbial activity. This potential change in EO and therefore volatile profile may produce a more dynamic and therefore desirable crop from the consumers point of view. Typically essential oils are extracted through a distillation mechanism (Kerrola and Kallio 1993). EO's are then assessed for composition through use of a Gas chromatography/mass spectrum device (GC-MS) . However, through use of solid-phase microextraction (SPME), non-destructive sampling of volatile compounds can be achieved, producing a clear, qualitative analysis of potential compounds found within EO's of target crops.

2.2.3 Volatile profile: Coriander

Much work has taken place regarding volatiles and their indication of essential oil composition and concentration from which they emit (Nematollahi, Kolev, and Steinemann 2018). The development of analytical profiles for herb volatiles is well established, with GC-MS/SPME being a preferred methodology due to its straightforward and relatively inexpensive operation. The profile of Coriander has been established in distinctive detail (Potter and Fagerson 1990; Ravi, Prakash, and Bhat 2007). The primary constituent is Linalool, a commonly occurring terpene alcohol found in a variety of flora. Linalool is primarily used for its scent which is a sweet, light smell and is consequently used predominantly within the cosmetics industry (Yazar et al. 2011).

2.2.4 Volatile profile: Basil

Basil is reputable for its fresh and almost minty flavour. The composition of basil is somewhat different to Coriander in the respect of the quantity of different cultivars available. The volatile profile therefore shifts somewhat depending on which variety is being investigated. However, there are x4 primary components regarding the aroma of the plant: Estragole; used heavily throughout the food and cosmetic industry. Linalool, allylveratrol; a compound with a clove-like taste, used extensively in the food sector. methyl-cinnamate has a distinctive fruity smell and is once more used broadly in the cosmetics and food sector (Klimánková et al. 2008). The variety of basil cultivars and potential flavour profiles therefore lends itself very well for further analysis regarding the influence of substrate and biological amendments, and any benefits they can bring in producing a better tasting product.

2.3 Microbial Amendments

Soils are huge ecological systems, with abundant biodiversity and species interactions. The plants inhabiting the soils are also interacting with these communities, and as such both benefit and suffer from them accordingly. Symbiosis is not a unique entity. It can span several species interactions. Symbiosis here is defined as 'the mutual benefit of species cohabitation'. The two most relevant symbionts here are AMF (Arbuscular Mycorrhizal Fungi) and PGPR (Plant Growth Promoting Rhizobacteria), which provide a variety of services to plants, namely their ability to provide alternative methods for approaching stress inducing scenarios such as mineral toxicity, nutrient depletion, drought and temperature fluxes.

The ability of AMF and PGPR's to obtain nutrients in the substrate and make them available for plants is well documented. The mode of action in which this achieved, although dependent on species, is typically by the exudation of extracellular enzymes or organic acids, thereby dissolving minerals and releasing less complex, more consumable mineral sources for plants (Priyadharsini and Muthukumar, 2016). These microbes in turn gain benefits from the plants by absorbing carbon, released by the root system in the form of simple sugars, this 'rhizodeposition' (Jones, Hodge, and Kuzyakov 2004) can be anywhere from 5% to 20% of the total photosynthetic carbon of the plant in question (Jones, Nguyen, and Finlay 2009). This symbiosis therefore offers a biological source of nutrient supply which maybe able to reduce some of the traditional, and costly inputs into modern horticulture, namely that of fertilizers. Fertilizer use has enabled the production of food to meet global demand, fuel growing economies and prevent mass starvation. The necessity of meeting global food security is evident in the necessity of increasing food production by at least 70% to meet the demands of an increased population by 2050 (Godfray et al. 2010). The process of achieving this is much debated however, as the Green revolution enabled the feeding of billions in the developing world due to increased integration of technological improvements such as mechanisation, pesticides, better crop varieties and a heavy reliance on fertilizer use. However, the production, use and need for fertilizers and pesticides has resulted in mass environmental pollution, has been shown as detrimental to human health and is a significant contributor to global warming (Horrigan, Lawrence, and Walker 2002; Townsend et al. 2003). This paradox of sacrificing environmental concerns for increased food security may be resolved with more sustainable and focused use of biological fertilizers and controls such as can be provided by PGPR and AMF.

2.3.1 Arbuscular Mycorrhizal Fungi

The potential and scope of mycorrhizal fungal for improving crop qualiy, health and resistance to external factors is large, and the knowledge surrounding these aspects is constantly evolving and will continue for decades to come. AMF are symbiont with *ca.* 80% of all vascular plants on the earth's surface (Smith and Read 2008). They have demonstrated to increase resistance against abiotic factors such as drought tolerance (Ruiz Lozano, Azcon, and Gomez 1995), poor soil structure (Miransari et al. 2008)(heavy metal tolerance (Upadhyaya et al. 2010), as well as biotic factors such as resistance to pests (Jung et al. 2012) .
Other than promoting plant health through increased resistance to stressful environmental conditions, AMF are well known for their ability to solubilize mineralised sources of phosphorus, and increase other nutrient uptake (Rasouli-Sadaghiani et al. 2010). These traits overserved through AMF symbiosis validate the potential they hold as a solution to out-dated agricultural practices, bio-remediators of contaminated land and climate changes mitigators (Stamets 2005).

2.3.2 Identifying AMF colonisation

The analysis of root colonisation by AMF is a critical process for understanding the effect of substrate, environmental conditions and crop type on the ability of AMF to operate and effectively colonise the target. Establishing the infectivity of mycorrhiza on plants has developed relatively little until the last decade or so, wherein molecular and image analytical techniques have started to flourish. Previously, these techniques have relied on visual quantification. From this colonisation analysis, efficacy of inoculation can be interpreted, crop quality can be assessed, and developmental work can begin. The variety of methods for establishing mycorrhizal colonisation is large, however there are several prominent and solidified methodologies frequently used for the visual estimation of mycorrhizal colonisation, and as such have been reviewed and compared several times (Brundrett et al. 1996; Gange et al. 1999; Vierheilig, Schweiger, and Brundrett 2005). Original developments of root clearing and staining procedures by Phillips and Hayman (1970) set the basis for the current procedures currently used. Use of heated KOH as a root clearing method to remove cytoplasmic and nucleic plant material, rendering roots translucent, enabling clear observation of fungal material and allowing a ‘canvas’ for subsequent dying.

Dying the processed root material post-clearing to highlight fungal material unaffected by the KOH allows for easier and more focused observation of fungal structures within the plant roots. The dyes used are varied and an often-debated topic between those who study mycorrhiza. The range of publications and techniques/dyes used was addressed by Gange et al. (1999), with the most prominent dye being Trypan blue (originally established by Philips and Hayman), followed by Acid Fuchsin, and then Chlorazol Black E. The evolution of stains from early research has omitted original dyes in favour of newer, more consistent and effective ones [Giovannetti1980]. The study also noted the range of results exhibited when using different techniques on the same sample. Another issue highlighted by (Gange et al. 1999) was the prominent toxicity/carcinogenic properties of dyes such as Trypan blue and the lack of uptake regarding other, less harmful dyes.

Further research into alternative staining techniques has been done focusing on more practical, cheaper, less harmful substitutes. Vierheilig et al. (1998) noted the ability for some inks to produce superb contrasts of plant material and fungal elements, and was followed by further research (Walker 2005) exhibiting the favourable results offered by inks. Methods are often adapted for target species of plant and mycorrhizal species and range in approach and reagents used, whilst retaining the foundation established by Phillips and Hayman (1970).

Conventional methodologies for determining AMF colonisation are dependent on observations of fungal structures directly using bright field microscopy (BFM). Combinative use of root staining and BFM has been used reliably for decades: An often referred and well trusted methodology is that suggested by McGonigle (McGonigle et al. 1990). This employs the use of a microscopy cross hair intercepting mycorrhizal features (gridline intersect method), recording what fungal feature occurs at each intersection. This method has been applied and modified in several studies (Cavagnaro et al. 2001), and is easily adaptable provided relatively large levels of sample are available. The gridline intersect method is a simple visual observation and allows estimation of colonisation of whole roots. Methods such as these, although effective, are dependent on operator bias and ability. Developments in accuracy and technology regarding quantification of mycorrhizal colonisation within plant roots is an ongoing process. For instance, use of image analysis software demonstrated by (Deguchi et al. 2017) proposes an accelerated method of analysis, whilst more molecular quantitative techniques are becoming more mainstream (Alkan et al. 2004).

2.3.3 Assessing AMF activity

The activity of AMF when colonised are varied and can be quantified in an assortment of ways. The solubilisation of immobilised phosphorous is a well established trait of AMF and can be used as a proxy of determining activity. An increase in plant available P/accumulation in vitro when introduced to inoculant (Jia, Gray, and Straker 2004) can be used as a direct measure of AMF activity and function. This can be achieved through the use of spectroscopy (flame absorbance for instance). A more recent development in assessing the potential of colonisation and activity of AMF is the use of tracking blumenols, an organic compound that accumulates in plant material when in symbiosis/as markers of colonisation (Wang et al. 2018). This method uses a highly automated (high-through-put) method for screening metabolites as proxies from the colonisation of AMF, specifically glucosides. This new development aims to revolutionise the process of establishing colonisation in plant species as it removes the need for destructive analysis. However, issues with initial calibration and standards are potentially very time consuming.

2.3.4 Plant Growth Promoting Rhizobacteria

PGPR are bacterial colonies found within the rhizosphere of plants and within the roots of plants. Alongside other soil bacteria, they are responsible for the mobilization of various nutrients and are a significant factor in the promotion of plant health (Ahemad and Kibret 2014). Soil bacteria have been observed as significant bio-remediators for toxins and pesticides (Myresiotis, Vryzas, and Papadopoulou-Mourkidou 2012), as well as increasing abiotic tolerances of plants to such things as increasing salinity (Mayak, Tirosh, and Glick 2004) and water-stress (Justine et al., n.d.). Another important benefit soil bacteria can confer onto plants is higher resistance to pests and pathogens (Chen et al. 2000). Typically, development and execution of PGPR inoculation has resulted in context specific results, similar to those exhibited by AMF, wherein complexities around biotic and abiotic factors has resulted in data representative of those particular circumstances (Bashan, Holguin, and De-Bashan 2004; Agrawal, Satlewal, and Varma 2015). This study aims to utilise specific bacteria under set, easily repeatable conditions in order to boost the quality of pot grown herbs in peat-free substrates.

2.3.5 Assessing PGPR colonisation

Assessing PGPR activity itself is difficult. The determination of colonisation or presence is comparatively simpler. The detection of bacterium though qualitative DNA extraction of roots or rhizosphere is typical, as is the extraction of bacteria from plant material in order to grow on a medium for further quantification (I.E. estimations of colony size), this can be achieved through N/P depleted media. Observation of PGPR activity in vitro is complex, often relying on electron microscopy or the gene editing of bacteria in order to place luminescent markers onto the targets, thereby highlighting location and activity. This is often achieved using something like the green fluorescent protein (gfp) (Bashan, Holguin, and De-Bashan 2004). However, the ability to observe bacterial colonisation and activity in vitro through root staining has been demonstrated (White et al. 2014). This method employs use of horseradish peroxidase (HRP) to react with a chromatic compound; 3,3'diaminobenzidine (DAB), producing a colouring on the target. In the case of White et al. (2014), this was the hydrogen peroxide produced by infective bacterial colonise in the root hairs. This process may also be adapted to target PGPR bacteria. However, this is entirely dependent on context and condition as not all PGPR will act as endophytes, with many species only found outside the root system.

2.3.6 Assessing PGPR activity

The functionality of PGPR's is varied. With activity not only being confined to N-fixation (Vessey 2003) or P-solubilisation (Somers, Vanderleyden, and Srinivasan 2004). N/P accumulation through PGPR activity can be quantified in the same manner as AMF nutrient activity . The range of activity so far observed includes promotion of phytohormones, detected through use of liquid-chromatography/mass-spectroscopy (Lugtenberg, Dekkers, and

Bloemberg 2001; Forcat et al. 2008) and induced systemic resistance to pests (Chandler et al. 2008), quantified by pest activity on inoculated plants.

2.3.7 Interactions of PGPR and AMF

The relationship of rhizobacteria and arbuscular mycorrhiza is often situation dependent, complex, and as a result, particularly difficult to quantify. There is a large body of research observing the benefits of utilising PGPR and AMF simultaneously, but fewer papers document the intricacies' of the relationship between the two, and these are significantly contextdependent, for instance; Mayo, Davis, and Motta (1986) observed that only in the presence of rhizobacteria did *Glomus versiforme* actually germinate, and when removed from the presence of the bacteria, the spores of *G. versiforme* remained inert. In contrary to this, (Walley and Germida 1997) observed the ability for rhizobacteria to inhibit *G. clarum* germination. The context of these two is entirely contrary. The former performed these tests under sterile, lab conditions, whilst the latter produced these results in vitro with a host plant. The difficulty therefore is assigning pre-observed data on AMF, rhizobacteria and conditions (such as host plant, climate and substrate type) to the context of the proposed study here; biological amendments in a potted plant with peat-free substrate. However, it can be stated that observing current research on a generalised, holistic level, it is apparent that rhizobacteria and AMF generally do not antagonise one another (Priyadharsini and Muthukumar 2016).

2.4 Horticulture and the Future

Many large-scale horticultural operations in glass houses have strictly regimented production cycles, rarely deviating from the system that provides consistency and profit. However, with increased pressure from governmental bodies, increasing awareness of environmental issues and consumer demand, the horticultural industry will inevitably shift towards a more environmentally enveloped methodology of produce procurement. It will be the early adopters of this new regime that will flourish. The rising popularity of alternative food production methods such as vertical farming, aquaponics, root mist/aeration systems and more extensive use of lowenergy LED systems (Despommier 2010) highlights this changing stance of prioritising environmentalism within horticulture. A recent example of this change can be seen within

Vitacress Ltd., and the switch from black plastic pots to grey. The use of black plastic pots is common due to the contrast highlighting the product (i.e. crop). However black plastic is very difficult to recycle. Thereby using grey, although aesthetically decreasing the value of the product, it is increasing the sustainability of it. Developments like these are progressive and necessary, as is the development of peat-free potting substrates and their implementation. The use of peat in horticulture will end, the time scale of this however is extremely fluid. This is especially true of the U.K. as priorities regarding ongoing relationship with Europe are foremost, leaving little resource to review and implement legislation regarding the issue of peat use. It is however important to continue driving the notion of peat-free substrates and sustainability forward, regardless of the current political and legislative landscape This study will provide a clear outline for addressing the issue of peat use in growth medias and generate data to support the use of peat-free medias with microbial amendments.