Figure 7: Shelf life test under low-level light conditions at ambeint temperatures

3.5 Trial set-up and preparation

Although each trial often catered to a specific investigative topic, the set up for each was broadly similar. Treatments were determined from each previous trial or indeed informed by secondary research elements. Trials were planned, with treatment randomisation (via a random number generator) and labels for blind assessments (labelled from 101, 201, 301 etc. depending on replicates).

3.5.1 Substrate Mixing

Each Peat-free substrate, unless otherwise stated, was mixed on-site with raw materials provided by Bulrush Horticulture, a co-sponsor of this project.

Substrate mixing was performed with a cement mixer running at 23rpm with a capacity of 134L (see Figure 8). Materials were measured volumetrically unless stated in componentry table (see table 2). Materials for each batch were added in an identical order and allowed to mix for 5minutes. Materials were then allowed to settle and then mixed briefly again before potting up.

(a) (b)

Figure 8: Use of cementmixer and volumetric measurements for mixing of growing media components

The wetter was added using a syringe during mixing to allow for maximum coverage of the substrate, however this method is ineffective compared to commercial scale substrate preparation. Potting fertilizer was Yara PG MIX 15-10-20: 15.0% N, 4.4% P (10.1% P2O5), 16.6% K (20.0% K2O). This fertilizer is a commonly applied potting amendment aimed at producing a favourable starting environment for crops planted in peat-based substrates

Table 2: Peat-Free substrate component and quantity table

	Quantity (ml) per pot
Coir	200
Forest Gold	200
Bark Fine	50
Loam	50
Lime	0.5
Urea	0.25
Fertilizer (NPK)*	0.375
Wetter	0.4

3.5.2 Potting

The pots used for all substrates were .48L capacity pots, with a 10.5 cm diameter, narrowing to 7.5cm at the base and 8cm deep. These were supplied by ScotPlantsdirect Ltd. and were chosen to match the specifications of a typical potted herb produced commercially. Pots were filled loosely with growing media by hand, tapped twice on a hard surface and the seeded. Coriander was seeded at *ca.* 1 cm from surface, with a covering of substrate bringing pot to capacity. Basil pots were filled completely, with seeds surface scattered. Basil pots were covered for *ca.* 3-5 days (until cotyledon emergence) as per commercial practices.

3.5.3 Plant species

Croppings selected for this research project were Basil (*Ocimum basilicum*) and Coriander (*Coriandrum sativum*). Both seeds were supplied from a commercial entity and supplied from field grown crops in Italy. The variety was "cruiser" (Coriander) and "marjoram" (Basil). The Coriander seeds were heat treated to prevent seed-borne pests, specifically Coriander Bacterial blight (*Pseudomonas syringae pv. coriandricola*). The heat treatment process involves heated water baths for the seeds, at 53°C for 10 minutes (Roberts and Green, n.d.). Basil seeds were treated with a fungicide; Metalaxyl. This systemic fungicide is used specifically for the control of Pythium, a water/soil borne pathogen that thrives in wet environments. Concerns regarding the ability of fungal inoculum to effectively colonise plant roots in the presence of Metalaxyl were addressed by the provision of previous research by the fungal supplier (Plantworks) which determined AMF would not be affected.

As per commercial growing standards, a relatively large quantity of seeds were used per pot. Coriander was planted at x25 seeds per pot. Basil was planted at *ca. 30* per pot. Due to the size of basil seeds (<2mm), counting or weighing was ineffective at producing consistent seed quantities for the volume of pots required per trial. This was remedied using a volumetric method (small cup attached to a spatula) that gave a consistency of 4 ± seeds (n=20). This was considered sufficient for trial purposes.

Commercial practices use large, expensive, automated machines to produce consistent croppings. For seeding, a suction plate with indentations to provide both an even pattern and

correct seed numbers. This approach was deemed impractical and time consuming for this project. All seeding was performed by hand.

3.5.4 Microbial Inoculation

Mycorrhizal inoculum (supplied by Plantworks LTD.) was a solid, granular format containing the spores, hypha and colonized root fragments from the original commercial propagation process. A total of six individual strains were used in this blended inoculum. These strains were selected on their ability to solubilize specific nutrients from soil and provide to the host plant. This inoculum was used as per the manufactures instruction (5ml of inoculum per 500ml of substrate), applied below seed level prior to planting.

The rhizobacterial mixes were also provided by Plantworks LTD. Four individual species of nitrogen fixing bacteria were used either individually, mixed or with other biological amendments (mycorrhiza). The inoculum was provided in a liquid suspension in an 2.5ml eppendorf tube, maintained at 4°C until use (no longer than 3 weeks). This was then mixed in a clean, sterile bucket with the required amount of water. A clean 50ml falcon tube was then used for application of the inoculum for individual pots. Extra care was taken to avoid dripping on other pots as to avoid cross contamination. As per the manufactures instruction, seeded pots were soaked with water until at container capacity, with the diluted liquid inoculum then poured in over the substrate for each pot.

The follow formula was used to determine targeted concentrations (5×10^9 cfu/pot) of rhizobacteria per pot:

$$N_p \times 100ml = D_i(+20\%) \tag{1}$$

Wherein,

N_p = Number of pots to treat with rhizobacteria.

D_i = Diluted inoculum.

Control pots were not inoculated, or indeed treated with sterilized, inert carrier material (mineral based). At 1% of total substrate material, this was not considered significant to

provide treatment effects from the carrier material alone. Additional water was added to untreated pots for bacterial inoculum. This was to maintain the same levels of saturation for all pots, and balance any potential material loss (leaching of nutrients) that may have effected results.

3.5.5 Substrate Measurements

Substrate characterization and categorization is imperative for effective crop production. Suitability to crop and environment dictate the attributes necessary for substrate to possess. In order to effectively determine these attributes, thorough assessment of the physical elements of the substrate must take place.

pH measurements were made regularly to ensure pots were at the correct levels (6.2pH) (check). This was achieved by calibrating the pH probe with standards prior to measurements. Distilled water was used to saturate 1g of substrate in a 50ml falcon tube, agitated briefly and left to settle for 5 minutes before measurements were taken.

Pots were filled to maximum capacity (.48L). They were then surface soaked with a watering can by hand until draining out the bottom. To calculate the saturated weight of the substrates (S_w), they were immediately weighed after soaking. To establish container capacity (C_c), pots were allowed to drain for 15mins and then weighed again. To calculate dry weight (D_w), pots were left in a dry environment at 26°C and weighed until mass remained constant (10days). Pot weight (P_w) was established by weighing pots (n=10) to determine the avg. weight ($8g \pm 0.1$).

Following equations derived from Bunt (1988).

Air porosity:

$$A_p(\%) = 100 \times (S_w - C_c)/(n \times V) \tag{2}$$

Water holding capacity

$$W_h c(ml/pot) = (C_c - A_d - P_w)/n \tag{3}$$

Water porosity:

$$W_p(\%) = 100 \times W_h c/V \tag{4}$$

Bulk density :

$$B_d = 1000 \times (A_d - P_w)/(n \times V) \tag{5}$$

wherein V= Volume

Total pore space:

$$T_p s(\%) = (1 - B_d/P_d) \times 100 \tag{6}$$

wherein P_d = Particle density*

*Particle density, the ratio of substrate to water.

3.6 Trial assessment

During the growth phase of each trial, numerous assessments were undertaken in order to quantify treatment effects from either amendments to growing media (fertilizer, microbial), growing conditions (irrigation rates) or different substrates, using crops as a proxy for treatment effects.

3.6.1 Phytometric

Phytometric measurements (read *Plant measurement*) are the foundation for plant based trials. Treatment differences are shown visually in the crop and are therefore easily quantifiable by measuring various parameters of the plants. The measurements taken for this research were broadly similar across all trials (n=25), performed in a controlled environment, with replicated substrates.

Phytometric measurements formed the base of all experimental data. Crop development and health was used as a proxy for substrate and amendment efficacy. Phytometric measurements

taken were crop height (see Figure 9), leaf diameter, yield (fresh and dry), shoot number, root length, RGB (red-green-blue) leaf values and crop count.

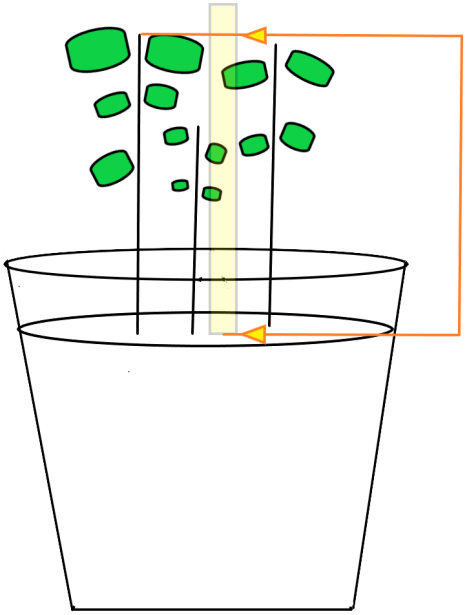

Figure 9: Diagram demonstrating the simple method of measuring plant height. The ruler (yellow) is set against the substrate and plant base, with the crop height being measured against the highest point

Crop height was measured using an inflexible ruler, from substrate to crop tip. This was repeated a minimum of 5 times per pot at random points. Leaf diameter was measured using a ruler across the center of true leaves, this was repeated 5 times per pot on a selection of random leaves. Yield was achieved by cutting each crop at substrate level (shoots and leaves), immediately weighing them and transferring into paper envelopes (Fresh weight). These envelopes were then dried at 40°C for 3-5 days until mass remained constant. The yield was then weighed again to determine dry weight. Shoot number was measured by visual observations, simply counting the number of horizontal off-shoots from the main stem for 5 plants per pot. Root length was measured by removing both substrate and crop from the pot, gently agitating under slow running water and briefly submerged in room temperature water to remove excess substrate. Roots were then laid out on a flat surface and measured from the point of chloroplasts cessation in the root stem. Each pot was measured 5 times. Crop count was achieved visually and was typically recorded from emergence (E0) to 7 days after

emergence (E+7d).

3.7 Assessing mycorrhizal root colonization

In order to confirm the presence and successful colonization of Arbuscular mycorrhizal fungi (AMF) in target crops, several methods of root staining, mounting and assessment were undertaken.

3.7.1 Root Staining

In order to confirm mycorrhizal colonization of the root structure, a simple and effective method is to clear and stain the roots of plants inoculated with fungi. This straightforward technique allows for visual confirmation as well as direct quantification of colonisation. The quantification of root colonisation may be used as an indication of AMF efficacy on crop growth, substrate suitability or indeed negative effects of AMF colonisation.

Roots were harvested by removing the plant from its pot, gently agitating under running water and briefly soaking to ensure a clean root. They were then chopped at stem level and transferred to 15ml falcon tubes with either 70% or 95% ethanol (Charoenpakdee et al. (2010)) . Roots were then rinsed under running water, cut to length and then placed in tissue cassettes.

Roots were then soaked in 10% KOH solution (10:90, KOH: dH2O) for clearing (Toussaint, Smith, and Smith (2007); Smith and Read (2008)), heated at 75°C for 10 minutes and then left over night at room temperature (see Figure 10). If the solution was saturated heavily with tannins (turning significantly yellow), this process was repeated. Once roots were successfully cleared (appearing translucent), root staining was performed. This solution was a slightly acidified, ink or phenol based product (84.4:15:0.6. dH20: 1% HCl: Ink Cotton blue dye) (Vierheilig et al. 1998). The process for root dying was similar to clearing, wherein the tissue cassettes were heated for 10 minutes and then left to cool overnight. Once this was completed, dye was safely disposed of and tissue cassettes were rinsed under gently running water to remove excess dye.

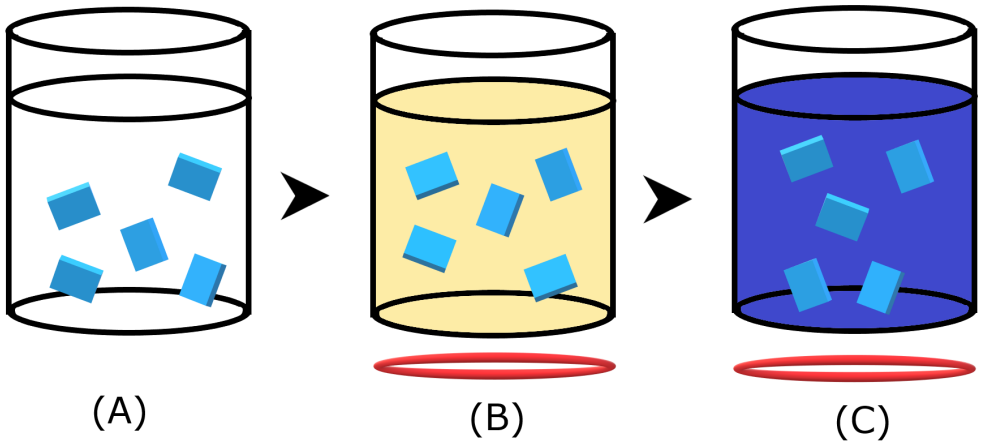

Figure 10: Simplified Illustration of root staining process. Tissue cassettes are rinsed under tap water to remove excess debris (A), followed by heating at 75 for 10 minutes in KOH (B) and left to cool for 24hrs. Followed by staining (C).

3.7.2 Root mounting

Microscope slides were used (see Figure 11) for mounting stained roots on. A polyvinyl-lacto-glycerol (PVLG: 100ml Lactic acid, 10ml Glycerol, Poly vinyl alcohol 16.6g, DH2O 100ml) (Koske and Tessier, n.d.; Treseder, Turner, and Mack 2007). Preservative/slide mountant was used to maintain root stability for a long term mounting, with the option to become permanent with the use of oven drying. Clear nail varnish was used to seal the slides and covers.

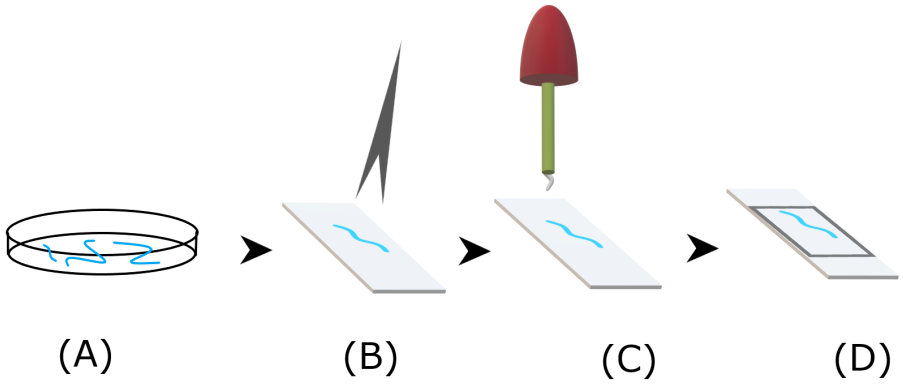

Figure 11: A simple illustration for the process of root mounting. A) floating root samples in an aqueous solution for easy picking by tweazers. B) tweazers allow placement of root onto slide. C) PVLG mountant solution added to slide. D) Slide cover put in place and sealed with nail varnish.

3.7.3 Root assessment

For viewing potential mycorrhizal colonisation, the cross hair method developed by McGonigle et al. (1990) was employed following Cavagnaro et al. (2001) adaption. Subsets of each root sample were applied to microscopy slides. Each slide was observed 100 times to produce % for root length colonisation. 3-5 roots samples were used per slide, 3.5 ± 1cm root samples fixed onto each slide. Hypha, vesicles and arbuscules were noted as present or absent at each intersect to determine % colonisation of each sub sample.

3.8 DNA isolation and Amplification

In order to confirm the presence and successful colonisation of plant material by mycorrhizal fungi, molecular techniques were employed to determine the efficacy of inoculate and support treatment responses. Similarly, growing media in close proximity to and around the rhizopshere was assessed for the presence of rhizobacterial DNA; as to ascertain the success of bacterial inoculum introduced to the croppings.

3.8.1 Mycorrhiza

Mycorrhizal DNA was obtained from the roots of croppings. Roots were harvested by pulling croppings gently out of pots, agitating and washing substrate from roots under gently flowing water and cutting surface vegetation at the base of the stem. Once clean, roots were submerged in a 95% ethanol fixiture in 25ml falcon tubes for preservation until analysed. Roots were then removed from fixature, thoroughly washed under flowing water and then frozen using liquid nitrogen. Roots were then homogenized using a pestle and motor wherein a DNEasy Qiagen plant kit was used for DNA extraction; an easy to use, standardised resource which includes the necessary reagents, methods and materials for DNA extraction. Primers for fungal DNA were selected based on successful alignments from NCBI database/GenBank.

3.8.2 Rhizobacteria

Primers selected for amplification of target DNA were obtained from literature. These were selected due to the likelihood of burden DNA likely found from the substrate extractions. Thereby reducing the efficacy of universal primers.

3.8.3 Primers

Primers were supplied by Sigma-Merch at a concentration of $0.05\mu M$. For AMF; Multiple segments of between 150-200bp were aligned for each species specific primer, from previously recorded species in the NCBI database. The universal primers were selected based on publications with successful amplifications from said primers. Futher to this, universal primers were also used to mitigate against high specificity demonstrated by species specific primiers (see Table 3). Master stock solution of 100 μM concentrations were prepared by using microbial grade RNA/DNA free water. This was further diluted to give 10 uM working solutions for each primer. These working solutions were further diluted to 1 μM for PCR mixes (1:9 $1\mu l$ of $10\mu M$ concentration to $10\mu l$ H20).

Table 3: Bacterial and Fungal Primer sequences

Kingdom	Target	Primer	Sequence 5'-3' Position (BP)	Tm	Ta	G:C %	Reference
Bact.	Pseudomonas fluorescens	16SPSEflu	TGCATTCAAAACTGA CTG	50.1	50.1	38.89	Dickson 2014
			AATCACACCGTGGTAACCG	57.5		52.63	
Bact.	Pseudomonas putida	Pp	CCAAAACTGGCAAGC TAGAGTA	58.9	57.9	45.45	Altinok 2011
			CATCTCTGGAAAGTTCTC TGC	57.9		47.62	
Bact.	Bacillus amyloliquefaciens	AmyE1	CCTCTTTACTGCCGTTATT	51.4	51.4	42.11	Wu et al. 2014
			ATGCCCGTAGTTAGAAGC	53.2		50	
Bact.	Azospirillum brassilense	Azo-2	GCGCGGGAAGTCCTGAAT	60.1	60.1	61.11	Stets 2015
			CCCTTCACCATCCAGTCGAT	59.1		55	
Fung.	Funneliformis mosseae	Fm	CGGGGAGTATGCCTGTTTGAG	62.8	60.4	57.14	
			GCTTTAATCGTACCGGATGGATG	60.4		47.83	
Fung.	Claroideoglomus claroideum	Cc	CGGGGAGTATGCCTGTTTGA	59.4	59.4	55	
			TGTTTAATTGCTCCATTCGGTCG	60.5		43.48	
Fung.	Glomus microaggretum	Gmcg	CATATGAAGGGGGATCGTGGA	61.5	59.6	52.38	
			CGGCATTGCTTAATATCACCGA	59.6		45.45	
Fung.	Rhizophagus irregularis	Ri	CCTTCATGCTTTGCATATTTGTG	57	57	39.13	
			AATCTCGTTCATCACATCTACCGA	60		41.67	
Fung.	Glomus geosporum	Fg	TGACTGGAGGAATGTGGCTTC	62	58.1	52.38	
			ATCATAAGCACGCTTTCGACAT	58.1		40.91	
Fung.	Diversispora	Div	ATGCTTGGTTGAGGGTCATTAAA	59.1	59.1	39.13	
			CAAGTTGTCAGCGAACCACAC	61.6		52.38	
Fung.	Universal	AML1	ATCAACTTTCGATGGTAGGATAGA	57.4	57.4	37.5	
		AML2	GAACCCAAACACTTTGGTTTCC	60.6		45.45	
Fung.	Universal	8F	AGAGTTTGATCCTGGCTCAG	56	54.6	50	
		1492R	CGGTTACCTTGTTACGACTT	54.6		45	

3.8.4 Reaction Mixture

REDTaq ReadyMix PCR Reaction Mix with MgCl2 (pre-loaded with dye, Sigma-Aldrich) was used. All reaction mixtures were prepared in 200μl thin walled PCR tubes on ice (see Table 4).

Table 4: Reaction Mixture

Reagent	Volume (ul)	Concentration
REDtaq Readymix	25	1x
Primer F	1	0.1 - 1.0 uM
Primer R	1	0.1 - 1.0 uM
Template DNA	1	-
Microbial grade H20	22	-
TOTAL:	50	-

3.8.5 Thermal cycling

As per recommendations, annealing temperature (Ta) was set -5 °C below melting temperature (Tm) of the selected primers. The cycling program included a 10-min incubation at 95°C , 40 cycles consisting of 95°C for 15 s and 60°C for 60 s followed by 72°C for 30 s, and an additional incubation at 72°C for 10 min. This was optimised to a 2-minute incubation at 95°C , 40 cycles of 95°C for 15s for denaturation, annealing temp at lowest Ta (forward and reverse for each primer pairing) for 15s and an extension step at 68°C for 40s. Incubation followed for 10 minutes at 68°C and then held at 15°C .

3.8.6 Gel Electrophoresis

TAE was created using 242.5g Tris base, 57ml acetic acid (99%) and 100ml of .5M EDTA solution (brought up to pH 8 with NaOH), made up to 1L volume using dH20 (TAE x50). This was then diluted for further working concentrations of x1 TAE (x50 TAE 20ml + 980ml dH20) for buffer and gel. Ethidium Bromide (EtBr) was used at a concentration of 10 mgml (stock) for bacterial samples, while SYBRSafe (Thermo Fisher Scientific) DNA gel stain and Diamond Nucleic Acid Dye (Promega) was used for mycorrhizal samples. This was added to both agarose gel and TAE buffer for a final concentration of 0.5 ugml of gel/buffer (5μl of stock EtBr/Other dyes) per 100ml gel-buffer. 12% Agarose gel (1/2g agarose +100ml TAE) was

used. Preparation was performed via microwaving for 40s, stirring and allowing to cool. EtBr was then pipetted into the agarose once cooled and then poured into the gel mold. A comb was added, and the gel was allowed to cool for ca. 20min. Comb was removed and reagents could then be loaded once submerged in buffer. Gel was run at 75v for 40 minutes. Ladders used were Bioline Hyperladder 1kb (200bp-10kb) and Hyperladder IV (100bp-1013bp). This was used at a rate of 5 ul per lane (loading). Both ladders were used to enable a large range of bp identification.

3.8.7 DNA concentration/Spectrophotometry

A Nano-drop ND-1000 Spectrophotometry device was used to determine concentrations of extracted fungal and bacterial DNA prior to sequencing. Samples used had been extracted via previously mentioned methods and amplified by PCR. This was performed by pipetting $2\mu l$ of DNA/RNA free water on the pedestal, performing a blank test, followed by a control extraction (free of DNA) and then the samples, wiping the pedestal between each sample with a lint-free tissue. Absorbency was set at 260nm (for Nucleic acids), with an expected ratio of -1.8 (260/280nm) for pure DNA samples.

3.9 GCMS

GC-MS: A polymer coated filament was employed for solid phase micro-extraction (SPME), a Hewlett-Packard 5890 GC-MS system was used with in conjunction with the filament absorbency tool to absorb volatiles produced from *C. sativum*. This was achieved by harvesting shoots and leaves, weighing to maintain an equal sample size (3g) and placing in an inert 'oven bake' plastic bag and leaving for 20minutes. After this time, volatiles in the bag would have accumulated allowing for collection via the filament. The running program for analysis was 50C initial temperature (held for 2mins) with a final temperature of 280°C, rising at 10°C minute.

GC/MS data was then analysed in open-chrom with publicly available libraries to identify volatile compounds. These libraries contain relevant data on a variety of compounds to allow for streamlined interpretation and processing of GC/MS outputs. This information was then compared to previous research on the phenolic composition of Coriander (Potter and Fagerson

1990). This data was extracted to .csv and quantified in excel for total volatile concentration for each identified compound.

3.10 Micro-controllers and Sensors

The micro-controller/sensor element of this project evolved out of the disruption to planned activities caused by Covid-19, necessitating adaptation, innovation and self-reliance for the continuation of several research elements.

The micro-controllers used for several trials were either clones or genuine Arduino Nano's. This micro-controller was selected due to cost effectiveness, compatibility with C++, small footprint and availability of digital, analogue and I2C and GPIO ports. The arduino Nano requires a 5V connection, which enables cross-platform compatibility with a range of USB port sockets or batteries (see Figure 12).

A range of sensors were employed to use with the Arduino Nano (see Figure 13). The ability of cost-effective sensors used in both industry and domestic applications is vast (Catini et al. 2019; Novianto, Setiyowati, and Purnomo 2019). The sensors selected in this research are done so for amplification and validation of previous research results.

Raspberry Pi's (4B) were also used in this trial. A small platform computer, running Raspbian (Linux), allowed autonomous data collection from the sensors, with the ability for remote monitoring of data through Secure Shell (SSH), accessing the Pi as a server. This function was abandoned during the trials due to continuous complications with localized VPN networks. The micro-controller element of the trial fed data to the Pi with a typical serial (USB) connection.

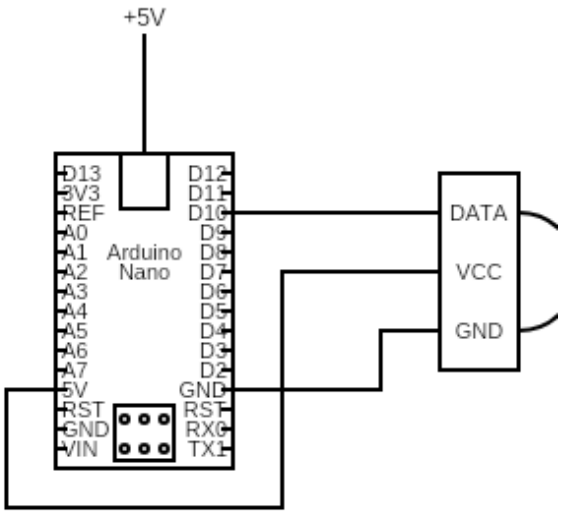

Figure 12: Simply circut diagram for the Arduino Nano microcontroller and generic sensor

Figure 13: An example of the sensors used throughout the latter phase of this reasearch.

3.10.1 RGB and luminosity

RGB values were measured using a sensor (Adafruit TCS34725) developed using a series of micro-controllers and batteries for increased portability. The sensor was placed *ca.* 1 cm away from the surface of 3 random leaves from each pot and left for 2 seconds to allow for processing. Each red-blue-green value was then recorded from an attached LCD screen (see Figure 14). Luminosity was also calculated using a sensor (Adafruit TSL2591). This was simply exposed to the glasshouse or ambient room (shelf life/supermarket) at crop height and left to self-calibrate for 5 minutes before results were recorded from an LCD screen. The use of RGB sensors was inacted to demonstrate alternative methods of assessing crop growth

(Seelye et al. 2011) and health (Zermas et al. 2015). The ranges of various colour values shown via sensors can indicate the concentration or absence of various phytochemicals such as chlorophyll (Yadav, Ibaraki, and Dutta Gupta 2010) or hormones like ethylene (Hofmann, Minges, and Groth 2021).

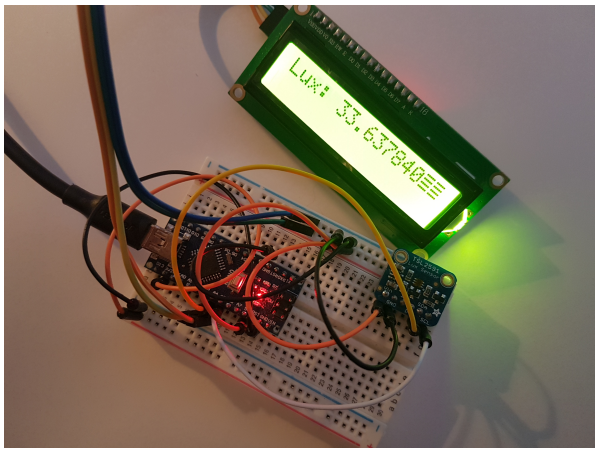

Figure 14: A prototyped board for Lux (luminisotity sensor)

3.10.2 Moisture

Soil moisture values were recorded using several capacitive soil sensors, calibrated against known values (full saturation at 100%, i.e. water, vs. air). Two sensors were used for each substrate and controlled via and Arduino Nano micro-controller, with data being fed into a raspberry Pi and recorded via Python/CSV. Sensors were buried until no capacitive material was exposed to air at a even distance between center of the pot and edge.

3.10.3 Humidity and Temperature

Humidity and temperature sensors, much like moisture and RGB sensors were operated in tandem with micro-controllers and Raspberry Pi's. Humidity and temperature sensors were primarily used for auto-calibration of SGP30 volatile gas sensors. This was achieved by a coded function to determine absolute humidity (AH, g/m^3) from relative humidity (RH, %) and temperature (°C) (Sensirion 2018). The sensors used to detect RH and temperature were cloned DHT22's/AM2302's.

3.10.4 Gas Sensors

Gas sensors (Pimoroni SGP30) were used to measure Total volatiles concentrations (TVoc), CO_2, H_2 and Ethanol. TVoc and CO_2 levels were determined via the H2 and Ethanol measurements, using the inbuilt algorithm stored in the EPROM (flash memory). Sensors were used in a series of 4/5 depending on trial. An atmospheric control sensor was set away from the trials by 50cm, other sensors were set in the middle of each treatment block, at just above pot height.

A multiplexer (TCA9548A clone) was used to split the I2C channels for SDA and SLC (Serial data line, serial clock line, respectively), as the SGP30 sensors shared the same I2C address.

3.11 Analysis and software

A variety of statistical and graphing packages were used in this study. The body of work was performed in R studio, Arduino IDE, Python 3.7.x., Openchrom, Excel, and Mike-Tex. Statistical analysis was performed mainly in R studio. Use of ANOVA, Tukey, Gini formed the basis for most analysis.

A large selection of packages and tools embedded in the RStudio IDE were used: R (Version 3.6.0; R Core Team 2019).