Final Report

Evaluation of alternative approaches for production of healthy PBTC mini tubers

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1. SUMMARY

1.1. Aim

Pre-basic tissue culture mini-tubers are the first tubers in the potato production chain and preservation of their high health status is of paramount importance to the industry and a requirement of the Seed Potatoes (Scotland) Regulations, 2015 and the Commission Implementing Directive 2014/21/EU. In the UK, PBTC mini-tuber production is mainly concentrated in Scotland. At present, UK mini-tuber production mainly relies on peat-based growing media, which is largely considered to be a low risk substrate for the presence of plant pathogens. However, there is a UK Government policy-led drive to reduce peat use by amateur growers by 2020 and by professional growers by 2030 to address environmental concerns. Growing media producers are reacting to this and introducing peat-free and peat-reduced alternatives for both amateur and commercial uses. This has resulted in a need to find alternative approaches that are suitable for producing disease-free mini-tubers in order to safeguard the future of the seed potato industry. This project investigated the use of peat-free and peat reduced growing media to produce disease-free potato mini-tubers and also considered the *in vitro* activity of a number of biological control agents to limit growth of important potato diseases.

1.2. Methodology

Peat-free and peat-reduced growing media were assessed for their ability to propagate disease-free potato mini-tubers in controlled environment cabinets, glasshouses and commercial site trials. Controlled environment cabinet experiments assessed plant growth rates in different growing media and the size of mini-tubers produced. Glasshouse and commercial site trials assessed plant tuber yields based on tuber number and total tuber weight per pot as well as individual tuber size and weight. In all three trial types, the mini-tubers produced were visually assessed for surface blemish disease symptoms and molecular diagnostics were used to detect any latent infections by *Pectobacterium atrosepticum*, *Spongospora subterranea*, *Streptomyces scabies*, *Polyscytalum pustulans*, *Helminthosporium solani* and *Colletotrichum coccodes*. The bacterial biological control agent (BCA) *Bacillus subtilis* and three isolates of a potential BCA *Aneurinibacillus migulanus* (Nagano, NCTC 7096 and E1) were assessed for their *in vitro* antifungal activity against a range of common potato pathogens using dual culture plate assays.

1.3. Key findings

Disease-free potato mini-tubers can be produced in peat-free growing media. However, tuber yield and individual tuber dimensions tend to be more variable in peat-free growing media with coir, pine bark and wood fibre typically yielding fewer tubers than peat. Wool compost has the most potential for producing mini-tubers in a peat-free system. However, as indicated by variation in tuber yield and quality parameters between glasshouse and the different commercial site trials, optimising this growing medium for use in commercial PBTC mini-tuber production is required, particularly with respect to watering regimes. Using peat-reduced growing media blends also shows promise to help limit the volume of peat used in PBTC mini-tuber production. None of the peat-free growing media alternatives appear to pose any additional risk to the plant health of PBTC mini-tubers compared to peat. In terms of biocontrol studies, *B*. *subtilis* showed stronger antifungal activity than the *A*. *migulanus* isolates *in vitro* studies.

1.4. Practical recommendations

- Peat-free and peat-reduced growing media can be used for propagating disease-free mini-tubers.
- Optimisation of these growing media and husbandry methods (nutrition and watering programmes) will be required to maintain current mini-tuber production levels.
- Bacterial biological control agents show potential to limit growth of potato pathogens.

2. INTRODUCTION

Seed (and ware) potatoes produced in the UK are descendants of pre-basic tissue culture (PBTC) mini-tubers, and it is essential that the high health status of these mini-tubers is maintained to ensure the health of future generations in the potato production multiplication chain (Figure 1). Currently there are six registered PBTC growers in the UK, five in Scotland and one in Northern Ireland, who produce mini-tubers from sterile microplants cultured in Scottish Government laboratories. Potato microplants are multiplied to required numbers in growers' own laboratories before being planted in secure polytunnels or glasshouses, protected from the outside environment, as well as pest and disease threats. PBTC facilities are subject to regular inspections by Scottish Government inspectors to ensure they meet the required standards to fulfil the demands of the Seed Potatoes Scotland (2015) Regulations (Anon, 2016) and ISPM 33 (Anon, 2015).

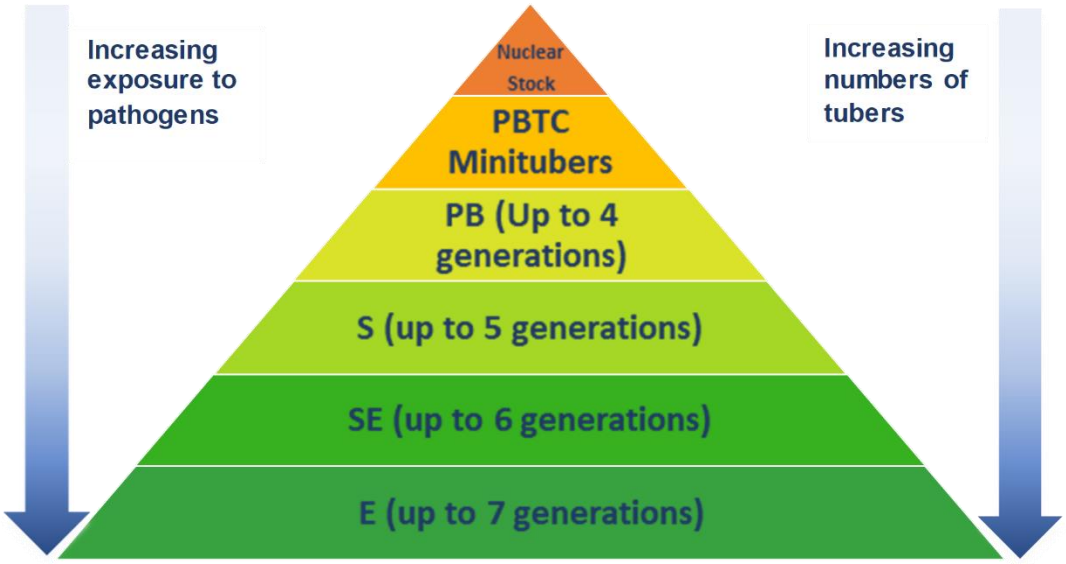

Figure 1. Potato mini-tubers are the first tubers in the potato production chain. Health of mini-tubers is essential to safeguard future generations to prevent the multiplication of seed-borne pathogens within the production system.

The Seed Potatoes (Scotland) Regulations 2015 (Anon, 2016) support EU Directive 2014/21EU (Anon, 2014) in advocating use of appropriate husbandry methods to keep mini-tubers free from disease and PBTC growers take every care to ensure optimal health of their mini-tubers. Microplants are usually transferred from culture medium to peat-based growing media to produce mini-tubers. At this stage, microplants could, potentially, acquire pathogens from the growing medium and some pathogens could persist in the multiplication chain.

Peat has a number of chemical and physical properties that makes it a suitable substrate for growing plants. It has low pH, low bulk density, good air-filled porosity and its low nutrient status means nutrition can be easily manipulated for specific plant needs (Alexander, 2014; Maher et al., 2008). Peat is widely considered to be pathogen-free (Schmilewski, 2008) which makes it an ideal growing medium for PBTC mini-tuber seed potatoes where disease avoidance is a requirement. However, there is a UK Government-backed drive to reduce the use of peat by 2030 for professional growers (DEFRA, 2011, 2018) and many growing media producers are developing alternatives. The horticulture sector is currently evaluating various growing media, assessing alternatives to peat to future safeguard the industry (Mulholland,

2016; Rivière et al., 2008). It is vital that the specific needs of potato mini-tuber production are considered, allowing growers to make informed choices as to the growing media they use. It is clear that the use of peat alternatives, in both professional and, especially, amateur markets, has increased in recent years. Denny and Waller (2015) reviewed peat use from 2012-2014 inclusive and found that, during that period, just over a third of growing media used by professional growers were peat alternatives whilst alternatives constituted around half of all amateur use. This contrasts to the relative amounts used in 1999 (Alexander, 2014) when peat alternatives were used much less frequently. Peat use by amateur growers was 15 times greater than alternatives and 19 times greater than alternatives in professional use.

The four most popular alternative growing media to peats are: wood fibre (33% of alternatives per volume supplied in 2014), bark (17%), green compost (22%) and coir (20%) (Denny and Waller, 2015). Wood-fibre based growing media are often, but not exclusively, derived from secondary processing of fresh or waste wood. Typically, wood-fibre is derived from tree species such as spruce (*Picea* spp.) or pine (*Pinus* spp.) and rarely contains bark (Maher et al. 2008). In regions where the large timber industries have thrived, pine bark has been an excellent renewable material for soilless growing media (Boyer et al., 2008). Wood-fibre is usually nitrogen poor although the nutritional status of the product will vary based on the processing method used (Maher et al., 2008). Bark from coniferous trees such Pine has been used in horticultural growing media for decades (Barrett et al., 2016). For use in growing media the bark needs to be screened down to particles of a desirable range from 5-20 mm. Pine bark usually requires composting to reduce phytotoxicity associated with secondary metabolites produced by the material and is usually low in nitrogen (Maher et al., 2008). Wood-based media can become nitrogen deficient due to issues with immobilisation of this element meaning these media would often require nutritional supplements (Buamscha et al., 2008; Jackson et al., 2009). Long term use of wood-based materials for growing media is unlikely to be sustainable given their use in bioenergy production and wood-based ethanol which would inevitability increase demand for the resource and therefore increase the economic value of forestry wastes (Barrett et al., 2016). Composted green waste is a highly variable growing medium with the chemical and physical properties of the product being dependent on the green waste used. It also suffers from issues with phytotoxicity due to incomplete composting (Maher et al., 2008). However, composted green waste has shown potential as a replacement for peat for some plant species (Zhang et al., 2013). Coir is a waste product of the coconut industry (Arenas et al., 2002). This by-product is derived from dust and short fibrous material from the thick mesocarp layer of the coconut fruit and is also known as coir pith, coir meal, coir dust and coco peat (Barrett et al., 2016). Coir has many similar physical and chemical properties to peat which has made it a potential replacement growing medium. However, despite some plant species being able to develop root systems more rapidly in coir (Maher et al., 2008), this product is typically low in nitrates and therefore when used alone in a peat-free growing medium can result in poorly performing plants without supplying the coir with additional nitrate (Drake et al., 2016; Scagel, 2003).

Despite the long-term approach to move away from peat as a horticultural growing medium there is still relatively little known regarding the production of disease-free plants from alternative growing media. During the processing of wood-fibre the materials are heated to 80-90°C meaning that the product should be free from plant pathogens (Maher et al., 2008). Coir has been shown to be rich in suppressive microorganisms (Hyder *et al*., 2009) that can counter some soil-borne pathogens, such as *Fusarium solani*. Wood-fibre and pine bark-based peat-free growing media can show higher levels of pathogen contamination compared to peat-based media although this can be variable from batch to batch (Drake et al., 2016). Green compost waste can be a source of pathogens. As the composting process doesn't involve sterilisation, some pathogens are able to survive it depending upon how long and how high temperatures are maintained during composting (Noble et al., 2009; Noble and Roberts, 2004). Compost moisture is also likely to be an important factor to eradicate pathogens during composting.

Should alternative growing media prove to be a potential plant health risk to PBTC mini-tuber production, then the industry will require additional disease management strategies to limit the risk to the crop. Fungicides are used to manage potato diseases during the growing season and post-harvest (Carnegie et al., 1990; Cayley et al., 1981; Hide et al., 1994; Hide and Cayley, 1980). However, the prolonged use of these synthetic chemicals has led to control problems in some cases. Dry rot is predominantly caused by the *Fusarium* species *Fusarium sulphureum*, *F. coeruleum*, *F. culmorum* and *F. avenaceum* and for a long time was controlled by post-harvest application of thiabendazole (TBZ) fungicides (Bojanowski et al., 2013). However, insensitivity to TBZ fungicides was soon observed in pathogen populations (Hide et al., 1992; Carnegie and Cameron, 1992; Carnegie et al., 1994) and the use of other fungicides has also resulted in issues of reduced chemical sensitivity in the population (Choiseul et al., 2007; Gachango et al., 2012). Issues with TBZ insensitivity have also been observed for skin spot (Carnegie and Cameron, 1992) and silver scurf (Hide and Small, 1993) control. Throughout agriculture there is a movement to reduce commercial reliance on the use of fungicides to maintain crop yields and quality (Lamichhane et al., 2016). Despite the widespread use of these agrochemicals there is still debate about their safe use within the modern environment. Reduced efficacy in disease control and with changing legislation soon likely to limit the number of products available for use to control crop diseases (Hillocks, 2012), there is a concerted effort to identify novel approaches to maintain food production.

Biological control agents (BCAs) offer alternative control strategies to fungicides for the management of a number of diseases (Fravel, 2005). For management of potato diseases there would be a requirement for BCAs to be effective against in-field developing diseases as well as those that affect the crop post-harvest. Investigations in the efficacy of potential BCAs as disease management options for potatoes diseases have provided varied results. Fungal endophytes such as *Trichoderma harzianum* (Wilson et al., 2008), *Trichoderma atroviridae*, *Epicoccum nigrum* and *Alternaria longpipes* (Lahlali and Hijri, 2010) showed potential as biological control agents for the control of black scurf in potato. Brewer and Larkin (2005) showed that a number of soil-dwelling bacterial and fungal micro-organisms reduced incidence and severity of stem canker and black scurf on potatoes in the field but disease control varied between trials. The search for effective BCAs that are able to reliably control a range of important diseases is still on-going. As such, very few identified BCAs have been commercialised. Two successes have been the bacterium *Bacillus subtilis* strain QST 713 and the fungus *Gliocladium catenulatum* fungal Strain J1446 which have provided disease control in a number of cropping systems (Ingram and Meister, 2006; McQuilken et al., 2001; Reiss and Jørgensen, 2017; Rose and Parker, 2003). The bacterium *Aneurinibacilus migulanus* (syn. *Bacillus brevis*, syn. *Brevibacillus brevis*; Shida et al., 1996), is a potential BCA that has shown some promise limiting growth of a number of pathogens (Alenezi et al., 2017; 2016a; 2016b; Edwards and Seddon, 2001) including fungal pathogens in other solanaceous protected crops (McHugh and Seddon, 2002). The effectiveness of *B. subtilis* and *A. migulanus* against the wide range of disease threats to potato remains to be determined.

This project will consider the use of peat-free growing media to determine alternatives to peat for use in PBTC mini-tuber production for the UK potato industry. The aim is to evaluate the potential to produce disease-free PBTC mini-tubers in a range of peat-free or peat-reduced growing media in controlled environment cabinet experiments, under glasshouse conditions and in trials at the premises of commercial PBTC mini-tuber producers. Tuber yields per plant will be assessed as will individual tuber quality parameters. Molecular diagnostics to detect potato pathogen DNA will be used to determine if peat-free growing media poses a greater plant health risk to mini-tuber production than peat. In addition, a number of biological control agents will be tested to ascertain whether or not they have action against a range of potato pathogens.

Objectives

Objective 1: Identify and source growing media suitable for PBTC production.
Objective 2: Test growing media for a range of pathogens using bait plants grown in microcosm experiments.
Objective 3: Assess cultivation of mini-tubers in growth media under glasshouse conditions.
Objective 4: Evaluate mini-tuber production in selected growth media in trials at commercial PBTC mini-tuber producers' premises.
Objective 5: Determine efficacy of biological control agents against pathogens of potatoes
Objective 6: Collate results, perform statistical analysis and complete final report

3. MATERIALS AND METHODS

Growing media

A range of alternative peat-free growing media were identified from commercial suppliers and compared against an industry standard peat-based growing medium (Fig. 1). Details of each specific growing medium are presented in Table 1.

Fig. 1 Growing media used in this project. (a) Peat, (b) Coir, (c) Peat/Coir (60:40 v/v), (d) Pine bark, (e) Wood fibre, (f) Wool compost

Table 1 Growing media and suppliers used in this research

Growing medium	Supplier
Peat-based mini-tuber mix	Sinclair Pro, Cheshire, UK
Coir	ICL Speciality Fertilisers, Suffolk, UK
Levington Advance, pine bark 0-8mm	ICL Speciality Fertilisers, Suffolk, UK
Levington Advance, FIBA GRO wood fibre	ICL Speciality Fertilisers, Suffolk, UK
Wool compost	Dalefoot Composts, Cumbria, UK

Nutrient levels, pH and conductivity of each growing media type (Table 1) was determined using the Horticultural loamless compost protocol of the Analytical Services group at SAC Commercial Ltd (https://www.sruc.ac.uk/info/120148/analytical_services_soils_feeds_etc). Two replicate samples per growing medium, sampled from different batches provided by each commercial supplier were tested.

Potato varieties

Potato varieties used in this project were selected based on their relevance to the industry. The total area on which each variety was grown in Scotland between 2014 and 2019 was determined from data collated on the SPUDS database (https://www.sasa.gov.uk/spcs-myspuds). Varieties cv. Hermes and cv. Maris Piper were planted over the greatest area in Scotland between 2014 and 2019 (Fig. 2).

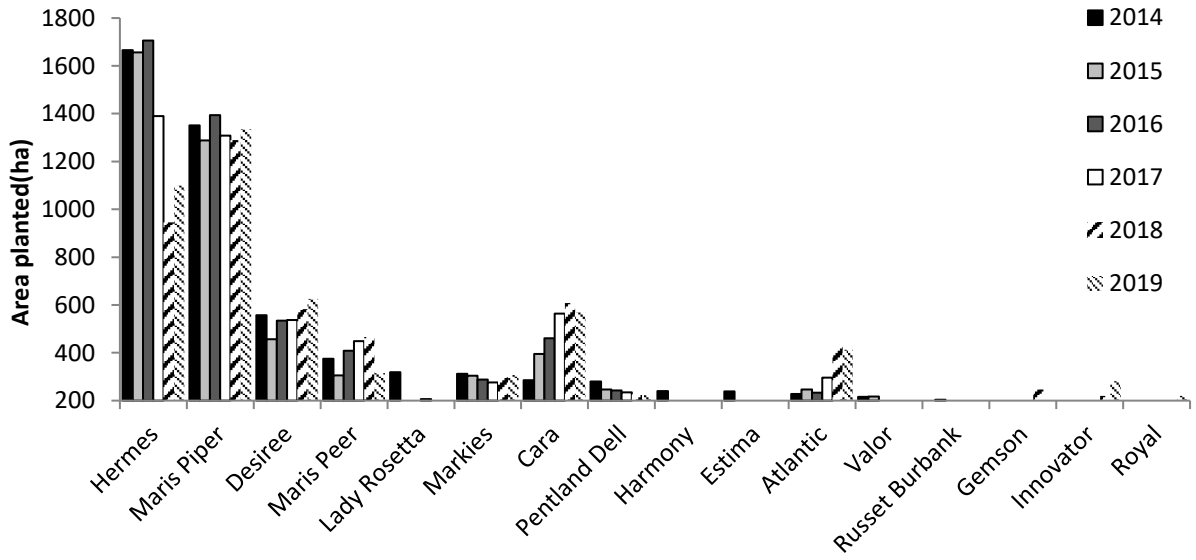

Fig. 2 Area planted with potato varieties in Scotland 2014-19. Only those varieties with total planting areas greater than 200 hectares grown are shown.

Both cv. Hermes and cv. Maris Piper are main crop maturity varieties. Disease resistance ratings vary between the two varieties with cv. Maris Piper particularly susceptible to common scab and powdery scab compared to cv. Hermes (Table 2).

Table 2 Disease resistance ratings for the potato cvs Maris Piper and Hermes from the AHDB Potato variety database

Disease (Pathogen)	**Maris Piper**	**Hermes**
Common scab (*Streptomyces scabies*)	1[a]	6
Blackleg (*Pectobacterium atrosepticum*)	5	6
Dry rot (*Fusarium sulphureum*)	2	
Dry rot (*Fusarium coeruleum*)	3	
Skin spot (*Polyscytalum pustulans*)	4	
Silver scurf (*Helminthosporium solani*)	4	
Black dot (*Colletotrichum coccodes*)	4	
Powdery scab (*Spongospora subterranea*)	3	8
Tuber blight (*Phytophthora infestans*)	5	4
Foliar blight (*Phytophthora infestans*)	4	3

[a] 9 = high resistance, 0 = low resistance http://varieties.ahdb.org.uk/

Microplant propagation

Microplants of cv. Hermes and cv. Maris Piper were obtained from the Nuclear Stock Initiation Unit at SASA, Edinburgh, UK. Stock microplants were subcultured on Murashige and Skoog medium (1 x Murashige and Skoog medium [MP Biomedical, California, USA], 3% sucrose w/v, 0.7% w/v Technical agar No. 3 [Oxoid, Thermo Fisher Scientific]) and grown for 21 days at 18°C with a 16:8 hour day:night cycle in a Fisons Fi-totron 600H (Weiss Technik, Loughborough, UK).

Microcosm experiments

Microcosms were prepared by placing two sterile 66 cm^3 plastic cups (Nupik-flo Ltd., West Sussex, UK) on top of one another in a sterile flow cabinet. The bottom of the microcosm was filled to a depth of 35 mm with growing media and a single microplant placed in to the growing media (Fig. 3). Growing media was watered to dampen the substrate but not result in oversaturation of the medium and the top of the microcosm attached to the bottom using micropore tape. Microcosm experiments tested how potato microplants developed in peat, coir, pine bark, wood fibre and wool-compost growing media, as well as blends of different growing media including peat/coir and wool/coir at ratios of 60/40 (v/) and 20/80 (v/v) and three-way blends of peat/coir/wool and ratios of 30/40/30 (v/v/v) and 20/40/40 (v/v/v).

Figure 3. Microcosm set up used for assessing the growth of microplants in different growing media. a=35 mm depth of growing medium; b=360 mm microcosm height.

For each growing medium tested, three replicate microcosms were prepared for both cv. Hermes and cv. Maris Piper. Microcosms were placed in a Fitotron SGC120 standard growth chamber (Weiss Gallenkamp Technik) and plants grown at 20°C with a 16 hour photoperiod (Figure 4). After 12 weeks growth, mini-tubers produced in each microcosm were washed free of growth media using sterile distilled water and stored in an extraction bag (BioReba AG, Reinch, Switzerland) at -20°C prior to DNA extraction.

Figure 4 Experimental setup for microcosm screening assays used to determine whether pathogen-free plants can be cultured in different growing media.

Glasshouse experiment

Based on the growth of potato plants in the microcosm bioassays, the nutrient analysis of growing media types and following discussions with mini-tuber growers and growing media produced, the peat, peat/coir blend and wool media were used with no additives whereas the

pine bark, wood fibre and coir media were modified to facilitate plant growth. To each of the pine bark, wood fibre and coir media, recommended rates of the following fertilisers and soil amendments were added and thoroughly mixed through the medium: Osmocote® controlled release fertiliser, Start n grow ® base fertiliser, lime and Micromax ® micronutrients (ICL Speciality Fertilisers). Each growing medium was placed into 5 L plastic pots. Four replicate pots were prepared for each variety and media combination and a single four-week-old microplant transplanted into each pot. The experiment was designed in randomised complete blocks with two treatment factors (variety and media) using Edgar v1.0 software (http://www.edgarweb.org.uk/). Microplants were grown to maturity in a temperature controlled glasshouse under a 16:8 hour day:night photoperiod set at 18°C during the day phase and 15°C during the night phase. Pots were watered as required with tap water, which was supplemented with 0.1% (v/v) wetting agent (H2Gro, ICL Speciality Fertilisers) every two weeks to optimise water management. After 16 weeks growth, watering was withheld from plants and haulms were removed to allow the tubers to mature as is standard practice in commercial mini-tuber production. Two weeks later tubers were harvested from each replicate pot, placed in to paper bags and stored at 5°C until processing. Each individual mini-tuber was measured from rose to heel end and weighed. The weight of all tubers from a single replicate pot unit was determined as the overall yield of each plant.

All harvested mini-tubers were assessed for disease, scoring the proportion of the tuber surface area covered with surface blemishes caused by powdery scab, common scab, silver scurf, black scurf, black dot, skin spot or the incidence of blackleg symptoms. Peel samples were taken from heel to rose end of tubers using a standard nylon handled potato peeler (Victorinox AG, Schmiedgasse, Switzerland), placed in an extraction bag (BioReba AG, Reinch, Switzerland) and stored at -20°C until required.

Trials at commercial PBTC mini-tuber sites

To evaluate the use of peat-reduced growing media, trials were conducted at the facilities of four commercial PBTC mini-tuber producers in Scotland. Due to the commercial sensitivity of PBTC mini-tuber production, trial sites were anonymised. Each producer was provided with microplants of cv. Hermes and cv. Maris Piper from the Nuclear Stock Unit at SASA, Edinburgh, UK. Microplants for each trial were subcultured following site-specific commercial protocols. Each site was also provided with the growing media to be tested: peat mini-tuber mix, wool compost and two peat/coir/wool blends (30/40/30 v/v/v and 20/40/40 v/v/v). Trial site A and D tested the four growing media with both varieties whereas trial site B and C tested cv. Hermes and cv. Maris Piper, respectively. Microplants were sown into 4 L poly pots containing growing medium and arranged in blocks (40-120 plants per block) at each site according to a randomised block trial design. Pots were watered following local procedures used at each commercial premises. Tubers were harvested after 12-16 weeks following standard practice at each commercial site. To harvest mini-tubers at each trial site, replicate samples were collected each consisting of the tubers harvested from ten randomly selected plants. A minimum of four replicates were collected from each site. After harvest, tuber numbers and total tuber yield were calculated per plant. Individual tuber length and weight were measured from a subset of four or five replicate samples from each variety and medium combination at each site. Tubers were graded in to four size categories: <15 mm, 15-20 mm, 20-30 mm and > 30 mm. Fifteen tubers were randomly selected from four replicates and visually assessed for surface area covered with surface blemishes caused by powdery scab, common scab, silver scurf, black scurf, black dot, skin spot or the incidence of blackleg symptoms. Peel samples were taken from heel to rose end from three individual mini-tubers sampled from each replicate pot for all variety and growing media combinations, placed in an extraction bag and stored at -20°C until required.

DNA extraction

Mini-tuber tissue was homogenized with buffer RLT (Qiagen, Hilden, Germany) and 0.01 % (v/v) antifoam B emulsion (Sigma-Aldrich, St. Louis, MO, USA) using a Homex 6 homogenizer (BioReba AG). The extract was aliquoted into 500 µL samples in 2 mL Eppendorf tubes and stored at -20°C until processing. Each sample was thawed and then incubated at 65°C for 10 minutes prior to centrifugation at 12000 rpm for 5 minutes. DNA was extracted from 300 µL of the extract supernatant using the BioSprint 15 DNA Plant kit (Qiagen) with a KingFisher™ mL Purification system (Thermo Fisher Scientific, Paisley, UK) following the manufacturer's instructions. *Pectobacterium atrosepticum* collected from Moray, Scotland, UK in 2013 was sub-cultured in liquid culture in pectate enrichment medium (12.4 mM K_2HPO_4; 16.3 mM $(NH_4)_2SO_4$; 5.3 mM $MgSO_4$; 0.34% (w/v) sodium polypectate) for 24 hours at 20°C in a Stuart SI5000 Shaking orbital incubator (Cole-Palmer Ltd., Staffordshire, UK) at 150 rpm. Cultures of *P. atrosepticum* were centrifuged at 10000 rpm for 5 minutes and the pellets stored at -20°C. *Spongospora subterranea* DNA was isolated from sporeballs from infected potato whereas DNA from fungal pathogens was isolated from mycelia collected from 14 day old cultures grown on potato dextrose agar (PDA). *S. subterranea* and fungal isolates were all taken from the SASA culture collection and respresent isolates collected from potatoes grown in Scotland. Fungal and *S. subterranea* samples were processed using a Tissuelyser II (Qiagen, Hilden, Germany) and stored at -20°C prior to DNA extraction. Pathogen DNA was extracted using the Illustra Nucleon Phytopure DNA extraction kit (GE Healthcare, Buckinghamshire, UK). DNA was quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific). All DNA samples were diluted to 10 ng μL^{-1} and 50 ng DNA added to each reaction.

qPCR diagnostic assays

Pathogen specific quantitative PCR (qPCR) assays were used to test for the presence of *P. atrosepticum* (Humphris et al., 2015), *S. subterranea*, *S. scabies* (Qu *et al*., 2011), *P. pustulans* (Lees *et al*., 2009), *H. solani* (Cullen *et al*., 2001) and *C. coccodes* (Cullen et al., 2002). DNA quality was assessed using a qPCR assay designed for the potato cytochrome oxidase (COX) gene (Weller et al., 2000). qPCR amplification of pathogen DNA from tubers was done using the Takyon™ Rox Probe Mastermix dTTP Blue system (EuroGentec, Liege, Belgium) following the manufacturer's protocol. qPCR conditions were as follows: 50°C for 2 minutes followed by an activation phase of 3 minutes at 95°C and then 40 cycles of 95°C for 10 seconds and 60°C for 1 minutes. All qPCR reactions were run on a CFX96 real-time PCR detection system (BIO-RAD, Hertfordshire, UK). Primers designed to detect the potato cytochrome oxidase (COX) gene were used as a positive control to confirm that amplifiable DNA had been extracted from tuber samples (Table 4).

Table 4 Primers used for qPCR diagnostics of potato pathogens in mini-tuber tissue

Target fungus (Disease)	***Oligonucleotide primers***		
		Final conc.	**Sequence 5'-3'**
Polyscytalum pustulans	**PPUSTF1**	0.3 µM	AGC GCC CCA CAG AAG CC
(Skin spot)	**PPUSTR2**	0.3 µM	GAC CGA ACT TCT CCG AGA GGT
	PPUSTPR1	0.1 µM	CGG CTC TAA ACC CTA CCG AAG TAG GGT AGC [a]
Colletotrichum coccodes	**CcTqF1**	0.5 µM	TCT ATA ACC CTT TGT GAA CAT ACC TAA CTG
(Black dot)	**CcTqR1**	0.5 µM	CAC TCA GAA GAA ACG TCG TTA AAA TAG AG
	CcTqP1	0.15 µM	CGC AGG CGG CAC CCC CT [b]
Streptomyces scabies	**StrepF**	0.1 µM	GCAGGACGCTCACCAGGTAGT
(Common scab)	**StrepR**	0.1 µM	ACTTCGACACCGTTGTCCTCAA
	StrepP	0.3 µM	TCGGTGATCCAGTACTTTCCGTCGGC [c]
Spongospora subterranea	**SponF**	0.25 µM	CTT TGA GTG TCG GTT TCT ATT CTC CC
(Powdery scab)	**SponR**	0.25 µM	GCA CGC CAA TGG TTA GAG ACG
	SponP	0.05 µM	TCT TTC AAG CCA TGG ACC GAC CAG A [a]
Pectobacterium atrosepticum	**EcaF**	0.3 µM	ACATTCAGGCTGATATTCCCCCTGCC
(Blackleg)	**EcaR**	0.3 µM	CGGCATCATAAAAACACGCC
	EcaP	0.1 µM	CCTGTGTAATATCCGAAAGGTGG [a]
Helminthosporium solani	**HsTqF1**	0.3 µM	GTT TCA GCG GCC GCA AG
(Silver scurf)	**HsTqR1**	0.3 µM	TTC AGA TAC AAG GGT TTA AGG GAT TC
	HsTqP1	0.1 µM	TCG GAA CCC TCT GTC TAC CTG TAC CAC TTG TT [a]
Cytochrome oxidase	**CoxF**	0.3 µM	CGT CGC ATT CCA GAT TAT CCA
(COX)	**CoxR**	0.3 µM	CAA CTA CGG ATA TAT AAG AGC CAA AAC TG
	CoxP	0.1 µM	TGC TTA CGC TGG ATG GAA TGC CCT [a]

Probe modifications: [a] 5' 6-FAM; 3' BHQ-1; [b] 5' HEX; 3' TAMRA; [c] 5' CAL Fluor Red610; 3' BHQ2

Biological control agents

Biological control agents were selected based on previous data. *Aneurinibacillus migulanus* has shown promise in field trials against diseases of solanaceous crops (McHugh, 2003; McHugh and Seddon, 2002) whereas *Bacillus subtilis* has been used as a commercial biological control agent in a number of agronomic and horticultural systems (Ingram and Meister, 2006; Reiss and Jørgensen, 2017). Three isolates of *A. migulanus* were provided by Prof. Stephen Woodward, University of Aberdeen, Scotland. *A*. *migulanus* Nagano and *A*. *migulanus* NCTC 7096 both produce a known antimicrobial metabolite, gramicidin S (Alenezi et al., 2016a). *A*, *migulanus* E1 is an N-methyl-N'-nitro-N-nitroguanidine mutant of *A*. *migulanus* Nagano which lacks a D-phenyl-alanine activating and or racemizing enzyme and does not produce gramicidin S (Iwaki et al., 1972). Isolates of *A*. *migulanus* were stored on tryptone soy agar (TSA) at 30°C. *Bacillus subtilis* strain QST 713 was isolated from the commercial biological control product Serenade® ASO (Bayer CropScience, Cambridge, UK). *B*. *subtilis* was isolated by serial dilutions from commercial product and incubation for three days at 30°C. Pure bacterial cultures were used for comparative purposes in experiments to remove any potentially confounding effects of the commercial formulation which may improve biological control activity. *B*. *subtilis* was kept on potato dextrose agar (PDA) plates for long term storage at 30°C.

Pathogen isolates

A range of fungal potato pathogens from the SASA culture collection (Table 5) were used to assess the antimicrobial effect of each BCA. All potato pathogens were stored on PDA plates at room temperature in the dark. The antimicrobial effect of *A*. *migulanus* and *B*. *subtilis* was also assessed against the fungal BCA *Gliocladium catenulatum*. *G*. *catenulatum* strain J1446 was isolated from Prestop® (Verdera Oy, Espoo, Finland). A 0.5% w/v solution of Prestop® was prepared and serial diluted on to PDA and incubated for 14 days at room temperature to isolate the fungus. The isolation of *G*. *catenulatum* was confirmed by PCR analysis (Paavanen-Huhtala et al., 2000).

In vitro dual culture plate assays

A. *migulanus* inoculum was prepared by transferring bacterial cells from stock TSA plates into 10 mL tryptone soy broth (TSB) and incubating at 30°C for 24 hours under ambient light levels and constant agitation at 180 rpm. After 24 hours growth, variation in inoculum concentration was assessed by determining the number of colony forming units (CFUs) estimated by serial dilutions of liquid cultures in TSB and plating on TSA. CFUs were counted after incubation at 30°C for 48 hours for *A*. *migulanus* and 24 hours for *B*. *subtilis*. Dual culture plates were prepared by streaking a vertical line of bacterial cells positioned 30 mm from the edge of a 90 mm diameter PDA petri dish and incubating at 30°C for three days for *A*. *migulanus* and one day for *B*. *subtilis*. Suspensions of *A*. *migulanus* contained concentrations of 10^7-10^8 cfu mL. *B*. *subtilis* suspensions contained concentrations of 10^8-10^9 cfu mL. Control plates were prepared by streaking a line of TSB. After incubation a 5 mm agar plug was excised from a 14 day old test pathogen PDA plate and placed on the TSA plate, 30 mm away from the bacterial BCA line. Dual culture plates were incubated at room temperature and pathogen radial growth was measured after 4, 7, 10, 14 and 21 days incubation for all isolates except the slower growing *H*. *solani* and *P*. *pustulans* cultures which were measured at 14, 28 and 42 days to assess potential growth inhibition (Dussart et al., 2018). Inhibition of fungal growth was determined using the formula: % inhibition = [(Radial growth on control plate - radial growth on BCA plate)/Radial growth on control plate] X 100. The assay assessed growth for each pathogen on three replicate dual culture plates with data collected from three independent experiments.

Table 5 Fungal isolates used in this study

Pathogen	Isolate	Year	Location
Botrytis cinerea	P5	2008	*
Colletotrichum coccodes	P74	2010	Scotland
Colletotrichum coccodes	P78	2009	Scotland
Fusarium coeruleum	P60	2008	Scotland
Fusarium coeruleum	P67	2009/10	Scotland
Fusarium culmorum	P13	Pre-2007	Scotland
Fusarium sulphureum	P28	2005	Scotland
Fusarium sulphureum	P62	2008	Scotland
Gliocladium catenulatum	J1446	2018	*
Helminthosporium solani	P27	Pre-2007	Scotland
Helminthosporium solani	P113	2017	Scotland
Phoma eupyrena	P70	2009	Scotland
Phoma eupyrena	P77	2009	Scotland
Phoma exigua	P53	2008	Scotland
Phoma exigua	P55	2008	Scotland
Phoma foveata	P26	Pre-2007	Scotland
Phoma foveata	P52	2006/2007	Scotland
Phoma foveata	P54	2008	Scotland
Polyscytalum pustulans	P1	*	*
Polyscytalum pustulans	P76	2010	Scotland
Rhizoctonia solani	P2	Pre-2007	*[a]
Rhizoctonia solani	P35	Pre-2007	Scotland [a]
Rhizoctonia solani	P106	2001	England [b]
Rhizoctonia solani	P109	Pre-2007	UK [c]

* Information not available for this isolate; [a] Anastomosis group AG3; [b] Anastomosis group AG4; [c] Anastomosis group AG8

Statistical analyses

All data was analysed using GenStat v.14 (Payne et al. 2009). A general linear model (GLM) was used to assess differences in pH, conductivity and nutrient levels between growing media with replicate and growing medium as factors. Changes in microplant height during growth in different growing media in microcosm experiments was assessed using linear mixed modelling of repeated measurements to evaluate differences between each variety, at the different time points grown in the various growing media. Fixed factors included experiment, variety, medium and days post transplantation as well as the interactions between these factors. The random factor was the individual microcosm-by-days post transplantation interaction term. An unstructured covariance matrix was used to fit the data to the model. Significant differences between plants propagated in each of the growing media at different days were subsequently assessed using a t-test. Variation in mini-tuber size produced in different growing media was assessed using a GLM with variety, growing medium and the interaction between these two terms as factors within the model. For the glasshouse experiment GLM analysis was used to assess variation in tuber number, tuber yield, tuber size and tuber weight attributed to the effects of experiment, variety, growing medium and the interactions between these terms. The contribution of site, medium, variety and the interactions between site and medium and variety and medium towards the variation observed for each measurement from the site trial experiments was assessed using a GLM. Variation in tuber grading size categories was assessed using a generalised linear model of binomial proportions testing the contributions of site, medium and variety as well as the interactions between and site and medium and medium and variety. To determine the effects of each BCA on *in vitro* growth of target pathogens a GLM was used assessing the contribution of the factors experiment, the day radial growth was

measured, the treatment (control or BCA) and the interaction between day and treatment. Significant differences between treatments at different days were assessed using subsequent t-tests.

4. RESULTS

Growing media nutrient analysis

Compared to peat, wood fibre had significantly (P = 0.020) lower pH whereas the pH of the wool compost was significantly (P = 0.016) higher (Fig. 5A). Conductivity was also variable between the different growing media with significantly (P < 0.001) lower conductivity recorded in pine bark medium and significantly (P = 0.035) higher values found in wool compost (Fig. 5B). Conductivity data could not be determined from the wood fibre medium. Significantly lower levels of nitrate (P <0.05; Fig. 6A), extractable calcium (P < 0.001; Fig. 6D) and magnesium (P < 0.001; Fig. 6E) were found in coir, pine bark, wood fibre and wool compost compared to peat. Phosphorous levels were significantly lower in coir (P = 0.005), pine bark (P = 0.004) and wood fibre (P = 0.001) but higher in wool compost (P = 0.001; Fig. 6B). Potassium levels were significantly higher in coir (P = 0.009) and wool compost (P < 0.001) compared to peat (Fig. 6C). There were also significantly higher (P = 0.005) sodium levels in coir compared to peat (Fig. 6E).

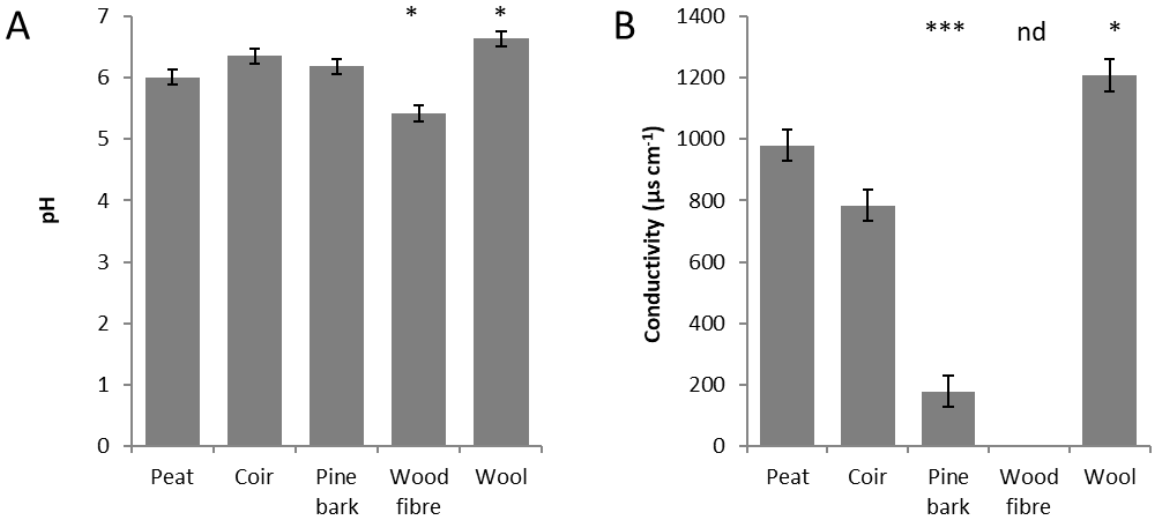

Fig. 5 Electrochemical properties of growing media. (A) pH and (B) conductivity.
*** = P < 0.001; * = p < 0.05 indicating a significant difference between growing media compared to peat. nd = not determined

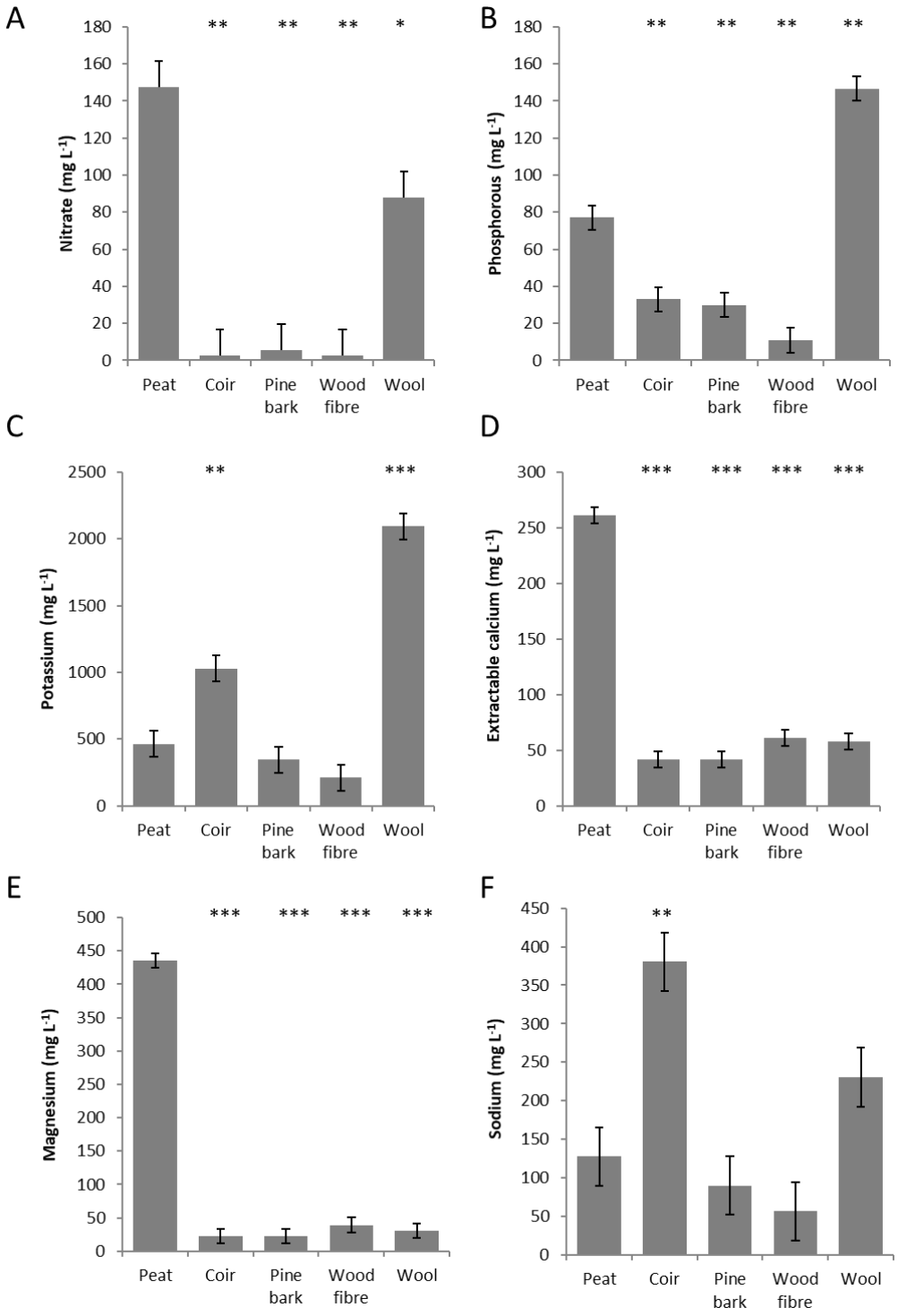

Fig. 6 Nutrient analysis of growing media. (A) nitrate; (B) phosphorous; (C) potassium; (D) extractable calcium; (E) magnesium; (F) sodium. *** = P < 0.001; ** = P < 0.01; * = P < 0.05 indicating a significant difference between growing media compared to peat.

Testing peat alternative growing media using microcosm controlled environment cabinet experiments

Microplant development in peat-based compost, coir, pine bark, wood fibre, wool compost and a blend of peat/coir (60/40 v/v) was assessed in microcosm experiments. Significant effects on plant height could be attributed to variety ($P = 0.008$), growing medium ($P< 0.001$), days post transplantation (dpt; $P< 0.001$) and the interactions between days post transplantation and the variety ($P = 0.033$) or medium ($P < 0.001$), as well as the interaction between variety and medium ($P = 0.029$). Within 14 days post transplantation (dpt) microplants of both varieties grown in pine bark medium appeared stunted, and spindly with occasional chlorotic lower leaves whereas those microplants grown in coir and wood fibre appeared healthy and green but were smaller than plants grown in peat, peat/coir (60/40) blend and wool compost which all looked healthy with green foliage. Differences in microplant development could be seen in plants by 28 days post transplantation (dpt) in to the different growing media (Fig. 7A and B). Microplants of both cv. Maris Piper and cv. Hermes looked green and healthy when grown in peat, the peat/coir (60/40) blend and the wool compost. However, microplants grown in coir alone had pale green foliage with some chlorotic older leaves which were particularly prevalent in cv. Hermes. Plants of cv. Maris Piper grown in pine bark medium had green upper leaves but chlorotic and senescent lower leaves compared to cv. Hermes whose leaves were generally chlorotic or senescent. Both varieties had dark green upper leaves when grown in the wood fibre medium with chlorotic older leaves. All plants grown in the peat-free growing medium, except the wool compost, were stunted compared to those grown in peat. Growth retardation continued in pine bark, coir and wood fibre from 42 dpt onwards for both varieties whereas microplants remained healthy in peat, wool and the peat/coir (60/40) blend. At 56 dpt some of the plants grown in pine bark medium were starting to show signs of hyphal colonisation along the stem. By 70 dpt plants grown in coir were senescing with little green leaf area (GLA) visible. Plants grown in pine bark medium were also senescent with hyphal growth clearly visible on the decaying stems. Both varieties were stunted and spindly when grown in wood fibre although more GLA was present in cv. Maris Piper plants compared to cv. Hermes plants. Plants grown in the wool compost were generally green and healthy at 70 dpt. However, some cv. Hermes plants had areas of chlorosis on lower leaves. Healthy looking plants were also noted in the peat/coir (60/40) blend despite cv. Hermes plants exhibiting a paler green coloration and some of the cv. Maris Piper plants showing chlorosis on the older leaves. The plants gown in peat were healthy with green foliage. At 84 dpt plants grown in peat looked the healthiest with most of the upper plant parts still green. Plants grown in the wool compost and the peat/coir (60/40) blend also appeared healthy although leaves were, in general, paler green with some of the older leaves showing signs of chlorosis. Both varieties grown in the other three growing media were senescent at the end of the experiment with evidence of hyphal growth visible on the decaying tissues.

To further assess plant development in each growing medium, the size of mini-tubers produced in each medium was measured (Fig. 7C). There was no significant difference in the size of mini-tubers produced by the two varieties ($P = 0.930$) but there were significant differences in tuber size when grown in the different growing media ($P < 0.001$). Mini-tubers produced in pine bark ($P = 0.023$) or wood fibre ($P = 0.016$) were significantly smaller than those produced in peat, whereas wool-grown plants produced larger mini-tubers than those from peat ($P = 0.004$). No interaction effect between variety and growing medium was observed ($P = 0.954$). None of the mini-tubers produced in any of the growing media showed signs indicative of surface blemish diseases. qPCR analysis confirmed that *P. atrosepticum*, *S. subterranea*, *S. scabies*, *P. pustulans*, *H. solani* and *C. coccodes* were not present in mini-tubers grown in any of the tested growing media (data not shown).

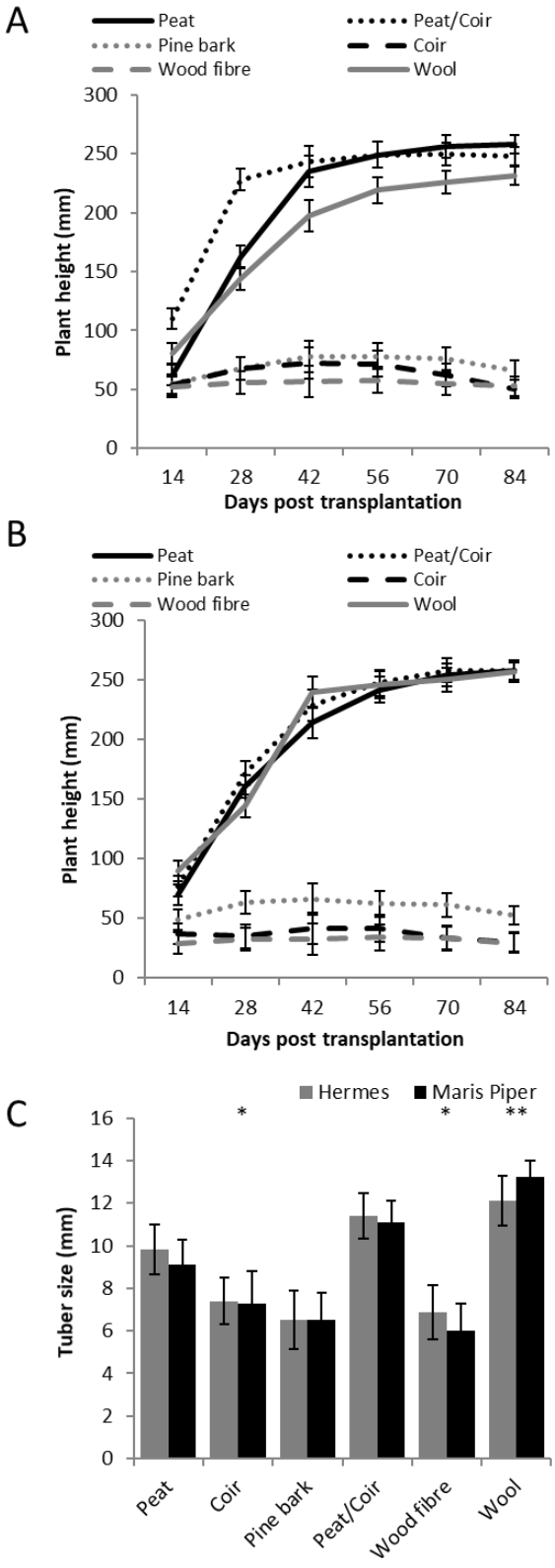

Fig. 7 Microplant development in peat and peat-free growing media. Plant height measurements for (A) cv. Hermes and (B) cv. Maris Piper and the size of mini-tubers (C) produced in each growing medium. ** = $P < 0.01$; * = $P < 0.05$ indicating a significant difference between growing media compared to peat. Peat/coir is at a 60/40 v/v blend.

Testing peat and wool compost blends using microcosm controlled environment cabinet experiments

Plants grown in peat and coir and wool compost and coir growing media blends were compared to growth in the peat-based or wool compost media. Peat or wool was blended with coir at either 60/40 (v/v) or 20/80 (v/v). No significant effects on plant height could be attributed to variety ($P = 0.783$). Growing medium ($P< 0.001$), days post transplantation ($P< 0.001$), and the interactions between days post transplantation and the variety ($P = 0.003$) or medium ($P < 0.001$), all significantly affected plant height. Despite some plants showing signs of senescence or chlorosis, plants remained healthy-looking for the duration of the experiment in the various growing media, except for those propagated in the wool/coir (20/80) blend.
At 14 days, microplants grown in all media were healthy and green which remained the case at 28 days post transplantation for all media except the wool/coir blends. In the wool/coir (60/40) blend cv. Hermes plants showed signs of senescence on some of the older leaves which was also observed in plants of both varieties grown in the wool/coir (20/80) blend which were also stunted. Although plants grown in peat or peat/coir (60/40) blend remained healthy and green at 42 days, differences in plant growth were evident in the other growing media. Plants grown in peat/coir (20/80) media had healthy green upper leaves whereas the lower leaves were paler green in colour. Plants grown in wool compost were for the most part still green and healthy looking similar to cv. Maris Piper plants grown in wool/coir (60/40) although cv. Hermes plants had paler green foliage with some older leaves having abscised. Plants of both varieties grown in the wool/coir (20/80) were pale, stunted and spindly with some signs of chlorosis visible by 42 days. Microplants grown in peat and the peat/coir (60/40) blend were still green at 56 dpt although some signs of chlorosis could be seen on some of the older leaves. However, plants in the peat/coir (20/80) were stunted, showing signs of senescence in the older leaves with the upper foliage remaining green with some signs of chlorosis at this time point. The microplants of both varieties grown in wool compost were still healthy and green at 56 dpt as were the cv plants grown in the wool/coir (60/40) blend although some plants had senescing lower leaves. Plants growing in the wool/coir (20/80) compost were stunted, spindly and chlorotic with the lower leaves senescent at 56 dpt. At 70 dpt peat-grown plants were still healthy and green as were those grown in the peat/coir (60/40), wool and wool/coir (60/40) although some plants in these peat-free or peat-reduced media were showing signs of chlorosis and senescence in the older leaves, particularly in cv. Hermes. Plants grown in peat/coir (20/80) were stunted and showed clear signs of chlorosis which was stronger in cv. Hermes whereas plants of both varieties were spindly, stunted and senescent in the wool/coir (20/80) blend. By 84 dpt the peat-grown microplants were still for the most part green and healthy looking similar to the first microcosm experiment although some cv. Hermes plants showed signs of chlorosis of the older leaves. Plants of both varieties grown in peat/coir (60/40), wool and wool/coir (60/40) were paler green than the peat grown plants with chlorosis of the older leaves. cv. Hermes plants grown in peat/coir (20/80) were chlorotic whereas cv. Maris Piper plants were pale green but the lower leaves of both varieties had abscised. Both varieties showed very poor growth in wool/coir (20/80) media at 84 dpt with plants looking spindly, stunted and most of the green leaf area had senesced (Fig. 8A and B).
There were significant differences in tuber size produced in the different growing media and between the varieties ($P < 0.001$). Overall, cv. Maris Piper tubers were larger than cv. Hermes tubers ($P < 0.001$), whereas tubers produced in the wool/coir (20/80) blend were significantly smaller than those produced in peat ($P = 0.004$). A significant interaction between variety and growing medium ($P = 0.041$) was also observed in this experiment. Mini-tubers of cv. Hermes produced in wool/coir (20/80) were significantly smaller than those grown in peat ($P = 0.001$) unlike cv. Maris Piper tubers which were of similar size in both media. cv. Maris Piper plants grown in wool compost produced larger mini-tubers compared to those from peat ($P = 0.004$) but no significant difference in tuber size was seen in cv. Hermes between these two media (Fig. 8C). None of the mini-tubers produced in any growing media showed signs indicative of surface blemish diseases.

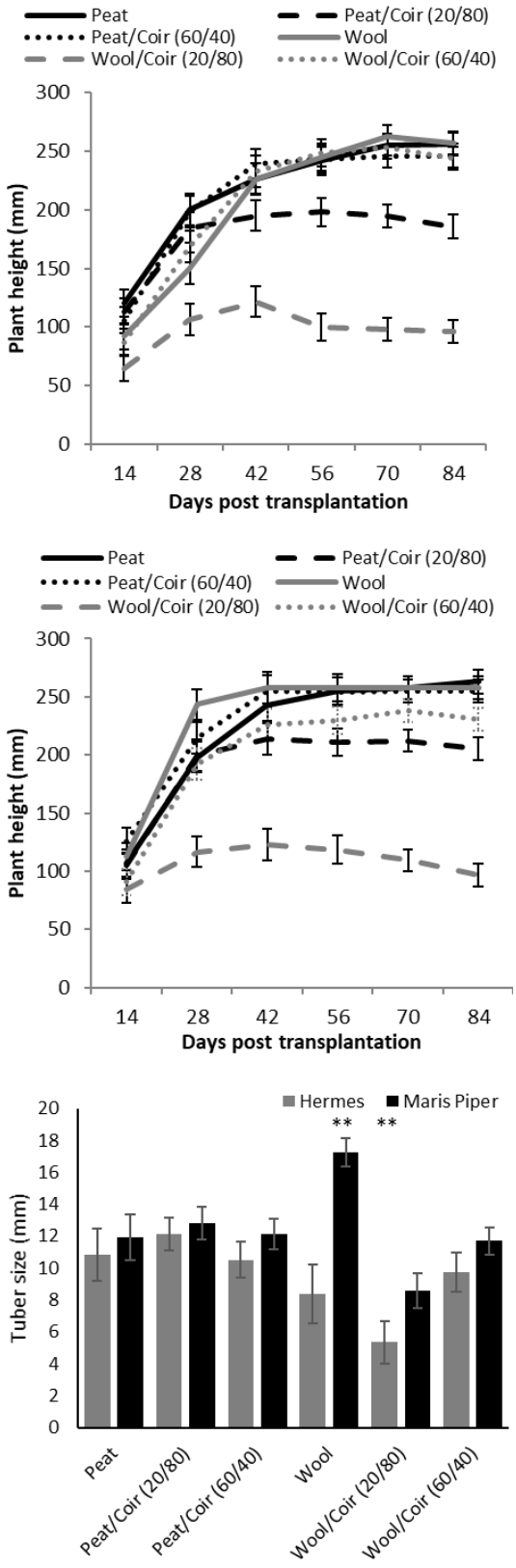

Fig. 8 Microplant development in peat, wool and blends of these two-growing media with coir. Plant height measurements for (A) cv. Hermes and (B) cv. Maris Piper and the size of mini-tubers (C) produced in each growing medium. ** indicates a significant difference ($P < 0.01$) in a specific variety between growing media compared to peat.

To further investigate the potential of blends for reducing peat content in growing media for mini-tuber production a third microcosm experiment was designed. This experiment compared two different blends of peat combined with coir and wool compost, 30/40/30 and 20/40/40 (peat/coir/wool v/v/v), with peat-based or wool compost media as sole constituents or mixed with coir (60/40 v/v). No significant effects on plant height could be attributed to variety ($P = 0.630$). Growing medium ($P= 0.02$), days post transplantation ($P< 0.001$), and the interactions between days post transplantation and medium ($P = 0.033$), and between days post transplantation, medium and variety ($P = 0.002$) all significantly affected plant height (Fig. 9A and B). All plants remained visually healthy until 42 dpt when the leaves of cv. Hermes plants grown in wool/coir (60/40) were paler green indicating early signs of chlorosis. This chlorosis in wool/coir (60/40) became more prominent by 56 dpt with many leaves in cv. Hermes having senesced. In the peat/coir/wool blends cv. Hermes plants started to show signs of senescence in the older leaves at this time point. However, cv. Maris Piper plants were still healthy and green in the 30/40/30 blend, while those plants grown in the 20/40/40 blend had paler green foliage. Plants grown in peat or wool-based composts were still healthy and green at 56 dpt. By 70 dpt plants in the wool/coir (60/40) blend were chlorotic with many leaves senesced on cv. Hermes plants. Plants grown in wool, peat/coir (60/40) or either of the peat/coir/wool blends showed some signs of chlorosis on the older leaves whereas plants grown in peat were still healthy and green. Plants grown in peat or wool compost remained green and healthy at 84 dpt. Peat/coir- (60/40) grown plants had pale green leaves with older leaves showing signs of chlorosis and senescence, whereas in the wool/coir (60/40) blend cv. Hermes plants were largely senescent at 84 dpt. This was unlike cv. Maris Piper plants which were chlorotic with only the older leaves senescing. The two peat/coir/wool blends yielded plants that were not as healthy looking as those grown in either the peat or wool compost these plants were general a paler green in colour with signs of chlorosis visible in the upper leaves.

There were no significant differences in tuber size produced in the different growing media ($P = 0.280$) and between the varieties ($P = 0.717$). However, a significant interaction *between* variety and growing medium ($P = 0.012$) was observed in this experiment. Mini-tubers of cv. Maris Piper produced in peat ($P = 0.033$) and the peat/coir/wool (30/40/30) blend ($P = 0.020$) were smaller than those of cv Hermes whereas cv. Maris Piper tubers produced in the peat/coir/wool (20/40/40) blend ($P = 0.032$) were larger than cv. Hermes tubers produced in that medium (Fig. 9C). None of the tubers showed signs of surface disease or internal rots.

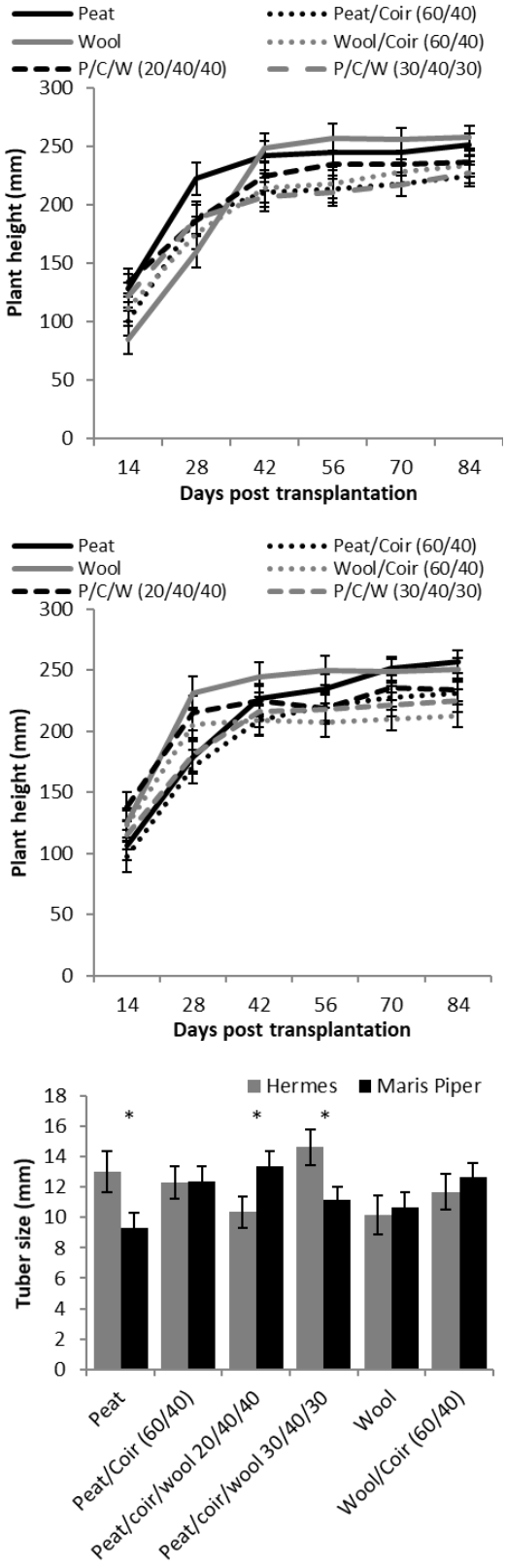

Fig. 9 Microplant development in peat, wool and blends of these two-growing media with coir. Plant height measurements for (A) cv. Hermes and (B) cv. Maris Piper and the size of mini-tubers (C) produced in each growing medium. P/C/W = Peat/coir/wool. * indicates a significant difference ($P < 0.05$) between cv. Hermes and cv. Maris Piper.

Testing peat alternative growing media under glasshouse conditions at SASA

Plant development varied between growing medium regardless of variety. Peat-grown plants were typically large and healthy looking throughout the trial with signs of chlorosis present from 15 weeks post transplantation (wpt). Plants grown in either coir or the peat/coir blend (60/40) were also large in stature similar to peat-grown plants. However, plants grown in coir or the peat/coir blend (60/40) showed earlier signs of chlorosis by 12 wpt and were senescent by 15 wpt. Plant growth in pine bark, wood fibre or wool-compost media was more variable across replicates but in general these plants were smaller than those grown in peat with some plants very stunted throughout the duration of the growing period. Plants grown in either the pine bark or wood fibre media were chlorotic by 12 wpt but those plants grown in wool compost showed no signs of chlorosis for the duration of the experiments (Table 6).

Table 6 Onset of foliar chlorosis and senescence weeks after sowing (n=4)

Growing medium	cv. Hermes	Maris Piper
Peat	15	15
Peat/Coir (60:40 v/v)	12	12
Coir	12	12
Pine bark	12	12
Wood fibre	12	12
Wool	No chlorosis	No chlorosis

Tubers produced in any of the growing media were generally quite healthy looking and showed no signs of surface blemish diseases or internal rots. Tubers harvested from pine bark or the wool compost usually had more growing medium adhered to the tuber surface than tubers harvested from the other growing media. However, this material could be easily washed off with water and the underlying skin finish of tubers produced in the pine bark or wool media was no different to those tubers produced in peat. There was no significant effect of experiment ($P=0.106$) or variety ($P=0.338$) on the number of tubers produced per pot but growing medium did have a significant effect ($P<0.001$) with all media yielding fewer tubers compared to peat except coir which yielded a higher number (Fig. 10A). Growing media also had a significant effect on total tuber yield weight per pot ($P<0.001$) with all growing media except coir yielding significantly lower than peat (Fig. 10B). Tuber yield was not affected by variety ($P=0.144$) or experiment. ($P=0.360$). Tuber size was significantly affected by experiment ($P=0.001$) with tubers produced in the second experiment larger than those in the first. Variety also had a significant effect on tuber size ($P=0.017$) with cv. Maris Piper tubers being larger than cv. Hermes tubers. Significant effects on tuber size were also observed for growing medium ($P<0.001$). Tubers produced in coir, wood fibre or wool were all significantly smaller than tubers produced in peat compost (Fig. 10C). The average tuber weight was significantly affected by experiment ($P<0.001$) with heavier tubers produced in the second experiment. There was no effect of variety on tuber weight ($P=0.936$) but growing medium did have a significant effect ($P<0.001$). Tubers produced in bark, coir, wood fibre and wool compost were significantly lighter than tubers produced in peat (Fig. 10D). No disease symptoms typical of surface blemish diseases were observed on the any of the mini-tubers produced in any growing media. qPCR analysis confirmed that mini-tubers produced in the different growing media did not contain DNA of *P. atrosepticum*, *S. scabies*, *P. pustulans*, *H. solani* and *C. coccodes*. *S. subterranea* was detected in one mini-tuber of cv. Maris Piper produced in the peat/coir blend (60/40). However, this pathogen was not detected in any of the other mini-tubers produced by the same plant. *S. subterranea* was not detected in mini-tubers produced in any of the other growing media tested.

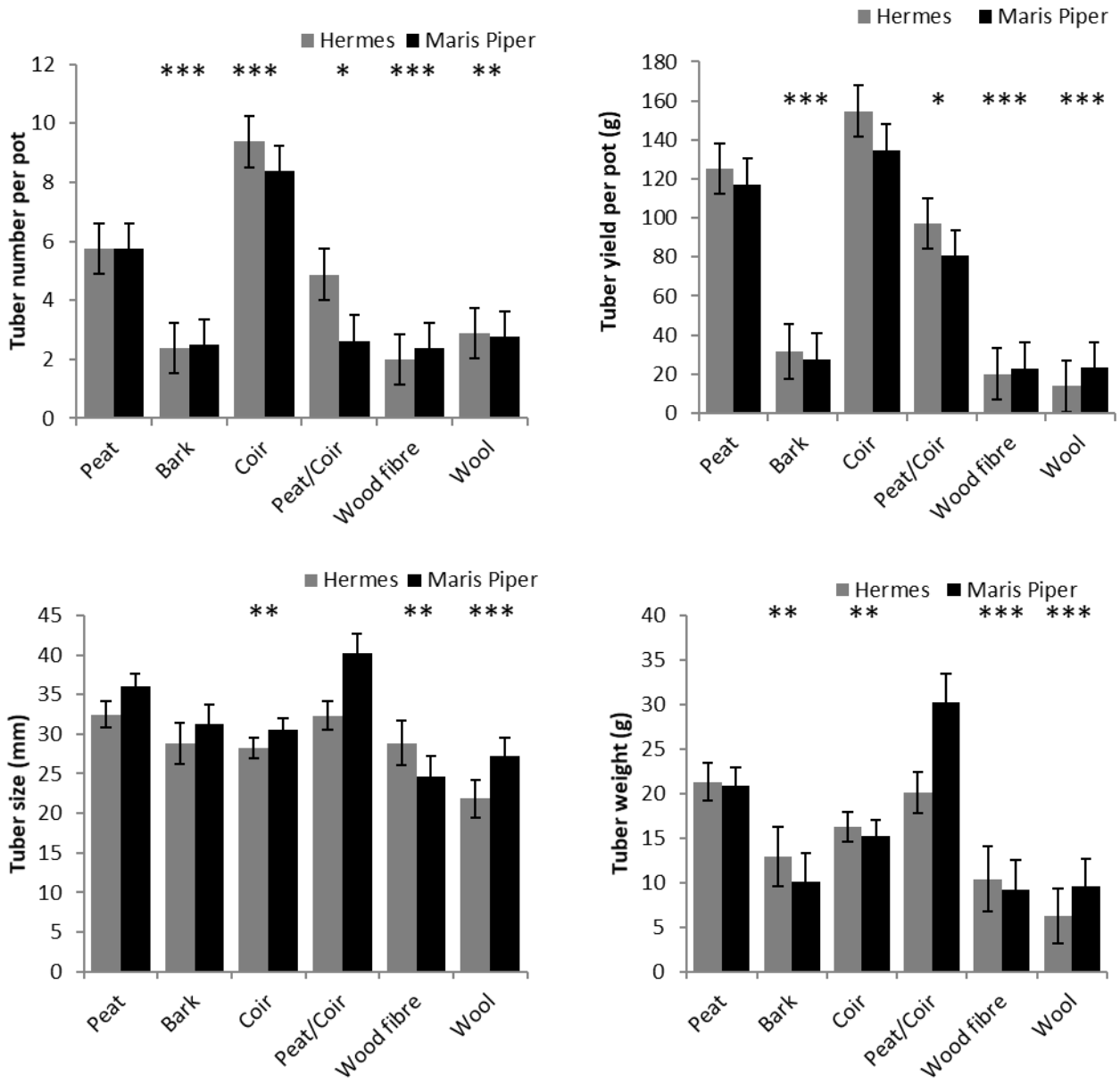

Fig. 10 Effect of different peat and peat-free growing media on tuber production under glasshouse conditions. (A) total number of tubers produced per pot; (B) total tuber yield produced per pot; (C) individual tuber size; (D) individual tuber weight. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$ indicating a significant difference in a specific variety between growing media compared to peat. Peat/coir is at a 60/40 v/v blend.

Testing peat and wool compost blends under glasshouse conditions at SASA

Plant development was very similar in peat and the two peat/coir/wool growing medium blends regardless of variety. Peat-grown plants were large and healthy looking throughout the trial with no signs of chlorosis visible on either variety. Plants of cv. Hermes grown in either of the peat/coir/wool blends showed signs of chlorosis between 12-13 wpt. Cultivar Maris Piper plants grown in the peat/coir/wool (20:40:40 v/v/v) blend showed signs of chlorosis from 13 wpt but plants grown in the 30/40/30 (v/v/v) blend did not go chlorotic during the course of the trials. Plant growth in wool-compost was more variable across replicates but in general these plants were smaller than those grown in peat. No signs of chlorosis were observed on the wool compost grown plants of either variety (Table 7).

Table 7 Onset of foliar chlorosis and senescence weeks after microplants were transplanted into growing medium (n=4)

Growing medium	cv. Hermes	Maris Piper
Peat	No chlorosis	No chlorosis
Peat/Coir/Wool (30/40/30 v/v/v)	12.57	No chlorosis
Peat/Coir/Wool (20/40/40 v/v/v)	12.63	13
Wool	No chlorosis	No chlorosis

There was no significant effect of experiment (P=0.190) or medium (P=0.160) on the number of tubers produced per pot but variety did have a significant effect (P=0.020) with more tubers produced for cv. Hermes (Fig. 11A). There was also a significant interaction between variety and growing medium (P=0.021) with cv. Hermes producing more tubers in wool than Maris Piper (P=0.017). Total tuber yield (Fig. 11B) per pot was also significantly affected by growing medium (P<0.001) with both cv. Hermes (P=0.038) and cv. Maris Piper (P<0.001) producing less total yield in wool compost whereas cv. Hermes yielded more than peat when grown in the 30/40/30 (v/v) blend (P=0.041). Tuber yield was not affected by variety (P=0.441) or experiment (P=0.937). Tuber size was significantly affected by variety (P<0.001) with tubers from cv. Maris Piper larger than those produced by cv. Hermes (Fig. 11C). Medium also had a significantly effect on tuber size with tubers of both varieties significantly smaller when produced in wool (P<0.001). Tubers produced in wool compost were significantly lighter for both varieties (P<0.001) compared to those grown in peat (Fig. 11D). Significantly heavier tubers were produced by cv. Maris Piper (P<0.001). Tubers harvested from the four growing media showed no signs of surface blemish diseases or internal rots. qPCR analysis confirmed that mini-tubers produced in the different growing media did not contain DNA of *P. atrosepticum*, *S. subterranea*, *P. pustulans*, *H. solani* or *C. coccodes*. However, *S. scabies* was detected in a single tuber of cv. Hermes grown in wool, in two tubers from two replicate plants of cv. Maris Piper grown wool and three tubers from one plant grown in peat in one of the replicate experiments.

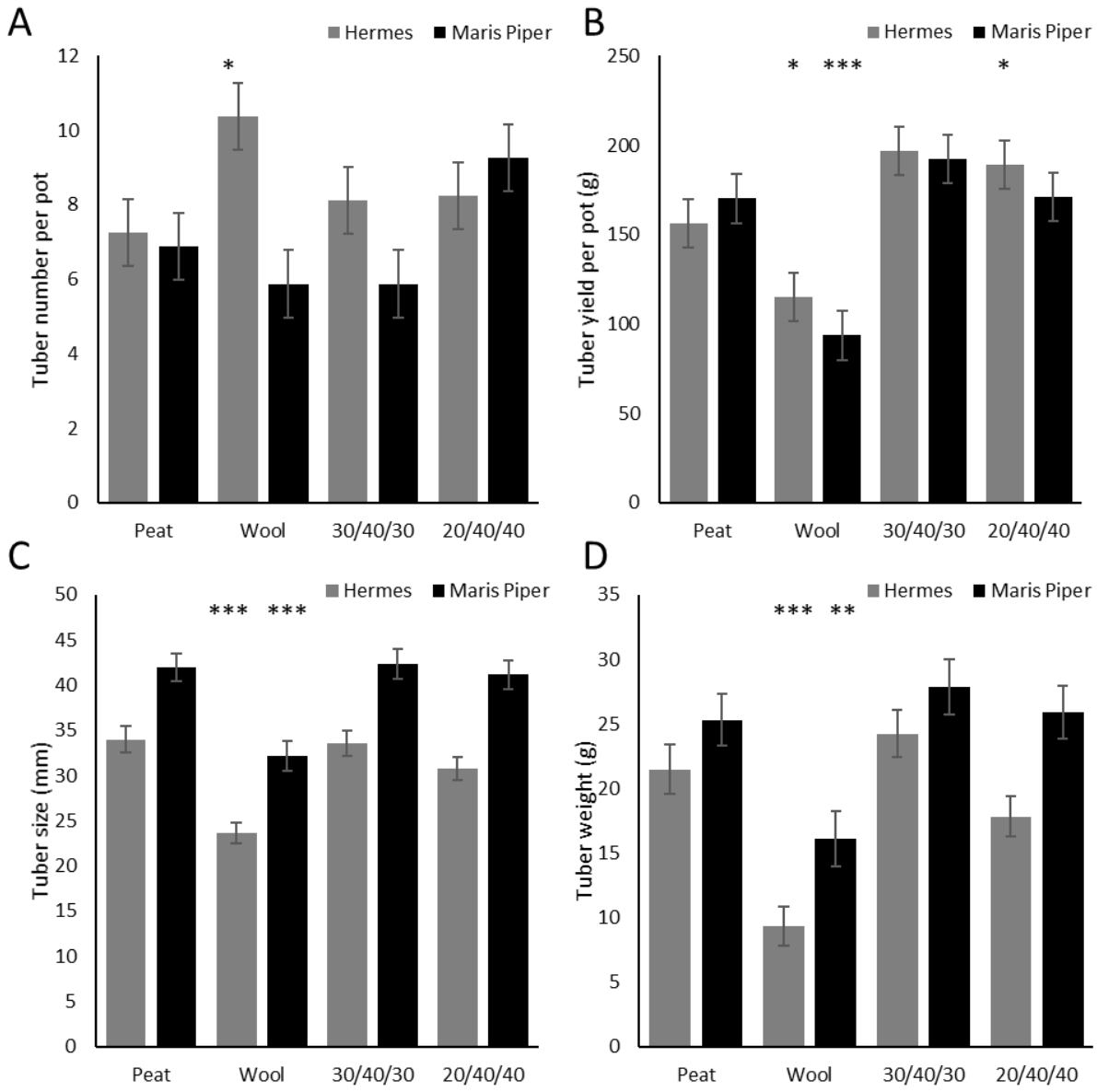

Fig. 11 Effect of different peat, wool and coir growing media blends on tuber production under glasshouse conditions. (A) total number of tubers produced per pot; (B) total tuber yield produced per pot; (C) individual tuber size; (D) individual tuber weight. 30/40/30 and 20/40/40 refer to the ratios (v/v/v) of the components in the peat/coir/wool blends. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$ indicating a significant difference in a specific variety between growing media compared to peat.

Testing peat and wool compost blends under commercial mini-tuber production conditions

The data generated from trials at site D was limited due to commercial pressures faced by the grower and have therefore not been included in the analysis. Tuber number per pot significantly differed between sites ($P<0.001$), between growing medium ($P=0.003$) but not between varieties ($P=0.988$). There were also significant effects for the interactions between site and medium ($P=0.002$) and between medium and variety ($P=0.023$). No difference in tuber number per pot was observed for cv. Hermes at Site A or B nor for cv. Maris Piper at Site C. However, cv. Maris Piper yielded significantly fewer tubers per pot compared to peat in both the 30/40/30 and 20/40/40 blends ($P<0.001$) at Site A (Fig. 12A). Total tuber yield per pot (Fig. 12B) did not significantly vary between trial sites ($P=0.108$) unlike growing medium, variety and the interactions between site and medium as well as medium and variety which all significantly affected this variable ($P<0.001$). No variation in total tuber yield per pot was observed for cv. Hermes at site A or site B. cv. Maris Piper plants yielded significantly less total tuber yield per pot in both blends compared to peat ($P<0.001$) at site A, whereas, at site C this variety yielded more weight in wool compost ($P=0.047$) but less in the 20/40/40 blend ($P=0.013$). Individual tuber size (Fig. 12C) was significantly affected by site ($P<0.001$), growing medium ($P=0.010$), variety ($P<0.001$) and the interaction between site and medium ($P<0.001$). There was no effect of the interaction between medium and variety on individual tuber size ($P=0.608$). cv. Hermes tuber size at Site A was not significantly affected by growing medium, whereas at site B cv. Hermes tubers produced in wool compost were smaller than those produced in peat ($P=0.015$). cv. Maris Piper tubers produced in wool compost at site A were smaller than those grown in peat ($P=0.001$) but not at site C ($P=0.108$). Tubers grown in the 30/40/30 ($P=0.009$) or 20/40/40 ($P<0.001$) blends were smaller than those grown in peat at site C. Individual tuber weight (Fig. 12D) was significantly affected by site ($P<0.001$), variety ($P<0.001$) and the interaction between site and medium ($P<0.001$). There was no overall effect of growing medium ($P=0.473$) or the interaction between medium and variety on individual tuber size ($P=0.563$). Tuber weight of cv. Hermes at site A or B did not significantly differ due to growing media. Contrastingly, cv. Maris Piper tuber weight was significantly lower at site A when produced in wool compost ($P=0.023$) but significantly higher in the 20/40/40 blend ($P=0.016$) when compared to peat grown tubers at that site. At site C, cv. Maris Piper tuber weight was significantly higher in the wool compost ($P=0.003$) but significantly lower in the 20/40/40 blend ($P=0.003$) when compared to tubers produced in peat.

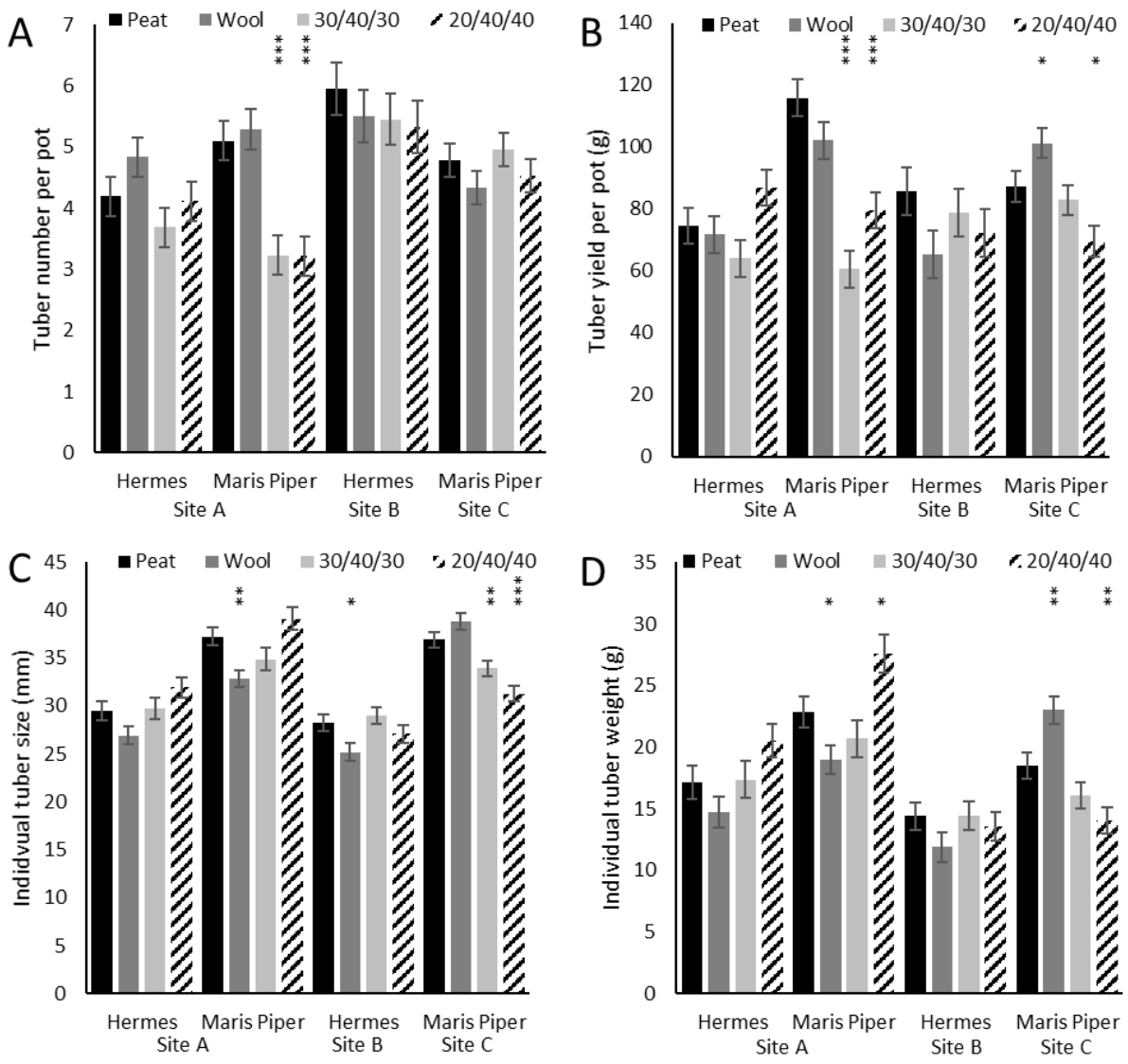

Fig. 12 Effect of different peat, wool and coir growing media blends on tuber production under commercial mini-tuber production conditions. (A) total number of tubers produced per pot; (B) total tuber yield produced per pot; (C) individual tuber size; (D) individual tuber weight. 30/40/30 and 20/40/40 refer to the ratios (v/v/v) of the components in the peat/coir/wool blends. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$ indicating a significant difference in a specific variety between growing media compared to peat.

Tubers were graded by size into four categories. Significant effects of site (P<0.001) and variety (P=0.006) were observed on the proportion of tubers in <15 mm category, whereas site (P=0.007), variety (P=0.014) and the interaction between site and growing medium (P=0.018) significantly affect the proportion of tubers in the 15-20 mm category. No significant differences in the proportion of the tubers in 20-30 mm category were observed however, site (P<0.001), medium (P=0.024), variety (P<0.001) and the interaction between site and medium all significantly affected the proportion of tubers in the >30 mm category (Fig. 13).

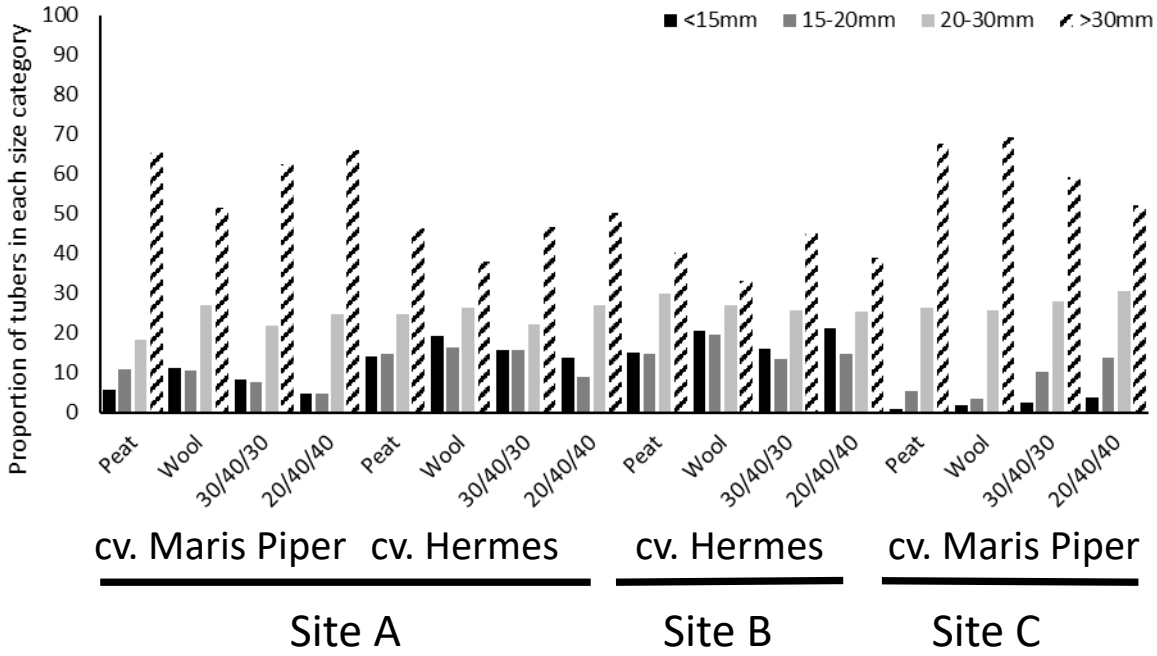

Fig. 13 Effect of different peat, wool and coir growing media blends on tuber grading proportions under commercial mini-tuber production conditions.

No signs of surface blemish diseases or rots were visible on any of the tubers produced at Site B or C. A small number of tubers (8.33%) of cv. Maris Piper grown in peat at site A displayed lesions typical of common scab (Fig. 14). Tubers produced in the other growing media at site A were disease-free. Molecular diagnostics confirmed that no pathogens were present on any of the tubers tested from Site C. *S*. *scabies* was detected in at least one tuber from each experimental replicate of cv. Maris Piper grown in peat, wool or the 30/40/30 blend from Site A. This pathogen was also detected in a single tuber from one replicate of cv. Maris Piper grown in the 20/40/40 blend. At this site *S*. *scabies* was also detected in tubers of cv. Hermes in the experimental replicates grown in wool compost but this pathogen was not detected in tubers produced in the other growing media. *P*. *pustulans* was detected in a single cv. Hermes tuber from one experimental replicate produced in the 20/40/40 blend at Site C. The pathogens *P*. *atrosepticum*, *C*. *coccodes*, *H*. *solani* and *S*. *subterranea* were not detected in tubers produced in any of the tested growing media at any trial site.

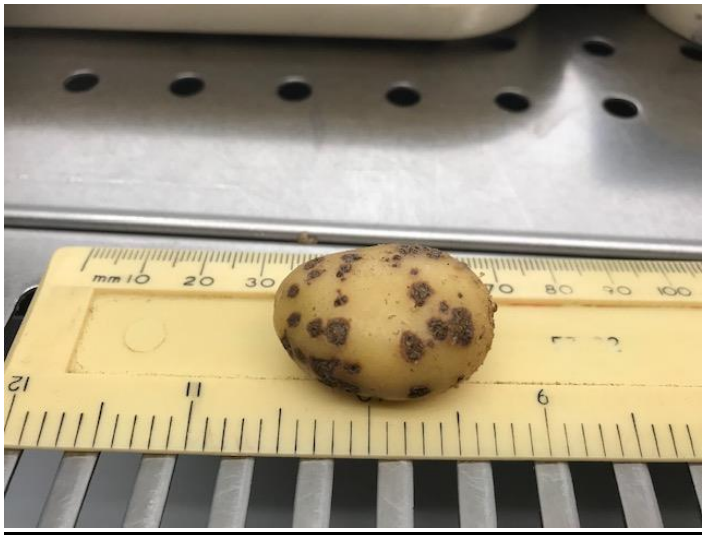

Fig. 14 Symptoms typical of common scab on cv. Maris Piper tuber grown in peat medium at trial site A

In vitro effect of *B. subtilis* on growth of potato pathogens

B. subtilis significantly inhibited growth of *B. cinerea* (P<0.001), *F. coeruleum* (Fig. 15; P<0.01), *F. culmorum* (P<0.01), *F. sulphuerum* P28 (Fig. 15; P<0.01) and *P. exigua* (P<0.001) from four days onwards and inhibited growth of *F. sulphureum* P62 (P<0.001), *P. foveata* (P<0.05) after seven days (Table 8). Radial growth of the fungal BCA *G. catenulatum* was also inhibited by *B. subtilis* from day 10 onwards (P<0.001). Growth of *H. solani* (Fig. 15; P<0.001) and *P. pustulans* P1 (P<0.05) were inhibited from day 14 onwards and *P. pustulans* P76 (P<0.001) growth was inhibited after 28 days growth (Table 9). *P. foveata* P54 growth was inhibited by *B. subtilis* from day 4 (P<0.001) whereas growth of isolates P52 and P26 was inhibited from day 7 (P<0.05). *P. eupyrena* (P70 and P77) growth was inhibited by *B. subtilis* from day 4 onwards (P<0.001). *R. solani* AG3 isolate P2 (P<0.001), P35 (P<0.001) and AG8 isolate P109 (P<0.05) cultures were significantly inhibited by *B. subtilis* from day 4 onwards whereas the AG4 isolate P106 was inhibited from day 7 (P<0.05). *C. coccodes* growth (P74 and P78) was significantly inhibited (P<0.001) from day 4 (Table 8).

Table 8 *In vitro* radial growth inhibition (%) of pathogen cultures in presence of *Bacillus subtilis*

	B. subtilis				
Day	**4**	**7**	**10**	**14**	**21**
Pathogen					
***B. cinerea* (P5)**	61.6***	73.0***	79.6***	72.8***	74.2***
***C. coccodes* (P74)**	72.0***	81.3***	86.2***	87.1***	87.7***
***C. coccodes* (P78)**	68.2***	80.5***	85.5***	87.6***	86.7***
***F. coeruleum* (P60)**	32.4***	53.6***	62.7***	71.0***	74.1***
***F. coeruleum* (P67)**	29.1**	54.0***	66.7***	72.1***	73.6***
***F. culmorum* (P13)**	80.0**	60.6***	50.4***	48.0***	44.0***
***F. sulphureum* (P28)**	30.7**	56.5***	63.5***	65.0***	61.2***
***F. sulphureum* (P62)**	30.2	52.8***	61.2***	55.7***	48.5***
***G. catenulatum* (J1446)**	25.9	25.7	45.9***	48.9***	59.9***
***P. eupyrena* (P70)**	49.5***	61.7***	70.8***	76.0***	78.4***
***P. eupyrena* (P77)**	42.7***	54.6***	63.6***	68.2***	75.6***
***P. exigua* (P53)**	46.0***	69.4***	74.5***	75.6***	72.4***
***P. exigua* (P55)**	50.9***	65.0***	70.1***	74.0***	76.8***
***P. foveata* (P26)**	31.0	43.5*	51.5***	64.8***	72.6***
***P. foveata* (P52)**	24.7	40.3*	56.8***	64.2***	75.5***
***P. foveata* (P54)**	53.5***	70.8***	79.2***	82.2***	82.0***
***R. solani* (P2)**	53.4***	71.9***	71.6***	71.5***	71.1***
***R. solani* (P35)**	57.7***	76.2***	76.2***	71.5***	71.1***
***R. solani* (P106)**	34.9	51.2*	63.9***	74.4***	73.3***
***R. solani* (P109)**	25.9*	52.7***	62.1***	57.4***	58.6***

*** = P < 0.001; ** = P < 0.01; * = P < 0.05 indicates significantly different growth compared to control plate at that time point.

In vitro effect of *A*. *migulanus* on growth of potato pathogens

Variation in pathogen growth inhibition was observed in response to the three *A*. *migulanus* isolates (Table 9 and 10). *A*. *migulanus* Nagano (P<0.001) inhibited *B*. *cinerea* growth from day 4 onwards, whereas NCTC 7096 (P<0.001) and E1 (P<0.001) were able to inhibit *B*. *cinerea* growth initially but this inhibition was no longer effective by day 21 or day 14, respectively (Table 10). *F*. *coeruleum* growth was inhibited from 10 days onwards (Fig. 15) by Nagano (P<0.001) and NCTC 7096 (P<0.001). Isolate E1 had no effect on *F*. *coeruleum* P60 growth and only inhibited growth of *F*. *coeruleum* P67 at 10 days (P<0.01). *F*. *culmorum* growth was restricted by Nagano (P<0.001) and NCTC 7096 (P<0.001) from day 7 onwards whereas E1 had no effect on the growth of this fungus. Nagano inhibited *F*. *sulphureum* P28 growth from day 4 (P<0.01) onwards and P62 from day 7 (P<0.001). NCTC 7096 inhibited growth of both *F*. *sulphureum* isolates from day 7 (P<0.001) whereas E1 only inhibited P62 at day 7 (P<0.001) but had no effect on P28 growth (Table 10; Fig. 15). Nagano and NCTC 7096 inhibited growth of both *C*. *coccodes* isolates from day 7 onwards (P<0.001). E1 only inhibited *C*. *coccodes* P74 growth at day 10 (P<0.001) and day 14 (P<0.05) and growth of isolate P78 was inhibited between day 7 to day 14 (P<0.001). Growth of *R*. *solani* AG3 isolates P2 and P35 was inhibited by Nagano (P<0.001) and NCTC 7096 from day 4 onwards (P<0.01). E1 inhibited *R*. *solani* P2 growth at day 4 (P<0.01) and day 7 (P<0.05) only but had no effect on the growth on isolate P35. *R*. *solani* AG4 isolate P106 was inhibited by Nagano (P<0.001) and NCTC7096 (P<0.05) from day 7 onwards and E1 inhibited growth of this isolate from day 10 onwards (P<0.05). Growth of *R*. *solani* AG8 isolate P109 was inhibited by Nagano and NCTC7096 (P<0.001) from day 7 onwards but E1 had no effect on radial growth of this isolate (Table 10). *P*. *foveata* P26 growth was inhibited by Nagano (P<0.001) and NCTC 7096 (P<0.001) at day 21 and by E1 from day 10 onwards (P<0.05). *P*. *foveata* P52 growth was inhibited by Nagano from day 7 onwards (P<0.05), by NCTC 7096 from day 10 onwards (P<0.01) and by E1 from day 14 onwards (P<0.05). Nagano (P<0.01) and NCTC7096 (P<0.05) significantly inhibited growth of *P*. *foveata* P54 from day 7 onwards unlike E1 which had no effect on the development of this isolate. *P*. *eupyrena* P70 was inhibited by Nagano from day 7 (P<0.05), by NCTC7096 from day 10 (P<0.001) and by E1 at day 10 only (P<0.05). Contrastingly, *P*. *eupyrena* P77 was inhibited from day 10 onwards by Nagano and NCTC7096 (P<0.001) whereas E1 significantly inhibited growth of this isolate on day 14 (P<0.05) but appeared to promote radial growth by day 21 (P<0.05). *P*. *exigua* was inhibited by Nagano from day 4 onwards (P<0.05), whereas NCTC 7096 inhibited *P*. *exigua* P53 from day 7 (P<0.01) and P55 from day 10 (P<0.001). E1 inhibited growth of *P*. *eupyrena* P53 at day 7 (P<0.001) but stimulated growth of this isolate at day 14 whereas growth of isolate P55 was significantly restricted at day 10 and 14 (P<0.05; Table 10). *G*. *catenulatum* growth was only inhibited at day 21 by Nagano (P<0.001), however NCTC 7096 did not significantly inhibit *G*. *catenulatum* growth nor did E1 which appeared to significantly stimulate growth of this fungus at day 21 (p<0.05; Table 10). *H*. *solani* P113 was inhibited after 42 days growth by Nagano (P<0.001) but no effects were observed for NCTC7096 or E1 (Fig. 15; Table 9). Contrastingly, *H*. *solani* isolate P27 growth was inhibited by Nagano from day 28 onwards (P<0.001) and by NCTC 7096 (P=0.001) and E1 (P=0.021) at day 42. No significant difference in growth of *P*. *pustulans* isolate P1 (P=0.114) or P76 (P=0.109) was observed when cultured in the presence any of the *A*. *migulanus* treatments compared to the control plates.

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Table 9 *In vitro* radial growth inhibition (%) of slower growing pathogen cultures in presence of potential biological control agents.

	***A. migulanus* Nagano**			***A. migulanus* NCTC 7096**			***A. migulanus* E1**			***B. subtilis***		
Day	**14**	**28**	**42**	**14**	**28**	**42**	**14**	**28**	**42**	**14**	**28**	**42**
Pathogen												
***P. pustulans* (P1)**	5.8	9.6	4.9	2.9	7.8	-2.7	3.9	10.2	-0.5	33.3*	46.5***	59.9***
***P. pustulans* (P76)**	6.1	12.2	6.8	4.0	5.8	5.2	6.1	5.8	6.3	28.1	44.7***	58.2***
***H. solani* (P27)**	-5.2	15.5***	26.3***	2.2	-3.1	6.5**	6.3	0	4.4*	71.5***	73.3***	77.7***
***H. solani* (P113)**	5.5	12.6	24.0***	-4.3	9.5	15.2	15.3	5	7.8	63.3***	67.6***	75.0***

*** = P < 0.001; ** = P < 0.01; * = P < 0.05 indicates significantly different growth compared to control plate at that time point.

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Table 10 *In vitro* radial growth inhibition (%) of pathogen cultures in presence of *A*. *migulanus* isolates.

	***A. migulanus* Nagano**					***A. migulanus* NCTC 7096**					***A. migulanus* E1**				
Day	**4**	**7**	**10**	**14**	**21**	**4**	**7**	**10**	**14**	**21**	**4**	**7**	**10**	**14**	**21**
Pathogen															
***B. cinerea* (P5)**	40.6***	63.1***	60.3***	51.1***	35.6***	36.9***	58.6***	48.3***	19.0***	0.0	31.1***	54.2***	31.5***	0.0	0.0
***C. coccodes* (P74)**	7.8	23.2***	42.7***	54.0***	56.9***	8.8	20.6***	38.7***	50.4***	53.9***	10.0	8.7	19.7***	5.9*	0.0
***C. coccodes* (P78)**	11.7	29.7***	47.7***	57.4***	54.8***	5.4	24.4***	41.7***	53.7***	45.7***	7.7	17.5***	14.0***	10.2***	0.0
***F. coeruleum* (P60)**	-1.4	13.3	39.4***	52.7***	60.5***	-1.4	3.9	27.8***	41.3***	31.0***	-5.5	-3.9	12.4**	-0.5	-1.9
***F. coeruleum* (P67)**	-5.6	12.3	37.0***	53.4***	61.1***	2.8	10.7	27.8***	39.3***	36.1***	-8.4	-8.2	8.4	0.0	0.2
***F. culmorum* (P13)**	19.2	40.8***	49.4***	51.0***	46.9***	15.4	38.7***	41.0***	30.5***	6.9	2.3	1.2	-4.6	-2.2	0.0
***F. sulphureum* (P28)**	19.1**	43.9***	51.2***	55.1***	53.7***	13.6	36.6***	47.3***	42.8***	9.1**	3.5	1.7	-1.1	0.0	0.0
***F. sulphureum* (P62)**	13.7	40.1***	48.3***	51.3***	48.8***	7.7	33.4***	36.7***	30.0***	4.6	1.7	14.8***	4.2	-0.6	-0.6
***G. catenulatum* (J1446)**	15.6	-3.8	4.8	9.9	29.8***	7.8	-3.1	-1.1	3.4	7.2	24.9	0.8	-4.3	-7.3	-8.3*
***P. eupyrena* (P70)**	0.0	22.8**	44.6***	56.7***	65.3***	-5.7	4.9	26.7***	43.7***	42.3***	-10.0	-0.5	10.5*	-4.9	0.0
***P. eupyrena* (P77)**	-3.1	15.4***	33.5***	47.0***	42.6***	-7.2	4.7	20.8**	31.3***	52.5***	-7.2	-3.5	7.2	-13.2*	-9.7*
***P. exigua* (P53)**	28.4**	48.8***	57.0***	57.6***	58.6***	21.3*	41.6***	48.7***	47.3***	43.4***	13.7	14.4**	-5.7	-8.6*	-1.6
***P. exigua* (P55)**	21.3*	29.2**	45.1***	51.4***	48.6***	11.2	21.1	33.6***	40.9***	44.0***	-2.6	-9.4	-11.1*	-13.6**	-6.9
***P. foveata* (P26)**	7.7	8.4	15.2	5.3	33.4***	12.5	13.7	15.2	12.6	35.2***	20.3	12.7	20.8*	18.5*	26.3***
***P. foveata* (P52)**	15.8	31.7*	43.0***	52.7***	53.7***	2.8	14.8	31.1**	37.7***	40.0***	-2.9	13.4	15.8	18.3*	19.6**
***P. foveata* (P54)**	6.6	30.1**	45.1***	49.4***	39.5***	-4.5	25.1*	39.1***	32.9***	24.4**	-7.8	17.3	1.8	0.0	-2.5
***R. solani* (P2)**	37.6***	61.0***	61.2***	59.6***	57.4***	36.4***	58.6***	57.2***	47.9***	37.4***	30.0**	11.0*	0.0	0.0	0.0
***R. solani* (P35)**	41.9***	64.1***	64.4***	62.4***	60.6***	31.0**	51.9***	47.7***	33.1***	20.0**	12.8	-0.2	0.0	0.0	0.0
***R. solani* (P106)**	32.7	46.5***	59.6***	66.5***	71.4***	0.0	26.2*	42.7***	51.9***	55.0***	-8.6	23.0	26.9**	25.0**	14.1*
***R. solani* (P109)**	18.3	47.7***	60.6***	60.0***	50.6***	7.7	44.1***	45.4***	37.4***	20.8**	0.8	-0.3	1.6	0.0	0.0

*** = P < 0.001; ** = P < 0.01; * = P < 0.05. indicates significantly different growth compared to control plate at that time point. - = negative growth inhibition resulting in increased growth of pathogen in presence of biological control agent

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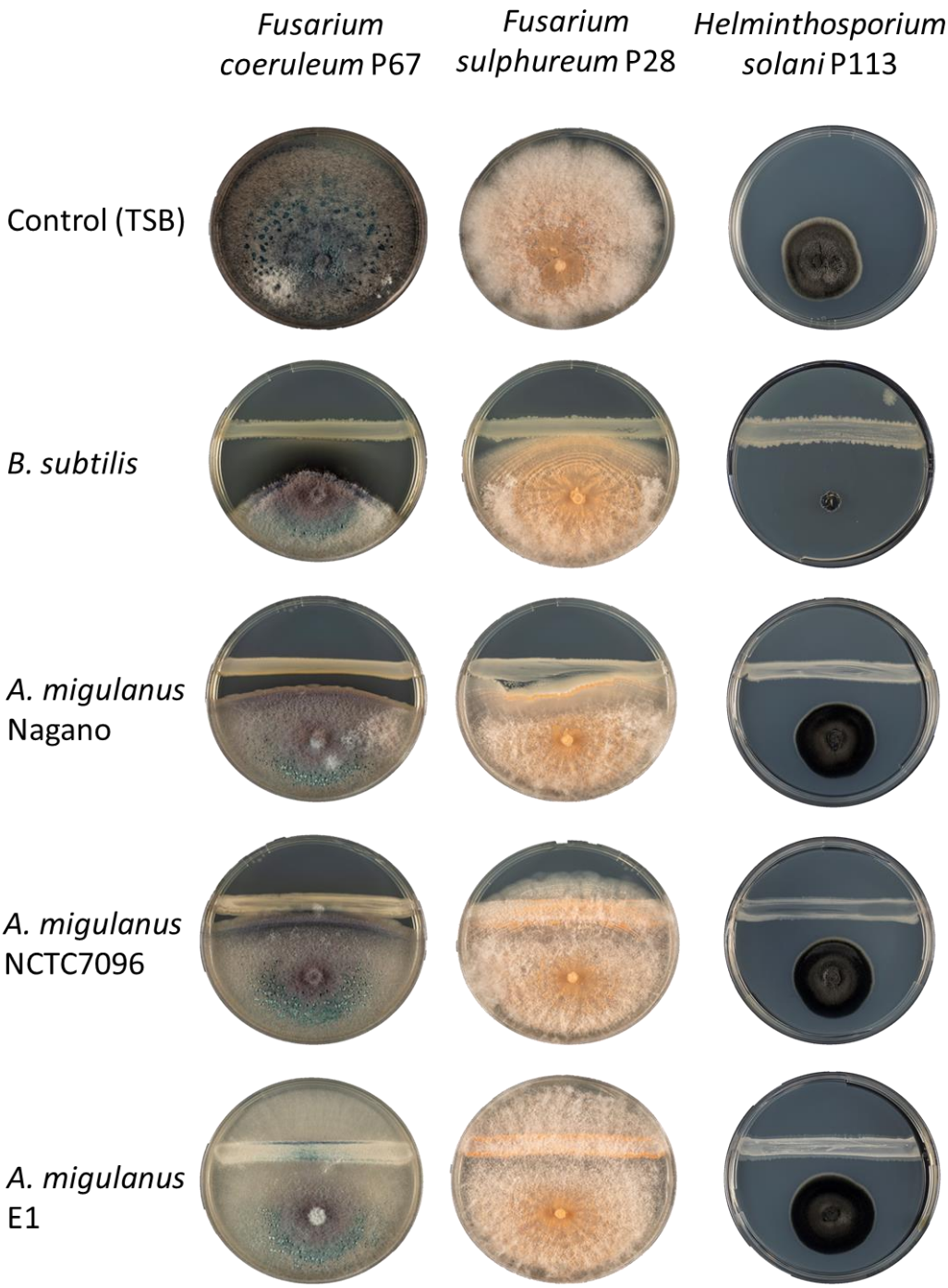

Fig. 15 Growth inhibition assays showing biological control activity of *Bacillis subtilis* and *Aneurinibacilus migulanus* against selected potato pathogens. Dual culture plates show growth of *Fusarium coeruleum* P67 and *F. sulphureum* P28 at 21 days and *Helminthosporium solani* P113 at 42 days in the presence of biological control agents or the tryptone soy broth (TSB) control.

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5. DISCUSSION

With Government focus on addressing the climate change emergency, there are moves to phase out the use of peat for horticultural use to protect peat bogs and allow restoration of these important carbon sinks. Therefore, the seed potato industry can no longer rely on the use of peat in the early stages of its production chain and must find alternatives. As the first commercially produced tubers in the potato production chain, ensuring that PBTC mini-tubers remain disease-free is critical to safeguard the industry. In the UK, PBTC mini-tubers are produced almost exclusively in peat-based growing media. Even those mini-tubers propagated using aeroponic systems require the use of peat plugs to culture the microplants prior to entry in to the aeroponic set up. Therefore, identifying peat-free growing media options that can be used to produce commercially viable disease-free mini-tubers is of the utmost importance to the seed potato industry. The research presented here shows that disease-free potato mini-tubers can be produced in a peat-free growing medium system. However, to keep production levels in line with the current commercial best practices with peat the use of peat-free or peat-reduced growing will require optimisation.

Controlled environment experiments demonstrated that potato plants could develop in peat-free and peat-reduced media and produce mini-tubers that appear free of surface blemish and rot disease symptoms and absent of pathogen DNA. However, plant development and mini-tuber size was negatively affected by some of the peat-free media. With the exception of the wool compost, potato plant growth in peat-free media without nutrient supplements result in less healthy-looking plants. In particular plants grown in coir, pine bark and wood fibre developed very poorly although mini-tubers were still formed in these media. Nutrient analysis of the raw ingredients of the peat and peat-free growing media indicated that the peat-free products have very different nutritional profiles compared to peat. The minimal nitrate levels in coir, wood fibre and pine bark would explain why plants grown in these media showed retarded growth in the microcosms. Coir is nutrient poor compared to peat, particularly with respect to nitrogen content (Scagel, 2003). However, when used as a growing medium amendment, coir can help improve the availability and uptake of other nutrients, which can improve development of some plant species (Scagel, 2003). Coir can have elevated salt levels depending on how it has been processed and these often need to be leached from the material prior to use as a growing media (Maher et al., 2008). As such coir from different sources can have highly variable levels of sodium and potassium. Wood-based media can become nitrogen deficient due to nitrogen immobilization although the addition of nutritional supplements may be able to alleviate these problems (Buamscha et al., 2008; Jackson et al., 2009). In particular, wood-fibre is usually nitrogen poor although nutrient levels will vary based on the processing method used whereas pine bark is also usually low in [i]nitrogen and can be associated with phytotoxicity caused by secondary metabolites produced by the material (Maher et al., 2008).

To take the lowered nutritional content of some of the peat-free growing media into account, the glasshouse experiment with peat-free growing media amended the wood fibre, coir and pine bark with a range of additives, tailored to each medium based on the results of the microcosm tests and nutrients analysis, to assess whether or not that would improve plant development and tuber production. Despite the additional nutrients added to the wood fibre, coir and pine bark, plants grown in these media all showed either accelerated foliar senescence or stunted growth. However, mini-tubers were still formed in all media but the yields assessed as total tuber weight, and individual tuber weight, number and size varied between the various growing media. Drake et al. (2016) showed that peat-free commercial growing media products could be used to grow the experimental model plant *Arabidopsis thaliana*. However, plants propagated in these products tended to yield less, which is likely to be an issue when trying to convince the industry to adopt peat-free growing media. Therefore, medium-specific nutrient additives for mini-tuber

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production may help facilitate the uptake of these products by commercial PBTC mini-tuber producers. The horticultural industry is currently investigating the potential of reduced-peat growing media and considering plant species-specific nutritional requirements will most likely be an essential part of that development (Mulholland, 2016).

Wool compost showed strong potential as a peat-free growing medium for mini-tuber production based on the microcosm experiments, but the tuber yields under glasshouse conditions were significantly lower than plants grown in peat. Wool-based products have shown some potential as amendments for producing various horticultural crops including tomato, pepper and aubergine (Gorecki and Gorecki, 2010). Sheep wool has high nutrient content, in particular nitrogen, and has been shown to improve plant yields for most crops tested (Böhme et al., 2012; Zheljazkov et al. 2005; Abdallah et al., 2019) when used as a fertiliser supplement. Wool decomposes slowly under glasshouse and field conditions acting as a slow release fertiliser (Zheljazkov, 2005). It was noted that in the glasshouse experiments the pots containing the wool compost were very heavy compared to the other growing media, which may suggest that the wool compost is able to retain water to a stronger degree compared to other growing media. Sheep wool pellets when used as fertilisers can rapidly absorb greater than 20 times their weight in water (Böhme et al., 2012). Using wool as a soil amendment has been previously reported to improve water holding capacity due to an increase in imbibition capacity (Mubarak et al., 2009). However, potato yields can be reduced by high soil moisture associated with poor soil aeration (Wolfe et al., 1983; Gausman et al., 1958). During the glasshouse trials, the wool compost pots appeared much wetter than the other growing media, despite being supplied with a similar watering regime, which may explain why tuber yields were particularly poor when grown in wool compost in these trials. Further analysis of the water holding capacity of the wool compost may help improve potato mini-tuber yields in this medium.

As none of the peat-free products tested in controlled environment and glasshouse experiments appear comparative to peat for the production of PBTC mini-tubers, an alternative method to reduce the use of peat in this industry could be the use of growing medium blends. Experiments showed that peat blended with coir at a 60/40 ratio produced fewer tubers than peat alone in the glasshouse experiment but the size and weight of the tubers are similar between the two growing media. These findings suggest that blends may offer a solution to reducing peat-use in mini-tuber production and aid eventual transition to peat-free growing media. Microcosm experiments showed that potato plants can grow well in a peat/coir blend of 20/80 and also in wool/coir blends of 60/40. However, the wool/coir 20/80 did not yield physiologically healthy plants and mini-tubers produced in this medium were significantly smaller. Three-way blends of peat/coir/wool showed much more promise in the controlled environment cabinet and glasshouse experiments with these growing media showing no negative effects on mini-tuber production compared to peat. This finding was further explored with site trials of the reduced peat-growing media blends at commercial premises. Despite some variation in yield performance between sites and varieties the trials of peat-free and peat-reduced growing media under commercial PBTC mini-tuber production, conditions look promising. The wool compost performed less well than peat at one site for cv. Hermes whereas the two peat/coir/wool blends appeared to be more affected by the variation in agronomic practices at the three different commercial sites. Due to the potential yield issues observed in the glasshouse experiments the blends with peat, wool and coir were developed as a way to reduce peat content in the medium without negatively affecting tuber quality. As the wool compost appears to hold water more in the glasshouse trials the addition of coir was tested to reduce the impact of water holding on tuber production. Coir has a lower capacity to hold total water (Abad et al., 2005) than peat and can be used to increase porosity in growing media. This may have helped improve any water holding capacity issues with the wool compost in the blends. Going forward, developing growing media blends may help lower the peat requirements for the mini-tuber production industry. As government policy aims to phase out peat use by professional growers by 2030 (DEFRA, 2011, 2018) the use of blends that reduce peat use in potato mini-tuber production may offer a pathway to encourage uptake of these alternatives to peat in the short term. The long-term aim could be to reduce the peat content of the blends over time resulting in a peat-free or peat minimal product for industry use by 2030. However, long-term consistency in growing media

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performance could be an issue with these blends. Coir from different geographical sources can have variable physical properties (Abad et al., 2005), which could impact on the use of this product in growing media leading to potential reliability issues for industry. Additionally, sheep wool residues can have variable effects of the physical and hydraulic properties of soil or growing media depending on the quantity of types of sheep wool residues used based on how the wool is processed prior to addition to the growing medium (Abdallah et al., 2019). If yield consistency proves to be linked to the sources of wool and how it is processed to prepare the growing medium then this may prove problematic if wool compost-based media were proposed as a replacement or substitute for peat for commercial PBTC mini-tuber production. To be able to recommend these peat-free and peat reduced growing media for mini-tuber production will require further optimisations of both the growing media and the agronomic practice required to achieve optimal plant development. Further testing of the nutritional requirements of peat-free media, such as the wool compost, and peat-reduced growing media blends will help to optimise these media specifically for PBTC mini-tuber production. Moreover, obtaining a better understanding of water management in these novel growing media should also allow improvements to made specifically tailored to producing high quality mini-tubers.

Amongst the many physical and chemical properties of peat that make it especially amendable as a growing medium, peat is generally considered to be pathogen-free (Schmilewski, 2008) which makes it highly suitable for PBTC mini-tuber seed potatoes which must be kept as free of disease as practicable. One of the key concerns with alternative growing media to peat is that these may pose a greater risk to the health of the mini-tubers which could be hugely problematic for the industry. Visual analysis of mini-tubers produced under controlled environment cabinet conditions in microcosms for surface blemish and rot diseases suggests that none of the growing media used poses a considerable risk of pathogen transfer. This was further supported by molecular diagnostics that confirmed the absence of pathogen DNA in these mini-tubers. These results suggest that the growing media themselves are unlikely to pose a risk to plant health directly, all though this cannot be totally excluded given the small sample sizes that have been tested in this study. This is promising for the industry and should not be too much of surprise considering the way some of these growing media are processed (Maher et al., 2008). Despite the absence of disease and detectable pathogen DNA in microcosm propagated mini-tubers, pathogens were detected at low incidence in the glasshouse and site trial experiments.

A single tuber of cv. Maris Piper from one plant grown in the peat/coir blend (60/40) from the glasshouse experiment undertaken at SASA was positive for *S. subterranea* DNA, whereas a small number of tubers of both varieties grown in peat or the wool compost in another of the glasshouse experiments were positive for *S. scabies* DNA. *S. scabies* was also detected in tubers from one of the commercial PBTC mini-tuber producer sites in the different growing media tested with a very small number of tubers presenting typical common scab symptoms. A single tuber at another commercial trial site was positive for *P. pustulans* DNA despite no visible skin spot symptoms. Whether or not this is due to pathogens being introduced to the system through the growing media is unclear. It is possible that pathogens are present in some growing media at very low levels which could explain why such small numbers of tested tubers were positive for pathogen DNA. However, as the controlled-cabinet experiments showed no pathogen DNA in tubers in any media tested it is more likely that the pathogens were introduced from external sources in the glasshouse and commercial site trials. For example, as *S. scabies* was detected in some tubers produced in peat, wool and the blends but as this detection was not uniform across varieties or trial sites it is difficult to ascertain whether or not this pathogen was present in the growing media or has been introduced by other means. *S. scabies* is typically soil-borne although it can be transmitted by infected seed potatoes, which is not a risk in our experimental system using *in vitro* microplants (Stead, 1999). The bacteria can be spread through water (Khatri et al., 2010) which may provide an explanation as to how *S. scabies* was detected on a small number of tubers grown in the different media at different trial sites. Infection by *S. scabies* can be negatively affected by soil water potential (Lewis, 1970) so it is plausible that variation in watering regimes at the commercial trial sites could have also impacted on host colonisation by this pathogen. What is clear is that the wool and reduced peat blends used in

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the commercial trials present no greater disease risk than peat based on the data presented here.

Even though peat-free and peat-reduced growing media do not appear to pose an increased disease threat to the plant health status of PBTC mini-tubers it will still be advantageous to improve the industries understanding of what control measures could be appropriate for maintaining mini-tuber health in case of a disease problem. Fungicides are traditionally used to control potato diseases in the field (Carnegie et al., 1990; Cayley et al., 1981; Hide et al., 1994; Hide and Cayley, 1980) but many of these products are no longer effective due to issues with pathogen insensitivity to the active ingredient (Hide et al., 1992; Carnegie and Cameron, 1992; Carnegie et al., 1994; Choiseul et al., 2007; Gachango et al., 2012; Hide and Small, 1993) and changes in legislation reducing the number of available products (Hillocks, 2012). As such there is a concerted effort across agriculture to reduce the reliance of synthetic fungicides for disease control (Lamichhane et al., 2016). The use of BCAs is often touted as a potentially acceptable method for disease control, particularly in closed environment propagation systems. Therefore, the activity of a number of bacterial BCAs were tested against a wide range of potato pathogens.

In vitro dual culture assays showed that *B subtilis*, the active ingredient of the commercial BCA Serenade® ASO, had strong antimicrobial activity against the different potato pathogens tested and *A*. *migulanus* also showed potential with control of some pathogens. *Aneurinibacillus migulanus*, though not commercially available is well studied (Edwards and Seddon, 2001; McHugh and Seddon, 2002; Seddon et al., 2007; Alenezi et al., 2016a/b) and, specifically, has shown potential in previous studies with protected solanaceous crops (McHugh and Seddon, 2002). Of the three isolates of *A*. *migulanus* tested, *A*. *migulanus* Nagano has typically showed stronger *in vitro* inhibition of fungal and oomycete pathogens than NCTC 7096 (Alenezi et al., 2016a) whereas Nagano reduces Dothistroma needle blight severity in *Pinus contorta* plants unlike NCTC 7096 which had no effect (Alenezi et al., 2016b). That observation was also noted against the potato pathogens with *A*. *migulanus* Nagano demonstrating a stronger, and in some cases earlier and longer lasting, inhibitory effect on the majority of the pathogens examined compared to NCTC 7096. *A*. *migulanus* Nagano and *A*. *migulanus* NCTC 7096 both produce the antibiotic cyclic peptide, gramicidin S, but this is unlikely to be the only mechanism by which the two isolates can inhibit pathogen growth. Genome analysis of the two strains suggests *A*. *migulanus* NCTC 7096 has a larger number of predicted secondary metabolite biosynthesis clusters implying that *A*. *migulanus* NCTC 7096 has the potential to produce a wider array of antimicrobial metabolites than *A*. *migulanus* Nagano (Alenezi et al., 2016a). However, the greater inhibitory action against fungi and oomcycete pathogens by *A*. *migulanus* Nagano has been associated with the enhanced production of gramicidin S and increased surfactant secretion by this isolate compared to *A*. *migulanus* NCTC 7096 (Alenezi et al., 2017).

Variability in the degree of control offered by different BCAs is common. A range of bacterial and fungal BCAs showed potential antagonistic activity against *R*. *solani* on potato under glasshouse conditions with much variation in disease reduction observed based on the BCA being used (Brewer and Larkin, 2005; Wilson et al., 2008). *A*. *migulanus* E1 showed much weaker inhibition of pathogen growth for the majority of species tested compared to Nagano and NCTC 7096 which is unsurprising as E1 is a mutant that does not produce gramicidin S (Iwaki et al., 1972) which has been shown to have an antimicrobial effect on fungal pathogens (Edwards and Seddon, 2001) *in vitro*. For some pathogens including *F*. *coeruleum*, *F*. *sulphureum*, *P*. *foveata* and *R*. *solani* growth inhibition was only observed for one pathogen isolate with no effect observed for the other tested isolates. This suggests that the weaker antimicrobial effect of *A*. *migulanus* E1 may be pathogen isolate-specific. Edwards and Seddon (2001) showed that *A*. *migulanus* E1 did not inhibit *B*. *cinerea* YCC conidia germination and resulted in very low levels of *in vitro* mycelia growth inhibition of this fungus compared to *A*. *migulanus* Nagano which strongly inhibited *B*. *cinerea* conidia germination and mycelia growth in a dose dependent manner. In our experiments *A*. *migulanus* E1 did inhibit *B*. *cinerea* P5. This may reflect different sensitivities to E1 between the pathogen isolates or may be caused by the higher E1 concentrations used in our experiments. We did note that *A*. *migulanus* E1 had an earlier and stronger effect on *P*. *foveata* P26 than Nagano and NCTC 7096 which was not

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observed in the interactions with the other two *P*. *foveata* (P52 and P54) with these BCA isolates, exemplifying the importance of tests with a range of pathogen isolates to determine reliability of BCAs. Such variation in control between pathogen isolates was not observed with *B*. *subtilis,* which has been previously shown to be effective against multiple pathogen isolates including different *R*. *solani* AG (Muzhinji et al., 2018) in glasshouse and field trials. *P*. *pustulans* was not inhibited by any of the *A*. *migulanus* isolates but growth was reduced in the presence of *B*. *subtilis* suggesting that this bacterium, which is commercially used as a BCA, may have a wider effective target range. *In vitro* challenge tests are the first step in determining efficacy of BCAs against target pathogens. Further tests are necessary to test *in planta* responses, especially within the growing environment, as BCAs can have a range of biocontrol properties such as biosurfactant production, a facet of *A. migulanus* isolates which was not tested in these experiments but has been shown to convey important biocontrol properties (Edwards and Seddon, 2001; McHugh and Seddon, 2002).

Potential BCAs can increase the growth of target pathogens which is sometimes dependent upon environmental conditions (Cray et al., 2016). Growth of *P*. *eupyrena* P77, *P*. *exigua* P53 and P55 and the BCA *G*. *catenulatum* was significantly increased in the presence of *A*. *migulanus* E1. The cause of this growth increase is unclear but it is reported that some bacterial metabolites can be utilised by fungi to promote growth (Hildebrandt et al., 2006). Whether *A*. *migulanus* E1 produces metabolites that promotes pathogen growth or if other mechanisms are at work here is a potentially interesting question that requires further research.

One of the major concerns about the use of BCAs in plant protection is that under field conditions the BCA is exposed to the natural environment which may result in loss of control if the local environment is not favourable to the development of the BCA. *B*. *subtilis* abundance declines significantly on plants grown in field environments compared to plants grown under protection (Wei et al., 2016) which may explain why biological control often provides variable levels of control in field grown crops. As potato mini-tubers are produced in protected facilities to minimise disease threats, and tubers are stored under controlled conditions to prolong tuber health, BCAs such as *B*. *subtilis* and *A*. *migulanus* are likely to provide more consistent disease control compared to that observed on field-grown potato crops. McHugh (2003) found that, in a polytunnel environment, *A. migulanus* populations declined less rapidly, corresponding to a recovery rate of around 10^4 – 10^5 cfu cm^{-2} immediately after spraying tomato crops and 10^2 cfu cm^{-2} 14-18 days later, meaning that populations can be maintained with a workable spray interval. With the number of fungicides currently available for post-harvest control of potato diseases limited (Cayley et al., 1981; Carnegie et al., 1990) and likely to be further reduced as pesticide regulations get updated (Hillocks, 2012) determining the value of BCAs to prevent potato losses to disease could be add an invaluable weapon to aid in the protection of potatoes. Combining BCA treatments with a range of modes of action and longevity, or using them with reduced fungicide application in an integrated control system, could aid this endeavour.

With government legislation looking to restrict the use of peat for horticultural purposes, the PBTC mini-tuber production industry requires alternative growing media to sustain current commercial production levels of disease-free mini-tubers to support the seed potato industry. The findings of this project highlight that disease-free mini-tubers can be produced in a peat-free system. However, use of peat alternatives requires further optimisation to be suitable for largescale commercial use. In particular, further work on the specific nutritional needs and watering regimes for peat-free growing media to support the production of mini-tubers is required. In the short-term, peat-reduced blends may be the way forward to start the process of reducing peat use in horticulture with the long-term goal of complete elimination. This work demonstrates that the industry could move to peat-free and peat-reduced growing media, although further research is needed to maintain current production levels.

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6. CONCLUSIONS

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- Disease-free and pathogen-free PBTC mini-tubers can be produced in peat-free growing media.

- Plant development is stunted in coir, wood fibre and pine bark growing media without additional nutrient supplements. Plants grown in wool compost or peat-reduced blends do not appear to show any serious growth retardation.

- Peat-free growing media have differential nutritional profiles compared to commercial peat growing media and it is likely that any alternative growing media would need to be optimised for nutrition to be suitable for commercial use.

- Under glasshouse conditions, tuber production in wood fibre, pine bark and wool compost was reduced compared to that achieved with peat-based media.

- Use of wool compost requires further optimisation with regards to plant watering to prevent tuber yield penalties.

- Wool compost and peat-reduced blends have strong commercial potential for producing PBTC mini-tubers

- Peat-free growing media do not pose a greater risk to the plant health of PBTC mini-tubers than peat.

- *Pectobacterium atrosepticum* was not detected in mini-tubers propagated in microcosms or in any of the growing media assayed.

- Bacterial BCAs show potential for limiting the growth of a wide range of potato pathogens in *in vitro* studies. *B*. *subtilis* shows the strongest activity against the widest range of pathogens. *A*. *migulanus* Nagano was effective against a number of pathogens.

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8. APPENDICES

If applicable, include information that supports your analysis, validates your conclusions or pursues a related point.

None

9. KNOWLEDGE EXCHANGE ACTIVITIES

Provide a complete record of any knowledge transfer activities undertaken during the reporting period (e.g. articles in technical or popular press; presentations; research papers; levy payer meetings attended). All items should be clearly dated.

PBTC Growers' Meeting October 2017:
McHugh, RC and Ormiston, N A project update was presented and a presentation given on visits to growing media producers together with a discussion around grower involvement.

Crop Protection in Northern Britain offered paper February 2018:
McHugh RC, Saubeau, G (2018) Development of new approaches for PBTC mini-tuber production. *Crop Protection in Northern Britain*.

SASA roadshows oral presentations March 2018:
McGrann GRD. Evaluation of alternative approaches for production of healthy PBTC (pre-basic tissue culture) mini-tubers

PBTC Growers' Visits October 2018:
McHugh, RC In a series of visits to growers, an update of project progress was given and grower involvement was discussed.

Nairn & District Gardening Club 2019:
McHugh, RC (2019) Potatoes: Production, Peat & Pathogens. A pre-festival event for the Nairn Book & Arts Festival.

PBTC Growers' Meeting October 2019:
McHugh, RC; McGrann and Feehan, L A project update was presented and a lab tour with a discussion on techniques used for project analysis.

Crop Protection in Northern Britain offered paper February 2020:
McHugh RC, McGrann, GRD (2020) Alternative growing media strategies for mini-tuber production. *Crop Protection in Northern Britain*.

Crop Protection in Northern Britain offered poster presentation February 2020:
Feehan L, McGrann GRD, Tsinganos A and McHugh, RC (2020) *In vitro* evaluation of two bacterial biocontrol agents against potato pathogens

Papers produced for all CPNB presentations. Several further journal papers are currently being prepared.

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